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REGULAR PAPER

The response of epilithic diatom assemblages to sewage pollution in mountain streams of the Czech Republic

Adéla Moravcová[1], Ota Rauch[2], Jaromír Lukavský[2] & Linda Nedbalová[1,2,*]

[1]Charles University in Prague, Faculty of Science, Department of Ecology, Viničná 7, CZ-12844 Prague 2, Czech Republic
[2]Institute of Botany, Academy of Sciences of the Czech Republic, Dukelská 135, CZ-37982 Třeboň, Czech Republic
*Author for correspondence: lindane@natur.cuni.cz

Background and aim – Mountain streams represent sensitive ecosystems of prime importance for biodiversity conservation. However, the knowledge of the impact of sewage pollution related to intensive recreational exploitation is still limited in these habitats. Our aim was to assess the response of stream diatom assemblages to sewage pollution in the Giant Mountains and the Bohemian Forest, and to find the main factors influencing the community structure.
Methods – Epilithic samples from sixteen streams were collected, both in unaffected stream sections and at sites downstream of sewage outflows from mountain cottages or small villages. Basic physical and chemical parameters were measured and relative abundances of diatoms were determined using light microscopy.
Key results – A total number of 153 diatom taxa belonging to 44 genera were identified. Based on species data, samples were divided into two groups: the first belonged mainly to sites influenced by pollution, whereas the second consisted of not or slightly affected sites. Both groups were further divided into several subgroups characterised by specific ecological conditions and assemblage composition. Canonical correspondence analysis (CCA) revealed five environmental parameters with significant influence on diatom species composition, some of which were related to sewage pollution (pH, water temperature, discharge volume and concentrations of nitrate nitrogen and organic nitrogen).
Conclusions – As a consequence of sewage pollution, oligo- to mesotraphentic taxa were outcompeted by pollution tolerant taxa. However, pollution had no significant influence on diatom diversity, which was similar at sites both upstream and downstream of the outflows. In this study, diatom assemblages performed well as indicators of sewage pollution, and biomonitoring proved to be a useful tool in the detection of environmental stress in mountain streams. Overall, the response of diatom assemblages showed that an increase of recreational activities might significantly alter the ecological status of these ecosystems.

Key words – Epilithic diatoms, mountain stream, assemblage composition, sewage pollution, recreation, nitrogen concentration, Giant Mountains, Bohemian Forest.

INTRODUCTION

The European Union's Water Framework Directive (2000/60/EC) was established as a framework for community action in the field of water policy (EU 2000). Its general objective is to achieve 'good quality' status for all surface waters throughout Europe by 2015. Water status monitoring programmes use both chemical and biological elements to assess water quality (Allan et al. 2006). Although chemical analyses are routinely used technique, this approach has several disadvantages, e.g. underestimation of variations occurring over short periods (Marker & Collet 1991) or the inability to detect all trace organic pollutants (Whitton 1991). Therefore, monitoring of indicator organisms has begun to be an important part of water management (Whitton & Kelly 1995, EU 2000). Algae respond rapidly to various pollutants and provide useful early warning signals about deteriorating ecosystem conditions (McCormick & Cairns 1994). Diatoms (Bacillariophyta) are routinely used algal indicators, which have a clear relationship to water quality (Cox 1991, Round 1991, Reid et al. 1995) and reflect environmental stress through shifts in the community species composition (Rott 1991). For taxonomic identifications, comprehensive diatom keys are available (e.g. Krammer & Lange-Bertalot 1986, 1988, 1991a, 1991b, Lange-Bertalot 2001, Krammer 2000, 2002, 2003), although recent diatom taxonomy is rather complicated due to the existence of many cryptic species and problematic species complexes (e.g. Mann et al. 2004, Potapova & Hamilton 2007, Vanormelingen et al. 2008).

There are many factors determining periphyton biomass and structure in rivers and standing waters, with nutrient status being one of the most important (Rosemond et al. 1993, Dodds et al. 2002, Carr et al. 2005, Veraart et al. 2008). Therefore, changes in diatom communities are used to indicate eutrophication (Sládeček 1986, Kelly 2003, Kelly & Wilson 2004, Poulíčková et al. 2004, Bellinger et al. 2006). Diatoms are also sensitive to water pH, and the occurrence of particular taxa can indicate acidification (Coring 1996, Passy 2006). As a result, they have successfully been used in the establishment of diatom-environmental transfer functions predicting pH values and nutrient concentrations (Cameron et al. 1999, King et al. 2000, Winter & Duthie 2000, Kovácz et al. 2006). The structure of diatom communities may also provide information about the impacts of heavy-metal pollution on freshwater ecosystems (Cattaneo et al. 2004, Duong et al. 2008). Many diatom based indices have recently been used in routine monitoring programmes in European Union's countries (Lenoir & Coste 1996, Kelly 1998, Rott et al. 2003, Rimet et al. 2005, Szilágyi et al. 2008, Kelly et al. 2008, 2009, Szczepocka & Szulc 2009).

Although extensive research into the impacts of eutrophication on diatoms has already been performed in lowland rivers and lakes (e.g. Lowe & McCullough 1974, Rott et al. 1998, Soininen 2002, Potapova et al. 2005, Camargo & Jiménez 2007), mountain stream assemblages have received much less attention. Algae from low order mountain streams, however, respond much more rapidly to pollution in comparison with algae from larger streams (Rott et al. 2006), and therefore they can quickly indicate actual environmental changes. This topic has recently been the subject of increased interest, mainly because of the continually expanding recreational activities within mountain ecosystems. A few studies have explored the impact of domestic sewage or agriculture on mountain stream periphyton or diatom assemblages in particular (Bombówna 1977, Kawecka 1977, 1980, 1981, Jüttner et al. 1996, 2003, Lukavský et al. 2004, 2006, Yu & Lin 2009), and showed a considerable shift in the species composition of attached algae from oligotraphentic towards eutraphentic taxa as the result of anthropogenic pollution.

This study investigates epilithic diatom assemblages from streams of the Giant Mountains and the Bohemian Forest (Czech Republic). Until recently, waters of both mountain ranges have been heavily impacted by anthropogenic acidification (Vrba et al. 2003, Sienkiewicz et al. 2006). Due to the marked decline of sulphur and nitrogen emissions in Central Europe during 1990s, a significant but slow recovery of water chemistry was observed (Vrba et al. 2003). Currently, increasing recreational activities are a new threat to the stability of these ecosystems. Mountain streams are among the first recipients of potential anthropogenic pollution (such as agricultural runoff, domestic sewage outflows, pasturage). Nevertheless, only few detailed studies using local stream periphyton as the indicator of water quality changes are available. Picińska-Fałtynowicz (2007) described the ecological preferences of diatom flora in selected streams of the Giant Mts. Lukavský et al. (2004, 2006) observed a significant influence of sewage outflows on periphytic assemblages of the Bohemian Forest streams.

Using a reference condition approach, the aim of our study was to explore the response of epilithic diatom assemblages to sewage outflows from cottages and small villages in selected mountain streams of the Giant Mts. and the Bohemian Forest. We have also tried to assess the main environmental factors influencing diatom assemblage composition.

MATERIALS AND METHODS

Sampling sites

We investigated sixteen streams in two Czech mountain ranges, the Giant Mts. (Krkonoše) and the Bohemian Forest (Šumava), both of which have the status of a national park. The Giant Mts. are situated in the north eastern part of the Czech Republic on the border with Poland. Twenty three sites on nine streams were sampled at elevations from 520–1420 m (fig. 1A, table 1). The geology of the area is composed of Krkonoše-Jizera Crystalline (mica schists,

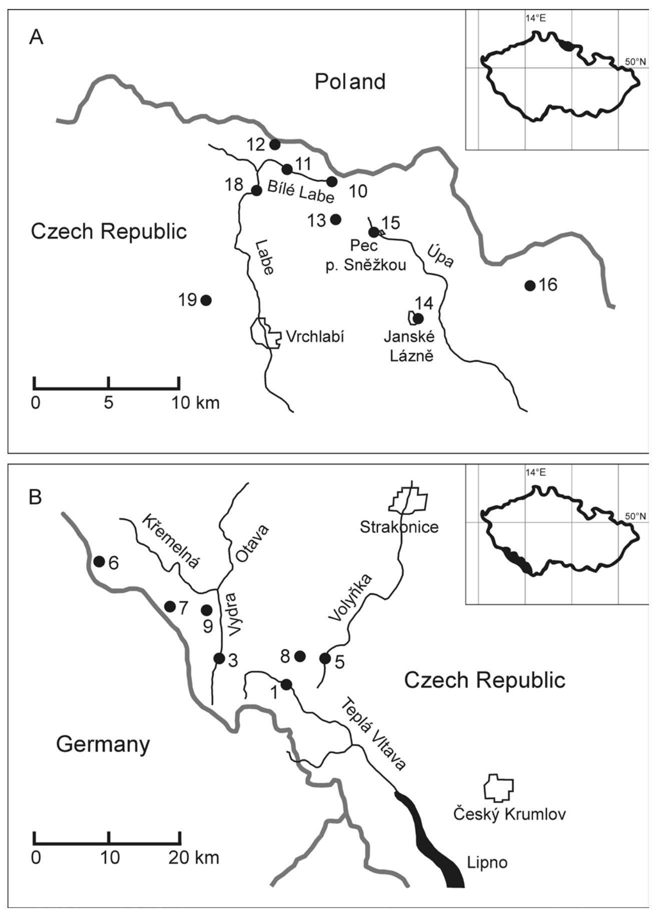

Figure 1 – Location of the investigated streams in: A, the Giant Mountains; B, the Bohemian Forest. Stream codes: 1 – Vltava, 3 – Vydra, 5 – Spůlka, 6 – Řezná, 7 – Prášilský potok, 8 – Vydří potok, 9 – Hrádecký potok, 10 – Bílé Labe (upper part), 11 – Bílé Labe (lower part), 12 – Hřímavá bystřina, 13 – tributary of Zelený potok, 14 – Jánský potok, 15 – Úpa, 16 – Kalná, 18 – Dolský potok, 19 – Žalský potok.

Table 1 – List of sampling sites.
BF: the Bohemian Forest; GM: the Giant Mountains. Codes in bold represent outflows of sewage treatment plants, asterisks mark sites where sampling was performed only once.

Range	Stream	Code		GPS		Altitude
				N	E	m a.s.l.
BF	Vltava	1	1.2	49°00’51.9”	13°34’38.4”	1048
			1.3	49°00’46.9”	13°35’04.6”	1030
			1.4	48°59’58.9”	13°37’57.3”	949
			1.5	48°59’40.0”	13°39’23.2”	904
			1.6**	48°59’23.5”	13°39’40.9”	894
			1.7	48°59’21.1”	13°39’43.0”	891
	Vydra	3	3.1	49°03’22.6”	13°30’42.7”	913
			3.2	49°03’33.6”	13°30’40.9”	911
			3.3	49°04’54.7”	13°30’54.3”	815
			3.4	49°04’56.8”	13°30’49.9”	814
	Spůlka	5	5.1	49°03’58.5”	13°37’57.2”	959
			5.2	49°04’23.2”	13°38’02.3”	883
			5.3	49°04’02.8”	13°37’58.4”	938
			5.4	49°04’56.7”	13°38’49.9”	812
			5.5*	49°05’00.1”	13°38’31.3”	863
	Řezná	6	6.0*	49°08’59.1”	13°15’18.2”	841
			6.1	49°09’35.0”	13°15’39.8”	880
			6.2	49°07’42.1”	13°13’06.4”	729
	Prášilský potok	7	7.0	49°05’47.8”	13°22’46.6”	891
	Vydří potok	8	8.0*	49°02’57.1”	13°39’51.2”	990
			8.2	49°02’57.4”	13°39’54.4”	992
			8.3*	49°02’57.4”	13°39’54.4”	993
			8.4	49°02’56.0”	13°39’54.9”	990
			8.5*	49°02’53.5”	13°39’58.1”	985
	Hrádecký potok	9	9.1	49°04’51.7”	13°29’06.6”	813
			9.2	49°04’51.6”	13°29’07.9”	812
GM	Bílé Labe (upper part)	10	10.1	50°44’03.9”	15°41’54.9”	1416
			10.2	50°44’05.1”	15°41’44.5”	1412
			10.3	50°44’06.7”	15°41’41.5”	1409
			10.4	50°44’10.4”	15°41’34.2”	1401
	Bílé Labe (lower part)	11	11.1	50°44’28.9”	15°38’51.1”	1012
			11.2*	50°44’28.8”	15°38’51.1”	1012
			11.3	50°44’28.6”	15°38’47.9”	1005
	Hřímavá bystřina	12	**12.1**	50°45’24.7”	15°38’04.0”	1116
			12.2	50°45’20.9”	15°38’00.9”	1081
			12.3	50°44’54.5”	15°37’33.9”	879
	tributary of Zelený potok	13	13.1	50°42’36.5”	15°41’54.9”	1200
			13.2	50°42’32.5”	15°41’54.4”	1116
	Jánský potok	14	14.1	50°37’50.4”	15°46’00.2”	710
			14.2	50°37’43.9”	15°47’22.6”	607
	Úpa	15	15.1	50°41’29.9”	15°43’47.3”	803
			15.2	50°41’42.8”	15°44’29.4”	781
	Kalná	16	16.1	50°36’58.7”	15°50’24.4”	520
			16.2*	50°36’59.1”	15°50’27.1”	517
		18	16.3*	50°37’01.0”	15°50’22.6”	519
	Dolský potok		18.1*	50°43’36.3”	15°38’50.2”	884
			18.2*	50°43’41.1”	15°36’41.1”	762
	Žalský potok	19	19.1	50°39’01.7”	15°32’51.8”	606
			19.2	50°38’53.3”	15°32’51.9”	598

phyllites, orthogneisses) and Krkonoše-Jizera Pluton (granite) (Chaloupský 1989). The most frequent vegetation types are spruce-beech, spruce, beech-spruce and mountain pine forests and artic-alpine tundra. The mountain range has periods of long-lasting snowfall, with snow cover persisting until late spring (especially above 800 m a.s.l.) and snow melt floods occur often. The annual flow regime is usually characterised by a spring maximum and an autumn-winter minimum (Dubicki & Malinovska-Małek 2007).

The Bohemian Forest lies in the south western part of the country along the Czech-German-Austrian border. Twenty six sites on seven streams were sampled at elevations from 730–1050 m (fig. 1B, table 1). The bedrock is formed by granite containing a large amount of gneiss, micaschist and phyllite xenoliths (Babůrek 1996). Spruce monocultures are the prevailing vegetation, with a lesser area covered by mountain pine and artic-alpine tundra.

Average annual precipitation is similar for both ranges (800–1600 mm).

Streams running through areas affected by recreational activities were selected (the vicinity of mountain cottages or small villages with large number of tourists). Altogether, 132 samples were collected both in unaffected stream parts and at sites downstream of sewage outflows, in order to analyse the influence of sewage pollution. Sampling was carried out in summer 2004 and twice a year in 2005–2006 (early spring – beginning of snow melt, summer – period without snow cover).

Physical and chemical variables

In the field, pH, conductivity, water temperature and concentration of dissolved oxygen were measured using handheld instruments from WTW (Wissenschaftlich-Technische Werkstätten GmbH, Weilheim, Germany). Discharge was estimated from water depths across the river profile and current velocity. In the laboratory, acid neutralisation capacity (ANC), nutrients and chloride concentration were analysed following the methods described in Grasshof (1983). For more details, see Lukavský et al. (2006).

Sample preparation and diatom identification

At each sampling site, epilithic diatoms were scraped from three randomly chosen submerged stones with a toothbrush and preserved in formaldehyde (4% final concentration). In the laboratory, samples were washed several times with distilled water and then boiled in hydrogen peroxide to eliminate organic matter. Clean diatom frustules were mounted using Pleurax (Fott 1954). To assess relative species abundances, up to 300 valves were counted using a Nikon ECLIPSE E400 light microscope. Nomenclature follows Krammer & Lange-Bertalot (1986, 1988, 1991a, 1991b), Lange-Bertalot (2001) and Krammer (2000, 2002, 2003). The type slide of *Gomphonema parvulum* Kütz. var. *exilissima* Grunow [Types du Synopsis des Diatomées de Belgique n° 220 (Arran, Scotland) present in the Van Heurck Collection at the National Botanic Garden of Belgium] was observed to compare the type population with the population of *G. parvulum* Kütz. from studied streams and to confirm the correctness of *G. parvulum* identification.

Data analysis

The Shannon diversity index and evenness of diatom assemblages were calculated according to Shannon & Weaver (1949). To assess the relationships between environmental parameters and diversity, a correlation matrix was calculated using STATISTICA software (version 7.0, StatSoft Inc., Tulsa, Oklahoma). Significant correlations were set with $p < 0.05$. Nonparametric tests (Mann Whitney or Kruskal-Wallis with Dunn's multiple comparison test) were applied for comparisons of environmental data sets. To perform multivariate statistical analyses, only those diatom taxa occurring at least in one sample with a relative proportion of 1% or more were included in analyses. At first, detrended correspondence analysis (DCA) was performed to assess the length of the gradient. Since it was longer than 2, non-linear methods (DCA, CCA – canonical correspondence analysis) were used in subsequent analyses (ter Braak & Prentice 1988). To eliminate the influence of general stream characteristics (e.g. location, bedrock etc.) and sampling season, covariates were established combining relevant data. We used step-forward regression to explore those environmental parameters significantly correlated with assemblage composition ($p < 0.05$, Monte Carlo randomization test with 500 permutations), and included them in the model. To classify similarities among individual sampling sites, we ran cluster analysis of species data using STATISTICA (complete linkage clustering with the City-block distances as dissimilarity measure) and indirect ordination analysis of species data. All ordinations were processed using CANOCO and CanoDraw software (ter Braak & Šmilauer 2002). Acronyms of diatom taxa were generated *ad hoc*.

RESULTS

Environmental characteristics of sites

We measured twelve chemical parameters of water together with water temperature and discharge. There were large gradients in nutrient concentrations (ammonium nitrogen (NH_4-N) 1–54298 µg l^{-1}, nitrate nitrogen (NO_3-N) 436–28059 µg l^{-1}), conductivity (10–690 µS cm^{-1}) and pH (4.7–8.2). Water temperature was related mainly to sampling season – the average in early spring was 2.3 ± 1.4°C, and in summer 11.8 ± 2.7°C. In spring 2005 and 2006, there were higher concentrations of NH_4-N and NO_3-N in comparison with summer. Besides temperature changes, it was the only consistent interseasonal pattern. Discharge also corresponded with season, but the differences were not significant. It was always very low at some sampling sites (< 20 l s^{-1}). There were significant differences ($p < 0.01$) in ANC, conductivity, chlorides and nutrients [NH_4-N, nitrite nitrogen (NO_2-N), total dissolved nitrogen (TDN), organic nitrogen, phosphate phosphorus (PO_4-P), total dissolved phosphorus (TDP)] between unaffected sampling sites and sites downstream of sewage outflows. Several environmental characteristics (organic nitrogen, TDP, dissolved oxygen (DO) and chlorides)

Table 2 – Medians of environmental variables and diversity index in the Giant Mountains (GM) and the Bohemian Forest (BF).
Asterisks indicate significant differences between both mountain ranges (* p<0.05, ** p<0.01, *** p<0.001). Minimal and maximal values are given in parentheses. Abbreviations: (ANC) acid neutralisation capacity, (NH_4-N) ammonium nitrogen, (NO_2-N) nitrite nitrogen, (NO_3-N) nitrate nitrogen, (TDN) total dissolved nitrogen, (PO_4-P) phosphate phosphorus, (TDP) total dissolved phosphorus, (orgN) organic nitrogen, (Cl^-) chlorides, (Temp) water temperature, (DO) dissolved oxygen, (Cond) conductivity, (Q) discharge, (H) Shannon diversity index.

	GM	BF
ANC (mmol l^{-1})	**0.2** (0.02–2.1)	**0.3** (0.1–1.4)
NH_4-N (µg l^{-1})*	**37** (6–54298)	**76** (1–15540)
NO_2-N (µg l^{-1})**	**4** (2–144)	**7** (0.5–132)
NO_3-N (µg l^{-1})	**597** (21–28059)	**447** (31–3436)
TDN (mg l^{-1})	**1.0** (0.4–56.3)	**1.1** (0.7–17.2)
PO_4-P (µg l^{-1})	**17** (0.01–14976)	**23** (1–2086)
TDP (µg l^{-1})***	**80** (48–15754)	**101** (60–3170)
orgN (µg l^{-1})***	**350** (136–5320)	**542** (179–6715)
Cl^- (mg l^{-1})***	**0.9** (0.2–65.7)	**1.9** (0.7–20.0)
Temp (°C)	**9.1** (2.3–18.4)	**10.5** (0.3–17.6)
DO (mg l^{-1})***	**8.6** (1.8–19.0)	**7.3** (1.2–11.9)
pH	**6.4** (4.8–7.8)	**6.7** (4.7–8.2)
Cond (µS cm^{-1})	**36** (10–690)	**30** (13–169)
Q (l s^{-1})	**43** (0.1–2000)	**100** (0.1–3200)
H*	**2.29** (1.08–3.03)	**2.40** (1.14–3.22)

differed significantly ($p < 0.001$) between both mountain ranges (table 2).

Diatom species composition and diversity

We identified 153 diatom taxa (including species and varieties) belonging to 44 genera. The most abundant taxa were *Achnanthidium minutissimum* (Kütz.) Czarn. sensu lato, representing 10% of all counted valves, *Eolimna minima* (Grunow) Lange-Bert. (9%), *Diatoma mesodon* (Ehrenb.) Kütz. (9%), *Fragilaria capucina* Desm. sensu lato (8%) and *Gomphonema parvulum* (7%). The genus *Eunotia* dominated in the samples (12%) and had the highest taxa number (17) – *E. exigua* (Bréb.) Rabenh. (5%) and *E. minor* (Kütz.) Grunow (3%) being most prevalent. Other important genera were *Psammothidium* (6%), *Planothidium* (5%), *Encyonema* (4%), *Cocconeis* (3%) and *Pinnularia* (3%).

The medians of the Shannon diversity index were similar within both ranges (table 2), as well as at polluted (2.2 ± 0.5) and unaffected (2.3 ± 0.4) sampling sites. Except for a weak negative correlation with chloride concentration ($r^2 = 0.2$), no significant ($p < 0.05$) correlations of diversity and environmental variables were found. However, there were differences in the relative abundances of individual taxa between both mountain ranges (table 3).

Grouping of diatom assemblages

Based on the species data (115 diatom taxa were included in the model), cluster analysis divided the 132 samples into two groups. A comparison of the environmental parameters of these groups clearly showed that the division was generated predominantly due to gradients in nutrient concentrations, pH and discharge (table 4). Group 1 contained 70 samples and mainly linked sites affected by sewage outflows. Group 2 consisted of 62 samples from non-affected or only slightly affected sites with lower nutrient concentrations. Conductivity, ANC, pH and concentrations of NO_3-N, TDN, PO_4-P and chlorides were significantly higher within Group 1 when compared to Group 2 ($p < 0.001$), indicating the influence of sewage pollution on sites from Group 1. Significantly lower discharges ($p < 0.001$) were also characteristic for Group 1 (table 4).

The division of samples into the two groups was confirmed by DCA (fig. 2). The first two canonical axes comprised 18.8% of total explained variability ($\lambda_1 = 0.117$, $\lambda_2 = 0.071$). The first axis separated Groups 1 and 2 to the right and left side of the graph and therefore seemed to correspond with the impact of sewage pollution. In the case of Group 2, the second axis could be taken to represent affiliation to a particular mountain range, since it separated the Giant Mts. sites into the lower part of the graph, and Bohemian Forest sites to the upper part.

Furthermore, cluster analysis of species data belonging to individual groups (not shown) separated several assemblage types (subgroups), which were characterized by different dominant taxa and specific ecological conditions. This subdivision is visualised using DCA (fig. 3). However, eight samples had an outlying position and could not be included into any of the subgroups. This was partly caused by the dominance (or at least high relative abundance) of taxa which were usually rare in other samples, such as *Mayamaea atomus* var. *permitis* (Hust.) Lange-Bert. (in samples with high concentration of TDN (~ 2800 and 1700 µg L^{-1}) and TDP (~ 240 and 110 µg L^{-1}) together with very low discharge (1 and 2 L s^{-1}), *Adlafia suchlandtii* (Hust.) Lange-Bert., *Meridion circulare* (Grev.) C.Agardh var. *circulare* or *Surirella roba* Leclerq. Within Group 1, four subgroups (1A–D) could be distinguished (fig. 3A, table 4):

Subgroup 1A – samples most affected by sewage pollution, collected downstream of the outflows (Vydří, Spůlka, tributary of Hřímavá bystřina). The eutraphentic taxon *Eolimna minima* dominated and the meso- to eutraphentic *Planothidium lanceolatum* (Brébisson) Round & Bukht. and *Reimeria sinuata* (W.Greg.) Kociolek & Stoermer had high abundances. High electrolyte content and concentrations of both nitrogen and phosphorus compounds together with very low discharges were characteristic for these samples. The highest median concentration of organic nitrogen ($p < 0.05$) was found in this subgroup (table 4).

Subgroup 1B – samples collected in five streams in the Giant Mts. (Hřímavá bystřina, Janský potok, Úpa, tributary of Zelený potok and Dolský potok). *Achnanthidium minutissimum* s.l. and *Diatoma mesodon*, generally considered as oligo- to mesotraphentic, were dominant.

Table 3 – List of the main diatom taxa in Group 1 (sites strongly influenced by sewage pollution) and Group 2 (sites with no or slight influence of sewage pollution), together with differences in their relative abundances between the Giant Mts. (GM) and the Bohemian Forest (BF).
Mean percentage abundances are shown as the symbols: X ≥ 10; 5 ≤ x < –10; 1 ≤ o < 5, + < 1; – absent.

Taxon name	Code	Group 1		Group 2	
		GM	BF	GM	BF
Achnanthidium minutissimum (Kützing) Czarnecki sensu lato	ACHNMINU	X	x	x	x
Adlafia suchlandtii (Hustedt) Lange-Bertalot	ADLASUCH	o	o	+	+
Amphora pediculus (Kützing) Grunow	AMPHPEDI	o	+	+	+
Brachysira brebissonii Ross	BRACBREB	+	+	o	+
Cocconeis placentula var. *euglypta* Ehrenberg	COCCPLEU	+	–	+	–
Cocconeis placentula var. *lineata* (Ehrenberg) Van Heurck	COCCPLLI	x	o	+	+
Diatoma mesodon (Ehrenberg) Kützing	DIATMESO	x	x	X	x
Encyonema minutum D.G. Mann	ENCYMINU	o	x	+	+
Eolimna minima (Grunow) Lange-Bertalot	EOLIMINI	X	X	o	x
Eunotia bilunaris (Ehrenberg) Mills	EUNOBILU	+	+	+	+
Eunotia exigua (Brébisson) Rabenhorst	EUNOEXIG	o	x	X	o
Eunotia incisa Gregory	EUNOINCI	+	+	x	o
Eunotia minor (Kützing) Grunow	EUNOMINO	o	o	o	o
Eunotia muscicola var. *tridentula* Nörpel & Lange-Bertalot	EUNOMUTR	–	+	–	+
Eunotia rhomboidea Hustedt	EUNORHOM	+	+	+	o
Eunotia tenella (Grunow) Cleve	EUNOTENE	+	–	o	+
Fragilaria capucina Desmasières sensu lato	FRAGCAPU	o	x	x	X
Fragilaria virescens Ralfs	FRAGVIRE	+	+	o	o
Frustulia erifuga Lange-Bertalot & Krammer	FRUSERIF	–	–	+	+
Frustulia saxonica Rabenhorst	FRUSSAXO	+	+	+	+
Gomphonema olivaceum var. *minutissimum* Hustedt	GOMPOLMI	+	o	+	+
Gomphonema parvulum Kützing	GOMPPARV	o	x	o	X
Gomphonema productum (Grunow) Lange-Bertalot & Reichardt	GOMPPROD	+	+	+	+
Gomphonema sp.1	GOMPSPE1	–	+	+	+
Gomphonema truncatum Ehrenberg	GOMPTRUN	–	+	+	+
Hantzschia amphyoxis (Ehrenberg) Grunow	HANTAMPH	+	+	+	+
Karayevia oblongella (Østrup) Aboal	KARAOBLO	o	o	+	x
Mayamaea atomus var. *permitis* (Hustedt) Lange-Bertalot	MAYAATPE	o	+	–	+
Meridion circulare (Greville) Agardh var. *circulare*	MERICICI	o	o	+	+
Meridion circulare var. *constrictum* (Ralfs) Van Heurck	MERICICO	+	+	+	o
Navicula cryptocephala Kützing	NAVICRYP	+	+	+	+
Navicula gregaria Donkin	NAVIGREG	+	+	+	+
Navicula lundii Reichardt	NAVILUND	+	+	+	+
Navicula rhynchocephala Kützing	NAVIRHYN	+	+	–	+
Naviduladicta seminulum (Grunow) Lange-Bertalot	NAVISEMI	+	+	+	–
Nitzschia dissipata (Kützing) Grunow	NITZDISS	+	+	+	+
Nitzschia fonticola Grunow	NITZFONT	+	+	–	+
Nitzschia frustulum (Kützing) Grunow	NITZFRUS	+	+	+	+
Nitzschia inconspicua Grunow	NITZINCO	+	+	–	–
Nitzschia palea (Kützing) W. Smith	NITZPALE	o	o	+	+
Pinnularia brebissonii var. *bicuneata* Grunow	PINNBRBI	+	+	+	+

Table 3 (continued) – List of the main diatom taxa in Group 1 (sites strongly influenced by sewage pollution) and Group 2 (sites with no or slight influence of sewage pollution), together with differences in their relative abundances between the Giant Mts. (GM) and the Bohemian Forest (BF).

Taxon name	Code	Group 1		Group 2	
		GM	BF	GM	BF
Pinnularia subcapitata Gregory	PINNSUBC	+	+	o	o
Planothidium lanceolatum (Brébisson) Round & Bukhtiyarova	PLANLANC	x	X	+	o
Psammothidium bioretii (Germain) Bukhtiyarova & Round	PSAMBIOR	+	+	o	+
Psammothidium subatomoides (Hustedt) Bukhtiyarova & Round	PSAMSUBA	o	o	x	x
Reimeria sinuata (Gregory) Kociolek & Stoermer	REIMSINU	o	o	+	+
Surirella roba Leclerq	SURIROBA	+	+	o	+
Synedra ulna (Nitzsch) Ehrenberg	SYNEULNA	+	+	+	+
Tabellaria flocculosa (Roth) Kützing	TABEFLOC	+	+	x	o

Subgroup 1C – samples from the same streams as in 1B, but only from sites downstream of sewage outflows. The abundance of meso- to eutraphentic *Cocconeis placentula* var. *lineata* (Ehrenberg) Van Heurck markedly increased. The influence of sewage pollution was indicated by significantly higher ANC, pH, electrolyte content and TDN concentration (p 0.05) within Subgroub 1C, when compared to Subgroup 1B (table 4).

Subgroup 1D – samples collected in the Bohemian Forest, at sites moderately influenced by sewage pollution (Vltava River below the Borová Lada village, sites from the stream Spůlka upstream or far downstream of the sewage outflow, Řezná). The eutraphentic taxa *Planothidium lanceolatum* and *Eolimna minima* dominated, but the oligo- to mesotraphentic *Diatoma mesodon*, *Fragilaria capucina* s.l., *Encyonema minutum* D.G. Mann and *Achnanthidium minutissimum* s.l. also had high relative abundances.

Within the "oligotrophic" Group 2, four subgroups (2A–D) could be distinguished (fig. 3B, table 4):

Subgroup 2A – all samples from the upper part of the Bílé Labe River. The oligotraphentic *Psammothidium subatomoides* (Hustedt) Bukhtiyarova & Round and oligo- to mesotraphentic *Diatoma mesodon* dominated. High relative abundances of *Eunotia incisa* Gregory and *E. minor* indicated more acidic conditions.

Subgroup 2B – all samples from the lower part of the Bílé Labe River, characterised mainly by high discharge and low nutrient concentrations. The acidophilic *Eunotia exigua* together with *Achnanthidium minutissimum* s.l. were the dominant taxa.

Subgroup 2C – parts of the stream Vydří far downstream of the sewage outflow. Low pH at these sites (pH ~ 5.3) was reflected by acidophilic assemblages, dominated mainly by *Eunotia* species (*E. rhomboidea* Hust., *E. minor*, *E. exigua*) and *Planothidium lanceolatum*. This subgroup was also characterised by low discharge (1 l s^{-1}).

Subgroup 2D – samples collected in four Bohemian Forest streams (Vydra and Vltava near the Kvilda village, Prášilský potok and Hrádecký potok). These streams were characterised mainly by high discharge and low nutrient concentrations. *Gomphonema parvulum* and *Fragilaria capucina* s.l. dominated in these assemblages.

While several assemblage types were identified in the Giant Mts. (separated as individual subgroups 1A,1B,1C,2A,2B), the majority of the Bohemian Forest samples was divided into only two subgroups (meso- to eutrophic subgroup 1D, oligo- to mesotrophic subgroup 2D), Subgroup 2C was an exception with specific environmental conditions (fig. 3A & B).

Overall, CCA demonstrated that concentrations of NO_3-N and organic nitrogen, pH, water temperature and discharge were the parameters having the most significant impact on species composition ($p < 0.05$). Altogether, they explained 15.8% of the cumulative variance in the species data. The relationships of the 34 main diatom taxa to these parameters, together with the position of subgroups along the environmental gradient, are shown in fig. 4. The abundant species *Eolimna minima* and *Planothidium lanceolatum* were clearly associated with eutrophic circumneutral conditions, while *Eunotia* spp. were characteristic for oligo- to mesotrophic conditions with lower pH.

DISCUSSION

Diatom species composition and diversity

Diatom species composition and richness were similar as in previous surveys performed in these mountain ranges. Picińska-Fałtynowicz (2007) studied epilithic stream diatoms in the Polish part of the Giant Mts. and the Sněžník Massif and identified 184 diatom taxa. Since her study focused only on streams with ultraoligo- to oligotrophic water, she did not find *Eolimna minima*, which dominated at sites strongly influenced by wastewater in our study (fig. 3A). Lukavský et al. (2004) identified 144 diatom taxa in streams in the Czech and German parts of the Bohemian Forest and their records correspond well with our results. Diatom assemblages with similar species richness have been found in other European (Kawecka 1981, Pfister 1992, Rott et al. 2006, Kawecka & Robinson 2008) and Asian mountain ranges (Jüttner et al. 1996, 2003, Yu & Lin 2009).

Table 4 – Medians and ranges of environmental variables and diversity index in Group 1 (sites strongly influenced by sewage pollution) and Group 2 (sites with no or slight influence of sewage pollution).
Values are given for all sites within the group (1 Tot and 2 Tot) and for individual subgroups (1A–D, 2A–D). The data were available for 119 samples. Asterisks indicate significant differences between the two groups (* $p < 0.05$, ** $p < 0.01$), *** $p < 0.001$). Concerning the variability within subgroups, values with different letters are significantly different ($p<0.05$). Minimal and maximal values are given in parentheses. Abbreviations: (ANC) acid neutralisation capacity, (NH_4-N) ammonium nitrogen, (NO_2-N) nitrite nitrogen, (NO_3-N) nitrate nitrogen, (TDN) total dissolved nitrogen, (PO_4-P) phosphate phosphorus, (TDP) total dissolved phosphorus, (orgN) organic nitrogen, (Temp) water temperature, (DO) dissolved oxygen, (Cond) conductivity, (Q) discharge, (H) Shannon diversity index.

	Group 1					Group 2				
	1 Tot	1A	1B	1C	1D	**2 Tot**	2A	2B	2C	2D
Number of samples	**63**	12	16	13	22	**56**	10	10	4	32
ANC (mmol l^{-1})***	**0.4** (0.1–2.1)	0.6 (0.1–0.9)^{ab}	0.2 (0.1–0.4)^{c}	0.9 (0.5–2.1)^{a}	0.3 (0.2–1.0)^{bc}	**0.2** (0–0.9)	0.2 (0–0.3)^{ab}	0.1 (0.1–0.2)^{a}	0.1 (0.1–0.2)^{a}	0.2 (0.1–0.9)^{b}
NH_4-N (µg l^{-1})*	**72** (4–54298)	524 (11–10211)^{a}	24 (4–72)^{b}	56 (23–54298)^{ab}	122 (11–6666)^{a}	**48** (1–1826)	55 (24–115)^{a}	29 (9–60)^{a}	174 (33–571)^{a}	49 (1–1826)^{a}
NO_2-N (µg l^{-1})*	**7** (1–144)	23 (6–132)^{ab}	2 (2–8)^{c}	6 (3–144)^{cd}	10 (1–66)^{bd}	**5** (1–29)	3 (2–7)^{a}	4 (2–5)^{a}	6 (6–18)^{a}	5 (0.5–29)^{a}
NO_3-N (µg l^{-1})***	**756** (23–28059)	1326 (31–28059)^{a}	450 (40–986)^{b}	790 (23–1865)^{ab}	715 (317–2048)^{ab}	**368** (131–1690)	223 (131–426)^{a}	488 (219–662)^{b}	284 (146–392)^{ab}	393 (275–1690)^{b}
TDN (mg l^{-1})***	**1.4** (0.4–56.3)	3.3 (1.4–43.6)^{a}	1.0 (0.4–1.5)^{b}	1.6 (0.8–56.3)^{ac}	1.4 (0.9–8.0)^{c}	**0.9** (0.4–6.1)	0.6 (0.4–1.2)^{a}	0.8 (0.4–1.0)^{ab}	1.1 (0.9–1.6)^{bc}	1.0 (0.8–6.1)^{c}
PO_4-P (µg l^{-1})***	**35** (0–14976)	241 (21–14976)^{ab}	13 (0–38)^{c}	35 (10–7372)^{cd}	43 (9–642)^{bd}	**15** (0–317)	15 (6–59)^{ab}	10 (0–20)^{a}	95 (19–127)^{b}	18 (1–317)^{ab}
TDP (µg l^{-1})	**95** (48–15754)	245 (83–15754)^{ab}	71 (49–126)^{c}	68 (48–7877)^{cd}	103 (61–711)^{bd}	**90** (48–418)	76 (48–104)^{ab}	74 (48–94)^{a}	170 (86–260)^{c}	94 (60–418)^{bc}
orgN (µg l^{-1})	**486** (167–6715)	1075 (445–6715)^{a}	353 (184–798)^{b}	404 (167–1996)^{bc}	529 (179–1014)^{c}	**413** (136–2538)	264 (136–843)^{a}	252 (171–388)^{a}	637 (562–733)^{b}	516 (273–2538)^{b}
Temp (°C)	**10.8** (0.5–18.4)	11.9 (2.5–18.4)^{a}	9.5 (0.6–14.7)^{a}	11.6 (3.2–17.6)^{a}	6.8 (0.5–14.3)^{a}	**8.9** (0.3–15.5)	6.7 (5.7–8.5)^{a}	9.1 (2.3–15.5)^{a}	5.7 (0.9–14.1)^{a}	10.9 (0.3–14.1)^{a}
DO (mg l^{-1})	**7.6** (1.2–19.0)	6.7 (1.2–16.8)^{a}	7.8 (4.9–19.0)^{a}	8.4 (1.8–17.8)^{a}	7.5 (2.9–11.5)^{a}	**7.5** (4.1–16.1)	8.8 (6.2–10.9)^{a}	10.2 (7.5–16.1)^{a}	5.2 (4.1–7.4)^{b}	7.4 (4.1–11.9)^{b}
pH***	**6.9** (4.8–8.2)	6.9 (6.2–7.4)^{ab}	6.8 (5.7–7.1)^{a}	7.5 (6.2–7.8)^{b}	6.9 (6.2–8.2)^{ab}	**6.5** (4.9–7.1)	5.9 (5.6–6.2)^{a}	6.1 (5.2–6.5)^{a}	5.3 (4.9–6.1)^{a}	6.6 (5.5–7.1)^{b}
Cond (µS cm^{-1})***	**72** (22–690)	92 (74–690)^{ab}	34 (22–69)^{c}	126 (51–538)^{a}	60 (27–159)^{b}	**21** (10–119)	18 (12–22)^{a}	19 (10–24)^{a}	26 (19–31)^{ab}	23 (13–119)^{b}
Q (l s^{-1})***	**25** (0.1–2000)	8 (0.5–100)^{a}	50 (4–2000)^{a}	25 (0.2–300)^{a}	35 (0.1–1550)^{a}	**500** (1–3200)	38 (25–55)^{a}	875 (100–2000)^{b}	1 (1–2)^{a}	900 (20–3200)^{b}
H	**2.4** (1.1–3.2)	1.9 (1.2–2.5)^{a}	2.0 (1.1–2.7)^{a}	2.5 (2.3–3.0)^{b}	2.6 (2.0–3.2)^{b}	**2.4** (1.3–3.0)	2.4 (1.6–2.8)^{a}	2.5 (1.8–2.8)^{a}	1.9 (1.3–2.1)^{a}	2.4 (1.3–3.0)^{a}

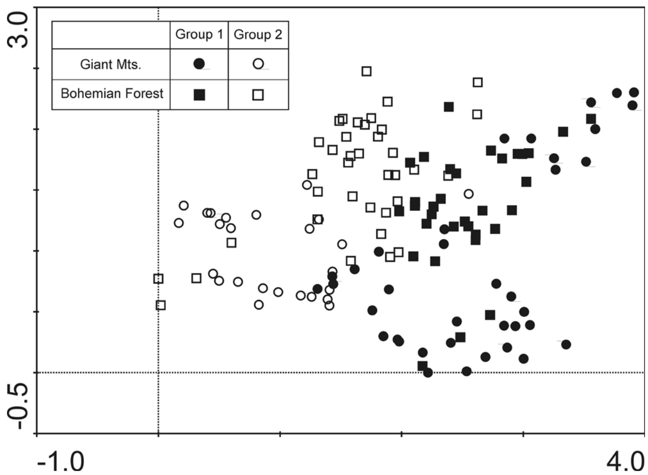

Figure 2 – Detrended correspondence analysis (DCA) of species data (n = 132).

Our study shows no significant pattern indicating the influence of sewage pollution on diatom diversity. Similarly, during our previous research in the Bohemian Forest, we did not observe any shifts in the overall phytobenthos species richness or diatom diversity in relation to sewage outflows (Lukavský et al. 2006). Bellinger et al. (2006) did not find significant differences in diatom species richness and diversity between deforested and forested watersheds from Gombe Stream National Park (Tanzania). On the other hand, Jüttner et al. (1996, 2003) observed significantly higher diatom species richness and diversity in agricultural Nepalese streams, where they were significantly correlated with water chemistry and stream habitat structure. Yu & Lin (2009) studied epilithic algae (mainly diatoms) from the mountain streams of Taiwan and found higher (though not significantly) species richness in assemblages from waters influenced by agriculture. Marcus (1980) found significant correlations between diatom diversity and nitrogen concentrations, with diversity first increasing and then decreasing as a function of nutrient enrichment. We did not see this pattern in our data, which could be affected by the choice of substrate for sampling, since various types of substrate can be inhabited by different diatom assemblages, giving dissimilar information about the environment (Cox 1991, Poulíčková et al. 2004, Besse-Lototskaya et al. 2006). Only epilithic diatoms were sampled, whereas epipelic and epiphytic assemblages were not included and therefore diversity measures did not reflect the full richness in these streams. In addition only 300 valves were counted which is insufficient to assess diversity. Other factors such as current velocity or substrate size and structure can also considerably influence diversity and composition of diatom assemblages (Cattaneo et al. 1997, Müllner & Schagerl 2003, Soininen 2004), but their effects on diversity were not studied in our survey.

Diatom responses to water quality

We found that the eutraphentic *Eolimna minima* indicated high concentrations of NO_3-N and organic nitrogen. This species is generally considered as resistant to organic pollution, tolerating α-meso- to polysaprobic conditions (Krammer & Lange-Bertalot 1986), and its increased abundance has been observed in other mountain streams with higher trophic levels (Jüttner et al. 1996, 2003, Gomà et al. 2005). Eutrophic conditions were also reflected by *Cocconeis placentula* var. *lineata*, *Mayamaea atomus* var. *permitis*, *Planothidium lanceolatum* and *Reimeria sinuata*. These taxa are common in lowland rivers (Krammer & Lange-Bertalot 1986, 1991b, Kim et al. 2008, Szczepocka & Szulc 2009), and have been often found in meso- to eutrophic conditions in mountain streams (Kawecka 1981, Jüttner et al. 2003, Gomà et al. 2005, Rott et al. 2006, Picińska-Fałtynowicz 2007). Even if elsewhere *Gomphonema parvulum* had higher abundances in samples with mean or high sewage pollution impact (e.g. Szczepocka & Szulc 2009), it predominated mainly in oligo- to mesotrophic conditions at sites in the Bohemian Forest (Subgroup 2D). This was unexpected, because the majority of diatom studies classify *G. parvulum* as a eutraphentic spe-

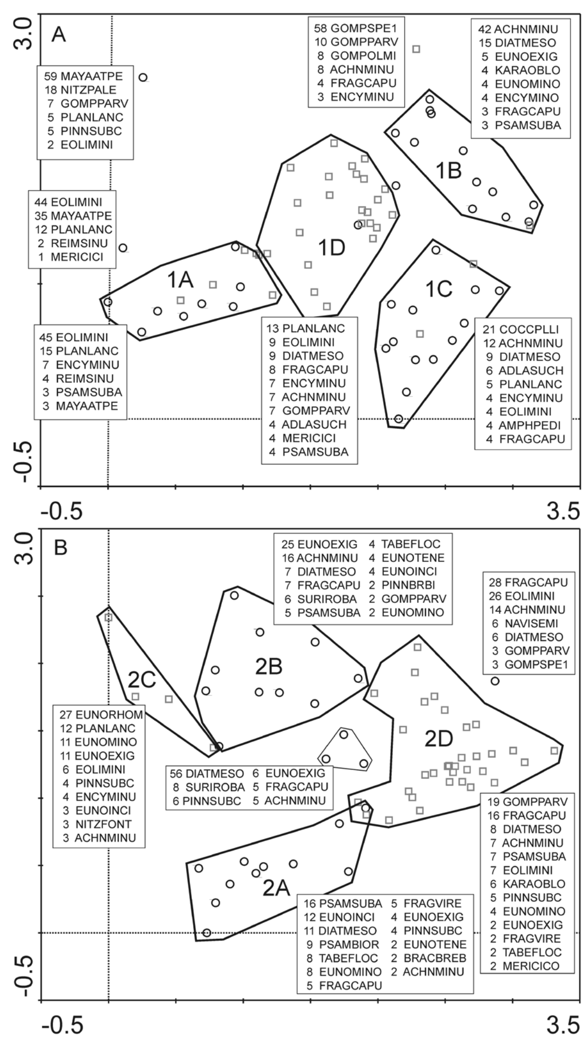

Figure 3 – Detrended correspondence analysis (DCA) of species data belonging to: A, Group 1, B, Group 2. Subgroups distinguished within both groups (1A–D, 2A–D), as well as several outliers are shown together with mean percentage abundances of dominating diatom taxa. For taxa codes see table 3. The Giant Mts. (circles), the Bohemian Forest (squares).

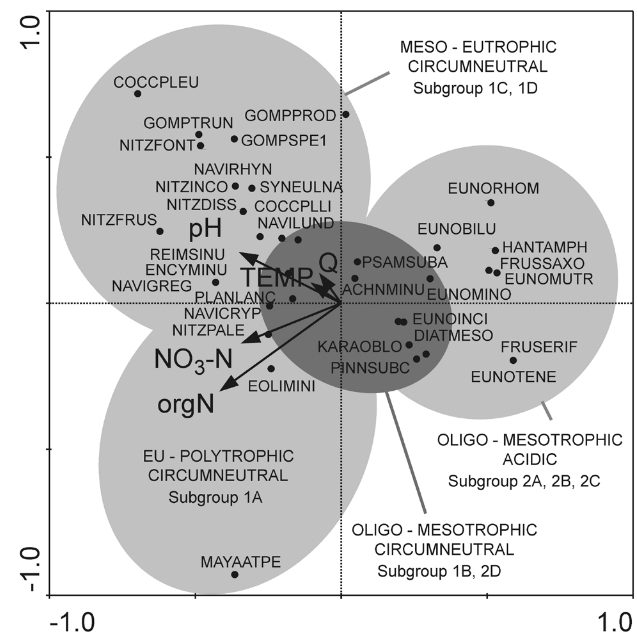

Figure 4 – Canonical correspondence analysis (CCA) – relationships of 34 main diatom taxa to environmental parameters with significant influence on assemblage composition. Taxa and ecological conditions in generated subgroups are shown. For parameter codes see table 2, taxa codes table 3.

cies (Rott et al. 1997, 1999, Kelly 2000, Gomà et al. 2005). In the study of Lowe & McCullough (1974), *G. parvulum* was the dominant species in assemblages intensively influenced by the effluent of sewage treatment plant. Nevertheless, it can be found in oligotrophic waters as well (Kawecka 1981, Picińska-Fałtynowicz 2007). Morphological variability within the *G. parvulum* complex is well-known and different species can be difficult to distinguish (Dawson 1972 Krammer & Lange-Bertalot 1986, 1991b, Salomoni et al. 2006, Tobias & Gaiser 2006). It is possible that previous specimens identified as *G. parvulum* belonged to other species, which would explain its wide ecological valence. However, further detailed research is needed to document these species and relate them to the environmental conditions in which they are found. *Gomphonema exilissimum* (Grunow) Lange-Bertalot & Reichardt is a species, which is morphologically very similar to *G. parvulum* and occurs mainly in oligotrophic waters (Krammer & Lange-Bertalot 1986). To confirm the correct identification of the populations in this study, a comparison with *G. exilissimum* type population was made. Since there were obvious morphological differences between studied populations and the type population (especially in length-width ratio, which was considerably higher in type population), we are confident about the identification of *G. parvulum*.

Similarly, more studies are necessary to correctly identify *Achnanthidium minutissimum* (Potapova & Hamilton 2007) and morphologically similar species. We found *A. minutissimum* s.l. predominantly at sites with oligotrophic conditions or with only slightly enhanced nutrient levels, which corresponds with other studies of mountain streams, lakes and springs (Jüttner et al. 1996, 2003, Gomà et al. 2005, Rott et al. 2006, Kawecka & Robinson 2008). Yu & Lin (2009), however, observed high abundances of this species in mountain streams with high nitrogen concentrations. Although *A. minutissimum* is considered a oligotraphentic species (Rott et al. 1999), it has also been reported as tolerant to slight organic pollution (Rott et al. 1997) and to grow in conditions with increased nitrogen levels (Fairchild et al. 1985). *Diatoma mesodon*, *Fragilaria capucina* s.l. and *Psammothidium subatomoides* were found predominantly at sites not or slightly -to- moderately affected by sewage pollution. These taxa are considered to be very good indicators of oligotrophic conditions (Kawecka 1981, Krammer & Lange-Bertalot 1991a, 1991b, Rott et al. 1997, 1999). However, *Fragilaria capucina* s.l. is another species complex which comprises taxa of contrasting ecological preferences. *D. mesodon* is one of the most common diatom species found in mountain water bodies (Krammer & Lange Bertalot 1991a, Cantonati et al. 2007). Species in the genus *Eunotia* are good indicators of water pH and were found at acidic sites. Veselá & Johansen (2009) considered *Eunotia* species as characteristic for acidic streams in the Elbsandsteingebirge region (on the Czech-German border west of the Giant Mts.). In our study, *Eunotia rhomboidea* and *E. exigua* were the most acid tolerant species and belonged to Subgroup 2C, where the lowest pH values (4.7–5.4) were measured (fig. 3B). These taxa are considered by Coring (1996) to be the dominant inhabitants of permanently acidic streams with pH < 5.5. Moderate acidic conditions were preferred by *Pinnularia subcapitata* Gregory, an oligotraphentic species (Rott et al. 1999) often used to indicate acidification (Coring 1996, Schaumburg et al. 2004, Kim et al. 2008).

Environmental parameters influencing diatom species composition

Sewage pollution at sites downstream of outflows markedly affected water chemistry and caused shifts from oligotrophic to eutrophic conditions. The effect of pollution on water chemistry was more important at sites with lower discharges. Since there were no significant interseasonal differences in discharges, higher spring concentrations of NH_4-N and NO_3-N were probably related to higher number of visitors (skiing season) in comparison with summer. However, no precise data about the number of tourists are available.

Concentrations of NO_3-N and organic nitrogen significantly influenced diatom species composition. Nutrient content (usually expressed as TDN and TDP) is often considered one of the main factors affecting epilithic diatom assemblages (Winter & Duthie 2000, Potapova et al. 2005, Lukavský et al. 2006, Salomoni et al. 2006).

Other significant parameters partly explaining the variability in species data were pH, water temperature and discharge. The range of pH in the dataset was relatively wide with the lowest values in stream sections flowing through peat bogs and the highest measured mainly at polluted sites. pH and ionic concentrations are important factors driving the structure of diatom assemblages (Coring 1996, Cameron et al. 1999, Kovácz et al. 2006, Kim et al. 2008). Responses of diatoms to temperature changes have been observed in several studies (Hieber et al. 2001, Lukavský et al. 2004, Gomà et al. 2005). Discharge values affected diatom assemblages mainly due to their impact on the total nutrient content, which highly depends on the volume of water in the stream channel.

It should be stressed that there was no significant difference in DO concentrations between unaffected sites and sites below sewage outflows. Since the decomposition of organic matter consumes DO and considerably influences the oxygen regime of the environment (Sládeček 1975), lower oxygen concentrations should be found at sites affected by pollution. However, this applies mainly for standing waters or rivers with low velocity (Allan & Castillo 2007). In the studied mountain streams, high velocity and low water temperature provided favourable conditions for the oxygen dissolution. Moreover, in the majority of cases, sewage water was aerated during the purification process in water treatment plants.

Differences between mountain ranges

The proportion of sites permanently affected by sewage outflows was the same in both mountain ranges (the Giant Mts. – 32%, the Bohemian Forest – 31%). However, this result was biased by our selection of sites. In fact, there are more recreation facilities (chalets, hotels, guest-houses) in the Giant Mts., one of the most frequently visited areas of the Czech Republic. In contrast, large parts of the Bohemian Forest were incorporated into the military frontier zone after World War II, and remained closed to tourists during the whole communist period (until 1989). Due to this fact, the recent impact of tourism in this area is lower. Nevertheless, at the sites investigated, the influence of sewage pollution had similar effects on benthic diatom assemblages in both mountain ranges. The mean percentage abundances of eutraphentic taxa represented about one third of all counted valves at sites from both the Giant Mts. and the Bohemian Forest.

The Bohemian Forest had more similar diatom assemblages in comparison with the Giant Mountains. We assume that the assemblages reflect the similarity (or variability) of streams and their general characteristics. Thus, the differences between the mountain ranges could be explained by a higher heterogeneity of habitats in the Giant Mts., where, in contrast to the Bohemian Forest, some sites were located above the timber line (e.g. upper part of the Bílé Labe River). Furthermore, differences in the extent of mountain glaciation during the Quarternary Ice Ages caused different geomorphological characteristics in each range – shallow, open valleys in the Bohemian Forest and deep, more isolated valleys in the Giant Mts. Spatial variability has been shown as an important factor driving the structure of benthic algal assemblages assemblages (Potapova & Charles 2002, Soininen et al. 2004). In the study by Potapova & Charles (2002), up to one third of total variability in diatom species data was attributed solely to geographical factors. Soininen et al. (2004) concluded that stream diatom assemblages incorporated a strong spatial component, since they explained almost 40% of species data variation through the combined effect of spatial and environmental variability. Our data set did not contain enough relevant information (limited number of sampling sites and areas to compare, no watershed and stream morphology characteristics) to allow an assessment of impacts of spatial variability. However, since the covariates accounted for 61.3% of the variability in the species data, it is likely that the spatial component was also important for diatom assemblage composition.

CONCLUSIONS

We found a considerable influence of sewage outflows from cottages and small mountain villages on water chemistry of mountain streams. This impact was more intensive at sites with low discharges, where the dilution effect was insufficient to adequately negate the effect of the pollutants. Markedly enhanced concentrations of NO_3-N and organic nitrogen had a significant influence on changes in diatom species composition. Natural diatom assemblages were replaced by pollution tolerant taxa that are common in lowland rivers. This diatom response shows that uncontrolled increases of recreation activities in these national parks might significantly alter vulnerable mountain stream ecosystems, which are often the first recipients of pollution. Therefore, suitable measures should be taken (which are unfortunately often neglected) by the owners of recreation facilities in these mountains.

Overall, diatoms performed well as indicators of environmental stress in mountain streams and diatom based biomonitoring should be considered an important supplementary method for assessing water quality in these ecosystems.

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