Comparison of the utility of a frequently used diatom index (IPS) and the diatom ecological guilds in the ecological status assessment of large rivers

Trábert, Zs.[*1], Kiss, K.T.[1], Várbíró, G.[3], Dobosy, P.[1], Grigorszky, I.[1,2], and Ács, É.[1]

[1]MTA Centre for Ecological Research, Danube Research Institute, 1113 Budapest, Karolina út 31, Hungary
[2]University of Debrecen, Department of Hydrobiology, 4010 Debrecen, Egyetem tér 1, Hungary
[3]MTA Centre for Ecological Research, Danube Research Institute, Department of Tisza Research, 4026 Debrecen, Bem tér 18, Hungary

Abstract

The Indice de Polluo-Sensibilité Spécifique (IPS) is one of the most frequently used diatom index. Beside this, according to some studies in streams and small rivers the diatom ecological guilds might be effective tools for ecological quality assessment in the future, but the usability of them is not always clear. Our main goal was to compare the robustness of the IPS index and diatom ecological guilds in a large river. For this i) a temporary study was carried out to investigate if there were some differences between how the nutrient content affected the IPS values and guilds proportions in the Danube River; ii) spatial studies were conducted to investigate the effects of the different water depths (different light intensity and current velocity) on biological metrics (IPS and guilds, as well); iii) we studied whether the IPS index or guild proportions were influenced more by the substrate type.
As for the results of the temporary study only the *motile* and *planktic guilds* had significant connections with phosphate-phosphorus concentration. However, the *high profile* and the *low profile* taxa showed correlations neither with nutrient concentration nor water discharge. Nevertheless, the higher abundances the *low profile guild* reached the lesser values of the IPS index were. In spatial studies the guild abundances (especially the *motile* taxa) altered in different water depths. This could be caused by

*Corresponding author. Tel.: +36 12793143.
E-mail address: trabert.zsuzsa@okologia.mta.hu, szilagyizsuzska@gmail.com (Zs. Trábert), kiss.keve@okologia.mta.hu (T. Kiss Keve), grigorszky.istvan@okologia.mta.hu (I. Grigorszky), varbiro.gabor@okologia.mta.hu (G. Várbíró), acs.eva@okologia.mta.hu (É. Ács).

different microhabitats characterised by disparate current velocity, and by different types of bed material along the cross-section. Contrary to guilds, there were no relevant differences in IPS values along the cross-sections. We experienced similarities in the study on shells and in spatial study: there were significant differences between guild abundances on different shell surfaces, but not so in the IPS. On the whole the IPS is robust enough to be a suitable index for the ecological water quality assessment and diatom ecological guilds could not be used instead of the diatom index (IPS) in large rivers. However, the trait based methods could be useful supplements of the ecological status assessment.

Key words: diatom ecological guilds, IPS, trophic level, flow disturbance, nutrients, Danube River

Introduction

Water Framework Directive (WFD; European Union, 2000) the third directive of the European Union (EU) requires to survey and assess ecological conditions of surface water bodies. Benthic diatoms are one of the most preferred organisms of creating biological indices and classifying the quality of waters. In case of the Danube River for water quality assessment the Indice de Polluo-Sensibilité Spécifique (IPS) is used (Liška et al. 2015) in order to detect basically the inorganic nutrient and organic loads in a river (Ács et al. 2003, 2006).

The diatom indices based on species level, according to the requirements of EU WFD however, because of several reasons (e.g. imprecisions or mistakes during the identification from lack of high level knowledge of diatom species) other simple metrics are proposed to complete the presently used biotic indices (Passy 2007a, b, Rimet & Bouchez 2011). Moreover these simple biological traits are said to be able to be used instead of the species-based diatom assessment tools (Berthon et al. 2011). The definition of ecological guild as a functional measure was introduced by Passy (2007a). Guilds consist of groups of taxa that live in the same environment, but may have adapted in different ways to abiotic factors. Passy groups three types of guilds by their profile. The *low profile guild* (LPG) favours in nutrient-poor and high disturbance habitats*; the high profile guild* (HPG) has the highest proportion in nutrient-rich sites and in conditions of low flow disturbance; and the *motile guild* (MG) increases in relative abundance with the increase of nutrient availability. Later Rimet & Bouchez (2011) recommended a new guild as well, the *planktic guild* (PG).

Until now experiments carried out in guild studies limited to small streams or rivers (Passy 2007a, b, Lange et al. 2011, Rimet & Bouchez 2011, 2012, Berthon et al. 2011, Stenger-Kovács et al. 2012, Marcel et al. 2013, Béres et al. 2014, Law et al. 2014). Other traits were also tested on benthic algae in 43 sites of the 85 km long Manuherikia River and its catchment, New Zealand (Lange et al. 2015) and found it a good tool for assessing the relationships between multiple anthropogenic stressors.

However, the large rivers can paint a totally different picture (e.g. because of the power from the significant amount and flow of water), the connections between guilds and other factors are not always unambiguous led us to investigate changes in guild abundances influenced by different factors in such a large river as e.g. the Danube River.

Our main goal was to compare the guilds and the IPS index to explore which one indicates some hydromorphological (like influences caused by the differences of substrates) and significant nutrient impacts. Hence, our intention was to test if connections between ecological guilds and nutrients can be shown in a large river. According to this the following hypothesis were tested: i) the connection between the *motile* and the *high profile guilds* and the nutrients should show significant positive correlations but the *low profile guild* should display a negative relation with the same factors; ii) significant negative correlations are expected between *motile, high profile guilds* and the IPS diatom

index, and a significant positive correlation between *low profile guild* and the IPS index; iii) the connection of the water discharge with the *high profile guild* would be significantly negative and with the *low profile guild* significantly positive.

Materials and methods

We performed three types of investigation in the Danube River at Göd (47°40'43.2" N, 19°07'30.1" E; 1669 rkm).

1) Temporal study

Monthly sampling was carried out in the littoral region of the Danube River during three years (from 2010 to 2012). Samples were taken from gravels scraping with toothbrush (CEN 2014). The water discharge highly fluctuated during the sampling period; in 2010 extraordinary floods were observed. In the year 2011 stream gauge was more balanced than in the other two years, and numerous but smaller floods were experienced in 2012. Due to floods in 2010 sampling failed in June and August and in June in 2012. Furthermore, the sample taken in October (2010) was not representative. Therefore we had altogether 32 samples.

The following physicochemical factors were measured every week (except in case of floods) at the sampling site in the framework of a water chemical monitoring: chemical oxygen demand (COD) was determined with the acidic potassium permanganate method (ISO 8467 1993), NO_3-N content was measured by the sodium salicylate method (Vijayasarathy 2011) and PO_4-P concentration with ammonium molybdate reagent and 10% ascorbic acid according to Eaton et al. (2005). Total organic matter was measured by applying a Multi N/C 2100S TC analyser (Analytik Jena, Germany) equipped with a non-dispersive infrared detector. Suspended matter and total dissolved matter content were determined gravimetrically. Turbidity was measured with a Lovibond PC Checkit portable meter, and water temperature, pH, conductivity, dissolved oxygen were measured with a WTW multiline portable meter. Values of water discharge were calculated from the water level measured at Vác, which is ca 7 km from the sampling station.

2) Spatial studies

The physico-chemical factors (conductivity (µS/cm), pH, temperature (°C), total dissolved solids (TDS, mg/l), chlorophyll *a* (µg/l), ammonium-nitrogen (NH_4^+), nitrate-nitrogen (NO_3^-)) of the cross-section were measured *in situ* by a YSI EXO2 Multi-Parameter Water Quality Sonde. The sonde was submerged to the bed directly before taking sample.

These studies were carried out in three times (23 May 2012, 13 May 2013 and 26 May 2014). The same month was chosen in order to prevention of the seasonal effects. Six sampling points were selected in every cross-sections, from different water depths (from the left side to the right side in the following depths: 1-2m, 2-3m, 4-6m, 4-6m, 3-5m, 1-2m– the values depended from water discharge). Water discharge was 1421 m^3s^{-1}, 2684 m^3s^{-1}, 2830 m^3s^{-1} respectively in three studied years. Samples were taken by a dredge (Szekeres et al. 2009) in five replicates from gravels, pebbles and sediments (in 2013 only from gravels and pebbles). In 2014 algae could have been detected only in 3 samples, so we exclude them from the statistical analysis. From gravels and pebbles algae were scraped with toothbrush, from sediment algae were extracted by shaking.

3) Studies on shells

We set up an *in situ* experimental system where the same environmental conditions were ensured (both physical and chemical variables) at every substrate. The differences were only in the smoothness and size of the substrates. There were 8 series (sets or samples) of valves, in total 80 shells. 10-10 polished and 10-10 ribbed valves of *Unio tumidus* (Linnaeus) (shells with size 'medium'), *Sinanodonta woodiana* (Lea) (shells with size 'big') and 20-20 *Corbicula fluminea* (O. F. Müller) (shells with size 'small') were fixed onto a single metal plate. Four plastic pots were bound to the four angles of the plate by ropes, and the plate was connected to the railing of a pontoon by means of a rope, so the metal plate was kept 20 cm under the water surface. 10-10 *Corbicula fluminea* (2 sets) were picked up after 1 week, and the rest of substrates (6 sets) were picked up after 4 weeks. Algae were scraped from the shells with toothbrush and 1-1 sets (10-10 shells) were treated as a composite sample. Altogether 8 samples were investigated with light microscope, but only 4 were used in statistical analysis, because of the small number of algae colonized on the surfaces.

Sample processing

The diatom frustules were cleaned with hydrochloric acid and hydrogen peroxide, subsequently washed in distilled water and mounted with Naphrax® medium (CEN 2014). An Olympus IX70 inverted microscope equipped with differential interference contrast (DIC) optics were used for LM observations. At least 400 valves were counted to estimate the relative abundance of each taxon in the sample. For a more accurate identification (first of all to identify the centric diatoms) we examined our samples with Zeiss EVO MA 10 scanning electron microscope. For SEM studies a part of the cleaned and washed samples was filtered through a 3 μm Isopore™ polycarbonate membrane filter (Merck Millipore), which was fixed onto a stub using double-sided carbon tape, and coated with gold using a rotary-pumped spatter coater Quorum Q150R S.

Diatom metrics

Species found in samples were grouped into four ecological guilds: *low profile guild* (LPG), *high profile guild* (HPG) and *motile guild* (MG) created by Passy (2007a), and *planktic guild* (PG) developed by Rimet & Bouchez (2011).
Furthermore, the biotic diatom index (IPS = Indice de Polluo-Sensibilité Spécifique) was calculated by OMNIDIA 5.3 (Lecointe et al. 2008). This index was used to assess the ecological water quality developed by Zelinka & Marvan (1961), then modified by Coste (Coste in Cemagref 1982).

Statistical analysis

Detrended correspondence analysis (DCA) was applied using CANOCO 5.0 (ter Braak and Šmilauer, 2012) to determine the appropriate response model (linear or unimodal) for diatom guilds and the environmental data. Response data are compositional and have a gradient 0.8 SD units long, therefore the linear method is recommended; therefore, redundancy analysis (RDA) was used to investigate the relationship between diatom guilds and environmental variables. For the RDA analysis, the environmental variables were $\log(x + 1)$ transformed. Significance was tested using Monte Carlo tests with 999 permutations (ter Braak and Smilauer, 2002). In species response curves (SRC) to reveal the general shape of the relationship General Additive Model (GAM) was used. The GAM algorithm used by the CANOCO 5.0 selects the best shape of given complexity (defined by degree of freedom) using the AIC. In our model the Gamma distribution and the canonical log link-function was used (ter Braak and Šmilauer, 2012). We calculated the coefficient of determination according to Nagelkerke (1991).

In spatial studies and in those ones shells served as substrates coefficient of variations (CV %) and standard error (SE) was computed to investigate how samples varied within a dataset (both in case of relative abundances of diatom ecological guilds and of the physical and chemical variables). It was calculated as follows: CV% = (standard deviation/mean)*100.

Results

Temporal studies

The range and CV of the variables can be found in Table 1. To summarise the monthly results of the three years, we examined the environmental variables. We found the CV values of suspended matter content, turbidity and phosphate-phosphorus concentration to be indicating the most heterogeneity (Table 1). Among the guilds only the *motile guild* presented stability (homogeneity) in the diatom assemblages (value of the CV was 22.16 %). The other three guilds proved to be quite labile, the relative abundances of them varied during the three years. Finally, the CV of the IPS indicated 'moderate lability' (Table 1).

The IPS showed significant correlations only with one guild (*low profile guild*) as can be seen in Table 2 and Table 5. Among the 11 variables the IPS had significant correlations only with the chemical oxygen demand at $p < 0.05$ significance level but it had correlations with temperature, nitrate-nitrogen, total organic and total dissolved matter content at $p < 0.1$. On this significance level it also showed relationship with the *motile guild*.

The IPS had a positive connection with the *low profile guild* and a negative connection with the *motile guild*. However, the connection between these two guilds was opposed. The *motile guild* also showed high negative correlation with the *planktic guild* (Fig. 1a).

As seen in Fig. 1a shows the *high profile guild* correlated significantly with two environmental variables, with the suspended matter content and turbidity (these were negative connections), however, there were not any strong relationships with water discharge and the nutrients (the summary of the RDA analysis total variance can be seen in Table 3 and Table 4).

The total variation was 183.12, explanatory variables account for 53.9% variation and the adjusted explained variation was 32.9%. The first axis contains 71.17 % of the explained variation. Significant variables were PO_4, COD, and DO. (Table 4.) The *low profile guild* had no connection with any variables. The *motile guild* correlated with two variables: the phosphate-phosphorus and chemical oxygen demand. Considering the significance level at $p < 0.1$, this *guild* also correlated with the dissolved oxygen, as well. (Table 2 and 3, Fig. 1a).The *planktic guild* displayed connections with more variables such as the temperature, phosphate-phosphorus, dissolved oxygen and chemical oxygen demand. At the same time, the ordination of samples was not influenced by nutrients (Fig. 1b).

Table 4 displays that the phosphate-phosphorus, the *motile* and the *planktic guild* having high correlations with Axis 1. Axis 2 indicated the highest correlations with the *high profile* and *low profile guild* and with the total organic matter content among the environmental variables. Furthermore, these guilds and the motile guild correlated with Axis 3, as well, but this axis had no correlations with any variables.

The *low profile guild* and IPS showed some correlations with Axis 4 but none with any variables.

Table 5 demonstrates that the phosphate-phosphorus concentration, the chemical oxygen demand, dissolved oxygen and turbidity were the explanatory variables.

The Table 6 and Fig. 2 also supported that the *motile* guild showed significant negative connection with the RDA axis but they did not display any significant relationships in case of *high profile* and *low profile guild*. The GAM model fitted the best in the case of the *planktic guild* though this connection was non-linear but polynomial and significantly positive.

5

As for the average taxa number in guilds the *motile guild* showed the most taxa in each season (the average taxa numbers were 22-29 during the seasons). There were less *low profile* species (9-11), and the least taxa belonged to the *high profile guild* (mean of the taxa number was 7).

The average taxa number of the *motile guild* increased continually from winter to autumn; while it was similar in the *low* and *high profile guild* during the sampling period. There were three dominant species in each season during the years. They were *Amphora pediculus (low profile guild)*, *Nitzschia dissipata var. dissipata* (*motile guild*) and *Nitzschia inconspicua (motile guild)*. Nevertheless, only a slight pattern can be seen by the seasons (Fig. 1.b).

Spatial studies

We investigated altogether 18 samples, however, only 13 of them could have been put into the diagram because of lack of algae in the other samples. Furthermore, only 11 samples were put into the statistical analysis because in 2014 just two samples were taken from gravels and only these ones contained diatoms in adequate number. So, CV had not been calculated with these two samples but we put them into the diagram to display the guild abundances. In 2013 diatoms occurred only in 5 samples instead of 6 in contrast to the year 2012.

The proportion of the *high profile guild* never reached the proportions of the *low profile guild* and the *motile guild*. Nevertheless, its highest relative abundance could be noticed in the deepest region of the cross-section where the *low profile guild* presented lower abundances in contrast with littoral regions.

The first year when samples were collected from different bed materials remarkable differences in the relative abundances of the *high profile guild* could be experienced (Fig. 3).

Compared to the proportions of the *low profile guild* in the three years, some trends are observed in the same zones. We saw the highest values of relative abundances of this guild in littoral habitats. Here these algae were liable to stronger physical effects as opposed to the other guilds, regardless of the year (namely, the type of substrate) (Fig. 3).

The proportions of the *motile guild* and the *planktic guild* changed the most excessively, though usually these changes moderated appreciably when substrates were the same. Besides, the relative abundances of the *planktic guild* were almost always higher in the greater depths than in the littoral regions (Fig. 3).

While there were considerable differences in guild proportions in the cross-segments, in the case of the IPS index the fluctuations were not so high as in the case of guilds. Some alterations could be observed but only in a given water qualifier category. In the view of nutrient content the cross-section was homogeneous. Namely, the detected environmental variables along a cross-segment of the Danube River allowed us to consider the cross-section as a stable (homogeneous) system (Table 8).

According to CV% of the guilds almost all data series could be considered extremely labile (Table 9). However, there were some differences between the CV% of the years (of the two cross-sections). For instance, in some cases the values of the CV% were much lower in 2013 (namely, in the year when we took samples from the same type of substrates) compared to values of that in the year of 2012 when we sampled from more types of substrates (Table 9). CV% values were also calculated in case of the IPS every cross-section (Table 9). In 2012 this value was higher than in 2013. Moreover it belonged to the category 'moderately labile', so based on the CV value of the IPS the cross-segment was not homogeneous in 2012 in contrast to 2013 when CV% value was under 10%, referring to a homogeneous system.

The CV % calculated by proportions of the *high profile guild* displayed the clearest difference between the year when samples were taken from more types of bed materials and the year when samples were taken from the same substrates. That means the CV % was very high in the first year, however, a great decrease could be observed in 2013 (Table 9). Similar shifting could be noticed in the case of the *low profile guild*. In respect to the CV% the *motile guild* did not follow the changes of the type of the

6

substrate. The *planktic guild* showed the opposite of what the *high profile* and the *low profile guild* revealed. It reached the least CV% value when the substrates were the same, and the CV% values were higher when samples were collected from different types of the bed material.

There were no dominant *high profile* species in any of the cross-sections. Among the *low profile* taxa *Amphora pediculus* reached the highest relative abundance in almost in every sample, but *Cocconeis placentula var. euglypta* was also dominant in many samples.
The dominant *motile* species were *Navicula cryptotenella, Navicula lanceolata, Navicula tripunctata, Nitzschia dissipata var. dissipata* and *Nitzschia inconspicua*.

Studies on shells

We detected diatoms in adequate number only in 4 polished series and in 1 unpolished (ribbed) sets but we checked the dominant taxa in the other 3 sample sets, as well. In all samples, the highest relative abundances were shown by taxa belonging to the high profile and motile guild. (Fig. 4 and Table 10). Among guilds only in case of the *low profile* guild we experienced that the ratio of this guild was the highest on the smallest shells (both in case of polished and unpolished shells) although these ratios proved to be exiguous as opposed to the *high profile* and the *motile* taxa (Fig. 4). In regard to the surface of the shells we did not get sharp differences in the biofilm community structure. However, at first on the polished shells there could be detected algae in adequate numbers.
Since one sample among the five samples, in which we found enough valves to count, was picked up only one week after put into the water, we ignored it when calculating the CV. The examined section of the river proved to be moderately labile regarding the values of the CV% of the *high profile guild* and the *motile guild*. In contrast, the *low profile* and the *planktic guild* presented extreme heterogeneity (Table 10), irrespectively of the physical and chemical variables being homogeneous in the microhabitat of the single metal plate on which shells were fixed. Considerable differences were experienced in variation coefficients of guilds while not so in IPS values (Table 10). In spite of that, the media, where diatoms developed, were homogeneous in view of environmental variables. Furthermore, there could not be found any tendencies in alterations of guilds proportions. Summarized, guild abundances varied in some extent among samples in spite of homogeneity of the light intensity, nutrient concentrations and current velocity.
Studying the dominant taxa in the samples, we found that except for the sample (polished and medium shells ('P, M')) the *Diatoma vulgaris* had reached high dominance in samples even in those which had included just a few diatom valves. The dominance of this taxa expressed in relative abundancy were 0.33 (in sample from polished and small shells after 1 week ('P, S_1')); 0.17 (in sample from unpolished and medium shells ('U, M')); 0.48 (in sample from polished and big shells 'P, B')) and 0.36 (in sample from polished and small shells after 4 weeks ('P, S_2')), respectively. The *Navicula recens* was also dominant in the samples – except for the sample from polished and big shells ('P, B') (in which it was only subdominant). Its relative abundancy values were 0.19 in sample from polished and small shells after 1 week ('P, S_1'); 0.05 in sample from unpolished and medium shells ('U, M'); 0.06 in sample from polished and medium shells ('P, M') and 0.08 in sample from polished and small shells after 4 week ('P, S_2').
Some species were subdominant or dominant in 'P, S_1' but they occurred just with low abundancies in other samples, or were missing completely: *Cocconeis placentula* var. *euglypta, Navicula cincta, Navicula radiosa, Ulnaria ulna, Ulnaria ulna* var. *acus*. Other taxa, however, could not be found in PS$_1$ but they were dominant or subdominant in the other ones; like *Gomphonema olivaceum* var. *olivaceum, Gomphonema parvulum* var. *parvulum* f. *parvulum, Gomphonema tergestinum, Navicula cryptotenella, Nitzschia dissipata* var. *dissipata*.
The averaged taxa number was the highest in the case of *motile* (12.8) and the lowest in the case of *low profile guild* (4.2).

Discussion

Temporal studies

It has been studied if human impacts could be detected by biological metrics (like diatom ecological guilds) also in a large river in natural circumstances.
Most publications in topic ecological guilds emphasize the connections between ecological guilds and nutrients and current velocity (Passy 2007a, b, Berthon et al. 2011, Rimet & Bouchez 2011, Stenger-Kovács et al. 2012). Our hypotheses in respect to the relationships between guilds and nutrients were supported partially. The *low profile guild* abundance had no connection with any of nutrients or the other environmental variables. Stenger-Kovács et al. (2012) revealed that the proportion of the *low profile guild* had increased with the decreasing total nitrogen and soluble reactive phosphorus. Lange et al. (2011) found that *low profile* taxa were more prevalent at ambient and medium nutrient concentrations compared to high and very high concentrations. In Passy's experiments the proportion of *low profile guild* decreased along the resource gradient (2007a, b) because even in eutrophic streams, where species found in the understorey of the community biofilm can experience reduced nutrient diffusion (Horner et al. 1990). Besides *low profile* taxa, as pioneer colonizers, are located in the lowest layer of the biofilm and have smaller cells opposed to the other taxa, consequently, they are impeded of nutrients (Pringle 1990). This is supported by our results. A negative relationship was shown between *low profile guild* and *motile guild* (Fig.1a). So, it is true that the *low profile* guild did not display significant connection with nutrients, however, it negatively correlated with the *motile* guild, which (the *motile guild*) had a strong relationship with phosphate-phosphorus concentration. Moreover, the negative connection of the *low profile guild* with *high profile guild* can refer to that if number of *high profile* taxa (which species can access phosphate simply) decreases influenced by appreciable amount of organic matters, then a faster diffusion of phosphate can begin toward the lower layers in the mat where *low profile* taxa are able to get nutrients in an easy way (Horner et al. 1990). The connection of *low profile guild* with water discharge was expected to be significant and positive, since it is resistant to the flood disturbances (Francoeur & Biggs 2006, Robinson & Rushford 1987). However, we saw no correlation as the other three guilds also had no relationship with water discharge. Increase of organic matter content resulted in rising number of *motile* and *low profile* taxa in Rimet & Bouchez's mesocosms experiment (2011) while Marcel et al. (2013) did not manage to detect this association. In his study only the *high profile guild* reacted on rise of organic matter content, positively. *High profile* taxa have more direct connections with the ambient water, which enable them to store more nutrients (Pringle 1990). At the same time these taxa are more susceptible to the shear forces of fast currents (Biggs et al. 1998b; Ghosh and Gaur 1998), and this group of algae prevailed in low velocities in Passy's experiment (2007a, b). According to Stenger-Kovács et al. (2012) in the summer-winter period the proportion of the *high profile guild* decreased due to recurrent floods but they carried out their investigation in a small stream, which may support entirely different circumstances from ours (large river). In our study the effect of water discharge (as disturbance gradient) is likely to be expressed indirectly, through the suspended matter content. Namely, in our investigations *high profile guild* had significant negative correlations with suspended matter content and turbidity. In those periods when the Danube River was characterized by (heavy) floods and its' suspended matter content increased, the penetration of the light to the algae was likely to be impeded. Lange et al. (2011) accounted that *high profile* taxa could not grow in decreased light intensity, and these taxa were more abundant when nutrients and light were not limited. Besides he recognized that light levels had the strongest effect on community composition. Hill et al. (2009) also observed in experiments that light affected algal community structure much more than the phosphorus enrichment utilized. In addition, although *high profile* taxa are able to form colonies, which give advantageous for

exploiting resources (Berthon et al. 2011), these colonies may be tumbled by gross amount of suspended matters in the river. Of course, this would mean that in case of floods with high suspended matter content there is a little light for all other diatoms, too, however, *low profile* taxa are relatively more common in shaded environments (Lange et al. 2011). By our results *low profile* taxa did not show correlation with suspended matter content, but the *motile* species had negative correlations with suspended matter content and turbidity, and the *motile* diatoms can also oppose such disturbances by moving rapidly not only from nutrient-poor microenvironments (Johnson et al. 1997) but also from ones with limited light (Consalvey et al. 2004). We also experienced that relative abundance of *motile* species increased along the phosphate gradient but they did not show any connections with nitrate. Stenger-Kovács e al. (2012) found that *motile guild* had shown the strongest correlations with inorganic total nitrogen but not with soluble reactive phosphorus. Nevertheless those samples, which included comparatively many *motile* taxa indicated a worst water quality by IPS index. Actually, the *motile guild* and the IPS values implied a slight negative correlation, and, as we mentioned above, with the *low profile guild*, as well, but the latter relationship was significantly positive. The explanation of this is likely to be that *motile* taxa have a role as competitors in habitats rich in resources (Fairchild et al. 1985, Van der Grinten et al. 2004) that may mean excess in nutrients or organic matters. The land use e.g. can also explain the connection between the IPS and the *motile* guild. Namely, these species have been especially useful for assessing siltation and land use impacts on diatom assemblages (Bahls et al. 1992, Fore & Grafe 2002, Smucker & Morgan 2010). Consequently, the more intensive the extent of the land use is, the worse the ecological state may be, the more the wideness of the natural shores may destroy, the more shores may be ready for erosion. This may result in an increase in the number of *motile* taxa because these species can leave. For instance, Stenger-Kovács et al. (2012) found the *motile guild* had positively associated to the water discharge, which had indicated their ability to change position in the biofilms.

Between the *low profile guild* and IPS index there was a significant positive correlation, as we expected. However, there was no correlation between *high profile* taxa and IPS whereas a strong negative correlation was expected to have been. Taxa belonged to the *planktic guild* was most effected by phosphate content but curiously, this proved to be a negative connection. Moreover, it was the *planktic guild* that contacted with most environmental variables, significantly.

Although the IPS had no significant relationships with the most variables, only with the chemical oxygen demand, and with the *low profile* guild among guilds, it introduced slight ($p < 0.1$) connections with the most variables (temperature, total organic matter, total dissolved matter, nitrate-nitrogen).

Furthermore, it is true that our results did not indicate significant connections between the IPS and nutrients and water discharge, however, in such a large river as the Danube (with constantly changing water discharge, hence constantly changing environmental variables) we cannot say a single factor that would be the main driver affecting the biological metrics and indices. In addition, the IPS showed correlations with those factors – if we consider $p < 0.1$ – which referred to more homogeneous environment (CV% in Table 1.). Besides, many countries also use the IPS index for the quality assessment of the Danube (final report of JDS3).

Accordingly, summarising the results of temporal studies the diatom ecological guilds showed some relationships with nutrients but they seemed to indicate these connections unstably. Namely, for example the *high profile guild* was expected to have (positive) connection with nutrients but it had no connections with nutrients. Contrary to the hypotheses (as for the literature) the *low profile guild* did also displayed any correlations neither with nutrients, nor with physical parameters. In contrast to the guilds the IPS proved to be more stable.

Spatial studies

The relative abundances of the *high profile guild* were the lowest in this sub-study. That is not surprising in a large river where the current velocity is always high. Furthermore, along the river bank,

water level fluctuations (as mechanical stress due to wave action and sediment relocation) are one of the key factors impacting the development of benthic algae (Bondar-Kunze et al. 2015). The *high profile* taxa with long mucous piles cannot withstand such disturbances (Passy 2007a, b). However, according to Leira et al.'s suggestion (2015) *high profile* forms may have advantages under low irradiance level caused by sediment resuspension. Although, it should be noted that Leira et al. got this result in Alpine lakes. At the same time, in our study, a finer trend could be also noticed in the case of the *high profile guild* along the cross-sections: they presented in higher amount in deeper zones than directly near the shores. The explanation of this is that the structure of the cross-segment between the two shorelines is not alike. This was also supported by different types of substrates. It may result in microhabitats where the water flow slowed from place to place. The deeper levels of water columns can also be considered such habitats near the bottom (Dussart 1992).

Moreover, we should consider the fact that in respect to CV% values were much higher in 2012 (also in case of *low profile guild*) than in that year when samples were taken from the same substrates, and the fact that the cross-segment was homogeneous in view of relevant variables (i.e. nutrients). This allows us to assume that it is not the nutrients inevitably which had the greatest influence on guilds proportions. Some cases can occur when such factors can have importantly roles like types of substrates or light intensity (Hill et al. 2009, Lange et al. 2011). For instance in some samples no diatoms were detected, or only just a few valves. Such microhabitats can be in the deeper zones where there is not enough light for diatoms to be able to propagate. Lange et al. (2011) accounted that the *high profile* taxa reached higher abundances when nutrients and light were not limited. Furthermore, light levels had the strongest effect on community structure. Consequently, diatom communities proved to be much more similar when substrates were uniform. At the same time, the values of the water quality index were always higher than the limit between 'moderate' and 'good'. In guilds proportions there were quite important differences while in the case of IPS values such varieties could not be experienced. IPS values did not indicate those distinctions that the ecological guilds did. So, ecological guilds may be interested in morphological investigations in watercourse, as usually there were not considerable connections between ecological guilds and nutrients during the temporary study.

In 2013 compared to the year 2012 the values of CV% of the *motile guild* were much smaller, and the relative abundance of that guild was also much lower in 2013 when samples were taken only from gravels. Passy (2007a) also stated that relative abundance of the *motile guild* had been considerably higher in the epipelon. She explained that algae had got frequently buried in the unstable epipelic substrates and the selection favoured *motile* species. Besides, bedrock and superficial geology strongly influence species composition of assemblages (Leland and Porter 2000).

The proportions of the *planktic guild* also showed characteristic distributions along the cross-section. The relative abundances of this guild were usually the highest in samples from the deepest zones. It may refer to that the velocity is slower in these habitats. Thus these diatoms may not readily be washed away and therefore they can settle in the biofilm. In 2012 the proportions of this guild were much higher in each sample than in 2013 when water discharge was also much higher. Consequently, in 2013 lesser *planktic* species could settle in the biofilm affected by the stronger water flow.

According to our results, the *motile guild* was proved to be sensitive to the substrate type, while the IPS index less. This is important because along such a large river as Danube River, the substrate type changes along the river (for instance it is stone in the upstream but only macrophyte or shell in the downstream). Consequently, to be able to compare the results of the water quality assessments there is a need for such an index, which is not too sensitive to the substrate type. The IPS has proved to be such a metric. The spatial study has another relevant conclusion. The IPS is not as sensitive to changes in water depth at least not to the extent that it to be able to influence the result of the water quality assessment. Namely, it is not so relevant from what depth the sampled substrate is taken, instead whether the water level can change still metres within a few days. It is the IPS that is robust enough to be able to a suitable index for the ecological water quality assessment in case of large rivers.

Studies on shells

In this study shells from mussels were used because sediment could only be found as substrate in some sections of the Danube River; and because previous investigations pointed out that there were not any remarkable differences in the IPS index values between the substrate type shell and pebble.

In the case of the spatial studies, we experienced that the samples without diatoms or with few numbers of diatom valves (this occurred not only in the case of samples taken from sediments but from gravels as well) could be caused by the different light intensity in different water depths. Furthermore, studying the cross-sections we found that the type of substrate also impacts the composition of the biofilm layer. Hence we were able to provide a system with shells where influences from different light intensity, current velocity and nutrient concentration could be excluded; and where there were differences only in the type of the substrate (differences in size and texture of substrate). Consequently, the substrate type might be responsible for any differences. In results Potapova and Charles (2005) found that the strengths of relationships between composition of algal assemblages and water chemistry parameters (conductivity, pH, total P and total N) had not differed consistently between substrate types.

The fact that there were differences only in the surface and the size of the substrate (ribbed and polished shells and three species with different shell size) suggests that the type of the substrate could cause the heterogeneity of the environment when using guilds. Although we did not get unambiguous connections between diatom taxa and substrate size and surface but slight connections there could be detected (for example in case of the connection of the *low profile guild* with the substrate size, or the connection of the smallest, polished shells with the taxa number). There have not been any publications available on comparisons the periphytic layers on shells with different surfaces. However, there are some studies when diatoms on different kinds of substrates were investigated. For instance, Patrick (1967) investigated the effect of the substrate size on periphyton community structure. She found more species on substrates with larger surface area. Similarly, Luttenton and Baisden (2006) established that the small tiles as substrates had supported fewer taxa than large tiles. Kröpfl et al. (2006) concluded that the substrate had strong influence on the colonisation of different algal species and this way on the biofilm formation. They used andesite, granite (polished and natural), Plexiglass and polycarbonate as substrates. Therefore the surface properties (polarity values, polar and dispersal components of the surface tension) were different.

In our study among the two dominant guilds (*high profile* and *motile guild*) the *high profile guild* reached big ratios (Fig. 4). This agrees what Lange et al. (2011) found, that is the *high profile* taxa could growth better at high light intensity.

The CV% values calculated from the relative abundances of the *low profile* and *planktic guild* referred to an excessively instable environment. Namely, these two guilds responded to the substrate effect in the highest degree. In case of the *high profile* and *motile guild* the CV% values were also high. It still showed the heterogeneity of the system (Table 10) in spite of the homogeneity of that in the view of nutrient content, light intensity and current velocity. So, guilds implied the heterogeneity of the environment while the IPS showed homogeneity as in the case of spatial studies as well.

Summary

Diatom ecological guilds showed connections with investigated variables only in some cases and these correlations were usually low. Furthermore, just the *low profile guild* and the *motile guild* had negative correlations with the IPS. Besides in our spatial study while the water quality did not show any changes, the guilds proportions did. Our results did not support Passy's statements (2007a) on the *high profile guild*. The IPS values did not display those distinctions that the ecological guilds. The IPS was not influenced enormously by any changes of physical variables and disturbances, but it was by the nutrient content. Although there were some guilds having connections with nutrients, there were some

guilds (e.g. *high profile guild*) whose ratios altered firstly due to changes of physical circumstances. Namely, in case of using guilds it is not sure that the natural effects can be differentiated from human impacts. These metrics (diatom ecological guilds) may be interested rather in hydromorphological point of view, but could not be used instead of the diatom index (IPS) in the ecological status assessment of large rivers, because the guilds are able to reflect the effect of substrate and the differences between flow regimes rather than nutrient loads. The IPS is robust enough, neither too sensitive to the substrate type, nor the light and nor the current conditions. For this latest feature, it can be use in the ecological status assessment of standing waters, as well, as it is used in Finland and Sweden already (Kelly et al. 2014).

Acknowledgment

References

Ács, É., Borsodi, A. K., Kiss, É., Kiss, K. T., Szabó K. É., Vladár, P., Várbíró, G. & Záray, Gy., 2008: Comparative algological and bacteriological examinations on biofilms developed on different substrata in a shallow soda lake. – Aquat. Ecol. **42**: 521–531.

Ács, É., Szabó, K., Kiss, K. T. & Hindák, F., 2003: Benthic algal investigations in the Danube River and some of its main tributaries from Germany to Hungary. – Biologia, Bratislava. **58/4:** 545-554.

Ács, É., Szabó, K., Kiss, K. Á., Tóth, B., Záray Gy. & Kiss, K. T., 2006: Investigation of epilithic algae on the Danube River from Germany to Hungary and the effect of a very dry year on the algae of the Danube River. – Arch. Hydrobiol. Supplement-Band: Large Rivers, **16/3:** 389-417. ISSN: 0945-3784.

Bahls, L. L., Burkantis, R. & Tralles, S., 1992: Benchmark biology of Montana reference streams: Helena, Montana, Dept. Health. Environ. Sci., Water Quality Bureau, 47. p.

B-Béres, V., Török, P., Kókai, Z., Krasznai, T. E., Tóthmérész, B. & Bácsi, I., 2014: Ecological diatom guilds are useful but not sensitive enough as indicatros of extremely changing water regimes. – Hydrobiologia **738**: 191-204.

Berthon, V., Bouchez, A. & Rimet, F., 2011: Using life-forms and ecological guilds to assess organic pollution and trophic level in rivers: a case study of rivers in south-eastern France. – Hydrobiologia **673**: 259-271.

Biggs, B. J. F., Goring, D. G. & Nikora, V. I., 1998: Subsidy and stress responses of stream periphyton to gradients in water velocity as a function of community growth form. – J. Phycol. **34**: 598-607.

Bondar-Kunze, E. & Tritthart, H. T., 2015: The influence of short term level fluctuations and desiccation stress on periphyton development at a riparian zone of a large regulated river. – Fund. Appl. Limnol. **186/4**: 283-296.

CEN (2014) Water quality – guidance for the routine sampling and preparation of benthic diatoms from rivers and lakes. EN 13946. Comité Européen de Normalisation, Bruxelles Geneva. 14 pp.

Coste, M. & Ayphassorho, H., 1991: Étude de la qualité des eaux du Bassin Artois-Picardie à l'aide des communautés de diatomées benthiques (Application des indices diatomiques). Raport CEMAGREF. Bordeaux – Agence de l'Eau Artois-Picardie, Douai, pp. 277.

Consalvey, M., Paterson, D. M. & Underwood, G. J. C., 2004: The ups and downs of life in a benthic biofilm: migration of benthic diatoms. – Diatom Res. **19**: 181-202.

Dussart, B., 1992 : L'étude des eaux continentales. – Limnologie. BOUBÉE, Paris. pp. 681.

Eaton, A. D., Clesceri, L. S., Rice, E. W. and Greenberg, A. E., 2005 : Standard Methods for the Examination of Water and Wastewater (21st ed.) – APHA, AWW, WEF, Washington, CC, USA

Fairchild, G. W., Lowe, R. L. & Richardson, W. B., 1985: Algal periphyton growth on nutrient-diffusing substrates: an in situ bioassay. – Ecology **66**: 465-472.

Fore, L. S. & Grafe, C., 2002: Using diatoms to assess the biological condition of large rivers in Idaho (USA). – Freshwater Biol. **47**: 2015-2037.

Francoeur, S. N. & Biggs, B. J. F., 2006: Short-term effects of elevated velocity and sediment abrasion on benthic algal communities. – Hydrobiology **561**: 59-69.

Ghosh, M. & Gaur, J. P., 1998: Current velocity and the establishment of stream algal periphyton communities. – Aquatic Aquat. Bot. **60**: 1-10.

Hill, W. R., Fanta, S. E. & Roberts, B. J., 2009: Quantifying phosphorus and light effects in stream algae. – Limnol. Oceanogr. 54: 368-380.

Hlúbiková, D., Makovinská, J., Fidlerová, D., 2015: Phytobenthos. – in: Joint Danube Survey 3. A Comprehensive Analysis of Danube Water Quality. ISBN: 978-3-200-03795-3.

Horner, R. R., Welch, E. B., Seeley, R. M. & Jacoby J. M., 1990: Responses of periphyton to changes in current velocity, suspended sediment and phosphorus concentration. – Freshwater Biol. **24**: 2115-232.

ISO 8467 (1993) Water Quality – Determination of Permanganate Index

Johnson, R. E., Tuchman, N. C. & Peterson, C. G., 1997: Changes in the vertical microdistribution of diatoms within a developing periphyton mat. – J. N. Am. Benthol. Soc. **16**: 503-519.

Kelly, M., Urbanic, G., Ács, É., Bennion, H., Bertrin, V., Burgess, A., Denys, L., Gottschalk, S., Kahlert, M., Karjalainen, S. M., Kennedy, B., Kosi, G., Marchetto, A., Morin, S., Picinska-Fałtynowicz, J., Poikane, S., Rosebery, J., Schoenfelder, I., Schoenfelder, J. & Varbiro, G., 2014: Comparing aspirations: intercalibration of ecological status concepts across European lakes for littoral diatoms. – Hydrobiologia **734**: 125-141.

Kröpfl, K., Vladár, P., Szabó, K., Ács, É., Borsodi, A. K., Szikora, Sz., Caroli, S. & Záray, Gy., 2006: Chemical and biological characterisation of biofilms formed on different substrata in Tisza River (Hungary). – Environ. Pollut. **144**: 626-631.

Lange, K., Liess, A., Piggott, J. J., Townsend, C. R. & Matthaei, C. D., 2011: Light, nutrients and grazing interact to determine stream diatom community composition and functional group structure. – Freshwater Biol. **56**: 264-278.

Lange, K., Townsend, C.R. & Matthaei, C.D., 2015: A trait-based framework for stream algal communities. – Ecology and Evolution **6(1)**: 23–36.

Law, R. J., Elliott, A. & Thackeray, S. J., 2014: Do functional or morphological classifications explain stream phytobenthic community assemblages? – Diatom Res. **29**: 309-324.

Lecointe, C., Coste, M. & Prygiel, J., 2008: OMNIDIA version 5.2 software for diatom-based water quality assessment. CD-ROM

Leira, M., Filippi, M. L. & Cantonati, M., 2015: Diatom community response to extreme water-level fluctuations in two Alpine lakes: a core case study. – J. Paleolimnol. **53**: 289-307.

Leland, H. W. & Porter, S. D., 2000: Distribution of benthic algae in the upper Illinois River basin in relation to geology and land use. – Freshwater Biol. **44**: 279-301.

Liška, I., Wagner, F., Sengl, M., Deutsch, K. & Slobodník, J., 2015: Joint Danube Survey 3 – A comparative analysis of Danube water quality. – ICPDR 2015. ISBN: 978-3-200-03795-3.

Luttenton, M. R. & Baisden, C., 2006: The relationship among disturbance, substratum size and periphyton community structure. – Hydrobiologia **261**: 111-117.

Marcel, R., Bouchez, A. & Rimet, F., 2013: Influence of herbicide contamination on diversity and ecological guilds of river diatoms. – Cryptogamie, Algol. **34/2**: 169-183.

Nagelkerke, N., 1991: A note on a general definition of the coefficient of determination. – Biometrika **78**: 691–692.

Passy, S. I., 2007a: Diatom ecological guilds display distinct and predictable behavior along nutrient and disturbance gradients in running waters. – Aquat. Bot. **86**: 171-178.

Passy, S. I., 2007b: Community analysis in stream biomonitoring: what we measure and what we don't. – Environ. Monit. Assess. **127**: 409-417.

Patrick, R., 1967: The effect of invasion rate, species pool, and size of area on the structure of the diatom community. – Natl. Acad. Sci., Proceedings **58/4**:1335-1342.

Potapova, M. & Charles, D. F., 2005: Choice of substrate in algae-based water-quality assessment. – J. N. Am. Benthol. Soc. **24/2**: 415-427.

Pringle, C. M., 1990: Nutrient spatial heterogeneity: effects on community structure, physiognomy, and diversity of stream algae. – Ecology **71**: 905-920.

Rimet, F. & Bouchez, A., 2011: Use of life-forms and ecological guilds to assess pesticide contamination in rivers: Lotic mesocosm approaches. – Ecol. Indic. **11**: 489-499.

Rimet, F. & Bouchez, A., 2012: Life-forms, cell-sizes and ecological guilds of diatoms in European rivers. – Knowl. Ma. Aquat. Ecosyst. **406**: 01.

Robinson, C. T. & Rushforth, S. R., 1987: Effects of physical disturbance and canopy cover on attached diatom community structure in an Idaho Stream. – Hydrobiologia **154**: 49-59.

Smucker, N. J. & Morgan, L. V., 2010: Using diatoms to assess human impacts on streams benefits from multiple-habitat sampling. – Hydrobiologia **654/1**: 93-109.

Stenger-Kovács, C., Lengyel, E., Crossetti, L. O., Üveges, V. & Padisák, J., 2012: Diatom ecological guilds as indicators of temporally changing stressors and disturbances in the small Torna-stream, Hungary. – Ecol. Indic. **24**: 138-147.

Szekeres, J., Molnár, M., Csányi, B. & Szalóky, Z., 2009: A Duna rajkai és szobi keresztszelvényének makrozoobenton vizsgálata mélységi kotort minták alapján. – Acta Biol. Debr. Oecol. Hung. **20**: 209-218.

Ter Braak, C. J. F. & Šmilauer, P., 2012: Canoco reference manual and user's guide: software for ordination, version 5.0. Microcomputer Power, Ithaca, USA 496 pp.

Vijayasarathy, P.R. 2011: Engineering Chemistry (2nd ed.). PHI Learning Private Ltd., New Delhi. 310 p

Van der Grinten, E., Janssen, M., Simis, S. G. H., Barranguet, C. & Admiraal, W., 2004: Phophate regime structures species composition in cultured phototrophic biofilms. – Freshwater Biol. **49**: 369-381.

www.hydroinfo.hu

Zelinka, M. & Marvan, P ., 1961: Zur Präzisierung der biologischen Klassification der Reinheit fließender Gewässer. – Arch. Hydrobiol. **57**: 289-407.

Figure captures

Figure 1. a. RDA between diatom ecological guilds and environmental variables. HPG: *high profile guild*, LPG: *low profile guild*, MG: *motile guild*, PG: *planktic guild*. T: temperature (°C), Turb: turbidity (FNU), SM: suspended matter content (mg/l), Cond: conductivity (μS/cm), COD: chemical oxygen demand (mg/l), DO: dissolved oxygen (mg/l), TOM: total organic matter (mg/l), TDM: total dissolved matter (mg/l), NO_3-N: nitrate-nitrogen (mg/l), PO_4: phosphate-phosphorus (mg/m^3). Total number of samples: 32.

Figure 1. b. RDA between diatom samples (temporal study) and environmental variables. T: temperature (°C), Turb: turbidity (FNU), SM: suspended matter content (mg/l), Cond: conductivity (μS/cm), COD: chemical oxygen demand (mg/l), DO: dissolved oxygen (mg/l), TOM: total organic matter (mg/l), TDM: total dissolved matter (mg/l), NO_3-N: nitrate-nitrogen (mg/l), PO_4: phosphate-phosphorus (mg/ m^3). Total number of samples: 32. Different symbols mark the seasons: '●': winter, '■': spring, '♦': summer, '▲': autumn.

Fig. 2. Distribution of response curves of MG: *motile* guild, PG: *planktic* guild along the 1st RDA axis (by the relative abundance of the guilds).

Fig. 3. Relative abundance of guilds (%) (axis Y_1) and IPS values (axis Y_2) across the cross-segment. Axis X shows the water depths from right site to left site, in 2012, 213 and 2014. In the name of the samples the first number refers to the year (12: 2012, 13: 2013, 14: 2014), the letter refers to the bank

of the river (R: right, L: left), the second number refers to the approximate depth (1: 1 m, 3: 3 m, 5: 5 m).

Fig. 4. Relative abundance of guilds (%) (axis Y_1) and the IPS (Y_2). The axis X shows from which surfaces and sizes of shells samples were taken. Abbreviations: 'P, S_1': polished and small shells (after 1 week), 'U, M': unpolished and medium shells, 'P, M': polished and medium shells; 'P, B': polished and big shells; 'P, S_2': shells with small sizes (after 4 weeks).