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Phytobenthos

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1 Introduction

Benthic algae (periphyton or phytobenthos) are the most successful primary producers in aquatic habitats. They are widely considered to be the main source of energy for higher trophic levels in many, if not most, unshaded temperate region streams (e.g., Minshall, 1978, Lamberti 1996). In large rivers, the leading role in primary production is governed by phytoplankton (Vannote et al. 1981). The specific conditions in such river types favour phytoplankton development and the algal biofilms are often restricted to the littoral zone because of limited light availability and high turbidity of the flow. Therefore, studies on phytobenthos from large rivers naturally refer to the river-bank area respectively visible and suitable for collecting samples. Nevertheless, phytoplankton as bioindicator mirrors environmental conditions in flows in short term, whilst attached benthic algae that are exposed to fluctuations of environmental factors and water chemistry within a period of time reflect a long-term status of aquatic health.

Phytobenthos together with macrophytes are identified as Biological Quality Element under the European Water Framework Directive (2000/60/EC), and as such need to be monitored to identify anthropogenic influences on aquatic ecosystems. Especially in the rivers, phytobenthos is considered to be a suitable parameter to determine the impact of nutrient pollution. Organisms are generally sessile and therefore reflect to the nutrients enrichment as well as to the other pollution.

In the Danube, nutrients have been identified as an important anthropogenic pressure threatening the quality of the river water (Danube River Basin Management Plan, 2009). In such conditions, benthic algae are an essential component of all bio assessment studies.

2 Methods

2.1 Sampling, sample processing, analysis

A segment of river (usually up to 50 m long) with a suitable substrate was chosen at each site for phytobenthos sampling.

Benthic diatoms and non-diatoms were sampled separately. Diatom sampling followed instruction of the CEN 13946 (2003), non-diatoms sampling was carried out according to CSN EN 15708 (2009).

In principle, at least five stones occurring in the euphotic zone down to 1m of depth (preferably cobbles with a diameter between 64 to 256 mm) were chosen at each site. Where hard substrata were absent, epiphyton was sampled following the CEN 13946 (2003), CSN EN 15708 (2009) and a Slovak Standard STN 757715.

On the stones selected, a chlorophyll-a concentration was measure in situ (see below for details). After the measurements, an area of minimum of 10 cm^2 was brushed thoroughly from each stone (as much concentrated as possible) into a plastic tray. The sample was transferred to at least two containers (for diatoms and non-diatoms analyses) and labelled. Relevant field information has been recorded to the standardised field protocol. Samples for benthic diatoms analyses were preserved by formaldehyde (final solution of 1 - 4 %) to allow a long term storage of samples.

Samples for non-diatoms analyses were refrigerated and analysed alive on-board. If any macroscopic algae were observed at site (e.g. Cladophora, Hydrodiction), a separate subsample was taken for easier determination.

2.1.1 Biomass measurements

Quantification of phytobenthos biomass has been done in situ on natural substrate by fluorescence fingerprint measurements using the BenthoTorch® (bbe Moldaenke) provided by Benten Water Solution (The Netherlands). On each of five or more stones (cobbles) five sub-areas were measured to obtain sufficient database of chlorophyll-a. Three main algal groups were distinguished: diatoms, green algae and cyanobacteria. For each of these groups and for total benthic algal biomass, the chlorophyll-a level was determined in µg/cm^2.

2.1.2 Non-diatoms

After sampling the microscopic analysis of non-diatom community has been performed using light microsopy at 400 x – 1000 x magnification. All important determination characteristics of the species were recorded using image analysis. All taxa were identified to the lowest taxonomical level possible using common determination keys for individual algal groups. The taxa identified were quantified on the scale 1 – 5 (1: rare, 5: dominant).

2.1.3 Diatoms

Epilithic diatom samples were collected and treated following the European standards CEN 13946 (2003) and CEN 14407 (2004). Minimum of five stones from the littoral zone occurring in the current, whenever possible, were brushed to collect diatom biofilm. Samples were preserved with formaldehyde at 4% concentration. Afterwards, samples were treated by hot hydrogen peroxide method to obtain the clean frustule suspensions. After eliminating the organic matter from the diatom suspension, diluted HCl was added to remove the calcium carbonate, very abundant in these waters, in order to avoid late precipitation, which could make frustule observation and counting difficult. Finally, the oxidised samples were rinsed with deionised water by decantation of the suspension several times, and permanent slides were mounted with Naphrax©. On average, 400 valves were counted on each slide in random transects with a Zeiss scope.A1 (Axio) microscope with 100x oil immersion objective. A list of taxa with relevant quantitative data was made from each slide and the counts were used to calculate species relative abundance (in %). These data were processed with the software OMNIDIA 5.3 (Lecointe et al. 1993, 1999, http://clci.club.fr/index.htm), which provided the values for diatom water quality indices.

2.2 Data treatment

Based on the diatom species data, 18 diatom indices were calculated with OMNIDIA ver. 5.3 (Lecointe et al 1993, 1999):

SLA	Sládeček Index (Sládeček 1986)
DES	Descy Index (Descy 1979)
L&M	Leclercq & Maquet Index (Leclercq & Maquet 1987)
SHE	Schiefele Index (Steinberg & Schiefele 1988, Schiefele & Schreiner 1991, Schiefele & Kohmann 1993)
WAT	DAIpo Diatom Assessment to Organic Pollution Index (Watanabe et al. 1988)
TDI	Trophic Diatom Index (Kelly & Whitton 1995, Harding & Kelly 1999)
GENRE	Generic Diatom Index (Rumeau&Coste 1988, Coste& Ayphassorho 1991)
CEE	Commission for Economical Community Index (Descy & Coste 1991)

IPS	Specific Pollution sensitivity Index (Coste in Cemagref 1982, OMNIDIA 5.2)
IBD	Biological Diatom Index (Lenoir & Coste 1996, Prygiel & Coste 2000)
IDAP	Artois-Picardie Diatom Index (Prygiel et al. 1996)
EPI-D	Eutrophication and Pollution Index with Diatoms (Dell'Uomo 1996, 2004)
DI-CH	Index DI-CH (Hürlimann & Niederhauser 2002)
IDP	Pampean Diatom Index (Gómez & Licursi 2001)
LOBO	Biological Water Quality Index BWQI (Lobo et al. 2004)
SID	Saprobic Index Diatom (Rott et al. 1997)
TID	Trophic Index Diatom (Rott et al. 1999)
IDSE	Saprobic/Eutrophication Index Diatom (Leclercq in OMNIDIA v. 5.2)

The community structure was expressed by calculating the proportion of species belonging to three ecological guilds (low profile, high profile and motile) adopted from Passy (2007) and Berthon et al. (2011) and to two life forms (planktonic, benthic). An ecological guild consists of species that live in the same kind of environment, but which may have adapted in different ways to survive there (Devito et al. 2004). The three ecological guild identified by Passy (2007) to classify diatoms were further extended by Berthon et al. (2011). The low profile guild consists of species of short stature, including prostrate, adnate, small erect, solitary centrics and slow-moving species (sensu Passy 2007). The second, the high-profile guild, consists of species of tall stature including large erect, filamentous, branched, chain-forming, tube-forming and stalked species and colonial centrics (sensu Berthon et al. 2011). The third motile guild contains fast-moving species (Hudon & Legendre 1987). Besides, proportions of centrics and pennates (in %) in each sample were calculated based on their relative abundance.

2.3 Statistical methods

Results of the chlorophyll-*a* measurements and analyses of species composition of non-diatoms and diatoms were treated separately. Environmental variables were standardized and log-transformed before the statistical analysis. Appropriate tests for normality were conducted using STATISTICA 10 (StatSoft Inc., 2011) on all environmental and biological data.

In total, 21 explanatory variables were treated for statistical analysis and comprised data on water chemistry (conductivity, temperature-t, pH, dissolved oxygen-O_2, total nitrogen-TN, total phosphorus-TP, nitrates- N-NO_3, phosphates-P-PO_4, potassium-K, calcium-Ca, sodium Na and dissolved organic carbon-DOC) and hydromorphological variables (discharge-Q, slope, granulometry-D16mm, D50mm, D84mm, mean velocity, suspended solids) and general descriptors such as river kilometre (riv.km) and 10 Danubian types (Moog et al. 2004) as follows Type 1: 2581 riv. km, type 2: 2415 – 2258 riv. km, type 3: 2204 – 2008 riv. km, type 4: 1942 – 1790 riv. km, type 5: 1761 – 1533 riv. km, type 6: 1481 – 1097 riv. km, type 7: 1071 – 954 riv. km, type 8: 926 – 378 riv. km, type 9: 235 – 130 riv. km, type 10: 107 – 26 riv. Km).

The chlorophyll-a content was correlated with environmental variables (water chemistry, river kilometres and hydromorphology) in order to identify the relationships between the algal biomass and environmental factors. Spearman correlation correlations were applied using STATISTICA 10 (StatSoft Inc., 2011).

Diatoms were evaluated based on the species composition related to environmental parameters and different diatom metrics. For species-based statistical analysis of non-diatoms, only 43 taxa were taken into account. For diatoms, only diatom taxa reaching a relative abundance of more than 3% in at least one sample were included in the statistics (86 taxa in total). Diatom species data were arcsin square root transformed prior to any statistical analysis, non-diatoms were not transformed.

The variance in diatom community regardless the environmental variables was explored by Detrended Correspondence Analysis (DCA, Hill and Gauch, 1980). The DCA was made using PC-ORD v. 6 (McCune and Mefford, 1999), rare taxa were downweighted and the randomization test was performed with 999 runs. The analysis of non-diatoms gave a gradient length of 3,382 SD (Axis 1), therefore a consequent a Canonical Correspondence Analysis was performed on non-diatoms and environmental variables to describe the relationships in the datasets. For diatoms, the DCA gave relatively short gradients of 2,199 for Axis 1 and of 2, 475 for Axis 2 indicating rather linear than unimodal response. For data with DCA gradients smaller than three turnover units (standard deviations), linear models are offered as preferable. However, according to Šmilauer & Lepš (2014), unimodal ordination can be successfully applied also to data with small gradient length produced by DCA, because they also have „a linear face“. Therefore, despite the short gradient lengths, a Canonical Correspondence Analysis was applied also to the diatom dataset in order to explore the relationship of between and among the diatom species composition at sites and environmental variables (terBraak and Verdonschot, 1995). Hill's scaling was chosen with focus on inter-sample distances. The CCA was run with manual forward selection, Monte Carlo permutation tests (full model, n=999) and Bonferroni correction of the significance levels to determine the factor significantly contributing to the model. Manual selection and Monte Carlo permutation test (999 runs) were used to reduce the environmental variables to those correlated significantly with the derived axes, at a cut-off point of P=0.05. Hill's scaling was selected with inter-sample distances. With regard of environmental parameters involved in the analysis, apart from water chemistry and land use practices, we also included general site descriptors such as distance from the source, altitude and stream order. As the multivariate statistics of species data (both DCA and CCA) showed that the diatom species composition differed between the Danubian types, the Indicator Species Analysis (Dufrêne & Legendre 1997) was applied to identify taxa characteristically found in the different types. Indicator species analysis allows to find species that are more consistently found within selected groups of samples. This analysis combines information on the abundance of species within a particular group and its frequency and it produces indicator values for each species in each group. Their significance was tested with a Monte Carlo technique (n=999), using the PC-ORD v. 6 (MacCune & Mefford 1999).

Relationships between the diatom metrics (diatom indices, diatom guilds and life forms) and all environmental variables (general, physico-chemical and hydromorphological) were assessed with the non-parametric Spearman correlations using STATISTICA 10 (StatSoft Inc., 2011). Samples from the right and left bank were treated separately in order to test whether the different banks influence the diatom assemblages in terms of their indication potential. In addition, a paired t-test was applied to the two groups (left and right banks) to see whether the diatom descriptors (diatom indices and ecological guilds and life-forms) differed between the two banks. The t-test was performed using STATISTICA 6.0 (StatSoft Inc., 2011).

Afterwards, values of diatom indices within the different Danubian types were compared with box-plots using Sigma Plot ver. 11.0 (Systat software, San Jose, CA). We further used a non-parametric Kruskal-Wallis one-way analysis of variance by ranks to evaluate whether the diatom indices differed significantly between the different Danubian types (N=108). The Kruskal-Wallis ANOVA by Ranks was performed using STATISTICA 10 (StatSoft Inc., 2011).

3 Results

3.1 Phytobenthos biomass

A total of 96 samples was evaluated for chlorophyll-*a* concentration on the hard substrate. The values of the total chlorophyll-*a* measured in situ varied between 0,06 - 7,19µg/cm^2 (Fig. 6). The highest values were detected in the upper Danube down to the station JDS10 (riv. km 1895) and started to increase again at JDS40 (riv. km 1107).

The phytobenthos structure evaluated via chlorophyll-*a* content was mainly formed by cyanobacteria and diatoms, green algae created only a minor part of the biofilm (Fig. 6). The cyanobacteria reached more than 50% of proportion in 52 samples, whilst diatoms prevailed in 37 samples. In general, diatoms prevailed in the upper Danube (down to JDS10 – 1895 riv.km).

With regard of the relationships of the chlorophyll-a concentration with other environmental variables, the Spearman correlations showed that it is most significantly related to the concentrations of suspended solids (Tab. 1). The negative correlation coefficient indicates that higher concentrations of suspended solids impede the phytobenthos development. This caused the low values of chlorophyll-a concentrations at sites in the type 6, which were proved to contain significant amounts of suspended solids. Furthermore, the chlorophyll-*a* was significantly positively correlating with phosphates and dissolved organic carbon (Tab. 1).

3.2 Non-diatoms

3.2.1 Species composition

In total 68 taxa were identified in 110 non-diatom samples of non-diatom community. Non-diatom species diversity was mainly created by species of cyanobacteria (Cyanophyta), green algae (Chlorophyta) and red algae (Rhodophyta).

Cyanobacteria were represented by filamentous genera *Calothrix* (C. *fusca*, C. *parietina*), *Heteroleibleinia* (*H. küetzingii*), *Homeothrix* (*H. janthina*), *Komphovoron*, *Leptolyngbya*, (*L. boryana*), *Lyngbya* (*L. major*), *Oscillatori*a (*O. limosa*, *O. formosa*, *O. sancta*, *O. redekei*), *Phormidium* (*P. amoenum*, *P. autumnale*, *P. breve*, *P. corium*, *P. chalybeum*, *P. chlorinum*, *P. tenue*, *P. targestinum*, *P. irriguum*), *Stigonema* and *Tolypothrix*. *Heteroleibleinia küetzingii* was growing on the filaments of green algae (e.g. *Cladophora*, *Spirogyra*). Coccal cyanobacteria were observed as well, mainly such genera *Chroococcus*, *Chamaesiphon*, *Geitlerinema*, *Geitleribactron*, *Pleurocapsa*, *Stanieria* were present. Together 40 taxa of cyanobacteria were found in the samples from the Danube and the tributaries.

Among green algae, a total of 24 taxa occurred at individual sampling stations. The most abundant filamentous species was *Cladophora glomerata* that was usually accompanying water macrophytes. *Cladophora glomerata* was found at 77 stations of the Danube and the tributaries. *Hydrodictyon reticulatum*, *Oedogonium* sp. and *Spirogyra* sp. were abundant in the shallow poles of the Danube river. Less frequent and abundant were taxa belonging to the genus *Enteromorpha*, *Stigeoclonium*, *Uronema* and *Zygnema*. Filamentous green algae were usually found epiphytic growing on anoher other greens such like *Characiochloris*, *Characium*, *Fernandiella*. Downstream of Novi Sad *Pseudendoclonium basiliense* was found quite often down to the Danube delta together with coccal cyanobacteria.

There were three taxa of red algae (Rhodophyta) found, *Bangia artropurpurea* (Roth) Aghard, *Hildebrandia rivularis* (Liebmann) Aghard and *Thorea* sp. Bory. *Bangia artropurpurea* has been identified in the Austrian stretch of the Danube upstream Abwinden-Asten dam (2120 river km) as well as near Klosteneuburg (1942 river km). *Hildebrandia rivularis* was recorded in the upper stretch of the Danube from Kelheim (Germany) up to Gabčíkovo (Slovakia). Macroscopic red algae *Thorea* has been found on the hard artificial substrate in the confluence of the Sava into Danube.

3.2.2 Relationships of non-diatoms and the environment

Distribution of non-diatom taxa in the Danube showed to depend on river kilometres, nitrates, velocity, pH, suspended solids, phosphates, potassium and DOC as showed by the CCA (Fig. 2). The most significant factors were river kilometres and suspended solids. However, the environmental variables tested explained only 21% of the total variance in the non-diatoms data. The two first axes accounted for 57% of the explained variance. The first axis clearly separated sites from the types 1-5 from the types 7-10 and represented changes in the longitudinal profile of the Danube related most strongly to river kilometres, velocity, phosphates and potassium content.. The different Danube types appeared gradually arranged along the axis 1 from the type 1 in the upper Danube with higher velocity, oxygen content down to the river mouth in the type 10 reflecting an increase of concentration of phosphates and potassium. The second axis allowed separation of the type 6 and was most strongly correlated with suspended solids.

3.3 Diatoms

3.3.1 Diatom species composition

A total of 318 diatom taxa belonging to 62 genera were detected in 108 samples. Among them, only 148 taxa reached a relative abundance of at least 1% at minimum of one site, 86 taxa with a relative abundance over 3% and only 61 species a relative abundance of at least 5%. With regard of the species frequency, only 28 species occurred at more than 50% of sites. The most frequent species detected in more than 75% of samples (more than 81) were *Amphora pediculus* (Kützing) Grunow, *Cocconeis placentula* Ehrenberg, *Cyclotella meneghiniana* Kützing, *Navicula cryptotenella* Lange-Bertalot, *Navicula recens* (Lange-Bertalot) Lange-Bertalot, *Nitzschia dissipata* (Kützing) Rabenhorst, *Nitzschia fonticola* Grunow in Van Heurck, *Nitzschia palea* (Kützing) W. Smith var. *debilis* (Kützing) Grunow in Cleve & Grunow and *Nitzschia palea* (Kützing) W. Smith. The most frequent and abundant taxa reaching a minimum relative abundance of 5% are listed in Table 2.

3.3.2 Relationships of diatoms and the environment

The variance in species composition in samples and species distribution in different Danubian types was assessed using a Detrended Component Analysis. The DCA gave a relatively short gradients (2,199 for Axis 1 and 2, 475 for Axis 2), which indicates that the compositional variation in the dataset was limited and suggests low data heterogenity. The ordination diagram of the DCA based only on the species composition showed that the samples differed between the different Danubian types (Fig. 3). Sites from the upper Danube (types 1-4) were clearly separated from the other types along the first axis. The second axis differentiated clearly between the types 6, 8 and 9. The overlaps observed between all the neighbouring types logically occur due to the natural connectivity of the sites and types studied.

The species composition was confirmed to differ between the different Danubian types also based on the Canonical Correspondence Analysis (Fig. 4). The first axis accounted for 12% of total variance, the second axis explained 7,6% of the data variance. All canonical axes accounted for a total of 37% of the variance in the species data. Axis 1 clearly represented the longitudinal gradient of the Danube and correlated significantly with river kilometres, slope, potassium and nitrates. Distribution of diatom samples along this axis clearly reflected the gradual changes in the longitudinal profile. The samples were arranged gradually along axis 1 from the type 2 to 10. Distinct overlap was found only for the lower Danube types 8-10. Besides, the species composition of the upper Danube types 2, 3 and 4 seemed to be influenced also by the calcium content. Second axis correlated with suspended solids and dissolved organic carbon (DOC) and showed to separate the diatom communities of the type 6. These assemblages were distributed in the ordination space mainly along the gradient of suspended solids, which according to the correlations results are positively correlated with proportion of centric diatoms in the samples. The proportion of centrics in samples from the type 6 reached an average relative abundance of 52% with a maximum of 83%, which is the highest proportion in the dataset (see also Fig. 5). These results confirm that the benthic algal communities at sites belonging to the type 6 are significantly influenced by higher rates of suspended solids that greatly increase the proportion of planktonic diatoms in the biofilms.

With regard of the results of multivariate statistics diatom assemblages were further explored using an Indicator Species Analysis. The analysis was applied to identify species that best characterize the different Danubian types. The analysis resulted in a numerous list of significant indicators for each of the type tested (except for the type No. 8) and showed indicator species to overlap among all types in the dataset. According to the results, the different types shared several indicator species. Diatoms identified as indicators for every out of the 10 types are presented in Table 2. The most characteristic species (with the lowest distribution among other types and lowest „sharing rate“) were identified in the type 10. In the contrary, there were no indicator species identified in the type 8, but the results indicate very similar composition with the type 9. The analysis confirmed systematically high similarity between the neighbouring types. For example types 2, 3 and 4 showed to have similar indicator species and also types 5, 6 and 7 shared a significant portion of indicators. There were only two species identified as indicators in one single type, e.g. *Achnanthidium atomoides* Monnier, Lange-Bertalot & Ector (Type 3), *Navicula riediana* Lange-Bertalot & Rumrich (type 7) and *Nitzschia clausii* Hantzsch (type 10) and three species in two types, e.g. *Achnanthidium lineare* W.Smith (types 3, 7), *Lemnicola hungarica* (Grunow) Round & Basson , *Fragilaria bidens* Heiberg (types 5, 7). In general, the best indicator species (Tab. 2) from the types 2 and 3 were mostly sesile, fast growing species adapted to fast flowing waters with relatively low antropogenic disturbance from the genus *Achnanthidium* Kützing, *Cocconeis* Ehrenberg or *Encyonema* Kützing, some very common and mostly euryvalent “Naviculoids” (e.g. *Navicula cryptotenella*, *N. gregaria* Donkin, *N. tripunctata* (O.F.Müller) Bory, *Nitzschia dissipata*) and a pollution tolerant *Luticola goeppertiana* (Bleisch) D.G. Mann in Round, Crawford & Mann. Type 4 was characterised by tube forming species from the genus *Cymbella* Agardh and stalked species from the genus *Gomphonema* Ehrenberg (e.g. *G. minutum* (C. Agardh) C. Agardh, *G. parvulum* Kützing, *G. tergestinum* (Grunow) Fricke in Schmidt et al.). In the contrary to the previous types with typically benthic assemblages, species best characterising the type 6 were all planktonic centrics from the genera *Aulacoseira* Thwaites, *Cyclostephanos* Round in Theriot, Håkansson, Kociolek, Round and Stoermer**,** *Cyclotella* (Kützing) Brébisson, *Discostella* Houk & Klee 2004 and *Stephanodiscus* Ehrenberg. Types 7-9 contained again purely benthic indicator species, all small pollution tolerant taxa e.g. *Eolimna subminuscula* (Manguin) Moser et al., *Mayamea permitis* (Hustedt) Bruder& Medlin, *Navicula veneta* Kützing and *Nitzschia inconspicua* Grunow. The last part of the Danube stretch belonging to the type 10 contained a specific set of motile pollution tolerant species often associated with find sediments mostly belonging to the genus *Nitzschia* Hassall (e.g. *Nitzschia clausii* Hantzsch and *N filiformis* (W.M.Smith) Van Heurck) and *Navicula* Bory de Saint-Vincent (e.g. *Navicula germainii* J. H. Wallace, *N. symmetrica* Patrick, *N .riediana*) (Tab. 2).

3.3.3 Diatom indices

The diatom guilds, life forms and diatom indices were evaluated by comparing their values between the different Danubian types and by correlating with environmental variables. The statistical analysis (paired t-test) did not prove any significant changes between the diatom indices and between the ecological guilds composition of the left and right banks.

All diatom indices except for the SLA index correlated significantly with river kilometres. Among all variables evaluated, the river kilometres were more closely related to diatom indices and were assigned the highest correlation indices (Tab. 3) indicating that all diatom indices decrease longitudinally from the upper Danube down to the mouth. The highest correlation coefficients were calculated for the GENRE, TID, SID and IPS. With regard of the water chemistry, the indices showed to be most significantly related to oxygen, pH, total nitrogen, total phosphorus, Ca, sodium and nitrates. The strongest correlations were detected between indices and total nitrogen and nitrates. However, the correlation indices calculated were positive and not negative as expected from an indicator whose value decrease with an increase of a pollutant. This confirms that diatoms as long term indicators might not reflect single values of chemical data gathered during the diatom surveys as they are adapted to a scale of values within a certain period. Since the correlations with water chemistry were incomparably lower than for river kilometres, it also indicates that diatom indices were more closely related to other parameters (other than water chemistry), which significantly change longitudinally. Therefore, the best performing indices (GENRE, IPS, TID), and the diatom guilds and life-forms were further correlated with hydromorphological variables.The three diatom indices (GENRE, IPS and TID) correlated significantly with most of the hydromorphological variables, the strongest correlations were detected for discharge (negative correlations) and slope (positive correlations) (Tab. 4). All these variables change naturally and gradually in the longitudinal profile.

Comparisons of indices values between the different Danubian types confirmed that there is a strong longitudinal gradient decreasing the diatom indices downstream (Fig. 6). All diatom indices differed between the 10 Danubian types at $p<0,001$ except for LOBO and IDP that differed at p $p<0,05$ (N=108). The indices decreased gradually downstream from the type 3. Interestingly, indices of the type 2 (JDS2: 2415 – JDS5: 2258 riv. km) were lower than those of the type 3 and 4 indicating an intensive degradation of the aquatic environment in the type 2.

Among the diatom indices available, the GENRE, IPS and TID appeared to be the most appropriate for further application in the ecological status assessment of the Danube. The GENRE index performed the best as it reached the most numerous and the highest correlation coefficients in the dataset. Both IPS and TID indices are widely used around Europe for ecological status assessment in rivers (Kelly et al. 2014) and were successfully applied during the intercalibration exercise (Kelly et al. 2009). The IPS index in particular, already proved to perform well in the Danube (Ács et al. 2006). However, the applicability of the GENRE index, which is based on the generic level only, is rarely been reported (for example see Kwandrans et al. 1998) due to its low specifity and taxonomical insufficiency. Among all, the GENRE usually does not yield reliable results, in so far as certain genera, such as *Navicula* Bory de Saint-Vincent and *Nitzschia* Hassall, contain species with a widely differing ecologies. However, it seems that in the Danube, these general ecological characteristics match the species requirements also on a generic level. The distinct longitudinal gradient of the Danube imply a distinct turnover of diatom genera and ecological guilds longitudinally and this makes such general generic index easily applicable. Contrary to very specific IPS, which is continuously being updated based on large datasets from different river types, although mostly much different from such like the Danube. Therefore, the specific indicator values assigned to a particular species based on data from various river types do not necessarily reflect the response of species in every condition. Consequently, it cannot be expected that any diatom index can be sufficiently precise in every river type even in case of such ubiquitous organisms like diatoms. Therefore a more general index like GENRE might be very useful for additional diatom-based assessment of the river Danube, although this requires more additional testing.

3.3.4 Diatom guilds and life-forms

Similarly to diatom indices, the ecological guilds showed to change significantly in the longitudinal profile. The distribution of diatom guilds among the sites investigated showed to change longitudinally. The high guild reached relatively higher proportion in the higher Danube, whilst the motile guild proportion increased significantly at sites in the lower Danube (Fig. 7).

There were strong positive correlations detected between all the three guilds with river kilometres, the high profile guild performed the best. Also, the guilds correlated significantly with oxygen, calcium, sodium, total nitrogen, nitrates, phosphates and DOC. With regard of their relation to hydromorphological parameters, the high profile guild was related to all variables tested except for suspended solids. In particular, the highest positive correlations were detected between slope and high-profile guild, which was also reflected in significant correlations with mean velocity. Similarly, significant negative correlations were detected between motile quild (and motile life-form) and mean velocity as well as slope. These results indicate that the diatom guilds composition reflect both chemical and hydromorphological variables. In general, the indication power of the three ecological guilds showed to be similar to the best performing diatom indices.

The two life-forms tested showed to strongly correlate with suspended solids (Tab. 4). There were high positive correlation coefficients calculated between the suspended solids and the proportion of planktonic diatoms in phytobenthos. This is most probably caused by the fact that planktonic diatoms, which do not move actively, are can be pulled down onto the river substrate by the sedimenting solids. Therefore higher rates of suspended solids, which imply higher rates of their sedimentation especially near the river banks, might have increased the percentage of planktonic species in phytobentos by purely accelerating their sedimentation. Moreover, such planktonic diatoms could further proliferate and develop successfully also in benthos if the benthic community occurred in favourable conditions. This might explain the high proportions of centrics reached in the biofilm at several sites mostly belonging to the type 6.

3.4 Indication of ecological status assessment

As diatoms have proved to be a reliable tool for phytobenthos-based assessment of river ecological status in Europe, they are being used as proxies for ecological status assessment by most of the member states of the European Union (see Kelly et al. 2009 and Kelly 2013) and a diatom-based assessment method was successfully intercalibrated on large rivers (Birk et al. 2012), diatoms alone were selected for an indication of ecological status assessment in the Danube. Among the diatom metrics most commonly used, the IPS complies the conditions of being used by most of the member states on national standardized level, it is regularly being updated and was applied in the intercalibration exercise of phytobenthos-based assessment of ecological status of rivers in Central Baltic Geographical group (Kelly et al. 2009) and large rivers (Birk et al. 2012). The IPS was previously applied to JDS2 results (Makovinská et al. 2008) and based on the results presented above it turned to be among the most appropriate indices for ecological status assessment of the JDS3. In order to confirm with the results of the intercalibration exercise, the ecological status was evaluated using the two intercalibrated boundaries between high/good and good/moderate status. For this purpose, the intercalibrated values of the IPS from the Slovak assessment methods were used (High ecological status: IPS>15.5, Good ecological status IPS>13.1). The entire Danube was assessed using the same classification scheme.

Based on this asessment approach the ecological status of most of the sites in the upper Danube down to Gabčíkovo reservoir in Slovakia (1852 riv. km) in the type 4 appeared in the high-good band (Fig. 8). The sites from Gabčíkovo down to Budapest (1632 riv. km) varied between good and moderate status and all sites downstream Budapest (downstream the 1852 riv. km) appeared consistently bellow the good/moderate boundary reaching a moderate or worse ecological status.

4 Conclusions

The Danube phytobenthos was mainly composed of diatoms and cyanobacteria, with the former prevailing in the upper Danube. The algal biomass showed to increase in the upper and lower Danube and was most significantly influenced by phosphates and suspended solids.

Both diatoms and non-diatoms in the Danube indicated that there is a strong longitudinal gradient of natural changes and anthropogenic disturbance in the Danube profile influencing algal biofilms. Both species composition of diatoms and non-diatoms as well as the diatom metrics reflected a distinct longitudinal pattern of environmental conditions and changed gradually downstream. The species composition of non-diatoms showed to change depending on the velocity and oxygen content on one hand and to be related to an increase of phosphates, suspended solids and potassium on the other.

Benthic diatom assemblages' structure as well as all diatom metrics tested (diatom indices, diatom guilds and life-forms) showed to change gradually in the longitudinal profile reacting on both natural changes of the Danubian typology and anthropogenic disturbance. The species composition showed to differ between the different Danubian types and the most important parameters influencing the species composition were phosphates, suspended solids, discharge, slope and velocity. Suspended solids showed to greatly influence the community structure by increasing the proportion of planktonic species in the biofilm and decreasing the overall biomass of the algal biofilm.

All diatom indices tested decreased gradually and significantly downstream reflecting the increase of general degradation of aquatic environment and natural longitudinal changes. The increase of general degradation in the longitudinal profile was well reflected by high correlation of diatom metrics with river kilometres as well as with water chemistry. Among the diatom indices available, the GENRE, IPS and TID appeared to be the most appropriate for further application in the ecological status assessment. The indication power of the three ecological guilds showed to be similar to the best performing diatom indices. The composition of the three diatom guilds as well as the life forms showed to be closely related to hydromorphology as well as water chemistry, the high profile guild performed the best.

The IPS-based ecological status assessment showed that the ecological status of sites above Gabčíkovo reservoir in the type 4 (1852 riv. km) varied between high to good. Sites downstream Budapest (after the 1852 riv. km) appeared consistently bellow the good/moderate boundary indicating that the ecological status of the middle and lower Danube is moderate and worse.

These results confirm that despite the methodological limitations related to phytobenthos in large rivers diatoms are valuable indicators of water quality and general degradation of the Danube and can be reliably applied to the assessment of its ecological status. Not only the diatom indices, but also the diatom guilds proved to provide a reliable reflection of the environmental conditions and supply an additional insight to the aquatic ecosystem functioning.

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7 Tables and figures

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Fig. 7. Distribution of diatom guilds among the sites investigated. River kilometres refer to the sites investigated. Fig. 8. Ecological status assessment using the two intercalibrated boundaries of IPS index of the Slovak assessment method: high/good (H/G) and good/moderate (G/M). Type 2: 2415 – 2258 riv. km, type 3: 2204 – 2008 riv. km, type 4: 1942 – 1790 riv. km, type 5: 1761 – 1533 riv. km, type 6: 1481 – 1097 riv. km.

Tab. 1. Spearman correlation coefficients between the chl-*a* biomass of green algae, cyanobacteria and diatoms and the total chlorophyll-*a* concentation. Correlations significant at p>0.05 (*) and p>0.001 (**) are shown.

Variable	Green algae	Cyanophytes	Diatoms	Total
River kilometer	-0,27*	ns	ns	ns
Conductivity	0,27*	ns	ns	ns
Dissolved oxygen	-0,28*	ns	ns	ns
pH	ns	ns	ns	ns
Temperature	ns	ns	ns	-0,24*
Total nitrogen	ns	ns	ns	ns
Total phosphorus	ns	-0,32*	-0,25*	-0,36*
Calcium	ns	ns	ns	ns
Magnesium	ns	0,23*	ns	0,28*
Potassium	ns	ns	ns	-0,22*
Sodium	0,33*	ns	ns	0,24*
Nitrates	-0,27*	ns	ns	ns
Phosphates	0,24*	ns	ns	0,23*
DOC	ns	ns	0,22*	0,25*
Discharge	ns	ns	ns	ns
Velocity	-0,24*	ns	-0,28*	ns
Granulometry (D16mm)	ns	ns	ns	ns
Granulometry (D50mm)	ns	ns	ns	ns
Granulometry (D84 mm)	ns	ns	ns	ns
Suspended solids	ns	-0,32*	-0,53**	-0,55**
Local slope	ns	ns	-0,27*	ns

Tab. 2. List of diatom species reaching a relative abundance (RL) of at least 5% at minimum of one site. The best type indicator is based on the results of the Indicator Species Analysis. The type number is assigned to a species that was determined as a type identifier for type with maximum observed indicator value and thus best characterised the particular type at p<0,001. Danube types 2-10 were considered in the analysis. Species with asterics were identified as indicator species in all the 10 types tested.

The most frequent and abundant taxa (with RL>5% at least at one site)	No of samples (out of 109)	Average RL (%) at sites were present	Max RL (%)	Nb of sites with RL >5%	Best type indicator (Type number)
Actinocyclus normanii (W. Gregory ex Grevile) Hustedt	40	1,06	6,57	3	**10**
Achnanthidium atomoides Monnier, Lange-Bertalot & Ector	4	3,95	10,39	1	**3**
Achnanthidium catenatum (Bílý & Marvan) Lange-Bertalot	7	2,13	8,87	1	
Achnanthidium eutrophilum (Lange-Bertalot) Lange-Bertalot	45	2,78	29,93	6	
Achnanthidium Kützing	31	0,98	8,56	1	
Achnanthidium lineare W.Smith	6	1,65	6,93	1	**3**
Achnanthidium minutissimum (Kützing) Czarnecki	47	3,17	14,15	11	**3**
Achnanthidium pyrenaicum (Hustedt) Kobayasi	19	1,67	15,07	2	**3**
Amphora montana Krasske	31	1,86	13,73	4	
Amphora pediculus (Kützing) Grunow in Schmidt et al.	94	7,30	50,97	40	**2***
Caloneis bacillum (Grunow) Cleve	34	1,16	5,17	1	**4**
Cocconeis pediculus Ehrenberg	59	1,40	16,52	4	**2**
Cocconeis euglypta Ehrenberg *sensu* Monnier et al.	91	1,83	17,47	4	**2***
Cyclostephanos invisitatus (Hohn & Hellerman) Theriot, Stoermer & Håkansson	70	1,41	6,77	3	**6**
Cyclotella atomus Hustedt	60	3,96	15,65	18	**6**
Cyclotella meneghiniana Kützing	92	8,49	40,00	42	**6***
Discostella pseudostelligera (Hustedt) Houk & Klee	79	6,66	69,66	25	**6**
Cymbella compacta Østrup	24	1,10	7,47	1	**4**
Cymbella excisa Kützing	23	1,39	10,14	1	**4**
Diadesmis confervacea Kützing	5	1,42	5,77	1	
Diatoma vulgaris Bory	53	0,78	6,31	1	
Encyonema minutum (Hilse in Rabenhorst) D.G. Mann	22	1,07	5,54	1	**3**
Eolimna minima (Grunow) Lange-Bertalot in Moser et al.	78	5,72	33,95	27	**7***
Eolimna subminuscula (Manguin) Moser et al.	45	2,15	10,14	3	**9**
Fistulifera saprophila (Lange-Bertalot & Bonik) Lange-Bertalot	22	1,43	6,67	1	
Gomphonema minutum (C. Agardh) C. Agardh	62	2,62	14,37	10	**4**
Gomphonema parvulum Kützing	68	1,81	13,81	6	**4***
Gomphonema tergestinum (Grunow) Fricke in Schmidt et al.	32	3,61	15,36	7	**4**
Gyrosigma acuminatum (Kützing) Rabenhorst	26	0,72	5,09	1	
Luticola goeppertiana (Bleisch) D.G. Mann in Round, Crawford & Mann	13	3,03	28,93	1	**3**
Luticola hlubikovae Levkov, Metzeltin & Pavlov	3	2,70	7,19	1	
Mayamaea permitis (Hustedt) Bruder& Medlin	52	2,33	8,70	4	**9**
Melosira varians C. Agardh	61	1,21	10,06	1	
Navicula amphiceropsis Lange-Bertalot & Rumrich	24	1,49	12,07	1	**5**
Navicula antonii Lange-Bertalot	67	1,24	5,33	3	**2**
Navicula capitatoradiata H. Germain ex Gasse	72	1,14	5,63	3	**5**
Navicula cryptotenella Lange-Bertalot in Krammer & Lange-Bertalot	95	5,68	35,68	36	**3***
Navicula erifuga Lange-Bertalot in Krammer & Lange-Bertalot	65	2,27	25,54	6	**10**

Navicula germainii J. H. Wallace	58	1,72	9,88	4	**10**
Navicula gregaria Donkin	31	1,23	5,68	2	**3**
Navicula lanceolata (C. Agardh) Ehrenberg	57	0,95	6,35	1	
Navicula recens (Lange-Bertalot) Lange-Bertalot	95	6,32	53,63	37	
Navicula rostellata Kützing	69	1,68	10,88	4	
Navicula symmetrica Patrick	22	1,53	6,42	2	**10**
Navicula tripunctata (O.F.Müller) Bory	73	1,97	15,01	9	**3***
Navicula veneta Kützing	49	1,24	6,49	1	**9**
Nitzschia amphibian Grunow	44	1,50	19,18	3	**7**
Nitzschia clausii Hantzsch	5	4,97	13,47	2	**10**
Nitzschia dissipata (Kützing) Rabenhorst	89	5,40	27,02	27	**3**
Nitzschia filiformis (W.M.Smith) Van Heurck	22	1,17	9,78	1	**10**
Nitzschia fonticola Grunow in Van Heurck	94	1,36	5,75	1	**7***
Nitzschia inconspicua Grunow	79	19,22	82,83	42	**9**
Nitzschia palea (Kützing) W.Smith var. *debilis* (Kützing) Grunow in Cleve & Grunow	92	2,41	17,61	12	
Nitzschia palea (Kützing) W.Smith	83	1,82	23,55	5	**5***
Nitzschia paleacea (Grunow) Grunow in van Heurck	59	1,02	6,34	2	**7***
Nitzschia sociabilis Hustedt	38	2,88	24,48	5	**10**
Reimeria uniseriata S.E. Sala, J.M. Guerrero & Ferrario	46	0,80	5,20	1	
Rhoicosphenia abbreviata (C. Agardh) Lange-Bertalot	62	1,23	7,84	2	**7***
Sellaphora seminulum (Grunow) D.G. Mann	7	2,06	8,48	1	
Stephanodiscus neoastraea Håkansson & B. Hickel	48	1,22	8,62	1	
Thalassiosira bramaputrae (Ehrenberg) Håkansson & Locker	20	0,79	6,89	1	**10**
Amphora meridionalis Levkov	28	0,98	4,15	0	**2**
Aulacoseira ambigua (Grunow) Simonsen	24	1,03	3,45	0	**6**
Aulacoseira granulata (Ehrenberg) Simonsen	10	0,75	1,9	0	**6**
Cocconeis placentula Ehrenberg	36	0,76	3,69	0	**7**
Cyclostephanos dubius (Fricke) Round	17	1,05	3,93	0	**6**
Cyclotella atomus var. *gracilis* Genkal & K. T. Kiss	50	1,18	4,82	0	**6**
Navicula riediana Lange-Bertalot & Rumrich	7	1,28	3,80	0	**5**
Fragilaria bidens Heiberg	4	1,81	4,61	0	**7**
Stephanodiscus alpinus Hustedt in Huber-Pestalozzi	10	0,82	4,51	0	**6**
Stephanodiscus tenuis Hustedt	10	1,22	4,19	0	**6**
Surirella linearis W. Smith	19	0,61	4,69	0	**10**

Tab. 3. Spearman correlation coefficients between diatom indices, diatom guilds, diatom life-forms and water chemistry. Correlations significant at p>0.05 (*) and p>0.001 (**) are shown. RKM: river kilometre, TN: total nitrogen, TP: total phosphorus, DOC: dissolved organic carbon.

	RKM	Cond	O2	pH	t	TN	TP	Ca	Mg	K	Na	NO3-N	PO4-P	DOC
SLA	ns	ns	ns	0,27*	-0,31*	ns	ns	ns	ns	-0,45**	ns	ns	ns	ns
DESCY	0,39*	ns	0,27*	0,39*	-0,33*	ns	ns	0,29*	ns	ns	ns	ns	ns	ns
IDSE	0,32*	ns	ns	0,35*	-0,37*	ns	ns	ns	ns	-0,3*	ns	ns	ns	ns
SHE	0,74**	-0,27*	0,54**	0,55**	ns	0,56**	0,25*	ns	-0,27*	ns	-0,67**	0,59**	-0,35*	-0,33*
WAT	0,39*	ns	0,27*	0,37*	-0,37*	0,27*	ns	0,32*	ns	ns	ns	0,34*	ns	ns
TDI	-0,47**	ns	ns	ns	ns	-0,47**	-0,28*	-0,37*	ns	ns	0,3*	-0,44*	ns	ns
GENRE	0,77**	-0,27*	0,46**	0,41*	ns	0,64**	0,35*	0,38*	-0,31*	ns	-0,69**	0,63**	-0,26*	-0,35*
CEE	0,65**	ns	0,42*	0,47**	-0,28*	0,55**	0,3*	0,41*	ns	ns	-0,45*	0,58**	ns	ns
IPS	0,64**	ns	0,41*	0,52**	ns	0,35*	0,36*	0,3*	ns	ns	-0,51**	0,42*	ns	ns
IBD	0,65**	ns	0,44*	0,45*	ns	0,44*	ns	0,31*	ns	-0,28*	-0,52**	0,52**	-0,27*	-0,28*
IDAP	0,61**	ns	0,38*	0,37*	-0,26*	0,54**	0,31*	0,39*	ns	ns	-0,39*	0,58**	ns	ns
EPI-D	0,56**	ns	0,37*	0,36*	-0,28*	0,39*	ns	0,41*	ns	-0,3*	-0,38*	0,45*	ns	ns
DI-CH	0,63**	ns	0,42*	0,42*	ns	0,46**	ns	0,27*	ns	-0,3*	-0,58**	0,58**	-0,27*	-0,44*
IDP	0,28*	ns	ns	0,4*	-0,36*	ns	ns	ns	ns	-0,25*	ns	ns	ns	ns
LOBO	0,43*	ns	0,26*	ns	ns	0,47**	ns	0,38*	ns	ns	ns	0,41*	ns	ns
SID	0,74**	-0,29*	0,49**	0,36*	ns	0,57**	ns	0,29*	ns	-0,27*	-0,63**	0,68**	-0,34*	-0,37*
TID	0,75**	ns	0,53**	0,41*	ns	0,67**	ns	0,35*	ns	ns	-0,6**	0,78**	-0,25*	-0,34*
Motile	-0,62**	ns	-0,35*	ns	ns	-0,6**	ns	-0,28*	ns	ns	0,56**	-0,56**	0,28*	0,34*
Low prof.	0,56**	ns	0,34*	ns	ns	0,56**	ns	ns	ns	ns	-0,5**	0,51**	-0,3*	-0,29*
High prof.	0,64**	ns	ns	ns	ns	0,45*	0,33*	0,28*	ns	ns	-0,55**	0,53**	ns	-0,42*
Planktonic	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	-0,26*	ns	ns	ns
Benthic	ns	ns	ns	ns	ns	ns	-0,29*	ns	ns	ns	0,33*	ns	ns	ns

Tab. 4. Spearman correlation coefficients between the best performing diatom indices, diatom guilds, diatom life-forms and hydromorphological variables. Correlations significant at p>0.05 (*) and p>0.001 (**) are shown. Q: discharge, D16-84 subtrate granulometry (diameter), Susp solids: suspended solids.

	Q	Velocity	D16 mm	D50 mm	D84 mm	Susp solids	Slope
GENRE	-0,44*	0,49**	0,35*	0,3*	0,3*	0,26*	0,52**
IPS	-0,56**	ns	0,41*	0,35*	0,33*	ns	0,37*
TID	-0,4*	0,42*	0,41*	0,32*	0,29*	ns	0,51**
Motile	ns	-0,49**	-0,27*	ns	ns	-0,35*	-0,36*
Low profile	ns	0,38*	ns	ns	ns	0,34*	ns
High profile	-0,36*	0,43*	0,39*	0,38*	0,38*	ns	0,61**
Planktonic	ns	ns	ns	ns	ns	0,5**	ns
Benthic	ns	ns	ns	ns	ns	-0,49**	ns

Fig. 1. The total biomass of chlorophyl-*a* (µg/cm^2) and distribution of different algal classes (green algae, cyanobacteria, diatoms) among the sites investigated. River kilometres refer to the sites investigated. Data from tributaries are not involved.

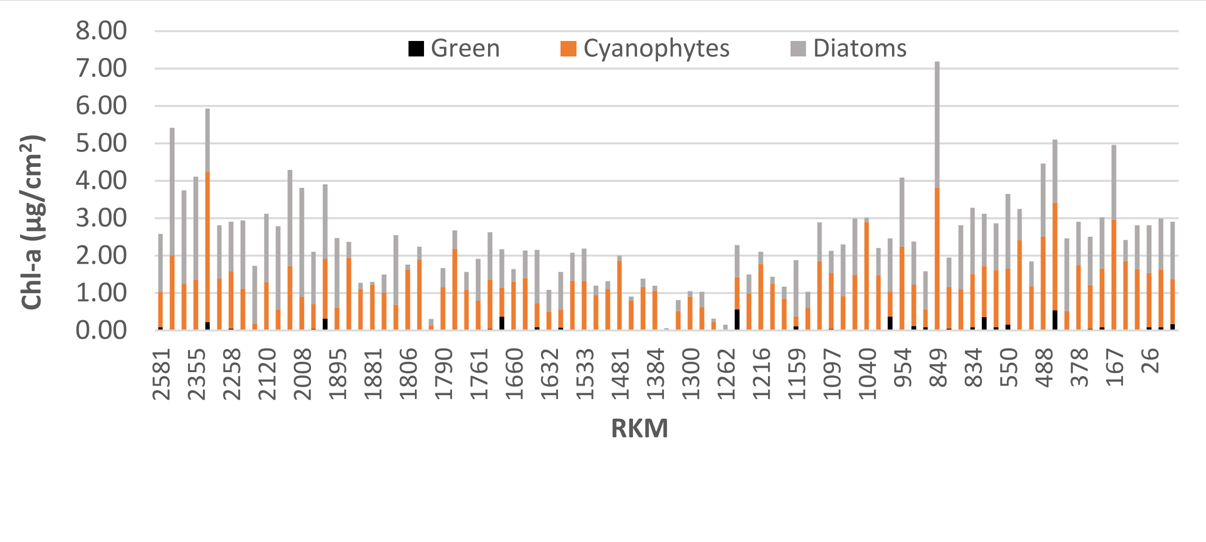

Fig. 2. The distribution of samples in the ordination space of a Canonical Correspondence Analysis based on non-diatoms. The different Danubian types and tributaries are differentiated. Type 1: 2581 riv. km, type 2: 2415 – 2258 riv. km, type 3: 2204 – 2008 riv. km, type 4: 1942 – 1790 riv. km, type 5: 1761 – 1533 riv. km, type 6: 1481 – 1097 riv. km, type 7: 1071 – 954 riv. km, type 8: 926 – 378 riv. km, type 9: 235 – 130 riv. km, type 10: 107 – 26 riv. km.

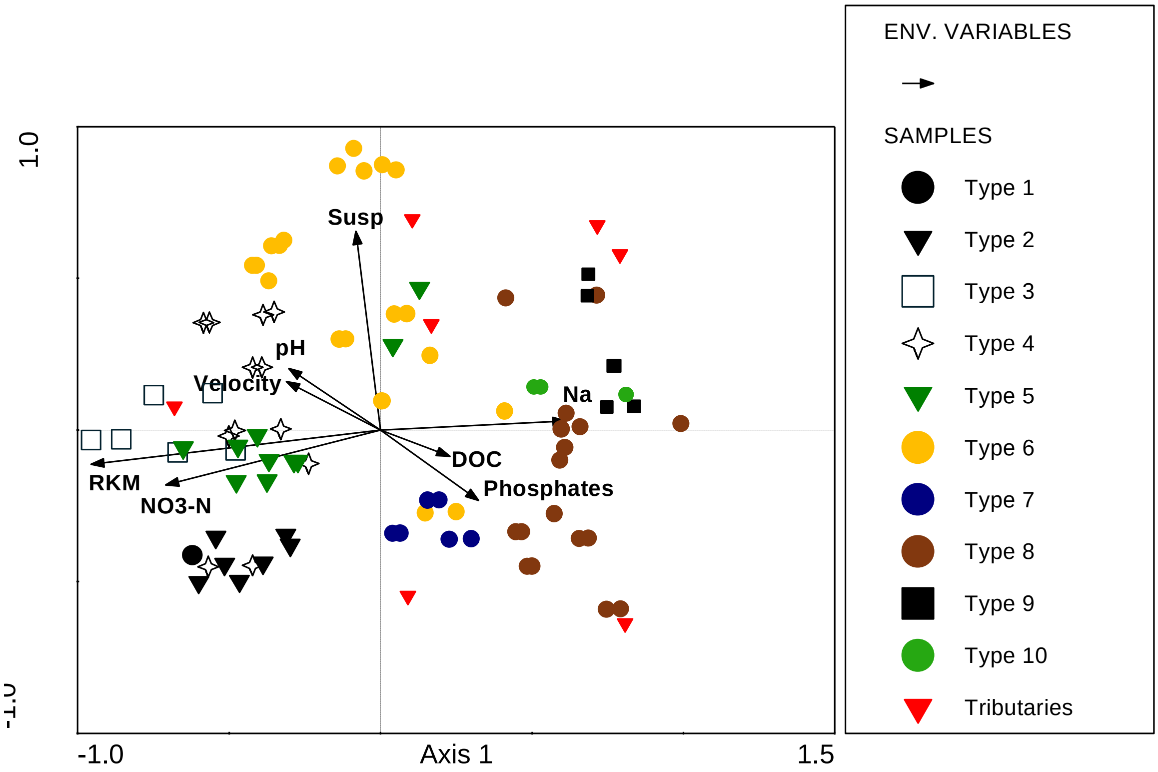

Fig. 3. The distribution of samples in the ordination space of a Detrended Correspondece analyses based on the species composition in the samples. The different Danubian types and tributaries are differentiated. Type 1: 2581 riv. km, type 2: 2415 – 2258 riv. km, type 3: 2204 – 2008 riv. km, type 4: 1942 – 1790 riv. km, type 5: 1761 – 1533 riv. km, type 6: 1481 – 1097 riv. km, type 7: 1071 – 954 riv. km, type 8: 926 – 378 riv. km, type 9: 235 – 130 riv. km, type 10: 107 – 26 riv. Km, type 11: Tributaries.

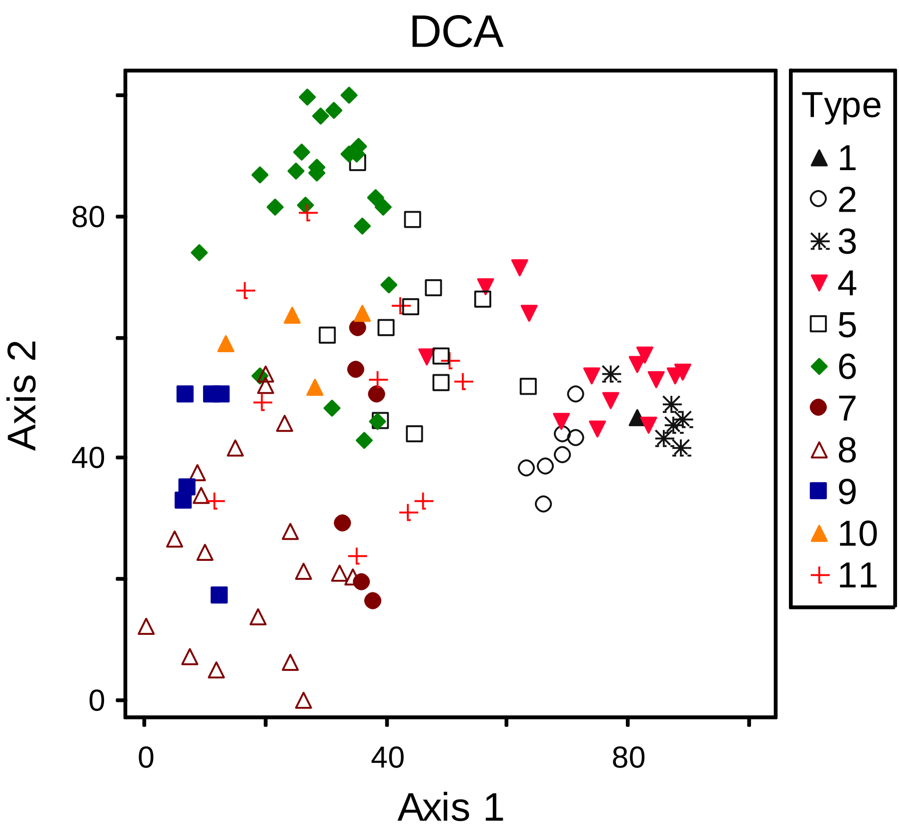

Fig. 4. The distribution of samples in the ordination space of a Canonical Correspondence Analysis based on diatom species composition. The different Danubian types and tributaries are differentiated. Environmental variables (arrows): Q: discharge, NO3-N: nitrates, PO4: phosphates, DOC: dissolved organic carbon, Ca: calcium, TP: Total phosphorus, O2: dissolved oxygen, RKM: river kilometer, Susp: suspended solids. Type 1: 2581 riv. km, type 2: 2415 – 2258 riv. km, type 3: 2204 – 2008 riv. km, type 4: 1942 – 1790 riv. km, type 5: 1761 – 1533 riv. km, type 6: 1481 – 1097 riv. km, type 7: 1071 – 954 riv. km, type 8: 926 – 378 riv. km, type 9: 235 – 130 riv. km, type 10: 107 – 26 riv. km.

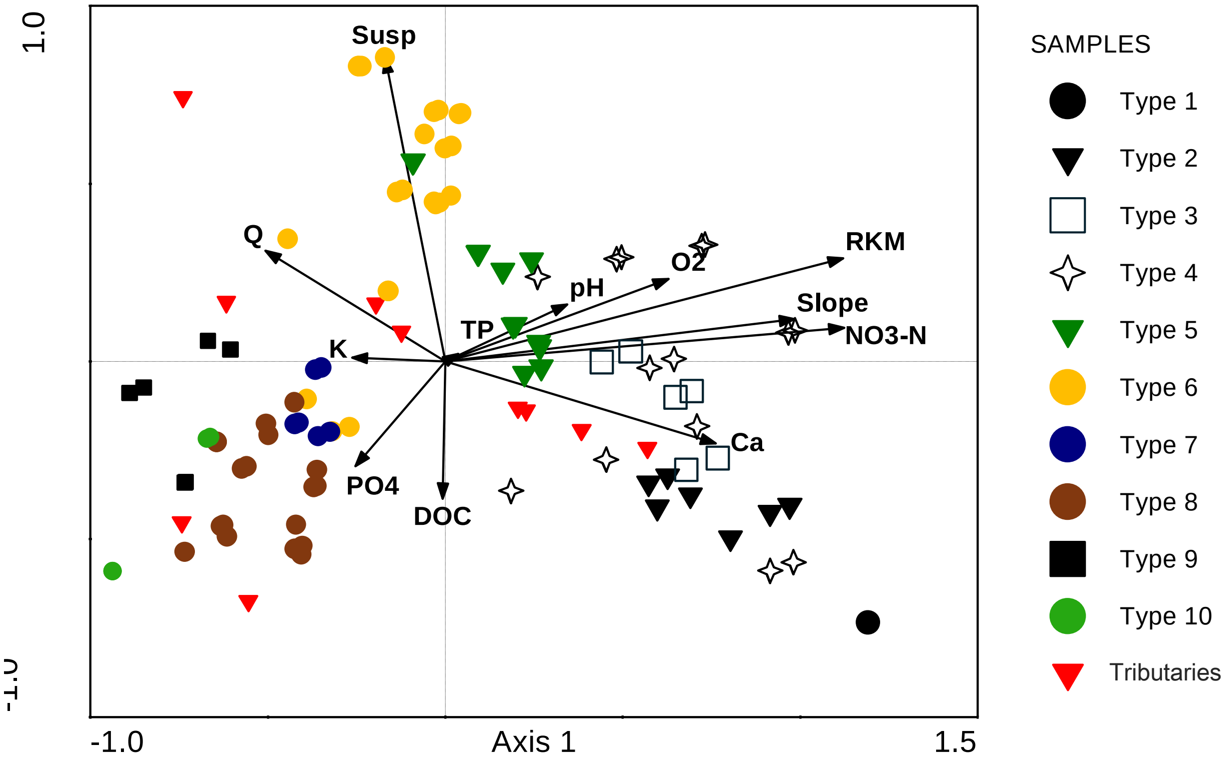

Fig. 6. Comparisons of indices values (GENRE, IPS, TID) in the longitudinal profile in the different Danubian types. Types 2-10 are compared.

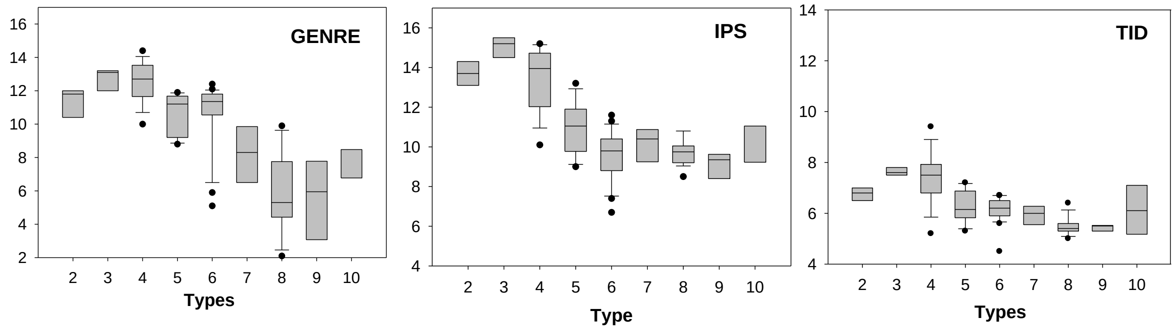

Fig. 7. Distribution of diatom guilds among the sites investigated. River kilometres refer to the sites investigated.

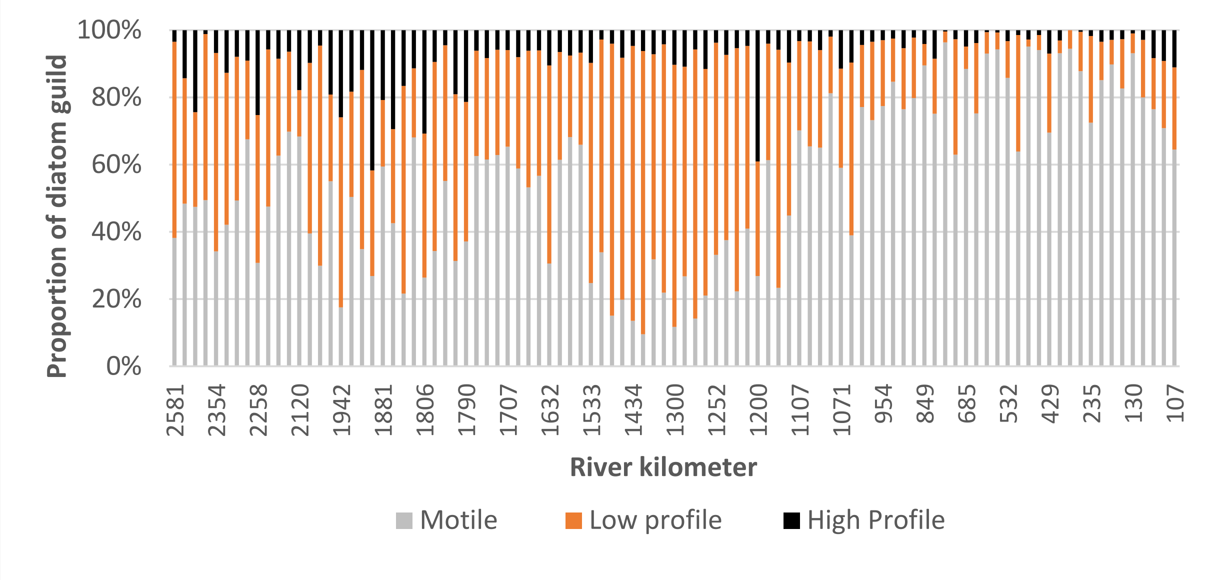

Fig. 7. Distribution of planktonic and benthic diatoms among the sites investigated. River kilometers refer to the sites investigated.

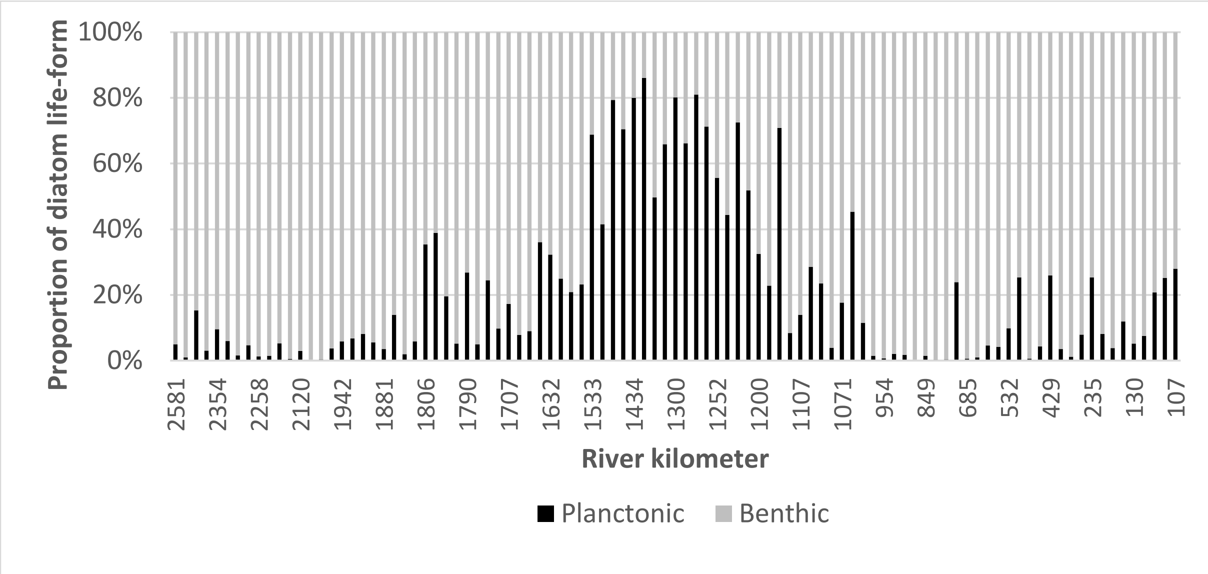

Fig. 8. Ecological status assessment using the two intercalibrated boundaries of IPS index of the Slovak assessment method: high/good (H/G) and good/moderate (G/M). Type 2: 2415 – 2258 riv. km, type 3: 2204 – 2008 riv. km, type 4: 1942 – 1790 riv. km, type 5: 1761 – 1533 riv. km, type 6: 1481 – 1097 riv. km.