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Relations between Benthic Diatom Community and Characteristics of Karst Ponds in the Alpine Region of Slovenia

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Abstract: The aim of this research was to investigate the structure of the benthic diatom community and its relations to selected environmental parameters. We collected samples in 16 karst ponds in the alpine region of Slovenia, where the Alpine karst is found. Since the predominating substrate in these ponds was clay, the epipelic community was analyzed. Hydromorphological characteristics, and physical and chemical conditions were also measured at each site. We found 105 species of diatoms, which belonged to 32 genera. The most frequent taxa were *Gomphonema parvulum* (Kützing) Kützing, *Navicula cryptocephala* Kützing, *Sellaphora pupula* (Kützing) Mereschkowsky (species group) and *Achnanthidium pyrenaicum* (Hustedt) Kobayasi. The pond with the lowest diversity was found at the highest altitude, while, on the other hand, the most species-rich pond was found at the lowest altitude. Regarding the ecological types, the most common were motile species. We confirmed a positive correlation between the number of diatom species and the saturation of water with oxygen, while correlation between species richness and NH_4-N was negative. The content of NO_3-N and NH_4-N explained almost 20% of the total variability of diatom community. Unlike our expectations, we calculated a negative correlation between the diversity of macroinvertebrates and diatoms, which is probably a consequence of different responses to environmental conditions.

Keywords: epipelon; diatoms; karst ponds; wetlands; southeast Alps

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1. Introduction

Ponds are water bodies ranging from 1 m^2 to 2 hectares, of natural or anthropogenic origin, with permanent or seasonal water [1]. Researchers used to treat them as lakes, but ponds differ from lakes due to several characteristics [2]: (a) smaller surface area and depth, (b) smaller ratio between the volume of water and the shore area, and therefore more direct contact with the terrestrial environment making them more susceptible to various influences; (c) smaller drainage basin and therefore bigger isolation [1]; (d) relatively small volume and water intake, which increases the connection between the sediments and water column and a more significant impact of sediment on the nutrient content in water, (e) due to the low water depth, the surface of the entire waterbody could be covered with macrophytes [3,4]. This is also the main reason why we consider ponds as a type of wetland. It is characteristic that their conditions change faster than in larger water bodies [5], which is reflected in large daily and seasonal fluctuations [1,5].

Ponds as a habitat have been neglected in ecological studies [6]. Today, we recognize them as an important carbon sink, pollution filter, and source of biodiversity, hosting several specialized and rare species [2,6]. For organisms living in the aquatic environment, ponds are refuges in degraded and inhospitable areas [1,7].

Karst ponds were made in areas with no surface water bodies (e.g., Karst), where people had problems with water supply [8]. Although they were used to water livestock and gardens, they lost their importance when water pipelines were constructed. However, today they represent an important source of biodiversity, like all other types of

ponds [1,7,9,10]. Smol and Stoermer [11] suggest that Karstic aquatic habitats are the most interesting environments in which to study algae, especially diatoms.

With their distribution, they form a network of aquatic ecosystems, which increases γ diversity [1,8]. Biodiversity and abundance of the biota in Alpine ponds significantly correlate with altitude—with it, the average air temperature decreases, the amount of local precipitation increases, and UV radiation is more intense. In addition, the organisms in these environments face high daily and annual temperature differences and have a short period suitable for growth, which gives cold stenothermic species a better chance of survival [12–14].

The substrate consisting of clay and silt mostly covers the entire bottom of these ponds. On such a substrate, an epipelic biofilm develops, which is dominated by diatoms constituting the basal trophic levels for extensive food webs [15]. Diatoms are present in different aquatic environments and their sensitivity to various environmental factors, makes them a good bioindicator of water quality [16]. Recent studies have highlighted the high level of cryptic diversity of diatoms [17]. The diatom community is influenced by several factors such as water chemistry (pH, nutrient concentration, and organic load), physical (electrical conductivity, temperature, light) hydromorphological characteristics (substrate, water regime), and biotic pressures such as grazing, competition, and parasitism [17–20].

Benthic diatoms are important primary producers in shallow waters where light penetrates to the bottom [21]. On a fine substrate, a specific epipelic diatom community usually forms, which is adapted to low light conditions, consisting mainly of motile taxa that can move through interstitial waters to avoid newly deposited sediments [22]. Due to their location between substrate and water, they play a fundamental role in various biogeochemical cycles and dynamics of aquatic ecosystems [23].

The biological characteristics of diatoms, such as cell size class and ecological types, give us information about the structure of the community [17,24], as well as environmental conditions. Low-profile diatoms are well adapted to physical disturbances and are more abundant in waters with low nutrient content [17,24,25]. For high-profile diatoms, the formation of colonies allows exploiting nutrients that are not available to other groups but are therefore more exposed to grazing [24,25]. Motile diatoms are fast-growing species. Their abundance increases with a higher concentration of nutrients and organic load. They are also well adapted to high physical disorders [24]. Planktic species are present in lentic water, where they float in the water column [25], but due to sinking they can also be abundant in phytobenthos [26].

Despite their import roles, karst ponds are disappearing due to the abandonment of their original use. In addition to natural processes such as overgrowing with plants, they are also threatened by anthropogenic factors, especially intensification of agriculture, abandonment of livestock farming, backfilling, the input of non-native species and chemical pollution [1–3]. Pollutants cannot be sufficiently diluted [27], and nutrients are retained and potentially recycled by internal processes, which is difficult in the affected ecosystem [4]. All this can be significantly reflected in the structure of the diatom community.

However, we have not found any published work on the epipelic diatom community in karst ponds. Even the studies of periphytic diatom communities in ponds are rare, which had been discovered by Šumberova et al. [28]. In central Europe, we have found one paper about epipelic diatoms in ponds [29], while in southern Europe there are some papers that analyze epipelic diatoms (e.g., [16,30–33]).

We measured physical, hydromorphological, and chemical factors in 16 ponds at various locations in the Alpine region and sampled the epipelon. In this paper, we focused primarily on their response to various environmental characteristics. The study aimed to determine the species composition of the benthic diatom community in the Alpine karst ponds, determine the relationships between the structure of diatom community and the studied parameters, and find out the significant correlation between them.

We hypothesized that: (a) the diatom's species diversity correlates with the diversity of macroinvertebrates; (b) the diversity of species will decline with altitude and declining

of ponds size; (c) the species composition will be significantly affected by the pH and electrical conductivity of the water and the land use in the drainage basin.

2. Materials and Methods

2.1. Study Sites and Sampling

We chose 16 karst ponds in the alpine region of Slovenia, which is a part of the South—Eastern calcareous Alps. Since limestone and dolomite are predominating rocks in this area, the Alpine karst is found there [34]. These water bodies are found in the area of the Julian Alps (Pokljuka, Jelovica, Ratitovec) and the Kamnik Alps (Krvavec, Velika planina, and Menina) (Figure 1). During the sample preparation we realized, that there were almost no frustules in samples from four ponds.

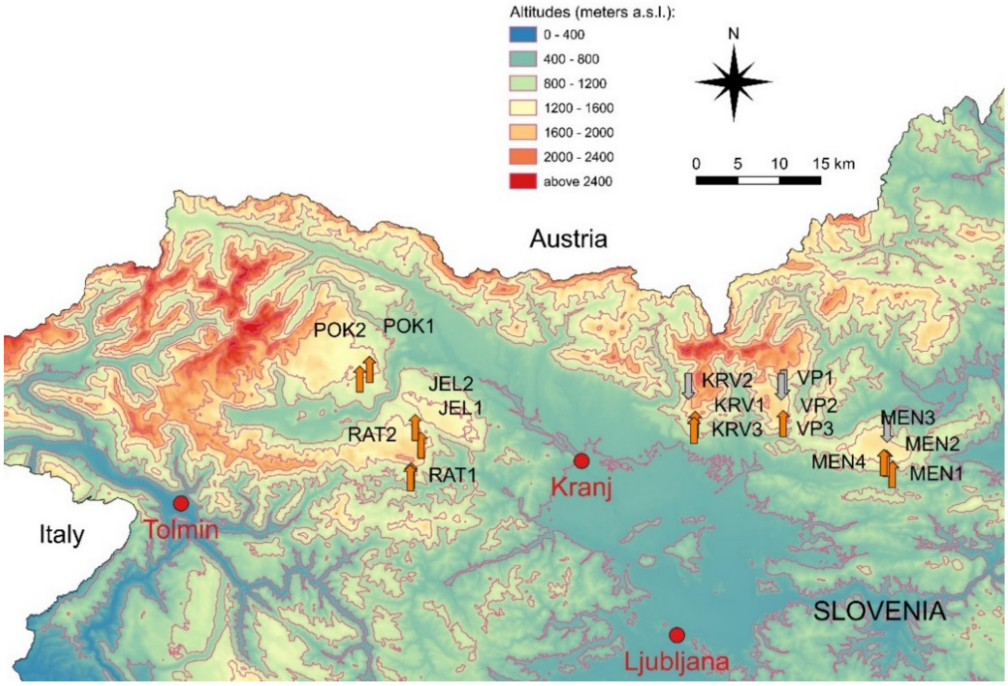

Figure 1. Map of sampled karst ponds. The arrow-tips indicate the localities of the studied ponds. Gray arrows represent ponds where samples contained low number of frustules. POK1, POK2—Pokljuka; JEL1, JEL2—Jelovica; RAT1, RAT2—Ratitovec; KRV1, KRV2, KRV3—Krvavec; VP1, VP2, VP3—Velika planina; MEN1, MEN2, MEN3, MEN4—Menina.

Mountain climate prevails in the area, where the average temperature of the coldest month is lower than −3 °C, and the average temperature of the warmest month depends on the altitude and location [35]. Macrophyte and macroinvertebrate communities were studied before in the same ponds and results were published in Zelnik et al. [10].

Sampling took place in August of 2016, during the peak pasture season. Argilal and clay, respectively, was the only type of substrate present in all sites, so we decided to sample epipelon. Since we experienced difficulties with cleaning the samples from four ponds as well as very poor presence of diatom frustules in them, the samples from 12 sites were studied only (Table 1).

Basic physical and chemical factors were measured with a portable multimeter (EUTECH, PCD 650). For each pond, we measured the pH and T of water (°C), electrical conductivity (µS/cm), total dissolved solids (mg/L), saturation with O_2 (%), and O_2 concentration (mg/L). For laboratory analyzes, a water sample (1 L) was taken at each site.

In the laboratory, the concentrations of NO_3-N (LCK 339), NH_4-N (LCK 304), TN (LCK 138), and orthophosphates (LCK 349) were determined using HACH Lange cuvette tests. Values were measured in individual samples with a HACH Lange LT 200 spectropho-

tometer. Dry mass and total suspended solids content (TSS) were determined by filtration and drying at 105 °C.

Table 1. Information about sampling sites.

Code	Karst Pond	Altitude [m]	Gauß-Krüger Coordinates		Precipitation per Year [mm]
			Y	X	
POK1	Pokljuka 1	1201	425202	134889	2200
POK2	Pokljuka 2	1302	424023	133737	2200
JEL1	Jelovica 1	1129	431399	125787	1900
JEL2	Jelovica 2	1138	430695	127923	1900
KRV1	Krvavec 1	1724	464378	128300	1650
	Krvavec 2	1509	463564	128355	1600
KRV3	Krvavec 3	1445	464227	127589	1600
RAT1	Ratitovec 1	1577	430192	122130	2100
RAT2	Ratitovec 2	1620	430104	121849	2100
	Velika planina 1	1434	475035	128689	1700
	Velika planina 2	1481	474750	128275	1700
VEL3	Velika planina 3	1454	474958	128408	1700
MEN1	Menina 1	1318	488084	122280	1250
MEN2	Menina 2	1403	487335	123639	1500
	Menina 3	1360	487473	123194	1500
MEN4	Menina 4	1419	487053	123695	1500

2.2. Biotic Analyses

Due to the absence of a firm substrate, diatom samples were taken from the surface of the loamy substrate. We scraped the top layer of argilal with an area of approximately 2 cm^2, with a spoon, at a 20–25 cm water depth. The samples were placed into bottles and 37% formaldehyde was added for fixation, in a ratio of 1:9.

Each sample was first homogenized with magnetic stirrer at a rate of 1200 rpm. We put 2 mL of the sample into a test tube and added 2.5 mL of 65% nitric (V) acid (HNO_3). The samples were heated over a fire until the smoke turned white to remove organic matter from the sample. After cooling the tube contents were centrifuged with a SIGMA 2-16PK centrifuge, 4 min at 4000 rpm, and the supernatant was discarded. The sample was further washed with distilled water. The resulting pellet was added to 2 mL of distilled water and mixed. We put single drops onto slides, dried them, and fixed them with Naphrax® mountant.

The prepared preparations were examined with an Olympus CX41 microscope under 1000× magnification, and the first 400 frustules of each sample were determined. Identification was performed using the keys of Hoffman et al. [36], Lange-Bertalot et al. [37], and in some cases Krammer and Lange-Bertalot [38–41].

2.3. Data Analysis

Correlation analysis was performed with PAST program [42]. Some data (land use, number of habitat types, turbidity) were of the interval type and thus not normally distributed, so we used Kendall correlation coefficients (tau).

Similarity in taxonomic composition of diatom community between the ponds was calculated using Sørensen similarity index. Diversity was calculated as Shannon-Wiener diversity index (S-WI) and Margalef diversity index. The trophic index (TI) was calculated according to Rott et al. [43].

The influence of individual factors on the composition of the diatom community was checked by direct gradient analyzes. First, we performed a detrended correspondence analysis (DCA) to determine whether the distribution of the diatom species along potential gradients is unimodal or linear. We found that the mentioned distribution was unimodal (Length of gradient: 9.7 S.D.), so we used Canonical Correspondence Analysis (CCA). All analyzes were performed with the Canoco 4.5 software package [44].

Environmental parameters were grouped into spatial variables (coordinates, altitude, annual precipitation, a distance from the next pond or road), substrate (inorganic and organic), chemical and physical variables, hydromorphological data, drainage basin etc. We used the method of forward selection to check the effect of individual environmental factors on the taxonomic composition. The program made 999 permutations in each round, three rounds were performed. In each next round, we considered only factors with p less than 0.1. In the last round, we considered the two most statistically significant factors, that were in fact marginally significant (p = 0.06 and 0.07). Based on two factors that had a marginally statistically significant effect on the structure of the diatom community, we also created an ordination diagram in which the ponds are distributed along gradients of environmental factors.

3. Results

3.1. Structure of the Benthic Diatom Community

A total of 105 species of diatoms were identified in 12 ponds (Table A1). Of these, most species-rich was JEL1 (43 species) and POK1 (30 species) (Figure 2). The pond with the lowest number of species was KRV1 (14 species). Dominant species and their proportions vary significantly between ponds (Table 2). *Navicula cryptocephala* Kützing was the most dominant in four ponds (POK1, JEL2 MEN2 and MEN4), and it was also present in a large proportion in RAT1. The pioneer complex *Achnanthidium minutissimum* (Kützing) Czarnecki was the most common taxon in three ponds (JEL1, RAT2 and MEN2). The highest dominance index is in POK2 and KRV1, where two dominant taxa represent 77% of the identified species (Table 2).

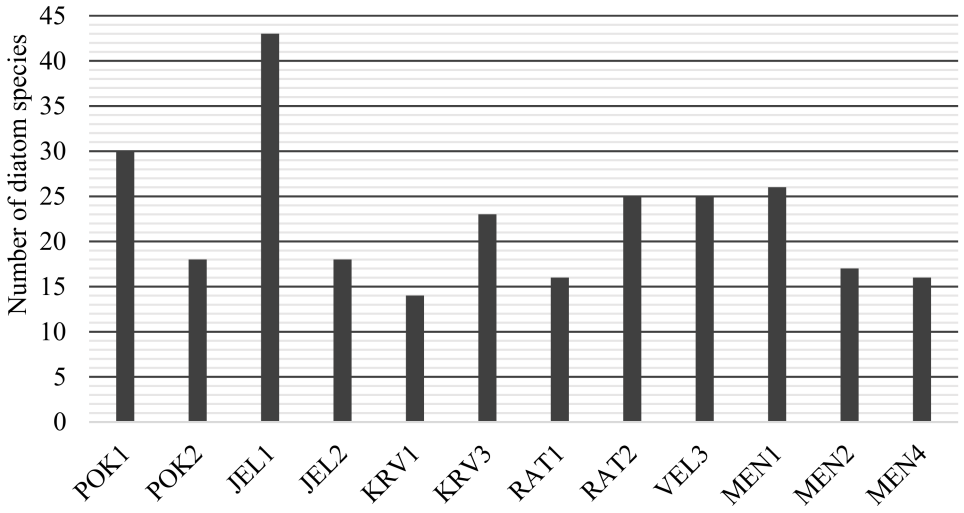

Figure 2. Number of diatom species in individual karst ponds.

Table 2. Dominance index (proportion in %) of the two most common species (highlighted in gray) in studied ponds. Diatoms that are not dominant in the sample but have a proportion ≥10% are also shown.

Species	POK1	POK2	JEL1	JEL2	KRV1	KRV3	RAT1	RAT2	VEL3	MEN1	MEN2	MEN4
Achnanthidium minutissimum			13					24			35	
Achnanthidium pyrenaicum				13				38			18	
Craticula accomoda						16						
Eucoconeis alpestris										10		
Eunotia bilunaris										19		
Eunotia tenella		41										
Gomphonema angustum									10	13		

Table 2. *Cont.*

Species	POK1	POK2	JEL1	JEL2	KRV1	KRV3	RAT1	RAT2	VEL3	MEN1	MEN2	MEN4
Gomphonema parvulum				11					13			19
Navicula cryptocephala	26			40			14				19	14
Navicula exilis							45					
Nitzschia acicularis					53							
Nitzschia adamata						16						
Nitzschia palea												
Nitzschia perminuta										16		
Nitzschia supralitorea					19							
Pinnularia interrupta		36										
Sellaphora pseudopupula				10								
Sellaphora pupula	28						17					10
Tabellaria flocculosa			15									
Dominance index	53.3	77.3	27.5	52.5	71.5	32.4	61.3	62.7	23.1	34.9	53	32.6

Ponds with the highest similarity of diatom community are POK1 and VEL3, although the huge distance between them (see Figure 1). On the other side there was POK2, which stood out the most in rare species—with four ponds (KRV1, KRV3, MEN2, and MEN4) had no species in common (Table 3).

Table 3. Similarity of diatom community between the studied ponds according to Sørenson index. The similarity indices >0.5 are in bold.

POK1	POK2	JEL1	JEL2	KRV1	KRV3	RAT1	RAT2	VEL3	MEN1	MEN2	MEN4	
	0.29	0.44	0.46	0.23	0.30	0.26	0.40	**0.62**	0.43	0.47	0.44	POK1
		0.13	0.11	0	0	0.06	0.05	0.23	0.18	0	0	POK2
			0.33	0.25	0.27	0.24	0.41	0.44	0.32	0.40	0.31	JEL1
				0.31	0.49	0.29	**0.56**	0.37	0.27	0.46	0.29	JEL2
					0.22	0.27	0.26	0.15	0.20	0.39	0.33	KRV1
						0.26	0.38	0.29	0.25	0.40	0.31	KRV3
							0.39	0.34	0.19	0.30	0.31	RAT1
								0.36	0.28	0.52	0.39	RAT2
									0.55	0.52	0.44	VEL3
										0.37	0.48	MEN1
											0.55	MEN2

Figure 3 shows the proportion of diatoms according to their ecological type. Motile and high-profile diatoms are present in all samples. Low-profile diatoms are absent in one pond, while in four ponds (KRV3, RAT1, MEN1, and MEN4) they are very rare. Their largest proportion is in RAT2 (68%) and MEN2 (52%). Planktic diatoms are present with a negligible proportion (JEL1, KRV3, and RAT1), except for KRV1, representing half of the specimens. The most common are motile diatoms. In POK1, JEL2, KRV3, RAT1, VEL3 and MEN4, they represent the majority proportion of diatoms.

Figure 4 shows the size classes of diatoms. The most common size class is 3, followed by 2 and 4. Members of size classes 1 and 5 are infrequent. Smaller diatoms (size classes 1 and 2) are dominant in RAT2, POK2, and MEN2. Data for POK2 are not representative, as 77% of specimens were not determined a size class due to lack of data in the literature. There is also a considerable proportion of unknown size classes in KRV1 and KRV3 (22% and 28%).

3.2. Effects of Environmental Factors on the Diatom Community Composition

The concentration of NO_3-N and NH_4-N in water explains almost 20% of the total variability of the diatom community in ponds (Table 4). The concentration of NO_3-N explains 10% of the variability, and the NH_4-N concentration in water 9.6%. The content of these two nutrients or nitrogen species is probably mainly due to the higher load in ponds

and their basin area with livestock. The same shows the ordination diagram based on CCA (Figure 5), where ponds are arranged according to the diatom taxonomic composition along the gradients of NO_3-N and NH_4-N concentration in water.

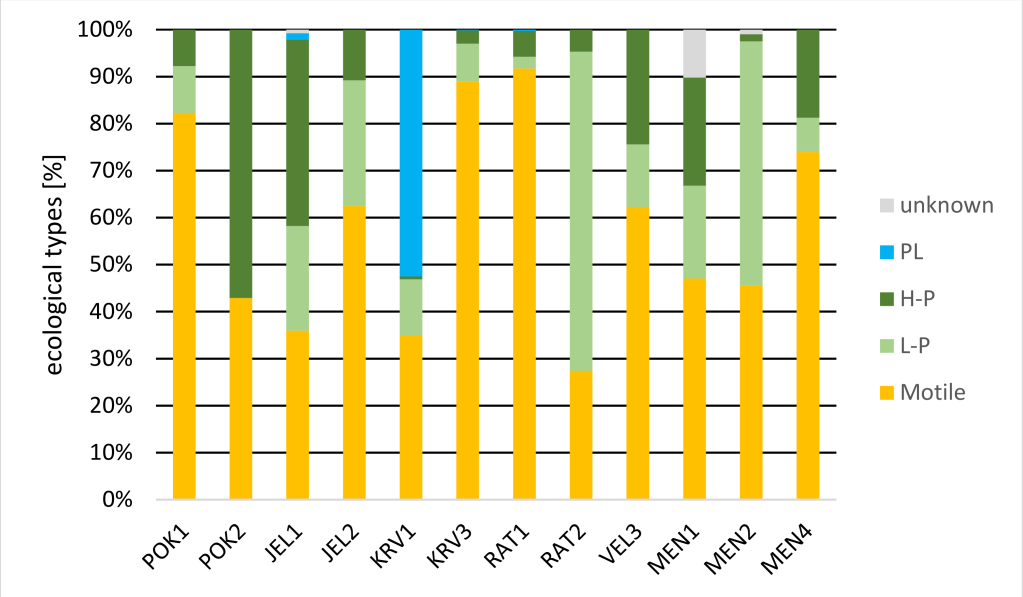

Figure 3. Diatoms according to their ecological type [in %]. (PL—planktic, H-P—high-profile, L-P—low-profile).

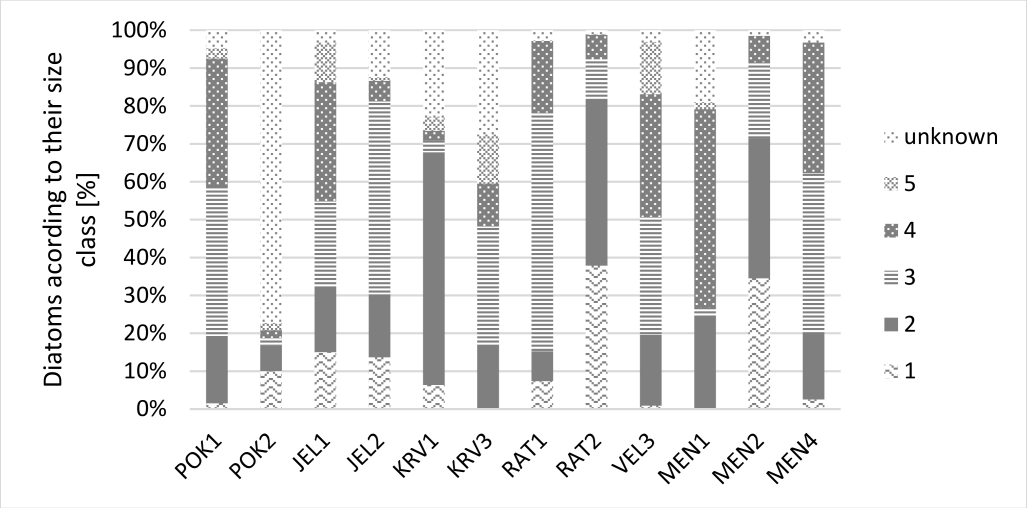

Figure 4. Diatoms according to their size class [%].

Table 4. Results of Canonical correspondence analysis (CCA) and forward selection. (% TVE-proportion of the explained variability by specific variable).

Variable	P	% TVE
NO_3-N	0.064	10.0
NH_4-N	0.072	9.6

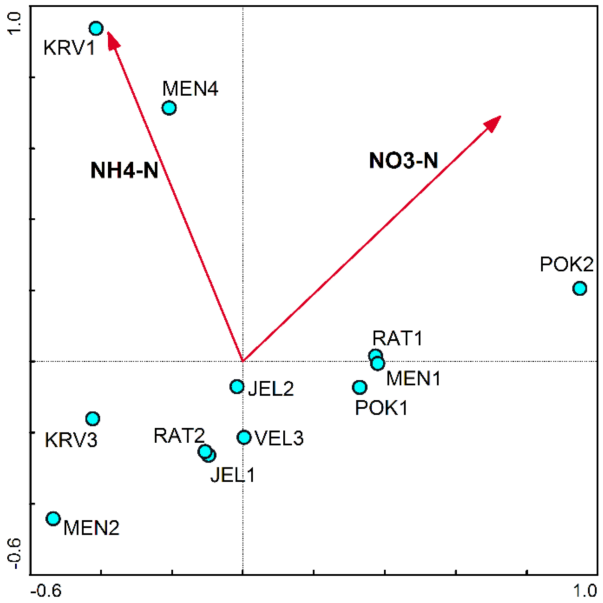

Figure 5. A CCA-based ordination diagram in which karst ponds are distributed along environmental gradients.

According to the S-WI index (Figure 6), the highest diversity is in JEL1, VEL 3 is next. The lowest diversity is in POK2, the lower diversity is also in KRV1 and RAT1. The Margalef index (Figure 7) showed a different assessment of diversity than S-WI.

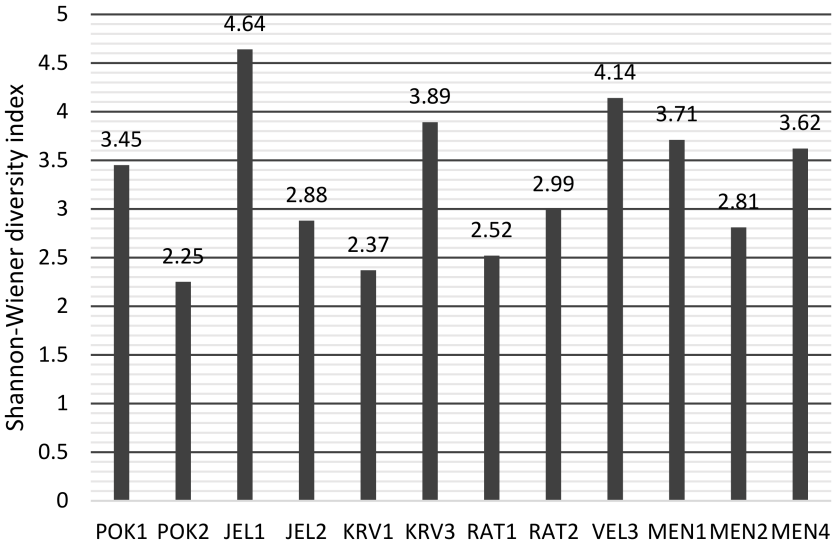

Figure 6. Shannon-Wiener diversity index values of diatoms in karst ponds.

JEL1 still has the highest diversity value (7.01), but the ponds with the lowest diversity are RAT1 and MEN4.

3.3. Environmental Factors and Diversity of Diatom Community

Kendall correlation coefficients showed that the number of diatom species is in a statistically significant positive correlation with oxygen saturation and a negative correlation with the concentration of NH_4-N (Table 5). The Margalef index was also positively correlated with oxygen saturation and negatively with NH_4-N concentration. A negative statistically significant correlation ($p = 0.05$) was calculated between altitude and the Margalef index.

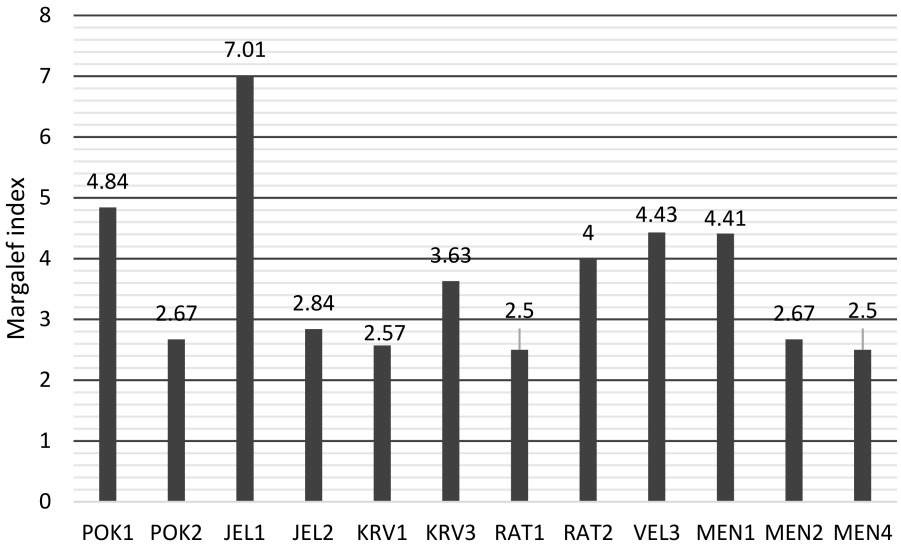

Figure 7. Margalef index values of diatoms in karst ponds.

Table 5. Kendall (tau) correlation coefficients between environmental factors and diversity parameters of diatom communities in ponds. Only statistically significant correlations (*—$p < 0.05$) and marginally statistically significant correlations ($p = 0.05$) are shown.

	No. of Diatom Species	Margalef Index
altitude [m]	n.s.	−0.431
O_2 saturation [%]	0.531 *	0.543 *
NH_4-N [mg/L]	−0.481 *	−0.492 *
SW_I h.taxa macoinvertebrates	−0.481 *	−0.492 *

We also found a negative correlation between the number of diatom species and S-WI and the Margalef index calculated based on the composition of the invertebrate community, which was contrary to our expectations.

Great differences in TI values were found between the ponds (Figure 8). The lowest TI value was in POK1 (ultraoligotrophic) and the highest in JEL2, KRV3, KRV1, POK1, and MEN4 (polytrophic).

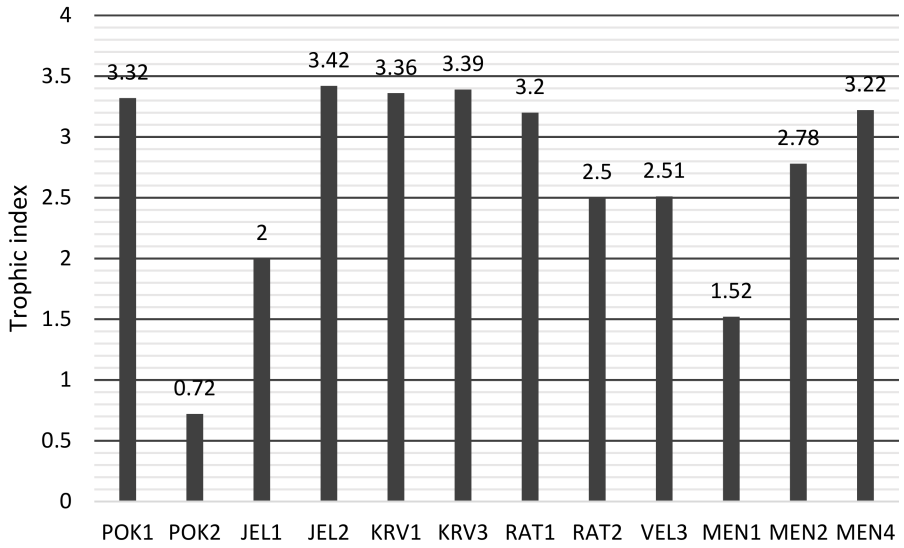

Figure 8. Trophic index values for sampled karst ponds.

4. Discussion

4.1. Structure of the Benthic Diatom Community

In total, 105 diatom species belonging to 32 genera were identified. The most common taxa were *Gomphonema parvulum* (Kützing) Kützing, *Navicula cryptocephala* Kützing, species group *Sellaphora pupula* (Kützing) Mereschkowsky (present in 10 sites). Almost half of the species (52) were present in only one site, from which we can assume that the composition of diatom communities differs much between the ponds. The genera with the highest number of species were *Nitzschia*, *Pinnularia*, *Navicula* and *Neidium*. The highest number of species was identified in the JEL1, whereas in KRV1, we found the lowest number of species, of which *Nitzschia acicularis* (Kützing) W. Smith represented more than half of the identified frustules. We expected lower diversity as well as variability of epipelic diatom community, as karst ponds are small water bodies with frequent disturbances, which make the conditions unfavorable. The number of species varied from 14 to 43, which is much higher than 11–26 taxa from ponds in South-eastern Alps reported by Cantonati et al. [29]. However, the mentioned researchers studied different type of ponds in alpine region.

Among the ecological types, the motile diatoms were the most common. They dominated in four ponds (POK1, KRV3, RAT1, and MEN4) and were codominant in another four ponds (Figure 2). Sites where deposition occurs are advantageous for motile diatoms [45–47] as well as nutrient-rich sites [48–50]. Typical representatives from genera *Navicula*, *Nitzschia*, *Sellaphora*, and *Surirella* [24] were also present in our samples. However, we did not calculate any significant correlation between environmental factors and the share of motile species. In ponds with higher trophic index values motile species dominated, which are well adapted to higher nutrient content. We expected that high-profile (H-P) diatoms would also be present here with higher proportion. However, they were probably not present in such high proportion due to physical disturbances.

High-profile diatoms, which are also common in nutrient-rich water but with fewer disturbances [48] are less common in our samples. The proportion of H-P negatively correlated with TSS ($p = 0.009$), which negatively influence light conditions with turbidity and deposition. On the other hand, we calculated positive correlation between proportions of H-P diatoms and argilal ($p = 0.029$). The typical genera of this group, which were also present in our samples, were *Eunotia*, *Fragilaria* and *Gomphonema*. The proportions of H-P diatoms were lower than motile, except POK2, where H-P represent two-thirds of the community. Disturbances and grazing, made motile species more efficient than H-P ones.

Low-profile (L-P) diatoms were rare, but in two samples (RAT2 and MEN2) they were dominant. Both ponds are fenced, so with no access of the cattle. Proportions of L-P diatoms negatively correlated with NO_3-N ($p = 0.023$) and positively with habitat diversity in the catchment area ($p = 0.039$), which actually means low density of the cattle. Typical representatives are from the genera *Achnantes*, *Achnanthidium*, *Amphora*, *Cocconeis*, and *Meridion* [24]. *Achnantidium minutissiumum* (Kützing) Czarnecki was the most dominant taxon in RAT2 and MEN2, as well as in JEL1. It seems that cattle cause problem for L-P diatoms due to high input of nutrients to ponds, to which L-P species are not adapted [48]. In some samples (JEL1, KRV1, KRV3, and RAT1), planktic diatoms were also present.

In ponds with higher concentrations of orthophosphates, we find mainly motile and H-P diatoms adapted to higher concentrations of nutrients [24,51,52] (Figure 3). In POK2 (0.3 mg/L of ortophosphate), MEN1 (0.92 mg/L) and VEL3 (0.23 mg/L) motile and H-P diatoms represent almost the entire sample, L-P diatoms are almost absent. However, the significant correlation between P and ecological types was not calculated. There was also no correlation between P concentration and diatom size classes, which also report Lavoie et al. [53].

The concentration of NO_3-N and NH_4-N in water explained almost 20% of the total variability of diatom community (Table 4). The concentration of NO_3-N explains 10% of the variability of the diatom community, and the concentration of NH_4-N 9.6% (Table 4, Figure 5). The ponds are arranged according to the taxonomic composition of diatom communities along the gradients of NO_3-N and NH_4-N concentration in water.

The results did not show statistically significant correlations between the composition of diatom community and concentrations of either orthophosphate or TP as expected, which is consistent with Soininen et al. [54]. This is probably because absorption rate for phosphorus from the water column by epipelon is lower than in other groups of primary producers [55].

Haubois et al. [56] report that large and small species do coexist within the epipelon. We found that size-class three had the highest proportion in five ponds, while size-class 2 and 4 in three ponds each (Figure 4). However, most of the identified frustules belonged to the middle-size class (3), which also report Lavoie et al. [53]. In ponds with higher biodiversity (JEL1, KRV3, VEL3, MEN1, and MEN4), size-classes 4 and 3 dominated.

4.2. Diversity of Benthic Diatom Community and Environmental Factors

In general, altitude affects biota in ponds as it affects temperature, precipitation, and radiation [12]. The results showed a negative correlation between altitude and the Margalef index, which is in line with our hypothesis and with the general rules in ecology [57]. The diatom species richness did not correlate with altitude, but pond at the highest altitude (KRV1) had the lowest number of species, while pond at the lowest altitude (JEL1) had the highest diversity. On the contrary for mountain ponds in Spain Blanco et al. [31] report positive correlation of diatom diversity with altitude.

The water depth in these shallow ponds is important mainly because of poor light conditions in turbid water. One of the dominant species was also *Nitzschia perminuta* (Grunow) M. Peragallo, which dominates in low light conditions [58]. Due to shallowness, there is no stratification during the summer [59].

We calculated no significant correlation between pH and diversity indices. The most extreme values were measured at POK2 (pH = 3.8) and MEN1 (pH = 9.6) (Table A2). The first is located in a coniferous forest and is a dystrophic system. Therefore, diatom species in this pond differed from others the most (Table 3). As reported in DeNicola [60] and Della Bella [16], we found there mainly species from the genera *Neidium, Eunotia, Pinnularia, Stauroneis*, and *Sellaphora*, which occurred in small numbers or were absent in other ponds. Diatom community from this pond had no species in common with four other ponds. This pond was more similar to the shallow ponds on mires presented in [29,61]. The lowest value of the electrical conductivity was also measured there (16 µS/cm), which coincides with the trophic index, which defines it as ultraoligotrophic.

We found a positive correlation between the number of diatom species and water saturation with oxygen and the Margalef index and water saturation with oxygen. The highest oxygen saturation was in MEN1 (almost 250%) due to intense photosynthetic activity of the phytoplankton, making the water very turbid.

In KRV1 and MEN4, a large proportion of N is in the form of NH_4-N, which can be explained by the high density of cattle in their catchments. Correlation coefficients showed a negative correlation between the Margalef index and the NH_4-N concentration. In ponds with a higher concentration (KRV1 and MEN4), the diversity was lower, while it was higher in ponds with lower NH_4-N concentrations (POK1, JEL1, RAT1, and VEL3). In contrast to NH_4-N concentrations, NO_3-N concentrations did not differ much between ponds. Values were 0.2–0.5 mg/L. NO_3-N and NH_4-N concentrations classify our ponds as eutrophic (POK2, JEL2, KRV3, RAT1), mesotrophic (POK1 and MEN1), or oligotrophic (JEL1, RAT2, and MEN2) [54]. In KRV1 and MEN4, the values of NH_4-H and NO_3-N were so high that they can be classified as hypereutrophic.

Cattle can have a substantial negative effect on the diversity of communities in ponds [62]. Trampling the bottom and the shore presents physical disturbances. In ponds with moderate intensity of trampling, the diatom diversity was higher than in those without trampling, which is consistent with the intermediate-disturbance hypothesis [63]. More important is the influence of the cattle as the source of nutrients and organic matter from their excrements. Smaller water bodies in the agricultural landscape are highly exposed to

influences from nearby agricultural areas, since they can be strongly affected by nutrient accumulation [4].

Based on the trophic index (TI), ponds vary from ultraoligotrophic to polytrophic. Della Bella et al. [30] report that trophic diatom index highly correlated with nutrient content, especially orthophosphate and NO_3-N in wetlands in central Italy. However, in our case orthophosphate concentrations were the highest where the TI values were low (POK2 and MEN1). According to TP concentrations and nutrient estimates for lakes [58], both ponds were hypertrophic, but TI classified them as ultraoligotrophic (POK2) and mesotrophic (MEN1). Due to the pH = 3.8, there were probably not enough basic ions in POK2, despite the high concentration of TP and NO_3^-. Insufficient amount of HCO_3^- was present at pH = 9.6, which reduced primary production and thus nutrient uptake, which was probably the explanation for the condition in the MEN1.

4.3. Correlations between Diatoms and Macroinvertebrates

We found a negative correlation between the diatom species richness and the S-WI, and Margalef index calculated on the base of the macroinvertebrate community, which was contrary to our expectations. Similar findings report also Gascón et al. [64], which found out that different aquatic communities respond differently to the environmental factors, so we could not generalize relations between parameters and diversity patterns. Due to the larger size of macroinvertebrates, they might be more susceptible to physical destruction of the littoral zone, and loss of mesohabitats due to trampling of the bottom compared to diatoms, whereas diatoms, as primary producers, are particularly sensitive to water chemistry and light conditions [16,65]. Another reason is probably grazing [66]. We should not neglect the fact that on the same substrate on which diatoms thrive, Chironomidae dominate, which graze on epipelon.

5. Conclusions

We found a negative correlation between species-richness and diversity of the diatom community and diversity of the macroinvertebrate community (S-WI, Margalef index).

Despite relatively small differences in altitude, the results showed a marginal statistical correlation between altitude and Margalef Index. No effect of the pond size on the diversity of diatom community was observed.

We did not calculate significant correlations between pH and diversity. Half of the species in most acidic pond POK2 were present only in this pond. Correlations between electrical conductivity, land use, and diversity of diatom community were not significant.

Motile diatoms were most common. They are adapted to high nutrient concentrations and disturbances and can migrate to the site with sufficient light or nutrients when the re-suspended substrate is depositing.

We found a positive correlation between the number of diatom species and O_2 saturation and the Margalef index and O_2 saturation. The pond with the lowest oxygen saturation value (KRV1) had the lowest species diversity.

The results also showed a negative correlation between the number of diatoms and NH_4-N concentration and the Margalef index and NH_4-N concentration. NH_4-N is probably present in the ponds due to the cattle grazing in the area in the summer. The concentrations of NO_3-N and NH_4-N explain almost 20% of the total variability of the diatom community.

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Appendix A

Table A1. The list of the names of diatom taxa found in studied karst ponds.

Achnanthidium pyrenaicum (Hustedt) Kobayasi
Achnanthidium minutissimum (Kützing) Czarnecki
Adlafia minuscula (Grunow) Lange-Bertalot var. *minuscula*
Amphora copulata (Kützing) Schoeman et Archibald
Amphora pediculus (Kützing) Grunow
Brachysira neoexilis Lange-Bertalot
Caloneis tenuis (Gregory) Krammer
Chamaepinnularia mediocris (Krasske) Lange-Bertalot
Chamaepinnularia muscicola (Petersen) Kulikovskiy, Lange-Beralot et Witkowski
Chamaepinnularia soehrensis (Krasske) Lange-Bertalot et Krammer
Cocconeis pediculus Ehrenberg
Craticula accomoda (Hustedt) D.G. Mann
Craticula ambigua (Ehrenberg) D.G. Mann
Craticula halophila (Grunow) D.G. Mann
Craticula molestiformis (Hustedt) Lange-Bertalot
Cyclotella stelligera Cleve & Grunow
Cymbopleura amphicephala (Nägeli) Krammer
Cymbopleura naviculiformis (Auerswald) Krammer
Diploneis krammeri Lange-Bertalot et Reichardt
Encyonema hebridicum Grunow ex Cleve
Encyonema minutum (Hilse) D.G. Mann
Encyonema silesiacum (Bleisch) D.G. Mann
Eucocconeis alpestris (Brun) Lange-Bertalot
Eunotia arcus Ehrenberg
Eunotia bilunaris (Ehrenberg) Schaarschmidt
Eunotia exigua (Brébisson) Rabenhorst
Eunotia minor (Kützing) Grunow
Eunotia paludosa Grunow
Eunotia pseudogroenlandica Lange-Bertalot et Tagliaventi
Eunotia subarcuatoides Alles, Nörpel et Lange-Bertalot
Eunotia tenella (Grunow) Hustedt
Fragilaria radians (Kützing) Williams et Round
Fragilaria tenera (W. Smith) Lange-Bertalot
Frustulia crassinervia (Brébisson) Lange-Bertalot et Krammer
Gomphonema acuminatum Ehrenberg
Gomphonema angustum (Kützing) Rabenhorst
Gomphonema calcifugum Lange-Bertalot et Reichardt
Gomphonema exilissimum (Grunow) Lange-Bertalot et Reichardt
Gomphonema occultum Reichardt et Lange-Bertalot
Gomphonema parvulum (Kützing) Kützing
Gomphonema sarcophagus Gregory
Hantzschia abundans Lange-Bertalot
Luticola nivalis (Ehrenberg) D.G. Mann
Luticola mutica (Kützing) D.G. Mann
Meridion circulare (Gréville) C. Agardh
Navicula antonii Lange-Bertalot

Table A1. *Cont.*

Navicula cryptocephala Kützing
Navicula cryptotenella Lange-Bertalot
Navicula exilis Kützing
Navicula menisculus Schumann
Navicula reichardtiana Lange-Bertalot
Navicula trivialis Lange-Bertalot
Navicula veneta Kützing
Navicula wildii Lange-Bertalot
Neidium affine (Ehrenberg) Pfitzer
Neidium alpinum Hustedt
Neidium ampliatum (Ehrenberg) Krammer
Neidium bergii (Cleve-Euler) Krammer
Neidium binodeforme Krammer
Neidium bisulcatum (Lagerstedt) Cleve var. bisulcatum
Neidium dubium (Ehrenberg) Cleve
Neidium iridis (Ehrenberg) Cleve
Neidium productum (W. Smith) Cleve
Nitzschia acicularis (Kützing) W. Smith
Nitzschia adamata Hustedt
Nitzschia angustata (W. Smith) Grunow
Nitzschia communis Rabenhorst
Nitzschia dissipata (Kützing) Grunow ssp. dissipata
Nitzschia fonticola Grunow
Nitzschia gisela Lange-Bertalot
Nitzschia palea (Kützing) W. Smith
Nitzschia perminuta (Grunow) M. Peragallo
Nitzschia pura Hustedt
Nitzschia pusilla Grunow
Nitzschia supralitorea Lange-Bertalot
Nitzschia umbonata (Ehrenberg) Lange-Bertalot
Pinnularia borealis Ehrenberg
Pinnularia gibba Ehrenberg
Pinnularia grunowii Krammer
Pinnularia interupta W. Smith
Pinnularia marchica I. Schönfelder ex Krammer
Pinnularia microstauron (Ehrenberg) Cleve
Pinnularia rupestris Hantzsch
Pinnularia sinistra Krammer
Pinnularia subcapitata Gregory var. subcapitata
Pinnularia viridiformis Krammer
Placoneis ignorata (Schimanski) Lange-Bertalot
Placoneis paraelginensis Lange-Bertalot
Planothidium lanceolatum (Brébisson ex Kützing) Lange-Bertalot
Psammothidium grischunum (Wunthrich) Bukhtiyarova et Round
Psammothidium helveticum (Hustedt) Bukhtiyarova & Round
Sellaphora pseudopupula (Krasske) Lange-Bertalot
Sellaphora pupula (Kützing) Mereschkowsky (species group)
Sellaphora stroemii (Hustedt) D.G.Mann
Sellaphora verecundiae Lange-Bertalot
Stauroneis acidoclinata Lang-Bertalot et Werum
Stauroneis anceps Ehrenberg
Stauroneis gracilis Ehrenberg
Stauroneis kriegeri Patrick
Stauroneis smithii Grunow
Stauroneis thermicola (Petersen) Lund
Stephanodiscus alpinus Hustedt
Surirella angusta Kützing
Surirella minuta Brébisson ex Kützing
Tabellaria flocculosa (Roth) Kützing

Table A2. Characteristics of karst ponds in the year 2016. * Secchi depth in most transparent ponds is the same as water depth; the bottom of the pond MEN2 was covered with plastic layer on which fine substrate deposited. + represents presence of substrate, cover <5%.

Sample	POK1	POK2	JEL1	JEL2	KRV1	KRV3	RAT1	RAT2	VEL3	MEN1	MEN2	MEN4
date	23.8.	23.8.	23.8.	23.8.	19.8.	19.8.	23.8.	23.8.	19.8.	18.8.	18.8.	18.8.
pH	5.9	3.8	6.5	6.4	6.7	8.3	7.4	6.5	5.9	9.6	7.2	6.2
T [°C]	17.5	12.2	14.1	9.8	14.9	15.3	7.7	10.2	17.3	17.7	17.9	16.0
Conductivity [µS/cm]	37	16	149	47	242	92	95	256	36	158	55	90
O_2 saturation [%]	75	53	56	62	10	69	56	74	100	244	90	25
O_2 [mg/L]	6.6	4.7	5.0	6.0	0.9	5.9	4.9	7.5	8.1	19.4	7.3	2.0
Secchi depth [cm]	25 *	30 *	60 *	55 *	30 *	13.0	20 *	30 *	35	10	56	36
depth [cm]	25	30	60	55	30	100	20	30	40	20	100	48
Turbidity [1–3]	1	1	1	3	3	3	1	1	3	3	1	3
Clay, silt [%]	100	100	100	90	80	5	100	100	100	95	-	100
Sand, gravel [%]	0	0	0	10	20	65	0	0	0	0	-	0
Pebbles [%]	0	0	0	+	0	30	0	0	0	5	-	0
Stones [%]	0	0	+	0	0	0	0	0	0	0	-	0
CPOM [%]	0	20	0	0	+	5	+	+	+	0	1	0
FPOM [%]	0	80	0	0	0	1	100	80	100	100	80	0
[%] of trampled shore	1	1	0	45	70	70	20	0	50	100	0	80
Intensity of trampled shores (0–5)	1	1	0	3	5	2	3	0	4	5	0	4
TP [mg/L]	0.17	0.34	0.03	0.05	0.28	0.07	0.07	0.06	0.23	0.92	0.08	0.15
PO_4^{3-} [mg/L]	0.17	0.30	0.02	0.02	0.07	0.03	0.01	0.001	0.23	0.92	0.05	0.02
TN [mg/L]	1.35	0.82	0.59	0.84	5.91	1.21	1.62	0.56	1.53	6.56	0.95	16.0
NO_3-N [mg/L]	0.39	0.52	0.30	0.34	0.42	0.26	0.41	0.30	0.32	0.40	0.21	0.42
NH_4-N [mg/L]	0.08	0.14	0.03	0.51	4.0	0.73	0.28	0.07	0.06	0.21	0.03	3.08
TDS [mg/l]	72	70	96	50	120	78	94	58	80	226	74	92
TSS [mg/L]	3	8	17	58	151	98	49	93	30	201	257	39

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