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ALGAE FOR MONITORING RIVERS

# The influence of substrates and physicochemical factors on the composition of diatom assemblages in karst springs and their applicability in water-quality assessment

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**Abstract** The methodological standards of monitoring programs recommend collecting material from stones, but this community is often absent in slow-flowing waters, whereas a great number of localities do offer bryophytic material for sampling. In order to investigate whether the kind of substrate (epilithic or bryophytic) influences the diatom-based biomonitoring results the structure of diatom assemblages from 13 anthropogenically altered springs were investigated. To examine differences between diatom assemblages, the species richness of diatom assemblages,  $H'$  (Shannon-Wiener diversity index), Trophic Diatom Index (TDI) and Specific Pollution sensitivity Index (SPI) were compared. The TDI and SPI differed significantly ( $P = 0.002$  and  $P = 0.004$ ) between springs located in villages and springs away from villages, and did not differ significantly between epilithic and bryophytic samples ( $P > 0.05$ ). No

significant differences in species richness or the diversity index ( $H'$ ) ( $P > 0.05$ ) were found between the epilithic and bryophytic diatom assemblages. This study suggests that both the kinds of substrates can be successfully used for diatom-based water-quality bio-monitoring in mesotrophic and eutrophic environments.

**Keywords** Bioindication · Diatoms · Epibryon · Epilithon · Krakowsko-Częstochowska Upland · Nutrients

## Introduction

Diatoms are one of the most commonly used organisms for assessing natural and human-related features of aquatic environments. Their value as bioindicators was appreciated long ago, and diatom analysis is still done for reconstruction of the paleoenvironment and for water quality/environmental health assessment. Their usefulness is due to the quick and precise response of the diatom assemblage structure to changing conditions (Stoermer & Smol, 1999). To standardize the methodology of using diatoms for water-quality assessment and to minimize substrate-related variability of diatom assemblage structure, it has been recommended to study communities developing on a single type of substrate; for example, in analyses of running waters the epilithon is recommended (e.g. Round, 1991, 1993; Kelly et al., 1998; Townsend & Gell, 2005).

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Once the general regularities of how specific diatom assemblages form in response to pH, salinity, trophic state and several other physicochemical parameters were recognized, a number of detailed studies of habitat–diatom assemblage relationships were undertaken. The relationships between benthic communities and substrate type have been the subject of several investigations and extensive discussion (Douglas, 1958; Stevenson & Hashim, 1989; Jüttner et al., 1996; Rothfritz et al., 1997; Sabater et al., 1998; Potapova & Charles, 2005; Townsend & Gell, 2005; Cantonati & Spitale, 2009). As the relationships between substrate and the organisms inhabiting it are much more complicated in benthic and periphytic communities (Cox, 1990; Burkholder, 1996; Townsend & Gell, 2005) than in plankton, the effects of different factors on assemblage structure in running waters are much less well understood. The influence of biotic and abiotic substrates on diatom assemblages has been studied in rivers on broad geographical scales (e.g. Jüttner et al., 1996; Rothfritz et al., 1997; Potapova & Charles, 2005; Townsend & Gell, 2005) but has received much less attention in springs (Cantonati, 2001; Cantonati & Spitale, 2009). Springs provide excellent opportunities for such studies because the fluctuation of water properties is minimal (Sabater & Roca, 1990; Cantonati et al., 2006; Dumnicka et al., 2007; Cantonati & Spitale, 2009) and the input of allochthonous organisms from other environments is limited. The available data indicate that the geomorphological features of an area and the water characteristics are major factors governing the diatom distribution in springs (Werum, 2001; Werum & Lange-Bertalot, 2004; Cantonati et al., 2006).

Most of the data on diatoms in springs come from mountain areas in natural or near-natural conditions (Sabater & Roca, 1990, 1992; Cantonati, 1998; Battegazzore et al., 2004; Werum & Lange-Bertalot, 2004), where the epilithon generally occurs. In lowland areas, the role of epilithic communities is smaller, because slow flow allows sediment to accumulate and become the predominant substrate in springs, together with submerged vascular and non-vascular macrophytes. Macrophytes such as bryophytes occur in most springs and are one of the most common habitats for a very diverse microflora (Cantonati & Spitale, 2009; Hájková et al., 2011). A comparison of the structure of diatom assemblages in bryophytic and epilithic communities from

non-mountain, mesotrophic springs can provide applicative data to use for water-quality biomonitoring; epilithic assemblages are recommended for water-quality assessment, but such assemblages are virtually absent in several aquatic lowland systems (e.g. Potapova & Charles, 2005). In order to investigate whether the kind of substrate (epilithic or bryophytic) commonly used for assessment of water quality diatom influences, e.g. Trophic Diatom Index (TDI; Kelly & Whitton, 1995) and Specific Pollution sensitivity Index (SPI; CEMAGREF, 1982), the diatom assemblages from 13 anthropogenically altered springs were investigated.

### Site description

The springs in question are situated in the Wyżyna Krakowsko-Częstochowska Upland in southern Poland (Table 1). This upland, covering 2,650 km<sup>2</sup>, extends from the Carpathian foothills in the vicinity of Kraków northward to the town of Częstochowa. It is ca. 80-km long and ca. 20-km wide, and rises to 350 m a.s.l. on average. Geologically, it is karst formed in Upper Jurassic calcareous rock up to 200-m thick and further altered since the Cretaceous (Rózkowski, 1996), partly covered by Quaternary loess in the southern part and by postglacial sands in the northern part (Dynowska, 1983). Most of the spring water belongs to the bi-ionic HCO<sub>3</sub><sup>-</sup> Ca<sup>2+</sup> category. Local hydrogeology is the main natural factor determining the spatial diversity of spring water chemistry (Siwek, 2004). The springs from the southern part of the upland are supplied from the Jurassic aquifer and are typically small, with discharge between 0.4 and 15 l s<sup>-1</sup> (Dumnicka et al., 2007). Rapid circulation in a shallow system of large karstic channels limits bedrock weathering there. The northern part of the study area belongs to the Cretaceous aquifer, and the springs supplied by it have greater discharge. The range of spring water chemistry is much broader there, due to diverse geology, several local water tables, and higher mineral dissolution of the bedrock (Siwek, 2004). The springs of the Krakowsko-Częstochowska Upland are very vulnerable to pollution because surface water flows rapidly into groundwater systems through natural conduits and is under significant anthropogenic pressure. Most of the springs in this study are located in highly populated areas. The selected springs differ in

discharge, type of surroundings, degree of artificial modification and ionic composition (Tables 1, 2).

**Methods**

Epilithic, bryophytic samples and water for physical and chemical analyses were collected in September–November 2004. Composite samples of at least four subsamples from submerged stones were collected by brushing the upper surface of submerged stones and squeezing from submerged bryophytes. Although previous studies did not show essential differences in the structure of epilithic diatom associations on stones of differing size in slow-flowing waters (Wojtal & Sobczyk, 2006), we collected the epilithon samples from stones of similar size. Some of the environmental variables were measured at the time of diatom sampling. Water temperature was taken and specific conductivity and pH were measured using an Elmetron CC-102 conductivity metre and a CC-104 pH metre. Other chemistry data were obtained by ion chromatography (Dionex 100), atomic absorption spectrophotometry and flame AAS in the Institute of Botany laboratory in Szarów, Poland.

To remove carbonates, 10% HCl was added to the samples in 250-ml glass beakers and boiled for 15 min after 24 h. Then the material was washed at least five times with distilled water, with 24 h settling time between each decanting. Then the samples were boiled in 30% H<sub>2</sub>O<sub>2</sub> with small amounts of KClO<sub>3</sub>, or using concentrated (>30%) acids (H<sub>2</sub>SO<sub>4</sub>, HNO<sub>3</sub>) to remove any leftover organic matter not digested by peroxide. After washing several times with distilled water the material was air dried on coverglasses and mounted in Naphrax<sup>®</sup> resin. The diatoms were observed with a Nikon Eclipse 600 light microscope with Differential Interference Contrast.

The relative abundance of particular taxa and the species richness of the assemblages were estimated on the basis of at least 300 diatom valves per sample. To examine differences between diatom assemblages, we compared the species richness of epilithic and bryophytic assemblages, and the following indices for assessing water quality were computed with Omnidia 4.1 software (Lecointe et al., 1993): *H'* (Shannon-Wiener diversity index), TDI and SPI.

Differences in Shannon-Wiener diversity index, TDI and SPI between bryophytic samples and epilithic

**Table 1** Morphological characteristics and classification of springs of the Krakowsko-Częstochowska Upland

Locality no.	1	2	3	4	5	6	7	8	9	10	11	12	13
Spring name	Ruskie	Hareczerza	Miłości	Kaplica	Cieplica	Józefa	Kobylanka	Imbramowice	Wielkanoc	Łany Wielkie	Dąbrowica	Węgrzynów	Siamoszyce
Location type	N	N	N	N	N	N	N	V	V	V	V	V	N
Insolation	HR	R	F	F	R	F	F	F	HR	F	R	F	F
Spring type	RH	RH	RH	RH	RH	RH	RH	LJ	RH	LI	H	RH	RH
Bottom cover	L + G	L + G	L + G	L + G	L + G	L + G	L + G	S	S	L + G	L + G	S	L + G
Discharge (l s <sup>-1</sup> )	>1	<1	<1	<1	<1	<1	<1	<100	<10	<10	>1	<10	>1

*N* low human-altered localities (away from villages), *V* localities under anthropopression (in or near villages), *F* full, *R* reduced, *HR* highly reduced, *RH* rheocrene, *H* helocrene, *L* limnocrene, *S* sand, *L* loess, *G* gravel

**Table 2** Biological, physical and chemical characteristics, and locations of springs of the Krakowko-Częstochowska Upland

Locality no.	1	2	3	4	5	6	7	8	9	10	11	12	13
Spring name	Ruskie	Harcerza	Miłości	Kaplica	Cieplica	Józefa	Kobylanka	Imbramowice	Wielkanoc	Lany Wielkie	Dąbrowica	Węgrzynów	Siamoszyce
Geographic coordinates N	50°12.7965'	50°12.3252'	50°11.7512'	50°12.8573'	50°10.2978'	50°09.7201'	50°09.3186'	50°18.1118'	50°20.2374'	50°31.0847'	50°33.7459'	50°33.8575'	50°30.8837'
Geographic coordinates E	19°48.1832'	19°48.8652'	19°49.7996'	19°49.7970'	19°37.2072'	19°37.9630'	19°45.3541'	19°51.7833'	19°54.9983'	19°51.0271'	19°50.1668'	19°53.2000'	19°35.3965'
Temperature (°C)	8.9	8.4	8.8	9.1	12.6	9.2	9.4	10.0	9.1	9.4	10.0	10.5	8.8
pH	7.0	7.1	7.2	7.4	7.1	7.4	7.3	7.6	7.2	7.2	7.2	7.0	7.7
Conductivity ( $\mu\text{S cm}^{-1}$ )	272	210	412	520	520	503	412	450	513	541	538	727	460
Oxygen (%)	96.2	92.0	87.0	66.1	66.1	66.5	54.1	67.0	62.0	72.5	68.7	49.0	87.9
BOD <sub>5</sub>	1.60	1.12	1.44	0.96	0.80	1.12	1.44	1.92	0.80	2.72	0.80	2.08	10.20
<i>Escherichia coli</i> (number of cells/100 ml)	0	0	0	0	28	0	16	4	0	500	370	446	10
Alkalinity (mval l <sup>-1</sup> )	4.9	4.8	4.2	4.5	4.6	4.1	3.4	4.2	3.7	3.5	4.0	5.1	3.6
NO <sub>2</sub> <sup>-</sup> (mg l <sup>-1</sup> )	0.027	<0.01	<0.01	<0.01	0.022	<0.01	<0.01	<0.01	<0.01	<0.01	0.051	<0.01	<0.01
NO <sub>3</sub> <sup>-</sup> (mg l <sup>-1</sup> )	10.71	22.15	12.50	13.99	20.23	23.01	16.68	14.61	16.57	38.18	34.49	34.25	35.20
PO <sub>4</sub> <sup>3-</sup> (mg l <sup>-1</sup> )	0.02	0.01	<0.01	<0.01	<0.01	0.01	<0.01	0.02	0.06	0.01	0.01	<0.01	0.05
SO <sub>4</sub> <sup>2-</sup> (mg l <sup>-1</sup> )	1.25	14.57	4.81	4.46	34.06	50.49	19.94	13.58	26.58	21.73	24.19	47.98	55.50
HCO <sub>3</sub> <sup>-</sup> (mg l <sup>-1</sup> )	3.74	4.44	3.90	4.46	3.44	3.24	3.24	3.48	3.16	2.88	3.32	4.46	2.82
Br (mg l <sup>-1</sup> )	<0.01	<0.01	<0.01	<0.01	0.03	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
F (mg l <sup>-1</sup> )	0.03	0.03	0.02	0.04	0.05	0.15	0.03	0.11	0.09	0.05	0.05	0.08	0.04
Cl (mg l <sup>-1</sup> )	18.63	9.62	4.76	4.53	9.98	8.13	6.80	6.51	10.21	12.60	14.06	13.56	14.20
Si (mg l <sup>-1</sup> )	7.34	7.68	7.79	8.84	8.18	7.68	9.98	10.18	24.25	24.08	25.45	9.20	6.51
Ca (mg l <sup>-1</sup> )	50.48	68.40	43.84	62.00	43.84	64.52	49.45	54.19	44.05	50.04	64.15	74.26	69.90
Mg (mg l <sup>-1</sup> )	1.10	1.86	1.97	1.73	21.67	11.40	2.96	5.11	11.16	2.86	1.61	3.53	1.90
Na (mg l <sup>-1</sup> )	6.37	4.48	4.53	3.44	7.60	5.56	3.79	2.69	3.33	2.13	0.99	2.25	3.20
K (mg l <sup>-1</sup> )	1.08	0.90	1.26	0.80	4.87	1.15	0.92	1.50	2.01	1.64	1.14	4.97	1.70
Cu (mg l <sup>-1</sup> )	0.44	0.78	0.58	0.86	1.28	3.27	0.87	0.64	0.89	0.54	0.77	1.02	7.88

Table 2 continued

Locality no.	1	2	3	4	5	6	7	8	9	10	11	12	13
Cd (mg l <sup>-1</sup> )	0.18	0.16	0.24	0.22	0.30	0.19	0.24	0.19	0.34	0.39	0.36	0.24	0.32
Pb (mg l <sup>-1</sup> )	0.98	0.88	0.94	0.83	0.90	1.13	0.65	0.73	0.36	0.68	1.57	0.39	3.54
Zn (mg l <sup>-1</sup> )	7.4	9.6	9.5	6.9	58.6	29.4	4.9	6.4	11.5	1.2	11.8	6.2	10.1
Fe (mg l <sup>-1</sup> )	18.6	11.8	9.0	16.7	7.2	8.3	13.1	7.8	15.1	11.3	13.6	10.4	15.9

samples and between springs under anthropogenic pressure (in or near villages) and springs away from villages were analyzed using ANOVA with three factors: substrate, spring and location type and springs nested in location type. Taxa accounting for >1% of the diatom assemblages at more than three of the studied springs, after logarithmic transformation (natural logarithm) were included in the ordination analyses. To explain variability in the species composition the data were analyzed by principal correspondence analysis (PCA) using correlation matrix. To explain species–environment relationship the species data were analyzed by redundancy analysis (RDA) (CANOCO v4.5 software; ter Braak & Šmilauer, 2002; Lepš & Šmilauer, 2003). The analyses were performed using the whole data set (bryophytic and epilithic diatom assemblages together) and then separately for bryophytic diatom assemblages only and epilithic diatom assemblages only.

## Results

The pH range was <1 U (pH 7.0–7.7) for the whole data set. All analyzed localities showed comparable calcium concentration, alkalinity and temperature (Table 2). There was a relatively high variation of specific conductivity in the springs, ranging from 210 (Harczerza spring) to 727  $\mu\text{S cm}^{-1}$  (spring in Węgrzynów), and considerable variation of nitrate concentration from 10.7 (Ruskie spring) to 38.2  $\text{mg l}^{-1}$  (spring in Łany Wielkie). The diatom assemblages were dominated by neutrophilous and alkaliphilous, mesotraphentic and eutrathentic,  $\alpha$ - and  $\beta$ -mesosaprobous diatoms (Table 3).

We identified a total of 136 diatom taxa, the vast majority of which were typically epilithic or typically epiphytic forms. Only 17 were common (i.e. present at >10% relative abundance in at least one sample); of those, 10 reached >20% relative abundance in at least one sample. Average species richness in epilithic samples was 18 diatom taxa, ranging from 5 to 34; the corresponding figures for bryophytic samples are 18.5 and 12–29. Average species richness differed between southern (away from villages; less eutrophicated) and northern (in or near villages; more eutrophicated) waters: 24 and 12, respectively, in the epilithon, and 19 and 18 in bryophytic samples. The Shannon-Wiener diversity index ( $H'$ ) did not differ significantly

**Table 3** Shannon-Wiener diversity index ( $H'$ ), trophic, saprobic and dissolved oxygen limit tolerances, pH preferences, and TDI and SPI index values calculated for diatom assemblages using Omnidia software

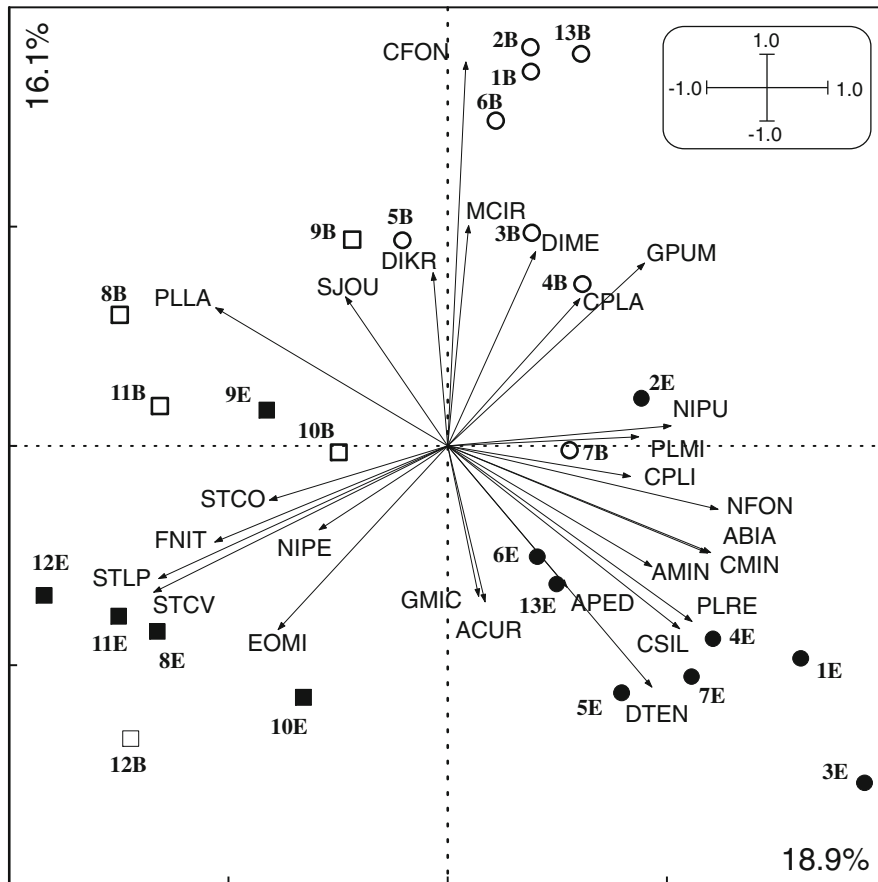
Locality no. and type of sample	1E	1B	2E	2B	3E	3B	4E	4B	5E	5B	6E	6B	7E
$H'$	3.7	2.5	3.7	2.2	3.3	2.6	2.1	2.5	2.4	2.6	2.3	2.5	3.3
Trophic tolerances	m-e	m	e	ind	e	ind	o	ind	e	ind	ind	e	m-e
Saprobic tolerances	$\beta$ -m	$\beta$ -m	$\beta$ -m	$\beta$ -m	$\beta$ -m	$\beta$ -m	mes	$\beta$ -m	$\beta$ -m	$\beta$ -m	$\beta$ -m	$\beta$ -m	$\beta$ -m
Oxygen limit tolerances (%)	50	50	75	50	100	100	100	75	100	100	100	50	100
pH preferences	alk	alk	alk	alk	alk	alk	alk	alk	neut	alk	neut	alk	alk
TDI	55.8	59	63	57	41.2	50	30	41.7	46	54	44	52	55.1
IPS	18	16	17	16	18	18	19	19	18	18	19	18	18.5
Locality no. and type of sample	7B	8E	8B	9E	9B	10E	10B	11E	11B	12E	12B	13E	13B
$H'$	3.8	1.6	1.6	0.9	1.7	2.6	1.6	2.9	3.7	2.8	2.7	3.1	3.1
Trophic tolerances	ind	e	ind	m-e	e	e	m-e	m-e	e	e	e	ind	m
Saprobic tolerances	$\beta$ -m	$\beta$ -m	$\beta$ -m	$\alpha$ -m	$\alpha$ -mes	$\beta$ -m	$\alpha$ -m	$\beta$ -m	$\beta$ -m	$\beta$ -m	$\beta$ -m	$\beta$ -m	$\beta$ -m
Oxygen limit tolerances (%)	75	100	100	50	50	100	50	100	100	100	100	100	100
pH preferences	alk	alk	alk	alk	alk	neut	alk	alk	alk	alk	alk	alk	alk
TDI	53	75	97	92.6	92	50	70	73	81	81	57	43	68
IPS	17.5	16	16	18	17	17	18	15	15	17	17	16.5	18

*E* epilithon, *B* submerged bryophytes, *o* oligotraphentic, *ind* of a broad spectrum of trophic conditions, *m* mesotraphentic, *m-e* meso-entraphentic, *e* eutraphentic, *mes* mesosaprobous,  $\alpha$ -*m*  $\alpha$ -mesosaprobous,  $\beta$ -*m*  $\beta$ -mesosaprobous, *alk* alkaliphilous, *neut* neutrophilous

between these groups, however. The different springs had similar total numbers of species, but the number of rare species (<1% relative abundance) shared among all 13 localities was low.

The diatom assemblages in both the bryophytic and the epilithic samples were dominated (>20% relative abundance) by *Achnantheidium minutissimum* (Kützing) Czarniecki, *Amphora pediculus* (Kützing) Grunow, *Planothidium lanceolatum* (Brébisson) Lange-Bertalot, *Staurosira venter* (Ehrenberg) Grunow and *Staurosirella pinnata* (Ehrenberg) D.M. Williams & Round. The diatoms that dominated exclusively in the epilithon were *Achnantheidium pyrenaicum* (Hustedt) H. Kobayasi, *Fragilariforma nitzschoides* (Grunow) D.M. Williams & Round and *Reimeria sinuata* (W. Gregory) Kociolek & Stoermer; in bryophytic samples they were replaced by *Caloneis fontinalis* (Grunow) Lange-Bertalot & E. Reichardt and *Meridion circulare* (Greville) C. Agardh. The other common diatoms (>10% relative abundance) were *Cocconeis placentula* Ehrenberg, *Denticula tenuis* Kützing, *Diatoma mesodon* (Ehrenberg) Kützing, *Gomphonema micropus* Kützing, *G. cf. pumilum* (Grunow) E. Reichardt & Lange-Bertalot, *Planothidium minutissimum* (Krasske) E. Morales and *P. reichardtii* Lange-Bertalot & Werum. The diatoms reaching >20% relative

abundance formed 67.3% of all valves identified in the counts of samples from epilithic substrates, and 74.4% of those from bryophytic substrates; the respective values increase to 95.8 and 96.0% when all taxa having over 10% relative abundance are included. Twenty taxa accounting for >1% of the diatom assemblages at more than three of the springs were found in the epilithon, and 13 such species in the epibryon; those species were included in the statistical analyses. The species–locality relationship analysis (PCA) of all the samples revealed that the first two principal components accounted for 18.9% (axis 1) and 16.1% (axis 2) of the total variation of species composition (Fig. 1). The first axis separates samples collected from springs situated in or near villages (mainly areas covered by postglacial sands) from those situated in localities less affected by human impacts (mainly loess-covered areas) (Table 1). Axis 2 separates samples collected from epilithic and bryophytic substrates. The main positive contributions to the results were from *Caloneis fontinalis*, *Meridion circulare*, *Planothidium lanceolatum*, *Gomphonema cf. pumilum*, *Diatoma mesodon* and *Diploneis krammeri* Lange-Bertalot & E. Reichardt, which were more abundant in bryophytic samples, and *Denticula tenuis*, *Eolimna minima* (Grunow) Lange-Bertalot, *Staurosirella pinnata*, *Staurosira venter*, *Planothidium reichardtii*, *Achnantheidium*



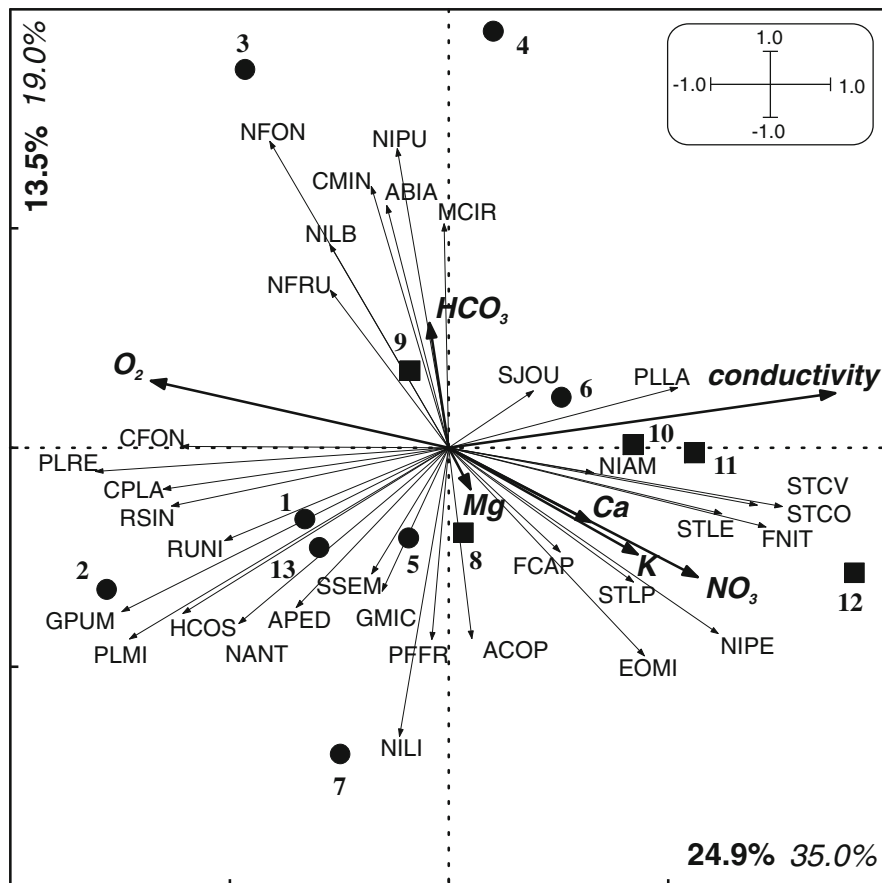
**Fig. 1** PCA ordination biplot. The first axis separates samples collected from springs in or near villages from those in less human-altered localities, away from villages. Empty circles Bryophytic samples, filled circles epilithic samples from springs located away from villages, empty squares bryophytic samples, filled squares epilithic samples from springs located in or near villages. Numbers localities. E epilithon, B submerged bryophytes, ABIA *A. pyrenaicum*, AMIN *Achnanthydium minutissimum*, ACUR *Psammothidium* cf. *curtissimum* (Carter) Aboal, APED *Amphora pediculus*, CFON *Caloneis fontinalis*, CMIN *Encyonema minutum*, CSIL *E. silesiacum* (Bleisch) D.G. Mann,

CPLA *Cocconeis placentula*, CPLI *C. placentula* var. *lineata* (Ehrenberg) Van Heurck, DIKR *D. krammeri*, DIME *Diatoma mesodon*, DTEN *Denticula tenuis*, EOMI *Eolimna minima*, FNIT *Fragilariforma nitzschioides*, GMIC *Gomphonema micropus*, GPUM *G. cf. pumilum*, MCIR *Meridion circularis*, NFON *Nitzschia fonticola*, NIPE *Nitzschia perminuta* (Grunow) Peragallo, NIPU *N. pura* Hustedt, PLLA *Planothidium lanceolatum*, PLMI *P. minutissimum*, PLRE *P. reichardtii* Lange-Bertalot & Werum, SJOU *Sellaphora joubaudii* (H. Germain) Aboal, STCO *Staurosira construens* Ehrenberg, STCV *S. venter*, STLP *Staurosirella pinnata*

*pyrenaicum* and *Gomphonema micropus*, which were more numerous in epilithic samples.

RDA based on combined data from epilithic and bryophytic assemblages showed that the first two axes accounted for 24.9% (axis 1) and 13.5% (axis 2) of the total variability in the species composition (Fig. 2). More than half of the total variability of the relationship between species composition and environmental variables was explained by the first two axes: 35.0% by axis 1 and 19.9% by axis 2, but these axes were not significant ( $P > 0.05$ ).

In an additional PCA restricted to the bryophytic diatom assemblages the first two principal components accounted for 31.8% (axis 1) and 21.9% (axis 2) of the total variation of species composition. RDA for these assemblages showed that the first two axes explain 29.7% (axis 1) and 9.4% (axis 2) of variance in species composition and 52.5% (axis 1) and 17.6% (axis 2) the relationships between species composition and environmental variables. The first axis (RDA) was significant ( $P = 0.008$ ) and correlated positively with dissolved oxygen concentration and negatively



**Fig. 2** RDA ordination triplot. Numbers localities. Arrows—species and environmental variables. In **bold**—% of variance in species composition, in *italics*—% of variance in species–environment relationship. *ABIA* *Achnanthydium pyrenaicum*, *ACOP* *Amphora copulata*, *APED* *A. pediculus*, *CFON* *Caloneis fonticola*, *CMIN* *Encyonema minutum*, *CPLA* *Cocconeis placentula*, *EOMI* *Eolimna minima*, *FCAP* *Fragilaria capucina*, *FNIT* *Fragilariforma nitzschoides*, *GMIC* *Gomphonema micropus*, *GPUM* *G. cf. pumilum*, *HCOS* *Hippodonta costulata* (Grunow) Lange-Bertalot, *MCIR* *Meridion circulare*, *NANT*

*Navicula antonii* Lange-Bertalot, *NFON* *Nitzschia fonticola*, *NIAM* *N. amphibia* Grunow, *NFRU* *N. frustulum* (Kützing) Grunow, *NILB* *N. liebetruthii* Rabenhorst, *NILI* *N. linearis*, *NIPE* *N. permitis*, *NIPU* *N. pura*, *PFFR* *Planothidium frequentissimum*, *PLLA* *P. lanceolatum*, *PLMI* *P. minutissimum*, *PLRE* *P. reichardtii*, *RSIN* *Reimeria sinuata*, *RUNI* *R. uniseriata* Sala, Guerrero & Ferrario, *SJOU* *Sellaphora joubaudii*, *SSEM* *S. seminulum*, *STCO* *Staurosira construens*, *STCV* *S. venter*, *STLP* *Staurosirella pinnata*, *STLE* *S. leptostauron* (Ehrenberg) D.M. Williams & Round

with specific conductivity and nitrate concentration. The first axis again separated the samples collected from springs with higher dissolved oxygen concentration and lower specific conductivity (Harczerza, Siamoszyce and Ruskie springs), with predominance of *Gomphonema cf. pumilum*, *Caloneis fontinalis*, *Cocconeis placentula* and *Diatoma mesodon*, which were replaced by *Planothidium lanceolatum* and *Eolimna minima* in springs with higher nutrient concentration and higher specific conductivity (in Wegrzynów and Łany Wielkie springs). Higher relative abundance of *Staurosirella pinnata* was related to springs with sandy

bottom and high discharge (e.g. Imbramowice spring); the occurrence of *Achnanthydium minutissimum*, *Meridion circulare* and *Amphora pediculus* was typical of small springs with gravel and silt bottom.

PCA analysis of epilithic diatom assemblages only revealed that the first two principal components accounted for 30.2% (axis 1) and 15.4% (axis 2) of the total variation of species composition. RDA for these assemblages showed that the first axes explain 23.3% (axis 1) and 11.4% (axis 2) of variation in a structure of diatom assemblages and 37.5% (axis 1) and 18.3% (axis 2) in the relationship between species



composition and environmental factors, but both axes were not significant ( $P > 0.05$ ). The first axis separated samples collected in springs with elevated specific conductivity, high nitrates, sulphates, calcium and orthophosphates, from those less eutrophicated, with higher dissolved oxygen concentration. The largest contribution to separation of the more eutrophicated springs was from *Eolimna minima*, *Planothidium lanceolatum*, *Staurosirella pinnata* and *Staurosira venter*. The rest of the springs with higher dissolved oxygen and lower specific conductivity were mainly colonized by *Achnanthydium minutissimum*, *Amphora pediculus*, *Planothidium reichardtii*, *Cocconeis placentula*, *Gomphonema cf. pumilum*, *Encyonema minutum* (Hilse) D.G. Mann, *Nitzschia fonticola* Grunow, *Denticula tenuis* and *Achnanthydium pyrenaicum*.

The diatom indices, TDI and SPI, differed significantly ( $P = 0.002$  and  $P = 0.004$ ) between springs in villages (no. 8–12, Table 1) and springs away from villages (no. 1–7 and 13, Table 1), and did not significantly differ between epilithic and bryophytic samples ( $P > 0.05$ ).

## Discussion

The minimal fluctuation of chemical parameters in springs (Sabater & Roca, 1990; Cantonati et al., 2006; Dumnicka et al., 2007), as compared to other freshwater systems and especially lakes, is related to the continuous renewal of the aquatic environment in springs. In karst areas, where pollutants are easily transported into the aquifer, spring water chemistry reflects changes in the whole area of the aquifer feeding the springs (Siwek, 2004). The composition of natural spring water is related to natural factors such as the weathering resistance of carbonate rocks, reflected mainly in total mineral content. High nitrate concentrations have been observed in several springs of this upland for decades, and are a permanent feature (Baścik et al., 2001; Siwek, 2004; Wojtal et al., 2009). The increased specific conductivity and  $\text{Na}^+$ ,  $\text{Cl}^-$ ,  $\text{K}^+$  and  $\text{SO}_4^{2-}$  concentrations are anthropogenic in nature (Rózkowski, 1996; Baścik et al., 2001; Siwek, 2004), attributable to animal husbandry, agriculture and the absence of sewage treatment systems in villages (Galas, 2005). The differences in water quality (Tables 2, 3) were clearly reflected in the composition of epilithic and bryophytic diatom assemblages. The

high bacterial contamination in springs in villages (Table 2) indicates pollution from wastewater.

Stones are one of the most common substrates in fast-flowing waters, but are much rarer in lowland areas. Boulders, cobbles and gravel were virtually absent in 9 of the 36 springs checked preliminary to the study, and at 3 more localities there were no epilithic diatom assemblages present, presumably as a consequence of insufficient light availability. Those springs were excluded from further study. In most springs, however, there were submerged bryophytes. Despite negative interaction—physical and sometimes chemical—between epiphytic algae and host macrophytes, epiphytic diatoms do secure a location for growth, improved access to light and shelter from scouring (Burkholder 1996). According to Burkholder (1996), loosely attached epiphytes generally do not exhibit substrate preferences. Moreover, the preferences of epiphytic algae for specific host plants decrease with increasing nutrient concentration (Eminson & Moss, 1980; Hall & Smol, 1999). The clearest relationships between hosts and diatom assemblages are known from oligotrophic waters, where nutrient limitation is one of the most important factors governing diatom distributions. In these conditions, macrophytes are an additional nutrient source (Wetzel, 1983) or even the main one (Eminson & Moss, 1980). Unfortunately, the data on the diatom floras of spring bryophytes are sparse (Cantonati & Spitale, 2009). Most epilithic and bryophytic diatoms share similar characteristics enabling them to adapt to these habitats, including small size and adaptations enabling adhesion. Some species seemed to prefer certain substrates (e.g. *Caloneis fontinalis* in submerged bryophytes, *Achnanthydium pyrenaicum* on stones) but the general response of diatom assemblages as a whole to water chemistry was very similar. The results are in agreement with those from other studies of running waters (Jüttner et al., 1996; Rothfritzt et al., 1997; Rott et al., 1998; Potapova & Charles, 2005) and springs (Cantonati & Spitale, 2009).

The relatively low species richness in the studied springs corresponds well with results from springs in the Pyrenees (Sabater & Roca, 1990, 1992) and Alps (Cantonati, 1998; Cantonati & Spitale, 2009), but could also be related to anthropogenic alteration of spring water quality in that upland, where some factor or set of factors limits the growth of the most sensitive taxa. In some springs, the epilithic diatom assemblages were dominated by the neutrophilous *Achnanthydium*

*minutissimum*, whereas bryophytic samples (*Fontinalis antipyretica* L.) were dominated by the alkaliphilous *Planothidium lanceolatum*. The differences might be related to host (bryophytes) photosynthetic processes, as host biological processes drive a rapid increase of pH (Wetzel, 1983) and precipitation of calcium carbonate (Burkholder, 1996). In addition, *Planothidium lanceolatum* prefers substrates that provide protection from abrasion (Biggs, 1996), and such conditions can be expected in submerged bryophytes (Cantonati & Spitale, 2009). Note that although the observed assemblages consisted of common diatoms having up to 96% relative abundance, of which several probably are habitat generalists; they represented only one-third of the total number of taxa identified in diatom counts. Those findings are in agreement with Townsend & Gell (2005) who observed the occurrence of common taxa in large numbers on all substrates, whereas taxa unique to a single substrate attained 0.1–2% relative abundance. In our study, the rest of diatoms (85 taxa) were rare (<1%), and their presence varied greatly among springs. Sometimes the more common diatoms also reflected the individual character of the assemblages in certain springs. For example, the spring in Węgrzynów was the only one where *Fragilariforma nitzschioides* was observed, reaching over 60% relative abundance. Unlike in springs in the Alps (Cantonati & Spitale, 2009), we did not find significant differences in species richness or the diversity index ( $H'$ ) between the epilithic and the bryophytic diatom assemblages.

The general variability of the aquatic environment and especially of total ionic strength is known to play a very important part in determining the distribution of diatoms (Sabater & Roca, 1990; Soininen, 2007; Cantonati & Spitale, 2009; Angeli et al., 2010). Potapova & Charles (2005) demonstrated that the effect of ionic strength is of much greater significance than the type of substrate colonized. According to Hall & Smol (1999), the diatom assemblages on various substrates become uniform in meso- and eutrophic waters, where nutrient limitation is weak. Our results support this. Changes in diatom assemblages were associated mostly with nutrient concentration, which was higher in springs in or near villages, as reflected in the TDI and SPI indices. Specific conductivity, dissolved oxygen and correlated factors had a strong effect on the structure of the diatom assemblages studied.

The methodological standards of monitoring programs recommend collecting material from stones (Round, 1991, 1993; Kelly et al., 1998), but this community is often absent in slow-flowing waters, and that limits the method to localities from which such material can be sampled. A great number of localities do offer bryophytic material for sampling. In our study, the data from both the epilithic and the bryophytic samples yielded diatom indices (TP, SPI) that reflected differences in water quality between springs, demonstrating that the two substrate types can be sampled for water quality assessment in slow-flowing mesotrophic and eutrophic waters.

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