Abundance and species composition of diatoms and invertebrates in the Sedemte Ezera Cirque, Rila Mts (Southwest Bulgaria)

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Abstract. A comprehensive research into a group of four lakes in the Sedemte Ezera cirque in the southwestern divides of the Rila Mts was carried out as part of a pan-European study of mountain lakes in order to provide comparable information about the current state of the selected lakes, as well as to add more biological data. The total number of diatom taxa observed in the epilithon and the sediment samples from the survey lakes is 223. Species assemblages showed high diversity (Shannon-Weaver Index). A total of 60 taxa, belonging to 15 groups of invertebrates (orders and classes) were established in the littoral zone of the studied lakes. The alterations in the quantitative and qualitative diatom and invertebrate composition in response even to insignificant changes of the water chemistry prove how sensitive are all these species to local environmental changes and their suitability for use in monitoring the mountain freshwater ecosystems.

Key words: diatoms, invertebrates, limnology, mountain lakes, water chemistry

Introduction

The Rila Mts, the highest massif on the Balkan Peninsula (peak Mousala is 2925 m a.s.l.), are situated in Southwest Bulgaria. Compared to other mountains, the Rila Mts are remarkable for their clearly outlined block-fault tectonic structure, high ridges rising above 2600–2800 m a.s.l. and widespread alpine and subalpine relief. Most characteristic hydrographic elements are the mountain lakes and the rivers that issue from them and flow radially to the Danube and the Aegean Sea (Zyapkov & Naidenow 2000). The total number of lakes in Rila is 140 (Ivanov & al. 1964).

Some of the first and basic records about the living assemblages and biological processes in the lake ecosystems of the Rila Mts were provided by Valkanov (1932, 1938), Petkoff (1939), and Vodenitcharov (1962). The first data about pH, dissolved oxygen, carbon dioxide, alkalinity and hardness were reported by Leutelt-Kipke (1935). A lot of taxonomical studies relating to different groups of algae and invertebrates have been recently published too (Yanev 1973; Kawecka 1974, 1976; Stoichev 1996a, b; Uzunov & Varadinova 2000; Janeva 2000; Vidinova & al. 2000, and Kumanski & Popov 2000). Stoyanova & al. (1995) and Botova & al. (1996) have reported chemical data on the samples from the surface around the shore lake. The paper of Botev (2000) seems to be the first more detailed study on the vertical distribution of chemical compounds in all seven lakes in the Sedemte Ezera cirque. The latest investigations focus on some more specific questions regarding the high mountain lakes, such as the phytoplankton structure (Beshkova 2000) and zoobenthos (Stoichev 2000), lake evolution and climate change (Lotter & Hofmann 2003).

However, to our knowledge, there have been no studies related to the benthic community structure and the composition of environmental components and their seasonal changes. The aim of this paper is to provide comparable information about the current state of four selected lakes and also to add some more biological data. It reports the results obtained on diatoms, invertebrates and water chemistry in the period 2000–2001 as part of a broader pan-European study of mountain lakes: the project EMERGE (**E**uropean **M**ountain lake **E**cosystems: **R**egionalisation dia**G**nostics & socio-economic **E**valuation).

Study site

The four selected lakes (with site codes) – Okoto-RI0008, Bubreka-RI0009, Sulzata-RI0010, Bliznaka-RI0011 – are situated above the timberline (in an altitude range 2243–2535 m) and are of glacial origin (Fig. 1). Their main river basin is that of river Struma which runs into the Aegean Sea in Greece. The lakes are covered with ice in winter and their ice-free period is usually from June to the end of October. The deep lakes have autumn and spring overturns of water, a marked summer thermal stratification and an inverse one in winter (Botev 2000). The shallow lakes undergo holomixing during the ice-free period.

The main morphometric characteristics of the lake group are given in Table 1. The maximum depth of Okoto is 37.5 m and it is the deepest natural lake in Bulgaria. Lake Bubreka ranks third among the natural Bulgarian lakes with regard to water volume. The water surface area of Bliznaka lake is 9.1 10^4 m^2 and it ranks fifth in terms of this morphometric dimension.

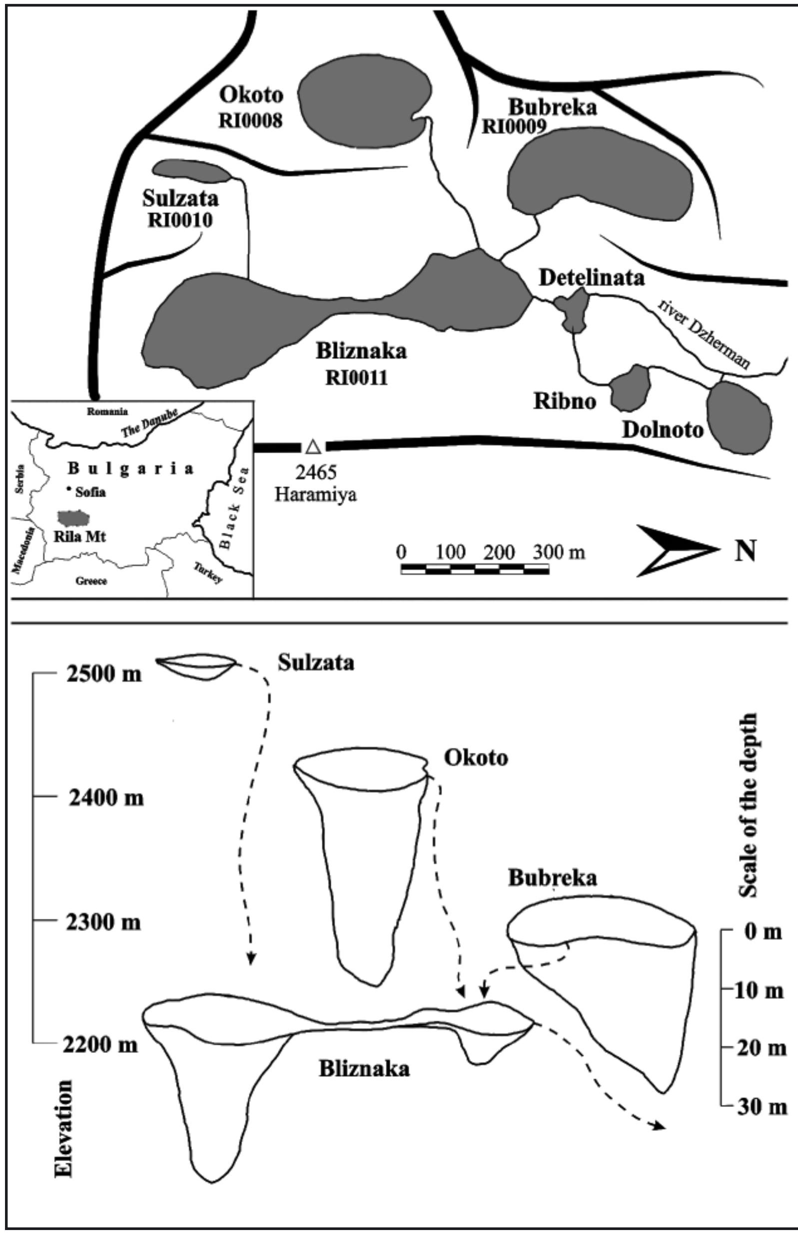

Fig. 1. Sketch map of the survey area in the Sedemte Ezera cirque, Rila Mts.

Table 1. Geography and morphometry characteristics of the Rila Mountains lakes studied.

Lake	Latitude N Degrees	Longitude E Degrees	Altitude* m a.s.l.	Catchment area* 10^5 m^2	Water surface area* 10^4 m^2	Water volume* 10^3 m^3	Maximal depth* m
Sulzata	42.19750	23.31028	2535	1.8	0.7	15	4.5
Okoto	42.19964	23.30584	2440	3.6	6.8	860	37.5
Bubreka	42.20556	23.30678	2282	5.6	8.5	1170	28.0
Bliznaka	42.20122	23.31497	2243	21.0	9.1	590	27.5

*Data according Ivanov & al. (1964)

Material and methods

Water chemistry was sampled from the surface at the deepest part of each lake in September of 2000 and in August and October of 2001, according to the EMERGE unified methods. The sampling and laboratory chemical analyses were carried out by application of the suggested procedures and methods described by Mosello & Wathne (1997) and Mosello & al. (1997).

Sampling of both living diatom communities (epilithon) and sediment diatom assemblages (top and bottom) was carried out in all surveyed lakes. A total of 48 epilithic samples (3 samples per lake) were collected in July, September 2000 and July, October 2001. Methods for epilithon sampling followed those used in the EMERGE programme described by Cameron (1997). Two sediment diatom assemblages have been analysed from the top and bottom of the short core (0–17 cm) retrieved from each survey lake. The samples were cleaned according to the standard methods described by Battarbee (1986). The cleaned diatoms were identified and counted under oil immersion at magnification of c. ×800 or ×2000, with a light microscope. A minimum of 500 valves was counted in every sample (Renberg 1990). In general, nomenclature followed Krammer & Lange-Bertalot (1986–1991) and Lange-Bertalot & Metzeltin (1996). The spectra of physicochemical tolerance of the identified diatom taxa were based mainly on Lowe (1974), Krammer & Lange-Bertalot (1986–1991) and Van Dam & al. (1994).

Qualitative benthic samples were taken three times from the littoral zones of the surveyed lakes as follows: September 2000, August and October 2001. The qualitative kick sample method was used (Frost & al. 1971), because the sampling sites were chosen to maximize habitat diversity. All samples were sieved through a collecting net with mesh size 250 μm and preserved in 70 % alcohol. Most of the invertebrate groups were identified to the level of species or the nearest possible taxa.

Besides qualitative and quantitative composition, the index of dominance and the frequency of occurrence index after De Vries (1937) have been determined too. Species diversity of the littoral diatom samples was calculated by the Shannon-Wiener Index (2 based logarithms, Shannon and Weaver 1963). The minimum-variance clustering (Ward's method) based on squared Euclidean distances has been used to group the lakes on the basis of their diatoms (epilithic, sediment) and invertebrates composition.

Results

Water chemistry

The values of chemical parameters are summarized in Table 2.

Table 2. Physico-Chemical parameters in pelagial of investigated four lakes: TDN – total dissolved nitrogen; TP – total phosphorus; DRSi – dissolved reactive silica; DOC – dissolved organic carbon.

Lake	Sulzata	Okoto	Bubreka	Bliznaka	Sulzata	Okoto	Bubreka	Bliznaka	Sulzata	Okoto	Bubreka	Bliznaka
Sampling period	09.2000	09.2000	09.2000	09.2000	08.2001	08.2001	08.2001	08.2001	10.2001	10.2001	10.2001	10.2001
Temperature (°C)	10.5	10.0	10.0	12.5	11.9	12.5	15.2	12.6	9.7	10.4	10.4	7.7
pH	6.98	7.18	6.62	6.82	6.36	6.69	6.68	6.77	6.27	6.45	6.47	6.76
Conductivity (μS/cm 25C)	35.3	25.9	25.8	32.5	20.6	23.2	23.5	28.8	20.9	24.0	24.8	31.1
NH_4-N (μgN l^{-1})	3	1	1	2	11	15	3	9	2	1	15	2
Ca^{2+} (mg l^{-1})	2.58	2.93	3.31	4.13	2.11	2.85	3.32	4.18	2.34	2.99	3.42	4.56
Mg^{2+} (mg l^{-1})	0.36	0.28	0.33	0.38	0.27	0.28	0.36	0.41	0.30	0.29	0.38	0.45
Na^{1+} (mg l^{-1})	1.81	0.85	0.63	1.09	0.62	0.58	0.40	0.49	0.83	0.52	0.41	0.51
K^{1+} (mg l^{-1})	0.60	0.63	0.31	0.47	0.48	0.62	0.32	0.46	0.45	0.70	0.33	0.48
Alkalinity (μeq l^{-1})	94.3	115.4	139.4	208.5	72.3	98.0	122.7	170.0	92.0	118.0	149.0	205.0
SO_4^{2-} (mg l^{-1})	4.46	3.78	2.95	2.99	3.18	3.46	2.81	2.87	3.55	3.58	2.83	2.98
NO_3-N (μgN l^{-1})	0	58	0	46	0	67	38	58	0	72	13	83
Cl^{1-} (mg l^{-1})	2.07	0.33	0.37	0.32	0.22	0.19	0.21	0.22	0.23	0.20	0.23	0.24
TDN (μgN l^{-1})	189	182	149	244	141	196	159	161	202	159	144	198
TP (μgP l^{-1})	17.6	7.1	14.7	7.1	4.9	1.7	1.9	2.2	7.9	1.4	2.6	2.9
DRSi (mg Si l^{-1})	0.72	1.26	0.84	1.20	0.82	1.14	0.68	0.89	0.84	1.14	0.66	0.95
DOC (mgC l^{-1})	–	–	–	–	0.91	0.74	0.56	0.52	1.17	0.33	0.56	0.48

The pH values varied between 6.27 and 7.18. The lowest values were measured by the authors in lake Sulzata in October 2001 and the highest in lake Okoto in September 2000. There has been a decrease in values during 2001 in all four lakes.

Only in lake Sulzata there was registered a change of conductivity (amounting to 14.5 µs cm^{-1}) between the first year and two months of 2001.

Alkalinity does not show any great changes between the two years for each separate lake. During the three measurements the values in lake Bliznaka were the highest, as compared to the other three lakes.

There have been no significant fluctuations between the two years of sampling in relation to Calcium, Potassium, Dissolved Reactive Silica (DRSi), Sulphate and Magnesium for each separate lake. In some lakes there were differences in the amount of Sodium, and even more significant differences in Chloride and Ammonium. For example: Chloride in lake Sulzata registered a difference of 1.85 mg l^{-1} between 2000 and 2001. The ammonium values in lakes Okoto, Sulzata and Bliznaka for August 2001 exceeded those in 2000 and in October 2001, while in lake Bubreka was registered a higher value in October 2001 than in the two other periods.

Diatoms

The total number of diatom taxa observed in the epilithon and the sediment samples from the surveyed lakes is 223. They refer to 35 genera, belonging to 15 families, six orders and the classes Centrophyceae and Pennatophyceae. Two forms could not be identified beyond the generic level, and they are listed as "sp." In terms of taxonomic diversity, the class *Pennatophyceae* prevailed (93.2 %). The family *Naviculaceae* Kütz.[1]* showed the greatest generic diversity. Twelve genera have been identified: *Navicula* Bory, *Luticola* D. Mann, *Cavinula* D. Mann & Stickle, *Sellaphora* Mereschk., *Brachysira* Kütz., *Stauroneis* Ehr., *Pinnularia* Ehr., *Caloneis* Cleve., *Diploneis* Ehr, *Neidium* Pfitzer, *Frustulia* Rabenh., and *Gyrosigma* Hassall. The most species-rich genera were *Pinnularia* (28 taxa), followed by *Eunotia* Ehr. (24), *Achnanthes* Bory (22), *Fragilaria* Lyngb. (20), *Cymbella* C. Agardh sensu lato (20), and *Navicula* Bory sensu lato (17). The greatest species diversity was identified within genus *Pinnularia*. Most of these species were well represented in almost all samples, but only some of them were dominants in the diatom association: e.g. *P. obscura* Krasske, *P. microstauron* var. *brebissonii* f. *diminuta* Grun., *P. brauniana* var. *amphicephala* (Mayer) Hust., *P. gibba*.

Identification of diatoms belonging to the genera *Achnanthes*, *Cymbella*, *Navicula*, *Pinnularia*, and *Gomphonema* followed the accepted names in Krammer & Lange-Bertalot (1986–1991). Further scanning electron microscopic studies will be necessary to revise all these forms and relate them to the newly described genera.

Biogeographical information was available for 199 (89 % of the total) taxa. The diatom flora was mainly composed of cosmopolitan species (64.3 %). Generally, 25.6 % of the taxa have been classified as "nordic-alpine" and only 10 % as boreal. As regards to general and specific habitats (Lowe 1974; Krammer & Lange-Bertalot 1986–1991; Van Dam & al. 1994), periphitic species dominated the flora; but there have been several (8.2 %) planktonic and euplanktonic species: *Aulacoseira alpigena* (Grunov.) Krammer *Cyclotella ocellata* Pant., *Cyclostephanos dubius*, *Actinocyclus normanii* (Greg.) Hust., *Asterionella formosa* Hassall, *Fragilaria ulna* Ehr., and *F. ulna* var. *danica* (Kutz.) Lange-Bert. Twenty-three taxa, "mainly occurring on wet and moist or temporarily dry places" (Van Dam & al. 1994) were found, f. e. *Eunotia bilunaris* var. *mucophila* Lange-Bert. & Norpel, *E. praerupta* var. *bigibba* (Kütz.) Grunov, *Hantzschia amphioxys*, *Luticola mutica* (Kütz.) D. Mann, *L. nivalis* (Ehr.) D. Mann and *Pinnularia obscura* Krasske. etc. *Pinnularia lata* (Bréb.) Rabenh., and *Navicula gallica* var. *perpusilla* (Grunov) Lange-Bert., found exclusively outside the water bodies, were also determined. Most of these species have lower relative abundance in the diatom association.

Among pH-indicator species, alkalophilous (37.8 %) and indifferent species (31.8 %) prevailed, while 26.9 % were acidophilous species with pH optima ranging between 5.4 and 6.9.

The total number of species found in the four investigated lakes ranged between 116 (lake Sulzata) and 154 (lake Bliznaka). Forty-two species recorded in all studied samples have had a frequency of occurrence over 50 % and appeared as constant species (Appendix 1). Some of them have not only had a very high frequency of occurrence, but also high dominance frequency, e.g. *Achnanthes minutissima*, *A. flex-*

* The authors' names are given in Appendix 1 or at the first mentioning of the diatom taxon in the text.

ella, *Cymbella minuta*., *C. cymbiformis*, *Fragilaria construens, F. construens* f. *venter,* and *Tabellaria flocculosa*. The incidental taxa (pF = 25–50 %) amounted to 57 species and varieties of diatoms, mainly belonging to the genera *Pinnularia* Ehr. (9), *Navicula* Bory (6) and *Eunotia* Ehr. (5). All the remaining taxa (124) were accidental, with a frequency of occurrence under 25 %.

According to the cluster dendrogram (Fig. 2), five main groups could be distinguished.

The first group of clusters combined all samples (epilithic and sediment) from lake Sulzata, the highest and shallowest lake in the cirque. All epilithic samples were mostly dominated by species belonging to genus *Achnanthes* (*A. minutissima, A. curtissima, A. subatomoides, A. helvetica*), as well as to genus *Cymbella* (*C. minuta* and *C. gracilis*), *Gomphonema* (*G. parvulum* and *G. clavatum*), *Fragilaria* (*F. construens* and *F. construens* f. *venter*), and *Tabellaria flocculosa*. Only in one autumn epilithic sample the highest domination frequency was due to *Diatoma mesodon*. There have been some differences in the species composition of the sediment samples. The most abundant species was the planktonic *Aulacoseira alpigena*, with subdominants *Pinnularia obscura*, *Navicula digitulus* Hust., *Cymbella naviculiformis*, etc. *Aulacoseira alpigena* was also dominant in some epilithic samples (epilithon sample 2, September 2000; July and October, 2001).

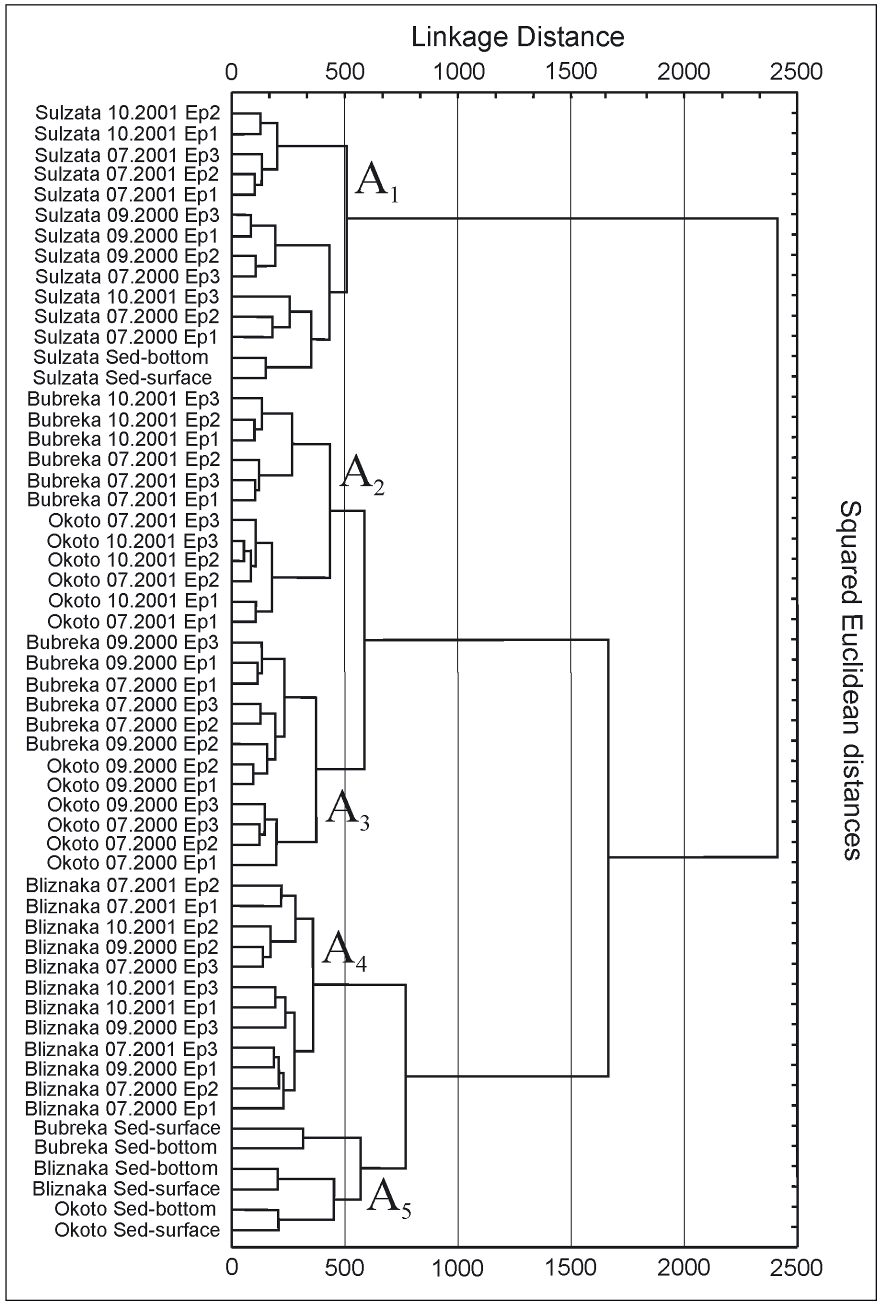

Fig. 2. Cluster analyses of all studied epilithic (Ep) and sediment (Sed) diatom samples.

Group A2 comprised all epilithic samples from lakes Okoto and Bubreka for the summer and autumn of 2001. They had a very similar taxonomical composition, which did not differ greatly. The dominant species belonged to the genera *Cymbella* (*C. minuta* and *C. cymbiformis*), *Fragilaria* (*F. construens*, *F. construens* f. *venter*, *F. tenera* and *F. ulna* var. *amphirhynchus*), *Achnanthes (A. minutissima* and *A. flexella*). *Gomphonema clavatum* was a subdominant species.

Group A3 included all other epilithic samples from lakes Okoto and Bubreka in the 2000 sampling. The most abundant species were *Cymbella minuta* and *Achnanthes minutissima*. Other important species were *Fragilaria pseudoconstruens* Marciniak, *F. ulna* var. *amphirhynchus*, *F. construens* f. *venter*, *Nitzschia palea*, *Brachysira neoexilis*, and *Tabellaria flocculosa*.

Cluster A4 comprised the most diverse assemblages from the epilithic samples of lake Bliznaka (the total number of determined diatom taxa ranged between 65 and 95). The assemblages were dominated by *Cymbella minuta*, *Fragilaria construens* and *Achnanthes minutissima*. Very important was *Brachysira neoexilis*, which was subdominant in almost all epilithic samples from this lake.

Cluster A5 consisted of all sediment samples collected from the different stratigraphic levels (top and bottom) in lakes Bubreka, Bliznaka and Okoto. Similarity among these samples was the highest, because the diatom flora in these sediments had a specific taxonomic structure. There have been determined again species of the genera *Achanthes* (*A. minutissima*, *A. curtissima*, *A. flexella* and *A. subatomoides*), *Cymbella*, and *Fragilaria*, but some other important species were: *Asterionella formosa*, *Navicula digitulus*, *Pinnularia obscura,* and *Eunotia bilunaris*.

Invertebrates

A total of 60 taxa belonging to 15 groups of invertebrates (orders and classes) were established in the littoral zone of the studied lakes (Appendix 2).

The total numbers of benthic taxa per lake were in close ranges. The richest was lake Okoto (30 taxa), followed by Sulzata (29) and Bubreka and Bliznaka (26).

The *Oligochaeta* class (14), *Chironomidae* family (*Diptera*) (8), *Nematoda* class and *Trichoptera* order (7) were most numerous both in taxa and abundance. Among these groups, only five taxa had frequency of occurrence over 50 % (*Dorylaimus stagnalis* (*Nematoda*), *Nais communis* and *Spirosperma (Peloscolex) velutinus* (*Oligochaeta*), *Limnephilidae* family (*Trichoptera*) and *Cryptochironomus* gr. *defectus* (*Chironomidae*, *Diptera*). Eleven of the established taxa had frequency of occurrence between 25–50 %, and only *Crenobia alpina* (*Turbellaria*), *Spirosperma (Peloscolex) ferox* (*Oligochaeta*) and *Corixidae* family (*Heteroptera*) were more abundant. The rest of the taxa were accidental, with frequency of occurrence under 25 % and represented by single specimens.

The cluster dendrogram (Fig. 3) shows two well distinguished lake groups. The first group is divided into two subgroups, each comprising Sulzata and Okoto respectively. Considering the differences in the species composition, some of the benthic groups were found only in these two lakes: *Turbellaria*, *Ostracoda* and *Heteroptera*. Besides the chironomides, which dominated all four lakes, more numerous and characteristic for lake Sulzata were *Crenobia alpina* (*Turbellaria*), *Tubifex tubifex* (*Oligochaeta*), *Drusus* sp. (*Trichoptera*), and the *Corixidae* family (*Heteroptera*). The last taxon was dominant in 2001, reaching over 500 individuals.

More abundant and in some cases dominating in lake Okoto were *Crenobia alpina* (*Turbellaria*), *Nais communis* (*Oligochaeta*), the *Limnephilidae* family (*Trichoptera*), and *Ostracoda*.

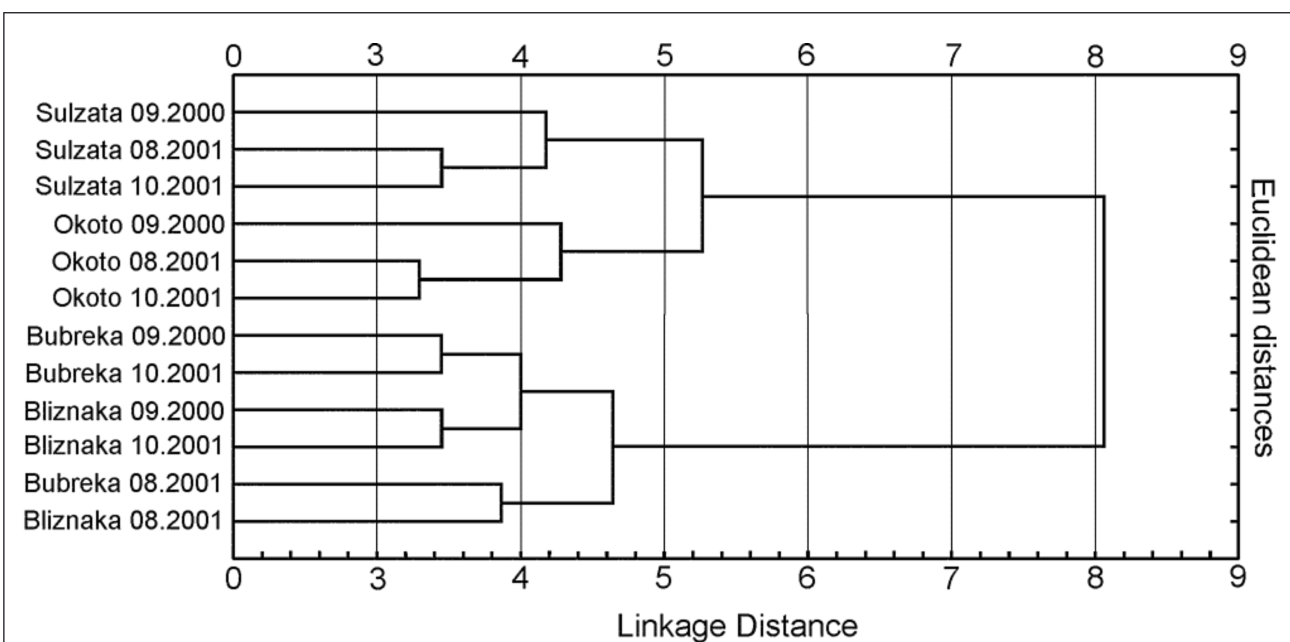

Fig. 3. Cluster analyses of all invertebrate samples.

The second group of clusters combines the data set of the other two lakes (Bubreka and Bliznaka), gathered in subclusters, more or less according to season. In total, 11 invertebrate groups have been established here, and such as *Gastropoda* and *Megaloptera* were found in these lakes only. More specific for lake Bubreka have been the oligochaets *Nais pseudobtusa* and the two species of the *Spirosperma* genus, as well as *Radix peregra* (*Gastropoda*) and the caddisfly, *Plectrocnemia conspersa* (found here only). The *Nemouridae* family (*Plecoptera*) was most abundant in October 2001. Bliznaka is a lake with the lowest number of individuals found during the study period. Except for the more abundant *Oligochaeta* and *Chironomidae* (*Diptera*), the remaining invertebrate groups were presented by single specimens.

Discussion

The results show slight variability of environmental conditions in the lakes during the two years of study (Table 2). The littoral diatom communities of the lakes were dominated by cosmopolitan, oligotraphenitic, and pH-indifferent and alkaliphilous taxa, mainly belonging to the genera *Fragilaria*, *Gomphonema*, *Cymbella* sensu lato, *Pinnularia*, and *Achnanthes*.

Cluster analyses of all samples and diatom taxa also indicate the important relationship between the environmental variables and diatom distribution. Best lake clustering results were obtained when all taxa were used (Charles 1985). When clustering was made with taxa >2% of the total count of the valves, the groups were generally similar to those produced when all taxa were used, but they were much more sensitive to different data transformation, and subgroup determination was not very good. There is only slight seasonal variability of the epilithon diatom flora, which proves a relatively stable physicochemical composition (Jones & Flower 1986). These small changes in the diatom flora have depended to a great extent on water quality (content of mineral components and pH) and on the changes in water level and conductivity. The lake Sulzata group is distinct from all others (Fig. 2). This lake is the shallowest of the surveyed lakes, of highest elevation, with the smallest catchment and surface area, and with a relatively lower pH in one autumn sample, October 2001, as compared to the other (Table 2).

The average pH value is very similar for all studied lakes (6.59–6.78). As most of the established benthic taxa tolerate pH in wider ranges (Raddum & Fjellheim 1984), it seems that a changing pH within close ranges

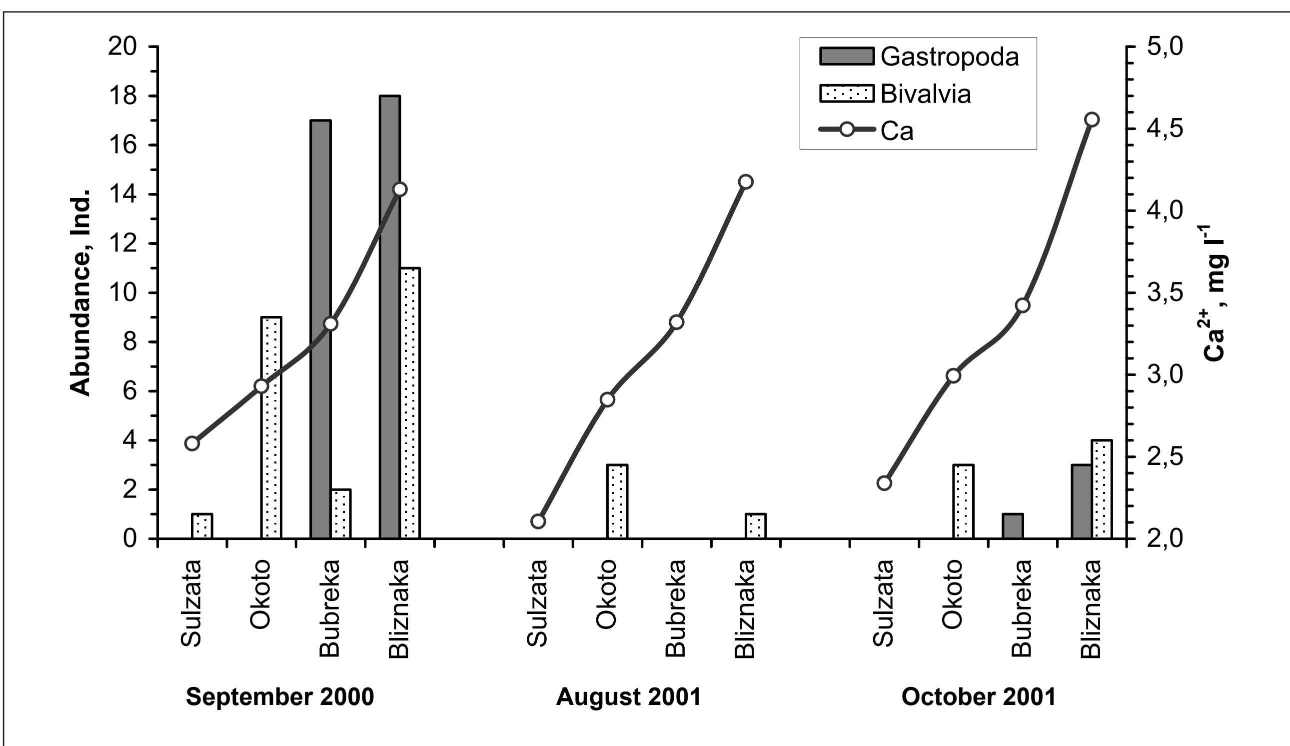

Fig. 4. Changes of the abundance of *Gastropoda* and *Bivalvia* in relation to Ca^{2+} concentration in September 2000, August and October 2001.

does not influence the invertebrate fauna significantly. Nevertheless, more sensitive to pH are the mussels of genus *Pisidium*, found in greater abundance in Okoto and Bliznaka lakes, where the values of pH were higher during our investigation.

The invertebrate fauna in clear-water localities varied considerably as a function of Ca concentration at pH > 5.5, because at higher Ca^{2+} concentrations a greater number of species of snails, mussels and mayflies are normally present (Økland & Økland 1986; Lien & al. 1996). The established taxa from *Gastropoda* and *Bivalvia* were found mainly in Okoto, Bubreka and Bliznaka lakes, where the concentrations of Ca^{2+} were higher than in lake Sulzata during the three months of investigations. The latter was well observed in autumn months, especially in September 2000, when the increased abundance of *Gastropoda* and *Bivalvia* apparently responded to the increased values of Ca^{2+} (Fig. 4).

The alkalinity values increase in reverse according to the altitude of lake locality. The alkalinity values in the four lakes have been ranging between 72.3–208.5 meq l^{-1} and, therefore, could be regarded as susceptible to episodic acidification (Camarero & al. 1995). The highest alkalinity was identified in lake Bliznaka, where the epilithon diatom flora has rich taxon diversity (Fig. 5). This environmental factor was determinant in the separation of cluster A_4 (Fig. 2). The higher values of alkalinity and conductivity do not always correspond to the higher number of invertebrate taxa.

The composition of the diatom benthic communities in the studied lakes has shown certain differences, both as regards to the number of species and to their percentage contribution. The same could be seen in relation to the various geomorphological and hydrological characteristics of the lakes. The geological characteristics of the basement are another very important factor, although the area of study was geographically limited only to the Sedemte Ezera cirque. The substrate roughness seems to be an important environmental factor influencing the species composition. The different types of substrate presume different species abundance and distribution. Stones, silt mud, gravel and sand are the main substrate types in the littoral of the studied lakes, and are represented to a different extent. The diatom distribution in lakes Okoto and Bubreka is very similar and this is also due to the stone substrate type (Fig. 2: Clusters A_2 and A_3). The diatom assemblages

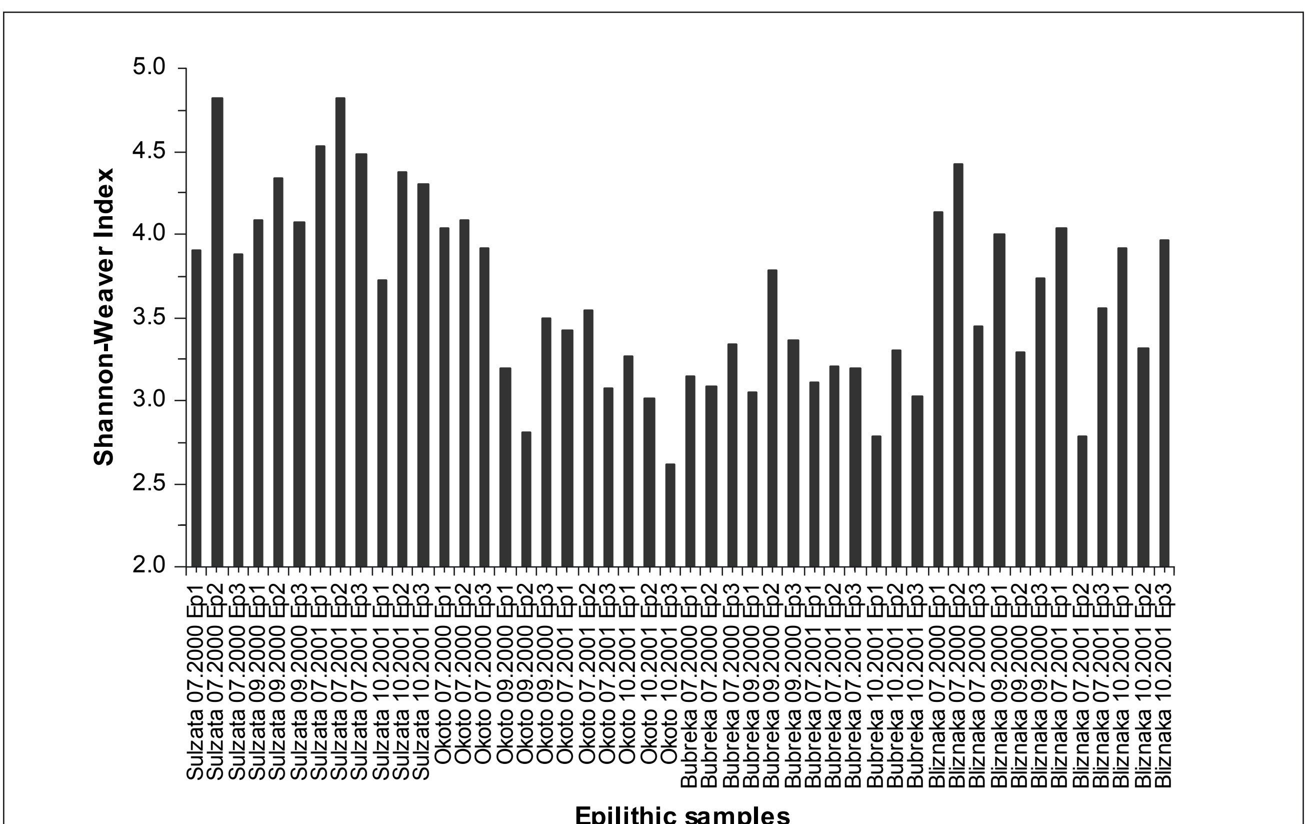

Fig. 5. The Shannon-Wiener Diversity Index of the studied epilithic diatom samples.

had relatively low species richness and were dominated by *Achnanthes minutissima*, *A. flexella*, as well as by different *Fragilaria* species: *F. construens*, *F. construens* f. *venter*, *F. tenera*, *F. ulna* var. *amphirynchus*, *F. pseudoconstruens*, and *Cymbella minuta* and *C. cymbiformis*. Lake Sulzata is characterized by a silt mud and gravel substrate type. All epilithic samples of this lake combine cluster A_1 in Fig. 2, where *Aulacoseira alpigena*, *Diatoma messodon*, *Tabellaria flocculosa*, *Cymbella gracilis*, and *C. naviculiformis* were most abundant.

Some invertebrate taxa also inhabit specific substrate types: the *Corixidae* family (*Heteroptera*), *Agabus* sp. (*Coleoptera*), *Tubifex tubifex* (*Oligochaeta*), and *Drusus* sp. (*Trichoptera*) favour silt mud and gravel (Sulzata lake); *Pisidium* sp. (*Bivalvia*), *Leuctra* sp. (*Plecoptera*) and *Cricotopus* gr. *sylvestris* (*Chironomidae*, *Diptera*) prefer stones, which were the only substrate type in lake Okoto and were presented to a greater extent in the other three lakes; *Monchystera filiformis* (*Nematoda*), *Plectrocnemia conspersa* (*Trichoptera*), and *Sialis lutaria* (*Megaloptera*) cling to a sandy substrate found out mainly in Bubreka and Bliznaka lakes. All these taxa have been more abundant or found predominantly in the respective substrate.

Conclusions

The alterations in the quantitative and qualitative diatom and invertebrate composition in response to even insignificant changes of water chemistry corroborate the sensitivity of all these species to local environmental changes and their potential for use in monitoring the mountain freshwater ecosystems.

Water chemistry parameters such as alkalinity and concentration of Ca^{2+}, which have statistically significant positive correlation ($R = 0.97$, $P < 0.0004$), are a very important factor for diatom and invertebrate composition. Another environmental factor, which influences the diatom and invertebrate species composition, seems to be the substrate roughness of the littoral of the studied lakes.

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Appendix 1: List of the epilithic diatoms with occurrence frequence more than 50 %:

Achnanthes curtissima Carter	*Diatoma mesodon* (Ehr.) Kütz.	*G. truncatum* Ehrenb.
A. flexella (Kutz.) Brun	*Eunotia bilunaris* (Ehr.) Mills	*Hantzschia amphioxys* (Ehr.) Grunov
A. minutissima Kütz	*E. glacialis* Meister	*Navicula radiosa* Kutz.
A. subatomoides (Hust.) Lange-Bert.& Archibald.	*E. implicata* Norpel, Lange-Bert. & Alles	*Neidium ampliatum* (Ehr.) Krammer
Asterionella formosa Hassall.	*E. minor* (Kütz.) Grunov	*N. bisulcatum* (Lagerst.) Cleve
Aulacoseira *granulata* (Ehr.) Simonsen.	*E. praerupta* Ehrenb.	*Nitzschia angustata* (W. Sm.) Grunov
A. valida (Grunov) Krammer.	*Fragilaria arcus* (Ehr.) Cleve.	*N. palea* (Kutz.)W. Smith
Brachysira neoexilis Lange-Bert.	*F. brevistriata* Grunov	*Pinnularia borealis* Ehrenb.
Caloneis silicula (Ehr.) Cleve.	*F. construens* (Ehr.) Grunov	*P. gibba* Ehrenb.
Cyclostephanos dubius (Fricke) Round.	*F. construens* f. *venter* (Ehr.) Hustedt	*P. microstauron* (Ehr.) Cleve
Cymbella cymbiformis C. Agardh	*F. tenera* (W. Sm.) Lange-Bert.	*Stauroneis anceps* Ehrenb.
C. gracilis (Ehr.) Kütz	*F. ulna* var. *amphirchynchus* (Ehr.) Valeva & Temniskova	*Tabellaria fenestrata* (Lyngb.) Kütz
C. minuta Hilse ex Rabenh.	*Gomphonema clavatum* Ehrenb.	*T. flocculosa* (Roth) Kütz
C. naviculiformis (Auersw.) Cleve.	*G. gracile* Ehrenb.	
C. silesiaca Bleisch.	*G. parvulum* (Kütz.) Kütz.	

Appendix 2. List of the invertebrate taxa and their abundances (number of individuals) in the years 2000 and 2001

Lake	Sulzata			Okoto			Bubreka			Bliznaka		
Taxa / Period of sampling	09 2000	08 2001	10 2001	09 2000	08 2001	10 2001	09 2000	08 2001	10 2001	09 2000	08 2001	10 2001
1	2	3	4	5	6	7	8	9	10	11	12	13
Turbellaria												
Crenobia alpina (Dana)	88	12	1	13	26	17	–	–	–	–	–	–
Nematoda												
Cylindrolaimus sp.	–	–	–	–	–	–	7	–	–	–	–	–
Dorylaimus stagnalis Dujardin	5	2	3	3	2	–	–	–	–	3	–	4
Dorylaimus montanus Stef.	1	–	–	–	–	–	–	–	–	–	–	–
Dorylaimus sp.	–	–	–	–	–	–	–	–	–	–	–	2
Monhystera filiformis (Bastian)	–	3	–	–	–	–	10	–	3	8	5	6
Monhystera sp.	–	–	–	–	–	–	–	–	2	–	–	–
Tripyla sp.	1	–	–	–	–	–	–	–	–	–	–	–
Indet.	–	–	–	–	–	–	–	4	–	–	–	–
Oligochaeta												
Lumbriculus variegatus Grube	–	–	–	–	4	–	3	6	–	2	–	2
Nais alpinus Sperber	–	–	–	–	–	–	1	–	–	53	–	–
Nais communis Piguet	102	36	–	45	9	113	1	–	24	–	–	–
Nais pseudobtusa Piguet	–	–	–	–	–	–	–	62	48	46	–	–
Lumbricillus sp.	–	–	–	–	–	–	–	3	–	–	–	–
Lumbriculus sp.	–	–	–	1	–	–	–	–	–	–	–	–
Spirosperma (*Peloscolex*) *ferox* (Eisen)	–	–	–	–	–	–	33	–	3	4	–	25
Spirosperma (*Peloscolex*) *velutinus* (Grube)	6	–	–	8	2	2	25	6	2	8	24	72
Stylodrilus sp.	–	–	–	1	–	–	–	–	–	–	–	–
Tubifex tubifex (O. F. Müller)	15	38	–	–	5	–	–	–	–	9	–	–
Lumbricidae - indet.	–	–	–	–	2	2	–	–	–	–	–	–
Lumbriculidae - indet.	–	–	–	–	–	–	–	–	1	–	–	–
Tubificidae - indet.	–	170	2	9	–	–	–	5	–	–	–	–
Enchytraeidae - indet.	5	–	–	–	–	–	–	–	–	–	–	–
Acari	2	11	1	–	18	3	–	5	1	8	5	9
Gastropoda												
Radix peregra (O. F. Müller)	–	–	–	–	–	–	17	–	1	1	–	3
Radix sp.	–	–	–	–	–	–	–	–	–	17	–	–
Bivalvia												
Pisidium spp.	1	–	–	9	3	3	2	–	–	11	1	4
Ostracoda	34	28	30	53	23	30	–	–	–	–	–	–
Ephemeroptera												
Cloeon simile Eaton	–	–	–	–	–	–	–	1	–	–	–	–
Plecoptera												
Isoperla sp.	–	–	4	7	–	1	–	–	–	–	–	–
Leuctra sp.	–	–	–	13	6	1	–	–	–	–	–	–
Nemouridae - indet.	–	–	–	–	–	–	–	–	234	3	–	–
Indet.	–	–	–	–	–	–	–	–	–	–	–	10
Heteroptera												
Corixidae - indet.	41	500	266	–	4	–	–	–	–	–	–	–
Indet.	–	–	–	–	–	1	–	–	–	–	–	–
Coleoptera												
Agabus sp.	3	–	8	–	–	–	–	–	–	–	1	–
Platambus maculatus (Linnaeus)	–	–	–	–	–	–	–	–	–	13	–	6
Dytiscidae - indet.	7	20	–	–	–	–	–	2	–	–	3	–
Indet.	3	16	7	–	–	–	–	–	–	1	–	–
Megaloptera												

1	2	3	4	5	6	7	8	9	10	11	12	13
Sialis lutaria Linnaeus	–	–	–	–	–	–	8	–	–	–	3	1
Trichoptera												
Chaetopteryx bosniaca Marinkovic	–	–	–	–	–	–	1	–	–	–	–	–
Drusus discolor (Rambur)	–	1	2	–	2	–	–	–	–	–	–	–
Drusus sp.	85	–	–	6	–	–	–	–	–	–	–	–
Limnephilus sp.	3	1	–	–	–	1	–	–	–	–	–	–
Plectrocnemia conspersa Curtis	–	–	–	–	–	–	17	–	12	–	–	–
Rhyacophila gr. *tristis*	–	–	–	–	–	3	–	–	1	–	–	–
Limnephilidae - indet.	–	5	6	37	71	48	1	3	1	9	1	–
Diptera-Chironomidae												
Chironomus sp.	–	1	–	–	–	–	–	–	–	–	–	–
Cricotopus gr. *algarum*	–	5	–	–	5	18	–	–	–	–	–	–
Cricotopus sylvestris (Fabricius)	–	–	–	100	–	–	–	–	–	–	21	–
Cricotopus gr. *sylvestris*	–	–	–	–	10	11	–	–	–	–	–	–
Cryptochironomus defectus Kieffer	60	–	–	150	–	–	–	–	–	–	–	–
Cryptochironomus gr. *defectus*	10	95	42	–	71	2	300	–	67	150	18	47
Psectrocladius simulans (Johannsen)	–	–	–	–	3	–	–	–	–	–	–	–
Indet.	–	–	–	–	–	–	–	200	–	–	–	–
Diptera – other												
Bezzia sp.	–	2	–	–	1	–	–	–	–	–	2	3
Dicranota spp.	–	–	–	1	11	1	–	–	–	–	–	–
Limoniidae - indet.	–	1	–	–	–	–	–	–	–	–	–	1
Muscidae - indet.	–	1	–	–	–	–	–	–	–	–	–	–
Indet.	–	–	–	–	–	–	–	–	–	–	1	–
Total number of taxa by sample	**19**	**20**	**12**	**16**	**20**	**17**	**14**	**11**	**14**	**17**	**12**	**15**
Total number of taxa by lake		**29**			**30**			**26**			**26**	

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