



Ecological background of diatom functional groups: Comparability of classification systems



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ABSTRACT

Functional classification based on species traits brought a revolution in community ecology, and also boosted phytoplankton and in phytobenton (diatom) research. Several studies stressed the usefulness of phytoplankton functional groups in ecological status assessment, and there is also a strong emphasis to use combined traits in ecological assessments of diatom assemblages. The Combined Eco-Morphological Functional Groups (CEMFGs) help to reveal species-environmental correlations, which can be hidden, controlling traits separately. Nowadays, there are three types of functional guild classifications simultaneously used in the literature: (i) the original classification proposed by Passy (O); (ii) Passy's classification complemented with a separated planktic guild (P); (iii) the refined guild classification by Rimet and Bouchez, also containing the planktic guild (RB). One of the most important criteria of the combined functional groups is the well-defined ecological frame of these combined groups; thus it is vital to harmonise the classification of the taxa into guild based combined eco-morphological functional groups (CEMFGs). In this study we tested the similarities and dissimilarities of the correlations between environmental factors and CEMFGs created in the (i)-(iii) classifications. Samples were collected in 138 sampling sites on lowland rivers and streams in the Hungarian Lowland Region. Strong correlations were anticipated between the functional groups and environmental factors due to the presence of common and/or abundant planktic taxa. Our results validated the necessity of a separated planktic guild in ecological assessments of diatom assemblages. Further relevant differences between the correlations of CEMFGs-P or CEMFGs-RB and abiotic factors were hypothesised in the case of those functional groups which contained reassigned taxa with high frequency and/or abundance. Our results confirmed this hypothesis. Furthermore, the present study also highlighted the relevance of well-defined trait classification. Abundant and/or frequent taxa, which are able to change their life forms, can modify significantly the relationship between the functional group containing them and environmental factors. In the future, both field and laboratory studies should focus on revealing the circumstances, which cause the mentioned changes in traits of diatoms.

1. Introduction

The definition of functional groups is based on the similarities in ecosystem functioning (Tapolczai et al., 2016), i.e. species classified into a functional group have similar morpho-physiological and/or ecological features. Functional classifications become widely used in ecology and habitat quality assessment. Comparisons based on functional classifications (i) helps in the joint analyses and evaluation of similar habitats with distinct species composition, and (ii) provide an

improved classification of habitats with a huge number of taxa and problematic species groups compared to the taxonomic approach (Salmaso et al., 2015). Moreover, the functional classification based approach is useful (iii) in water quality assessment (B-Béres et al., 2016) and (iv) in the detection of overall changes in ecosystem functions (Török et al., 2016).

The most widely known functional classification of diatoms is based on diatom guilds (Passy, 2007). Taxa are assigned to a guild based on similar resource use capability and disturbance tolerance. There are

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three diatom guilds formed: the low profile, high profile and the motile guild (Passy, 2007). Taxa in the low profile guild are resistant to high water flow conditions and capable of colonising rapidly on bare surfaces and substrates. In contrast, high profile taxa are sensitive to disturbances (Stenger-Kovács et al., 2013). The ability of motile guild taxa to adapt to active movements allows them to select the most appropriate microhabitat (Lengyel et al., 2015). Until now, the correlations of ecological guilds and environmental factors and the usefulness of the functional approach in ecological status assessment were only partially discussed (Berthon et al., 2011; Stenger-Kovács et al., 2013), but troubles with this approach were also emphasized (B-Béres et al., 2014, 2016; Rimet and Bouchez, 2012; Tapolczai et al., 2016). Rimet and Bouchez (2012) suggested a re-classification of taxa by the creation of a fourth guild containing planktic diatoms, and also changed the guild-assignment of several taxa. Studies proved that the joint use of the planktic guild, together with Passy's original groups, can help to explain the dynamics of this guild in the case of a medium sized lowland river or a lowland stream (B-Béres et al., 2014, 2016). But it has to be emphasized that the guilds themselves do not seem to be robust enough (i) to display and explain highly stochastic processes, like colonisation and re-colonisation after disturbance (B-Béres et al., 2016), or (ii) to explain changes of taxa composition in harsh and unpredictably changing physical and/or chemical environments (B-Béres et al., 2014; Tapolczai et al., 2016).

Not only ecological guilds but other easy-to-measure traits (e.g. cell size or biovolume) can support the understanding of the relationship between environmental factors and diatom assemblages. There are strong correlations between these single traits and (i) nutrient uptake and efficiency (Tapolczai et al., 2016), (ii) trophic levels or organic pollution (Berthon et al., 2011; Kókai et al., 2015; Lange et al., 2016), (iii) physical disturbances (Tapolczai et al., 2016), or (iv) salinity and conductivity (Kókai et al., 2015). However, similarly to guilds, single traits, like biovolume classes, are not robust enough by themselves to display stochastic processes appropriately (B-Béres et al., 2016).

Combining of various traits to explain changes in ecological processes and to characterise a habitat type was proven a vital idea in phytoplankton research (Reynolds et al., 2002; Salmaso and Padisák, 2007; Padisák et al., 2009), and several studies stressed for example the usefulness of phytoplankton functional groups in ecological status assessment (Krasznai et al., 2010); or in the detection of assembly changes following anthropogenic pollution (Bácsi et al., 2016). The trait-based analyses for benthic algal and cyanobacterial assemblages started very recently (simultaneous interpretation of different traits – Lange et al., 2016 and combined eco-morphological functional groups of diatoms – B-Béres et al., 2016). One of the most promising approaches is the combination of guilds with cell sizes by creating combined eco-morphological groups (B-Béres et al., 2016). Using combined eco-morphological groups was reported to be a powerful and robust method displaying quantitative and qualitative changes in diatom assemblages (Tapolczai et al., 2016). However, the crucial points of the utility of the combined groups are that (i) subgroups have to be clearly defined and their relationship with the habitat environmental factors needs to be validated, and (ii) the functional role of subgroups in a respective diatom assemblage has to be clearly specified (Tapolczai et al., 2016).

Nowadays, there are three types of guild classification simultaneously used in the literature: (i) the original classification proposed by Passy (2007); (ii) Passy's classification complemented with a separated planktic guild (e.g. B-Béres et al., 2014); and (iii) the refined classification by Rimet and Bouchez (2012), also containing the planktic guild. To reach a generally useful practical application of diatom classifications, as with phytoplankton functional group classification, it is important to compare the existing functional classifications and to also use a unified system based on clear criteria and well-defined functional groups. Therefore, it is vital to harmonise the classification of the taxa into guild based combined eco-morphological functional groups (CEMFGs). In our study we compared the three classification systems

combined with cell sizes within the eco-morphological functional group classification (CEMFGs) to test and validate their usability under the same abiotic and biotic circumstances. Our final aim was to choose the ecologically most relevant classification system of these three types and to recommend a unified classification method for global use. We hypothesised the following: (i) Planktic taxa hypothesis: There is no statistical validation of a planktic guild, although Rimet and Bouchez (2012) separated planktic taxa into an independent guild, and many studies used an independent planktic guild. We supposed that the presence of common and/or abundant planktic taxa strongly affect the relationship of the functional groups and environmental factors. Thus, the planktic taxa should be treated as a separated planktic guild in the functional-classification-based analyses. (ii) CEMFGs hypothesis: We hypothesised that the widely accepted refinement by Rimet and Bouchez (2012) on guilds affected the correlation between CEMFGs and abiotic factors only for those particle groups which contained reassigned taxa with high frequency and/or abundance.

2. Materials and methods

2.1. Sampling and measuring of environmental factors

Altogether 865 diatom samples were collected in 138 sampling sites on small, medium and large sized lowland rivers and streams in the Hungarian Lowland Region (Fig. 1) between 2007 and 2015 from the beginning of April to the end of October, usually at least twice a year (spring and autumn). This is one of the largest datasets covering benthic diatom assemblages ever collected and analysed for Central-Europe. There were 11 environmental factors measured in all sampling points (Appendix A). Conductivity (COND – $\mu\text{S cm}^{-1}$), pH, dissolved oxygen concentration (DO – mg L^{-1}), and water temperature (T – $^{\circ}\text{C}$) were measured with a portable-multiparameter digital meter (Multi 350i-WTW, Germany) in the field. The water samples were kept at 4 $^{\circ}\text{C}$ in a cooler bag during transportation to the laboratory for further spectrophotometric analysis of $\text{NO}_3^{-}\text{-N}$, $\text{NO}_2^{-}\text{-N}$, $\text{NH}_4^{+}\text{-N}$, Cl^{-} (mg L^{-1} ; ISO 15923-1; 2013) and $\text{PO}_4^{3-}\text{-P}$ ($\mu\text{g L}^{-1}$; ISO 15923-1; 2013), respirometric analysis (Biological Oxygen Demand – BOD in mg L^{-1} ; MSZEN -1; 2000; MSZEN 1899-1; 2000; MSZISO 6060; 1991) and gravimetric analysis (Total Soluble Solids – TSS in mg L^{-1} ; MSZ 260-3; 1973).

2.2. Sample collection and preparation

The European guideline (EN 13946) was used during the sampling and preservation. Diatom valves were prepared by the hot hydrogen-peroxide method (EN 13946). Naphrax synthetic resin was used for embedding. The Leica DMRB microscope with 1000–1600-fold magnification was used for identification of diatom taxa. At least 400 diatom valves were counted (EN 14407). We used Krammer and Lange-Bertalot (1997a, 1997b, 2004a, 2004b), Potapova and Hamilton (2007) and Bey and Ector (2013) for diatom identification.

2.3. Data processing and analyses

Diatom taxa were classified to combined eco-morphological groups based on B-Béres et al. (2016) using biovolume classes and diatom guilds.

(i) For the biovolume class assignment we used Berthon et al. (2011) where diatom taxa were classified into five biovolume classes S1–S5 (S1: 5–99 μm^3 , S2: 100–299 μm^3 , S3: 300–599 μm^3 , S4: 600–1499 μm^3 , S5: $\geq 1500 \mu\text{m}^3$).

(ii) For the guild classification we used the following three well-known classification schemes (See also details in Introduction and Appendix B): – a) We classified diatom taxa into the original low profile (L), high profile (H) and motile (M) guilds according to Passy (2007). Combining this guild classification (Passy, 2007) with the biovolume classes (Berthon et al., 2011) we created 15 combined eco-

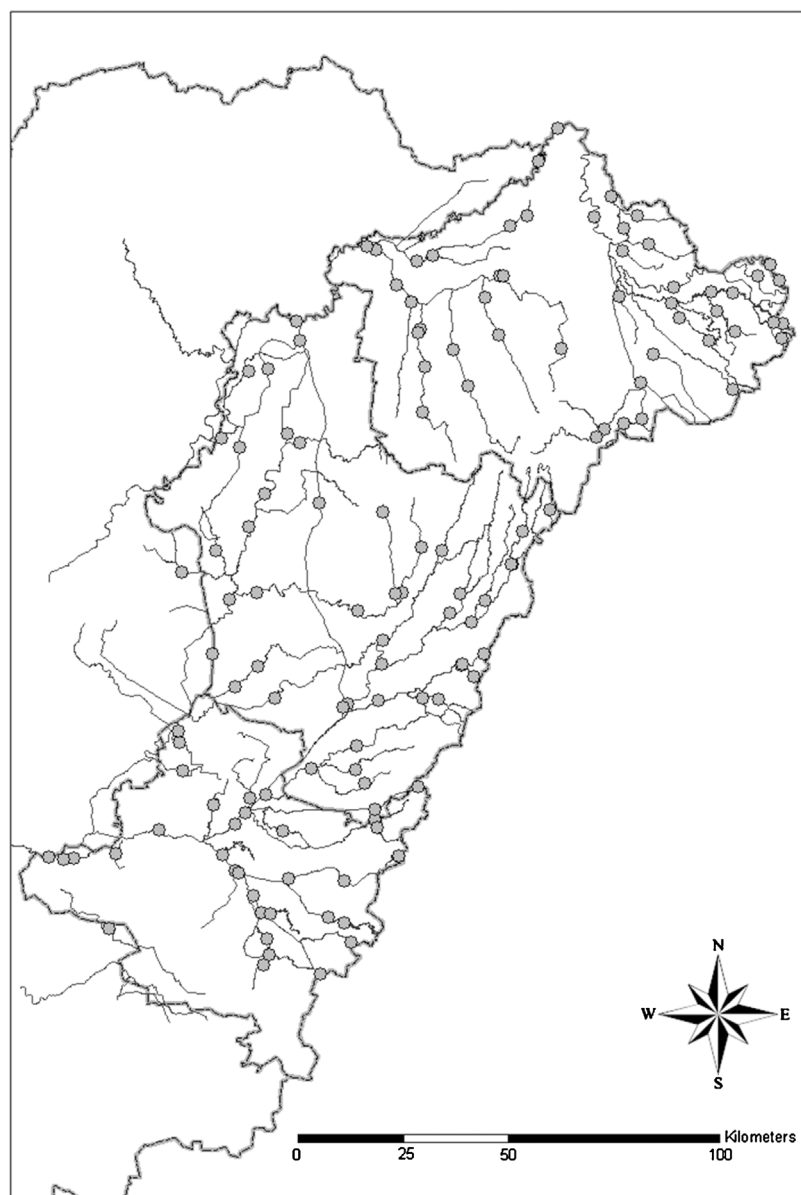


Fig. 1. The study area on *trans-Tisza* region of Hungary: (a) localization of the study area (marked with grey borders); (b) sampling sites on the rivers and channels marked with dots, grey lines: borders of the study area, black lines: the rivers and channels.

morphological functional groups (CEMFGs-O; LS1-LS5, HS1-HS5, MS1-MS5-Appendix B, C). – b) According to our knowledge, a separated planktic guild firstly appeared in the work of Rimet and Bouchez (2012). Planktic taxa were taken out from Passy's original guilds and were put together into a separated guild. The second type of classification occurred when Passy's original guild classification was completed by this planktic guild (B-Béres et al., 2014). Combining this guild classification (B-Béres et al., 2014) with the biovolumes (Berthon et al., 2011) we created 20 combined eco-morphological functional groups (CEMFGs-P; LS1-LS5, HS1-HS5, MS1-MS5, PS1-PS5-Appendix B, D).- c) Rimet and Bouchez (2012) not only created the planktic guild, but they revised Passy's original classification. Some taxa were reclassified from one guild to another based on the authors' experiences. This is the third type of classification of diatoms into guilds. The combination of this guild classification (Rimet and Bouchez, 2012) with the five biovolume classes (Berthon et al., 2011), resulted in 20 combined eco-morphological functional groups (CEMFGs-RB; LS1-LS5, HS1-HS5, MS1-MS5, PS1-PS5-Appendix B, E).

To analyse the relationship between CEMFGs-O, –P and –RB and eleven environmental factors (COND, pH, DO, T, NO_3^- -N, NO_2^- -N, NH_4^+ -N, PO_4^{3-} -P, Cl^- , BOD and TSS) Canonical Correspondence

Analyses (CCA) was performed, where environmental factors were added by weighted averages (ter Braak and Smilauer, 2002).

3. Results

In the analysed 865 diatom samples, altogether 495 diatom taxa were identified. Among them, 481 taxa were identified at least at the species level, while only 14 taxa were identified at the genus level. With the exception of the barely identifiable small centric diatoms in large or medium sized rivers, the relative abundance of taxa identified at genus level did not exceed 2.9%.

3.1. The relationship of environmental factors and CEMFGs-O

The Canonical Correspondence Analysis (CCA) accomplished with the 11 environmental factors and the 15 CEMFGs-O explained 83.3% of the variance of group-environment relation in four axes. The Monte-Carlo permutation test indicated the pattern displayed by the CCA differed significantly from a random pattern ($N = 499$; $p = 0.002$ for the first and $p = 0.002$ for all canonical axes). The CCA revealed that the factors with the highest correlation were conductivity (0.3375), Cl^-

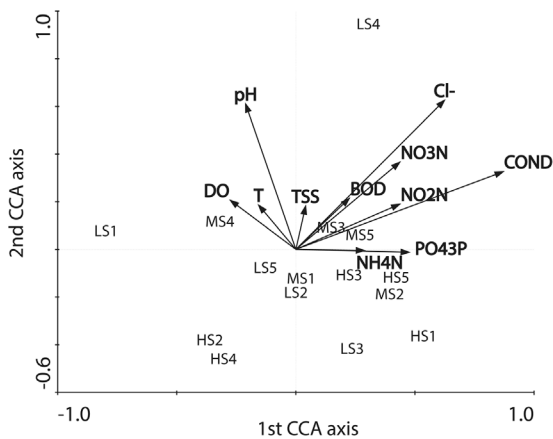


Fig. 2. Relation of the Combined Eco-Morphological Functional Groups based on the original classification proposed by Passy (CEMFGs-O) and the environmental variables displayed by CCA based on groups' abundances. Combining the original guild classification with biovolume classes 15 combined eco-morphological functional groups were created (LS1-LS5, HS1-HS5, MS1-MS5). Cumulative percentage variance of the species-environment relation was 34.3 and 57.9 for the first and second axis, respectively.

(0.2420), $\text{PO}_4^{3-}\text{-P}$ (0.1845), $\text{NO}_2^{-}\text{-N}$ (0.1690) and $\text{NH}_4^{+}\text{-N}$ (0.1115) with the first axis; pH (0.2217) with the second axis; dissolved oxygen (DO; 0.1285) and $\text{NO}_3^{-}\text{-N}$ (-0.1772) with the third axis and water temperature (T; 0.1059) with the fourth axis.

Most CEMFGs-O were negatively correlated with the environmental factors (Fig. 2). In contrast, there were positive correlations: the medium sized and the largest sized motile groups (MS3 and MS5) and the LS4 low profile group showed positive correlation with nutrient content and salinity/conductivity factors (Fig. 2). Furthermore, MS4 and LS1 groups correlated positively with pH, water temperature (T), and dissolved oxygen (DO).

3.2. The relationship of environmental factors and CEMFGs-P

There were significant correlations between the 11 environmental factors and the 20 CEMFGs-P based on the results of CCA analysis. The group and environmental correlation was 85.5% in the four axes. The Monte-Carlo permutation test indicated a significant difference from a random pattern for the pattern displayed by the CCA ($N = 499$; $p = 0.002$ for the first and $p = 0.002$ for all canonical axes). The relevant factors were Cl^{-} (0.3946), conductivity (0.3621), $\text{NO}_3^{-}\text{-N}$ (0.2204), $\text{NO}_2^{-}\text{-N}$ (0.1899), BOD (0.1667) and $\text{PO}_4^{3-}\text{-P}$ (0.1663) to axis one; pH (0.1967) to axis two; dissolved oxygen (DO; 0.1313) to axis three and temperature (T; 0.1317) to axis four.

With the exception of PS4, planktic groups correlated positively with pH, T, and DO (Fig. 3). Small and large sized low profile groups (LS1, LS4 and LS5) correlated negatively with conductivity and nutrient content (Fig. 3). Medium and the largest sized high profile groups (HS3 and HS5) correlated positively with these environmental factors. Other high profile groups showed positive (HS1) or contrariwise, negative (HS3 and HS4) correlation to water temperature (T) and dissolved oxygen (DO). With the exception of MS4, the motile groups correlated positively to the nutrient content (Fig. 3).

3.3. The relationship of environmental factors and CEMFGs-RB

Significant correlation was indicated by CCA between the 11 environmental factors and the 20 CEMFGs-RB. The group and environmental correlation was 86% in the four axes. The Monte-Carlo permutation test indicated a significant difference from a random pattern for the pattern displayed by the CCA ($N = 499$; $p = 0.002$ for the first and $p = 0.002$ for all canonical axes). The factors with the highest correlation were Cl^{-} (0.3946), conductivity (0.3780), $\text{NO}_3^{-}\text{-N}$

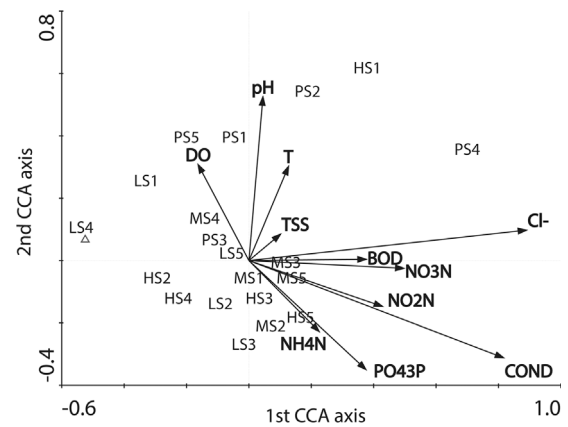


Fig. 3. Relation of the Combined Eco-Morphological Functional Groups based on the original classification proposed by Passy complemented with a separated planktic guild (CEMFGs-P) and the environmental variables displayed by CCA based on groups' abundances. Combining this guild classification with biovolume classes 20 combined eco-morphological functional groups were created (LS1-LS5, HS1-HS5, MS1-MS5, PS1-PS5). Cumulative percentage variance of the species-environment relation was 38.0 and 62.2 for the first and second axis, respectively.

(0.2247), $\text{NO}_2^{-}\text{-N}$ (0.1896), $\text{PO}_4^{3-}\text{-P}$ (0.1708), BOD (0.1611) and $\text{NH}_4^{+}\text{-N}$ (0.1034) with the first axis. The most important factor to axis two was pH (0.1776), while dissolved oxygen (DO; 0.1703) and temperature (T; 0.1182) showed the highest correlation to axis three and axis four, respectively.

With the exception of LS4 and HS4, the distribution of the groups along environmental factors was similar to those CEMFGs-RB which were classified by the original guilds completed by planktic guild (Fig. 4). The differences were that LS4 showed the strongest negative correlation with water temperature (T) and pH, HS4 showed a weaker negative correlation with these factors (T and pH) and a stronger negative correlation with nutrient content and conductivity (Fig. 4).

4. Discussion

4.1. Relevance of a separated planktic guild

In the original guild classification by Passy (2007) planktic taxa were classified mostly in low or high profile guilds. But planktic taxa

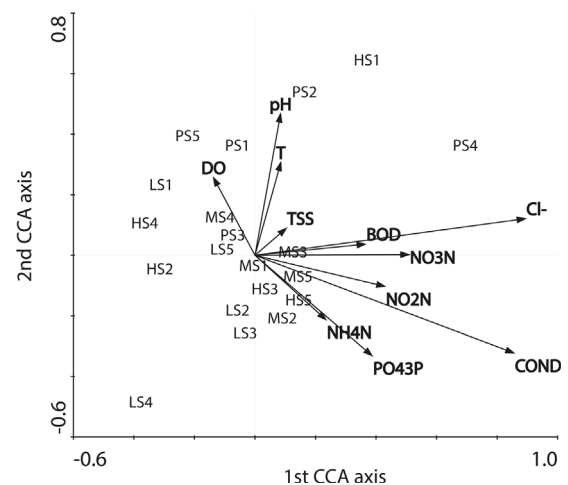


Fig. 4. Relation of the Combined Eco-Morphological Functional Groups based on the refined guild classification by Rimet and Bouchez, also containing the planktic guild (CEMFGs-RB) and the environmental variables displayed by CCA based on groups' abundances. Combining this guild classification with biovolume classes 20 combined eco-morphological functional groups were created (LS1-LS5, HS1-HS5, MS1-MS5, PS1-PS5). Cumulative percentage variance of the species-environment relation was 38.7 and 64.1 for the first and second axis, respectively.

are not steady members of benthic assemblages. According to Rimet and Bouchez (2012) these taxa are morphologically adapted not to benthic but to lentic environments that make them able to resist sedimentation. In the benthic assemblages they can appear even in relatively high proportion (i) after floods (B-Béres et al., 2014); (ii) in sections of watercourses close to reservoirs (Szabó et al., 2004); (iii) in the late successional stages of matured biofilm (Stevenson et al., 1996); and (iv) they can settle down due to reduced flow rate. Thus, the presence of planktic guild taxa in benthic assemblages is not affected by the same abiotic and biotic factors as the members of the other guilds.

Rimet and Bouchez (2012) argued that formation of a separated planktic guild was necessary because planktic taxa are usually not adapted to those circumstances (disturbances, nutrients, etc.), which basically determine the presence of the other three guilds in benthic assemblages. However, the differences between the original classification (without planktic guild) and the classifications with a separated planktic guild were not statistically analysed and validated before. Our results supported the hypothesis that the planktic taxa should be treated as a separated planktic guild in the functional-classification-based analyses. Most of the planktic groups showed positive correlation with temperature and/or nutrient content (Figs. 3 and 4). Classifying highly abundant planktic taxa into low or high profile groups may hide the real ecological character of these benthic groups. In our study, the total number of planktic taxa was relatively high (~8% of total taxa number). The most common and relatively abundant planktic species was only the S4 sized *Cyclotella meneghiniana* Kützing. In the case of the other planktic taxa, only local accumulation was observed. Thus, only the removal of *C. meneghiniana* from LS4 to PS4 had significant effect on the correlation of CEMFGs and environmental factors. The halophilic character of this taxon (van Dam et al., 1994) explained the positive correlation of LS4 and chloride ion in the case of CEMFGs-O classification (Fig. 2). In contrast, the removal of the taxon from LS4 resulted in negative correlation of this low profile group with chloride ion in the case of CEMFGs-P and CEMFGs-RB (Figs. 3 and 4). The removal of small and medium sized (PS1 – PS3) or large (PS5) centric diatoms from low or high profile guilds to planktic ones did not change significantly the position of CEMFGs in the matrix. This phenomenon can be explained by the low abundance of these taxa. Namely, it is recommended to use separated planktic functional groups, especially when planktic taxa are abundant in the samples.

4.2. Similarities and discrepancies between the guild classification systems

We hypothesised that the correlation between groups and abiotic factors change only in the case of those groups, which contain reassigned taxa with high frequency and/or abundance. Our findings supported this hypothesis. The most pronounced differences between CEMFGs-P and CEMFGs-RB were the positions of LS4 and HS4 in the matrix (Figs. 3 and 4). It was basically due to the removal of tube-forming taxa (e.g. *Encyonema mesianum* (Cholnoky) D.G. Mann, *Encyonema silesiacum* (Bleisch in Rabh.) D.G. Mann) from low profile to high profile guild (Rimet and Bouchez, 2012). The group containing them showed negative correlation with inorganic nutrients (LS4-Fig. 3, or HS4-Fig. 4). These tube forming taxa are often abundant and common in the studied watercourses and they are able to produce extracellular enzymes similarly to motile taxa (Tapolczai et al., 2016). This ability offers advantage in inorganic nutrient poor environments. Namely, this ability allows the mobilization of other nitrogen and phosphorus-forms due to the lack of inorganic ones for these taxa. But this phenomenon should be proved by measurements of organic nitrogen and phosphorus forms, so to ascertain the real importance of extracellular enzymes in diatom assemblages needs further field and laboratory investigations.

Usually, abundance of HS4 group containing *Diatoma*, *Eunotia* and *Fragilaria* taxa was high in early spring or in autumn. Autumn is the slow flowing period of the studied watercourses, so it ensures “low

disturbance-circumstances” for colonial taxa (mentioned above). Furthermore, Rimet et al. (2016) also presented the dominance of these high profile taxa in early spring. But they emphasized the effects of low nutrient availability as a key factor. Namely, these colonial and/or filamentous taxa are able to extend the biofilm thickness contributing to the nutrient access into the mat. Completion of HS4 group with tube-forming taxa in CEMFGs-RB increased the negative correlation with nutrient and conductivity in our study. In addition, LS4 group without tube-forming taxa in CEMFGs-RB showed strong negative correlation with temperature (Fig. 4). This was due to one species, *Meridion circulare* (Greville) C.A. Agardh, which prefers low water temperature (Stenger-Kovács et al., 2013). *M. circulare* was present in 16% of the samples with high abundance (maximum relative abundance was 41%). This species is usually common in winter or in spring in the watercourses, it tolerates high disturbance well (Stenger-Kovács et al., 2013). Based on the detailed comments above, reclassification of tube-forming taxa from LS4 to HS4 seems to be ecologically justified.

Similarly to the tube-forming HS4 taxa, the motile group with the same size (MS4) also showed negative correlation with nutrient content (Figs. 3 and 4). One reason could be the ability of tube-forming HS4 and MS4 taxa mobilizing organic nutrient forms, but it needs further confirmations (see above). Furthermore, it should also be taken into account that certain tube-forming taxa (e.g. *E. silesiacum*) are able to change their life form character and they can be motile also (Rimet and Bouchez, 2012; Tapolczai et al., 2016). So it is possible, that the strong negative correlation of S4 groups containing tube forming taxa with inorganic nutrients may be due to changing their life form to motile. Therefore, the strong negative correlation of S4 groups with inorganic nutrient content needs further investigations.

In the case of S1-S3 and S5 groups, there were no significant differences in classification between CEMFGs-P and CEMFGs-RB. Due to this fact, these groups correlated similarly with environmental factors (Figs. 3 and 4). LS1 correlated negatively with nutrient content in both cases (Fig. 3 and 4). This group includes the attached and/or pioneer taxa (e.g. *Achnanthydium minutissimum* (Kütz.) Czarnecki, *Amphora pediculus* (Kütz.) Grunow; Rimet and Bouchez, 2012; B-Béres et al., 2016). Due to this pioneer character (high disturbance tolerance, and high efficiency to nutrient uptake) the position of this group was expected. There was strong positive correlation between temperature and HS1: these taxa (e.g. *Pseudostaurosira parasitica* (W.Smith) Morales var. *subconstricta* (Grunow) E.Morales.) were usually abundant at the end of spring or at the beginning of autumn in the studied watercourses, when the low biotic or abiotic pressures (low grazing in spring, or low share effects in autumn) did not suppress their populations' growth. With the exception of HS2, S2 and S3 groups correlated negatively to dissolved oxygen and water temperature, but the correlation of these groups with nutrient content was rather positive (Figs. 3 and 4). Positive correlation of S2 and S3 groups with different nutrient forms were also justified by Berthon et al. (2011).

Although the correlation between MS1 and environmental factors was weak, the position of this group connected to nutrients (Figs. 3 and 4). The strongest correlation appeared between nutrients and MS2-MS3 groups (Figs. 3 and 4). Motile taxa have many adaptation advantages to dominate the assemblages in nutrient rich environment (e.g. secretion ability of extracellular enzymes, nutrient storages, ability to choose their microhabitats by motility, Berthon et al., 2011). Due to their motile character, these groups are able to find and reach faster the well-resourced habitats than the other groups. Our data confirmed again, that taxa belonging to the same biovolume categories could be separated from each other by their other features, like relation to nutrients and disturbances (belonging to guilds; B-Béres et al., 2016).

Among groups composed of large species, LS5 showed negative correlation with inorganic nutrient content, while HS5 and MS5 correlated positively to inorganic nutrients in both CEMFGs-P and CEMFGs-RB (Figs. 3 and 4). Representatives of LS5 group are common and abundant adnate taxa in the studied watercourses (e.g. varieties of

Cocconeis placentula Ehrenberg). Despite adnate taxa showing strong positive correlation to total nitrogen content (B-Béres et al., 2014), they have to adapt or tolerate the strong interaction caused by high profile guild in thick biofilm (Tapolczai et al., 2016). So, their real nutrient preference is masked in thick biofilm, and appears only in physically disturbed environments. But these two key factors (high nutrient content and high physical disturbance together) are not common in the studied watercourses. It has to be emphasized, the advantages of adnate taxa could appear in the presence of physical disturbance if two differently disturbed segments of the same river were studied (so there were similar nutrient regime; B-Béres et al., 2014).

Both HS5 and MS5 groups have abilities, which provide them advantages in nutrient rich environments. The position of high profile taxa in the upper part of biofilm allows them to access faster the dissolved nutrients than the other groups, especially the low profile taxa positioned in the bottom layer (Stenger-Kovács et al., 2013; Tapolczai et al., 2016). In turn, motile taxa are able to appear quickly in the most appropriate microhabitats (high nutrient content and moderate disturbance – Tapolczai et al., 2016). It seems that the key factor, which will effect to the ratio of these large sized taxa is the physical disturbance in lowland watercourses. This requires further investigation focused more on the role of physical disturbance among nutrient rich circumstances.

5. Conclusions

The statistical analyses of the differences between the original guild classification of diatoms (CEMFGs-O) and the classifications with separated planktic guild supported the ecological importance of the separate planktic guild in assessment of diatom assemblages. The most pronounced differences between the CEMFGs-P and CEMFGs-RB were the relation of LS4 and HS4 to environmental factors. It was basically due to the removal of common and usual dominant tube-forming taxa from LS4 to HS4 groups. Considering the ecological characters of these differently classified morpho-functional groups, we propose the general use of CEMFGs-RB for classification of diatoms. This study also highlighted that abundant and/or frequent taxa, which are able to change their life forms can modify significantly the relation of the functional group containing them with environmental factors. In the future, both field and laboratory studies should focus on revealing the circumstances which cause the changes in traits of diatoms.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.ecolind.2017.07.007>.

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