Recommendations for ecological status assessment of Lake Balaton (largest shallow lake of Central Europe), based on benthic diatom communities

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RECOMMENDATIONS FOR ECOLOGICAL STATUS ASSESSMENT OF LAKE BALATON (LARGEST SHALLOW LAKE OF CENTRAL EUROPE), BASED ON BENTHIC DIATOM COMMUNITIES

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DIATOMS
ECOLOGICAL STATUS ASSESSMENT
EU WFD
BALATON
SUBSTRATE
DIATOM INDICES

ABSTRACT. – Diatom communities of Lake Balaton, the largest shallow lake of Central Europe, were studied between 2006 and 2008, with the purpose of establishing an initial database for a monitoring system for the lake in accordance with the EU Water Framework Directives. Four basins of the lake were investigated at several sampling sites; diatoms were sampled from four different substrates (reed, stone, sediment, and artificial substrates) in summer and in autumn. The goals of the investigation were to choose sampling sites, to select the most suitable substrate, and to identify an adequate index for monitoring studies of Lake Balaton in the future. 101 samples were collected and 289 diatom species were identified. The most abundant species were *Achnanthidium minutissimum, Amphora pediculus, Cymbella exigua, Encyonopsis minuta, Staurosira grigorszkyi, Navicula cryptotenella* and *Nitzschia dissipata*. Data sets were analyzed with multivariate statistical methods. Cluster analysis, Principal Component Analysis (PCA) and Self Organizing Map method indicated a clear separation between the northern and southern shore of the lake. Taking diatom samples at several sites from each shore of the lake is suggested in the future. It was shown that reed is the most suitable substrate for studying diatoms of Lake Balaton. Our results have indicated that artificial substrate is an adequate alternative, since its epiphytic diatom biota is quite similar to reed. The water quality of the lake was generally good. The Keszthely basin is the most impacted area of the lake (caused by the nutrient load of River Zala), where diatoms indicate a moderate water quality. Using IBD along with TDIL seems to be the best method of assessing the water quality of Lake Balaton.

INTRODUCTION

EU Water Framework Directive (WFD) requires from its Member States that all inland and coastal waters should reach good ecological status by 2015. Benthic diatoms are an important group for the evaluation of the ecological status of surface waters. Numerous studies have been published about the application of benthic diatoms in rivers (see e.g., Kelly *et al*. 1998). Publications about the role of benthic diatom communities in lentic ecosystems are scarcer, but both should be improved (Solimini *et al*. 2006). Diatom communities are one of the examined parameters in the case of German qualification methods, since this group reacts rapidly to environmental changes (Poikane 2008).

Hungarian studies on benthic diatom communities of Lake Balaton are sporadic. Pantocsek (e.g., 1902, 1913) investigated the sub-fossil sediment and recent diatom flora of Lake Balaton. Uherkovich investigated the sediment and the periphytic communities of reed in the lake (e.g., Uherkovich 1988, Uherkovich & Csermák 1992). Despite the widespread application of benthic diatoms to water quality assessment at a regional level, there is no standard European sampling protocol or associated assessment metrics in lakes (Besse-Lototskaya *et al*. 2006).

The most appropriate substrate for sampling is not determined in the case of running waters or lakes. The selection of substrate plays a very important role in the water quality assessment of rivers (Snoeijs 1991, Rolland *et al*. 1997, Kelly *et al*. 1998) and lakes (Kitner & Poulíčková 2003, Poulíčková *et al*. 2003, 2004). Regarding lakes, there are several controversial results in the literature. Hofmann (1994) used benthic diatoms as indicators to assess water quality of the trophic and of the saprobic state. In her opinion, differences in epiphytic and epilithic diatom composition do not induce remarkable difference in index values. In contrast, several authors have found differences in diatom composition on different substrates. Schaumburg *et al*. (2005) worked out a sampling protocol for German lakes where stone and sediment are proposed as appropriate substrates to collect benthic diatom samples. King *et al*. (2006) pointed out that diatom composition of sediments is mainly determined by chemical properties of interstitial water and not by open-water. Besides, epipelon is often dominated by small *Fragilaria* sensu lato species which have a broad tolerance to different nutrient-concentrations, thus they are quite bad indicators of water quality. Consequently, the authors recommend using the most characteristic substrate of the littoral region of the given lake (King *et al.* 2006). Pouličková examined benthic diatom communities of eutrophic shallow lakes in Czech Republic (Kitner & Pouličková 2003), and several alpine lakes in Austria (Pouličková *et al.* 2004). They collected samples from stone, reed and silt and found that species composition was different depending on the substrates. This fact is reflected in the calculated value of diatom indices. Sediments contain species washed out from epiphyton and species settled out from plankton and metaphyton. The quantity of these effects depends on physical factors (e.g., fluctuation) rather than on chemical variables. In the case of stone species composition has a great variability, and it is difficult to collect representative samples.

Another essential point of water qualification based on diatoms is to select a suitable index that fits best with the physicochemical parameters of the given water. Diatom indices are mainly based on benthic diatom communities of running waters but there are some studies in the literature which proved that some of them can be used in the case of lakes as well (e.g., Blanco *et al.* 2004). Besides, special indices for lakes have been worked out in some countries in order to maximize the effectiveness of water quality assessment in lentic ecosystems. For example in Germany, Schaumburg *et al.* (2005) worked out DI Seen for lakes which is an elaborated version of the Trophic Index (TI) of Hofmann (1999). In Hungary, Stenger-Kovács *et al.* (2007) developed a trophic index for lakes (TDIL), based on transfer functions widely used in paleolimnology.

The main purpose of the present study was to develop the basis of the monitoring system for Lake Balaton in accordance with prescriptions of EU Water Framework Directive. Our main goals were (1) to find out if there is a difference in benthic diatom composition between the four basins of Lake Balaton and to appoint sampling points for future monitoring studies, (2) to examine which is the most appropriate substrate, from which to collect diatom samples in the case of Balaton and (3) to analyze the applicability of different diatom indices for being able to characterize the ecological status of the lake which is the only large, shallow permanent lake with medium depth (type 16 according to national lake typology) in Hungary.

MATERIALS AND METHODS

Study site and sampling methods: Lake Balaton is the largest shallow lake of Central Europe with a surface area of 593 km^2, catchment area 5182 km^2, length 77 km, width ranging from 4 to 14 km. Its surface is at 104 meter ASL. Its average depth is 3.2 m with a maximum depth of 12.2 m. Because the lake is quite shallow, even a breeze can stir the water. Due to geological features, Balaton is divided from west to east into the following

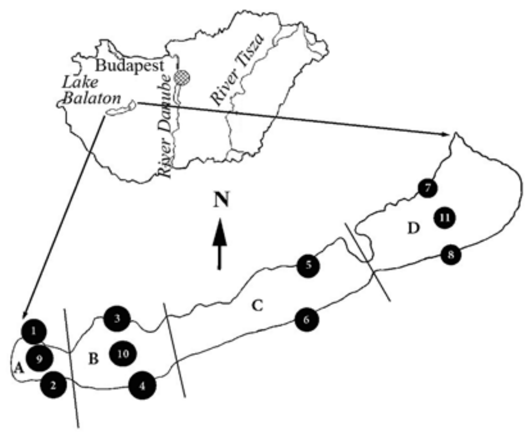

Fig. 1. – Four basins of Lake Balaton, and sampling points, A = Keszthely-, B = Szigliget-, C = Szemes-, D = Siófok basin. Further abbreviations see Table I.

four basins: Keszthely-, Szigliget-, Szemes- and Siófok basins. The river Zala discharges into the lake at the south-western part of Keszthely. The river provides the largest inflow to the lake and carries a considerable amount of nutrients. For this reason the nutrient- and phytoplankton chlorophyll *a* concentration diminish from west to east (Vörös *et al.* 2006). Water level is regulated by the Sió canal, the only outflow of the lake. Biogenic lime precipitation can be observed in the lake as a consequence of the unique calcium-magnesium-hydrocarbonate character of the water, and the CO_2 consumption of algae. Due to this phenomenon the water appears azure-opaque. Secchi-transparency is low and rarely exceeds 1 m. We studied the four basins at eight sampling sites in the shallows of the southern and northern shore of each basin in 2006 and 2007 (Fig. 1). In 2008 we investigated all selected sites but the Szemes basin because there did not exist a discrete diatom biota, which is necessary for future monitoring studies. Additionally in 2008 artificial substrates were exposed in the pelagic zone of Keszthely-, Szigliget- and Siófok basin and in the littoral zone of Siófok basin (Table I) to compare the diatom composition of the littoral and the pelagic zone. Samples were collected from reed in June and September 2006, from reed and stone in June and October 2007 and from reed, stone, sediment, and artificial substrates in June and late August 2008. In order to identify the best time to characterize the diatom composition of the autumn the last date of sampling varied within the years. Epiphytic samples were taken from green reed stems (*Phragmites australis* (Cav.) Trin. ex Steud.) at the open water side of the reed belt. Stems were collected in five replicates and gathered as a composite sample. Twenty cm sections of the stems were cut starting 10 cm below the water surface. Epiphyton was removed using a stiff bristled brush and tap water. Each epilithon sample was gathered from five cobbles. Epilithic biofilms were brushed off from the vertical, open water side of submerged cobbles. Loosely attached algae were removed from the surfaces by washing the substrate briefly in the lake water. For sediment sampling an Eikelkamp sediment sampler was used, at 60 cm depth. Five replicates of 20-30 cm sediment cores (diameter 20-30 cm) were taken; the upper 0.2 m

Table I. – Sampling points, sampling dates, sampled substrates and abbreviations for samples collected in 2006-2008 at Lake Balaton. Sample code characters were made as follows: 1-2: basin, 3: shore, 4: substrate type, 5-6: year, 7-8: month.

Basin	Shore	Abbreviation	No. on Fig. 1	Sampling Point	GPS-N	GPS-E
Keszthely	North	KEN	1	Gyenesdiás	46.76’126”	17.29’05”
Keszhely	South	KES	2	Balatonberény	46.71’57”	17.32’509”
Szigliget	North	SZN	3	Szigliget	46.78’531”	17.43’396”
Szigliget	South	SZS	4	Balatonfenyves	46.71’756”	17.48’929”
Szemes	North	SEN	5	Balatonakali	46.88’208”	17.76’003”
Szemes	South	SES	6	Balatonszemes	46.81’521”	17.77’681”
Siófok	North	SIN	7	Alsóörs	46.59’016”	17.58’481”
Siófok	South	SIS	8	Siófok	46.90’491”	18.03’145”
Keszthely	Middle	KEM	9	-	46.44’011”	17.17’305”
Szigliget	Middle	SZM	10	-	46.47’120”	17.35’401”
Siófok	Middle	SIM	11	-	46.56’538”	18.01’403”

Type of substrate	Sediment	Stone	Reed	Artificial
Abbreviation	1	2	3	4

Sampling date	23.06.2006	26.09.2006	21.06.2007	11.10.2007	30.06.2008	25.08.2008
Abbreviation	0606	0609	0706	0710	0806	0808

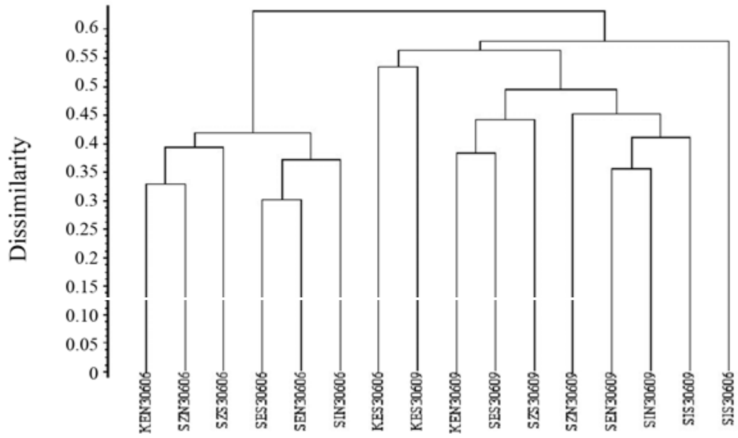

Fig. 2. – Samples collected from reed in 2006. Bray-Curtis index, UPGMA, data standardized with log2. Sample codes characters were made as follows: 1-2: basin, 3: shore, 4: substrate type, 5-6: year, 7-8: month. For further abbreviations see Table I.

section of each was used for further examinations. We used matted glass bottles (0.33 l) as artificial substrate in five replicates. They were placed into a frame and fixed with piles 0.1 m below the water surface in the pelagic zone (at the middle of cross-section of the basins) and littoral zone (near the reed belt), 10 cm below the water surface. Artificial substrates were exposed six weeks before sampling.

Laboratory analysis: All samples were preserved with buffered formaldehyde and transported to the laboratory where they were treated with H_2O_2 and HCl (according to CEN 2003), washed five times with distilled water and mounted with Naphrax® mounting medium. An Olympus IX70 inverted microscope equipped with differential interference contrast (DIC) optics was used for diatom identification. At least 400 valves were counted to estimate the relative abundance of each taxon of each sample. For the identification of critical taxa we examined our samples with a Hitachi S-2600N scanning electron microscope (details see Ács *et al*. 2009). Conductivity and pH-value were measured in situ, and a water sample for further analysis in the laboratory was taken at the each sampling date (details see Ács *et al*. 2009). The data of chemical analysis of the interstitial water of the sediment analysis were used to calculate the correlations between diatom indices and chemical parameters.

Statistical analysis: The data were analysed using several statistical methods. In order to compare samples collected at different sampling sites and different substrates we performed cluster analysis (Bray-Curtis index, UPGMA) with program Syn-tax 2000 (Podani 2001). Principal Component Analysis was used to determine the main ecological factors which have the greatest effect on variability of samples. With the aim of exploring and visualizing differences and similarities of sample groups we used Principal Coordinates Analysis (PCoA). We used the Kohonen Self Organising Map (SOM) (Kohonen 1982, 2001) in order to classify our samples. As a part of the SOM method Structural Index values were calculated for each species in order to determine species importance. The following diatom indices were calculated with the software OMNIDIA 5.2 (Lecointe *et al.* 1993, 1999, 2008): CEE (European Index, Descy & Coste 1991), IPS (Index Polluosensitivity Specific, Coste in Cemagref 1982), IBD (Indice Biologique Diatomées, Lenoir & Coste 1996), IDAP (Diatom Index Artois-Picardie, Prygiel *et al.* 1996), EPI-D (Eutrophication Pollution Index Diatoms, Dell'Uomo 1996, 2004), LOBO (Lobo's Index, Lobo *et al.*1996). Index TDIL (Trophic Diatom Index for Lakes, Stenger-Kovács *et al.* 2007) was calculated with a self-developed program (Hajnal *et al.* 2009). Differences of H-values were tested with Kruskal-Wallis ANOVA test. Pearson correlations were calculated to verify the significance between index values and measured chemical variables.

RESULTS

Differences in diatom composition between basins

Within the investigation period 101 samples were collected, and 289 diatom species were identified. 27 % of the taxa were represented by only one or two individuals. Generally, the most common species were *Achnanthidium minutissimum* (Kützing) Czarnecki, *Amphora pediculus* (Kützing) Grunow, *Cymbella exigua* Krammer, *Gomphonema pumilum* (Grunow) E. Reichardt & Lange-Bertalot, *Navicula cryptotenella* Lange-Bertalot, *Nitzschia dissipata* (Kützing) Grunow, *Pseudostaurosira elliptica* (Schumann) Edlund, E. Morales & Spaulding, *Staurosira grigorszkyi* (Pantocsek) Ács, E. Morales & Ector and *Staurosira pinnata* Ehrenberg.

Species number varied from 12 (Keszthely basin, sediment) to 65 (Siófok basin, stone). Shannon diversity ranged from 1.6 (Siófok basin, reed) to 5.5 (Siófok basin, stone). Average diversities in the four basins were quite similar. Differences between basins were not significant.

Multivariate analysis does not separate the four basins in each year clearly. In 2006 the samples were arranged

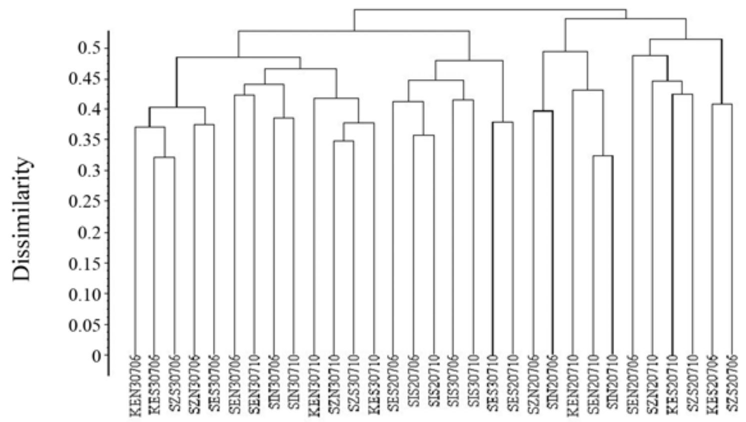

Fig. 3. – Cluster analysis of samples collected from reed and stone in 2007. Bray- Curtis index, UPGMA, data standardized with log2. Sample codes characters were made as follows: 1-2: basin, 3: shore, 4: substrate type, 5-6: year, 7-8: month. For further abbreviations see Table I.

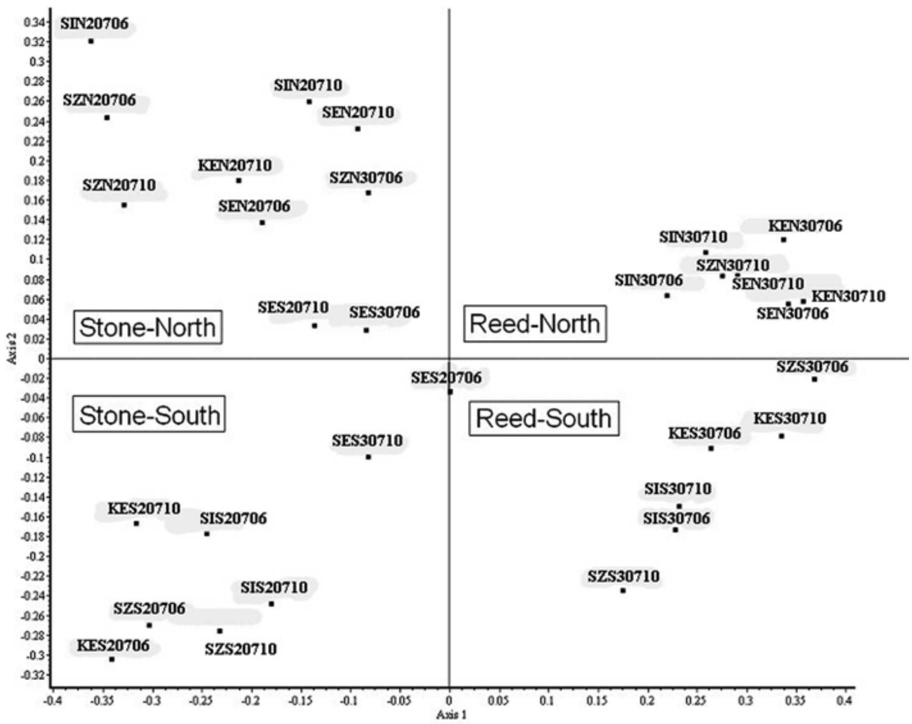

Fig. 4. – Results of PCoA analysis on samples collected from reed and stone in 2007. S = South, N = North. Sample code characters were made as follows: 1-2: basin, 3: shore, 4: substrate type, 5-6: year, 7-8: month. For further abbreviations see Table I.

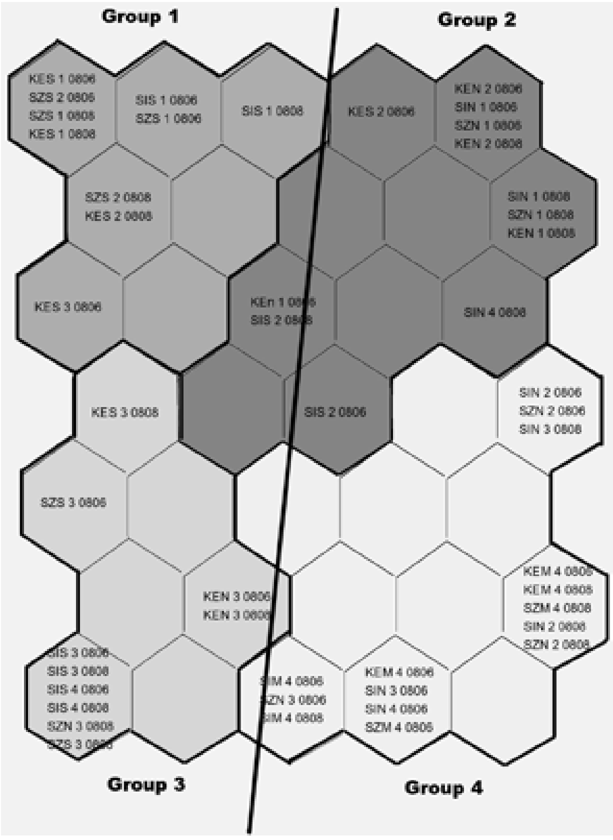

Fig. 5. – Arrangement of samples collected in 2008 in the SOM-map. The closer the samples are, the more they are similar in species composition and proportion. Group 1: Samples collected from sediment and stone in the southern shore, Group 2: samples collected from sediment and stone from the northern shore, Group 3: samples collected from reed and artificial substrates from the southern shore, Group 4: samples collected from reed and artificial substrates from the northern shore and the middle. Sample codes characters were made as follows: 1-2: basin, 3: shore, 4: substrate type, 5: sampling date, where s = samples collected in June 2008 and a = in August 2008. For further abbreviations see Table I.

primarily by the date of sampling, the species compositions of the summer and of the autumn samples were definitely different. The samples from the northern and southern shore were separated only slightly (Fig. 2). This little separation between the two shores of the lake is also apparent in the results of the cluster analysis of the data from 2007 (Figs 3, 4). Surveying the results of 2006-2007, it can be ascertained that benthic diatom communities of the basins are quite different, all of them have a characteristic biota, except for the Szemes basin samples which were similar to Siófok and Szigliget basin as well. Thus sampling of the Szemes basin was omitted in 2008.

The SOM analysis of the samples of 2008 has confirmed our results regarding the distinction of the northern and southern shore. The SOM map shows clearly this spatial pattern and the differences between the investigated substrates as well (Fig. 5). Samples collected in the pelagic zone from artificial substrates are separated from each other, especially in the case of Keszthely and Siófok basin. Diatom biota of these samples shows higher similarity with samples of northern shore than those of southern shore.

Species composition was also different in the two sampling periods (summer and autumn in each investigated year) but this difference was less marked in 2008. Consequently, samples collected in late August should be considered as summer biota.

Diatom composition on different substrates

In order to be able to choose the most convenient substrate for monitoring studies we compared diatom communities of reed, stone, sediment and artificial substrates in 2008.

Samples collected from reed and artificial substrates are located on the same area of the map (Fig. 5), while material collected from stones and sediment are found on the other part of the map. The segregation of the northern from the southern shore is distinctly visible on the map as well.

Based on all investigated samples, the most characteristic benthic diatom species of Lake Balaton are *Staurosira grigorszkyi* (FHUN), *Achnanthidium minutissimum* (AMIN), *Nitzschia dissipata* (NDIS), *Pseudostaurosira elliptica* (PSEI), *Amphora pediculus* (APED), *Cymbella exigua* (CEXI), *Navicula cryptotenella* (NCTE), *Staurosi-*

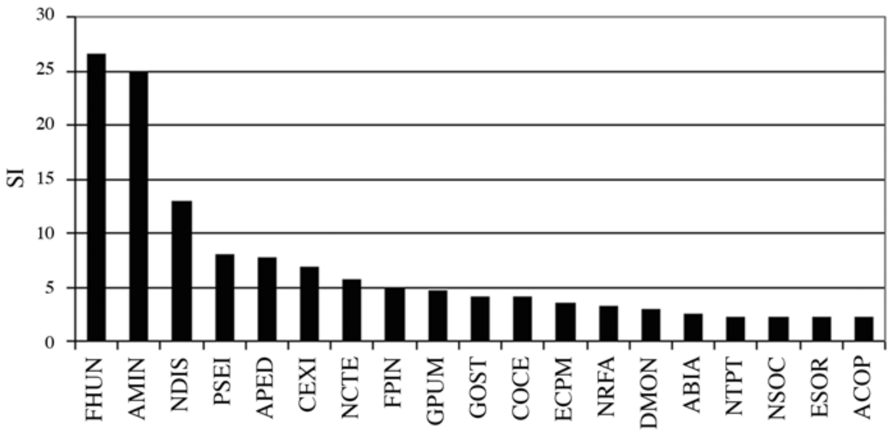

Fig. 6. – Characteristic species in Lake Balaton based on Structural Index (SI) calculation. See abbreviation in the text.

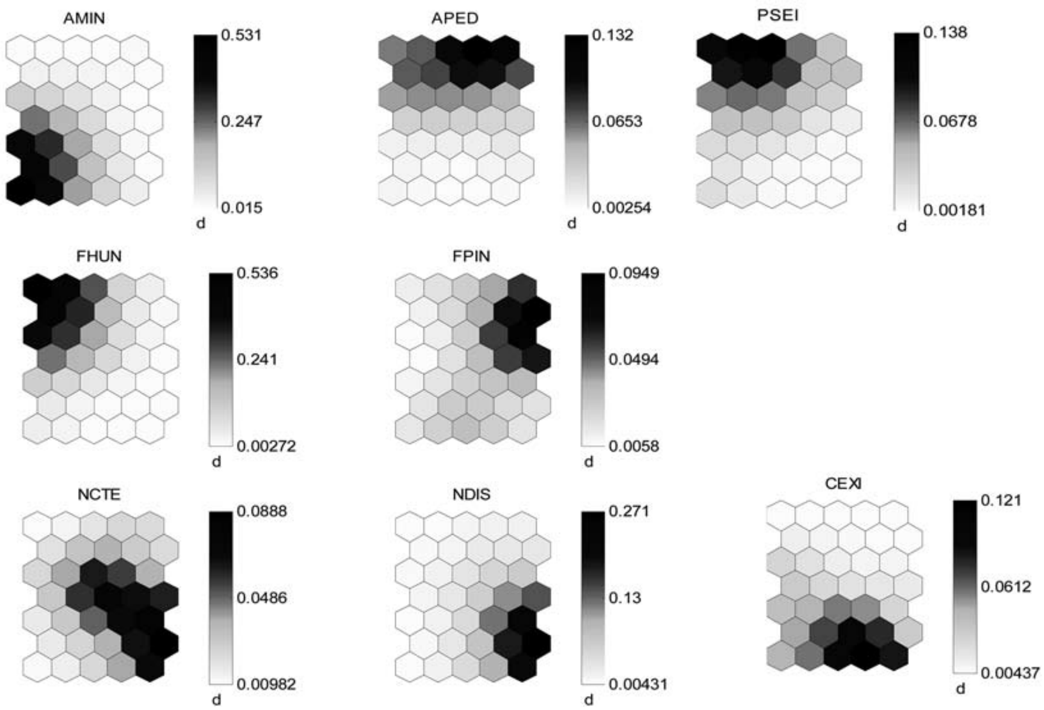

Fig. 7. – Arrangement of the 8 species with highest SI-values on the SOM-map. See abbreviation in the text.

ra pinnata (FPIN), *Gomphonema pumilum* (GPUM), and *Encyonopsis minuta* Krammer & E. Reichardt (ECPM). *Cyclotella ocellata* Pantocsek (COCE), which is the most characteristic species of phytoplankton of Lake Balaton, is often settled down to periphytic habitats in the lake (Fig. 6).

Achnanthidium minutissimum and *Cymbella exigua* are characteristic diatoms of reed and artificial substrates. *Staurosira grigorszkyi* and *Pseudostaurosira elliptica* are characteristic for stone and sediment in the southern part of the lake while *Staurosira pinnata* dominates the stones of the northern part. *Staurosira grigorszkyi* is also characteristic on reed, but only in the southern part. *Navicula cryptotenella* is a common species in northern areas, first of all on stones. *Amphora pediculus* and *Nitzschia dissipata* are frequent on stones in northern and southern parts of the lake as well and the latter was found on artificial substrates in the middle of the Keszthely and Szigliget basin (Fig. 7).

Diatom indices and water quality

Correlation between diatom indices and main chemical parameters was quite different in the case of different substrates (data not shown). Generally, correlation was highest in the case of reed, and lowest in the case of stones. Diatom indices calculated from stone showed weak correlations with main chemical variables while in the case of reed several indices (CEE, IPS, IBD, IDAP, EPI-D, LOBO and TDIL) correlated significantly or strongly with these variables. From these indices LOBO works only with 10 % of the benthic diatom species of Lake Balaton, CEE and EPI-D works with less than 70 % of the species. IPS takes at least 75 % of the species into consideration while IBD works with more than 80 % of species. TDIL is an index developed for Hungarian lakes, now it works with about 40 % of the species found in Lake Balaton but this proportion can be better and the index itself can be improved in the possession of a larger dataset.

Those indices can be considered as suitable ones for ecological status assessment of waters, which correlate with chemical variables and which take into consideration the highest percentage of relevant species during qualification. From the indices that can be calculated with OMNIDIA software, IPS and IBD met these criteria in the case of Lake Balaton. Therefore, here we analysed these two indices later and also TDIL. Regarding samples from reed, all of the indices showed high correlation with values of TP. Besides, the highest proportion of values was included in the confidence interval in the case of IBD (Fig. 8).

Qualification with different indices has resulted in different categorization of sampling sites (Fig. 9). Regarding the dataset of the three years, IPS and IBD classified 37 % of the samples into the same category, 61 % of samples were classified into a higher category by IPS, the rest (2 %) were categorized higher by IBD. IPS and TDIL also classified our samples differently. They arranged 49 % of the samples into the same category, IPS categorized 49 % of the samples into a higher category, and the opposite happened in 2 % of the cases. Contrarily, TDIL and IBD categorized similarly: in most cases (80 %) they arranged samples into the same category, in the 14 % of the cases

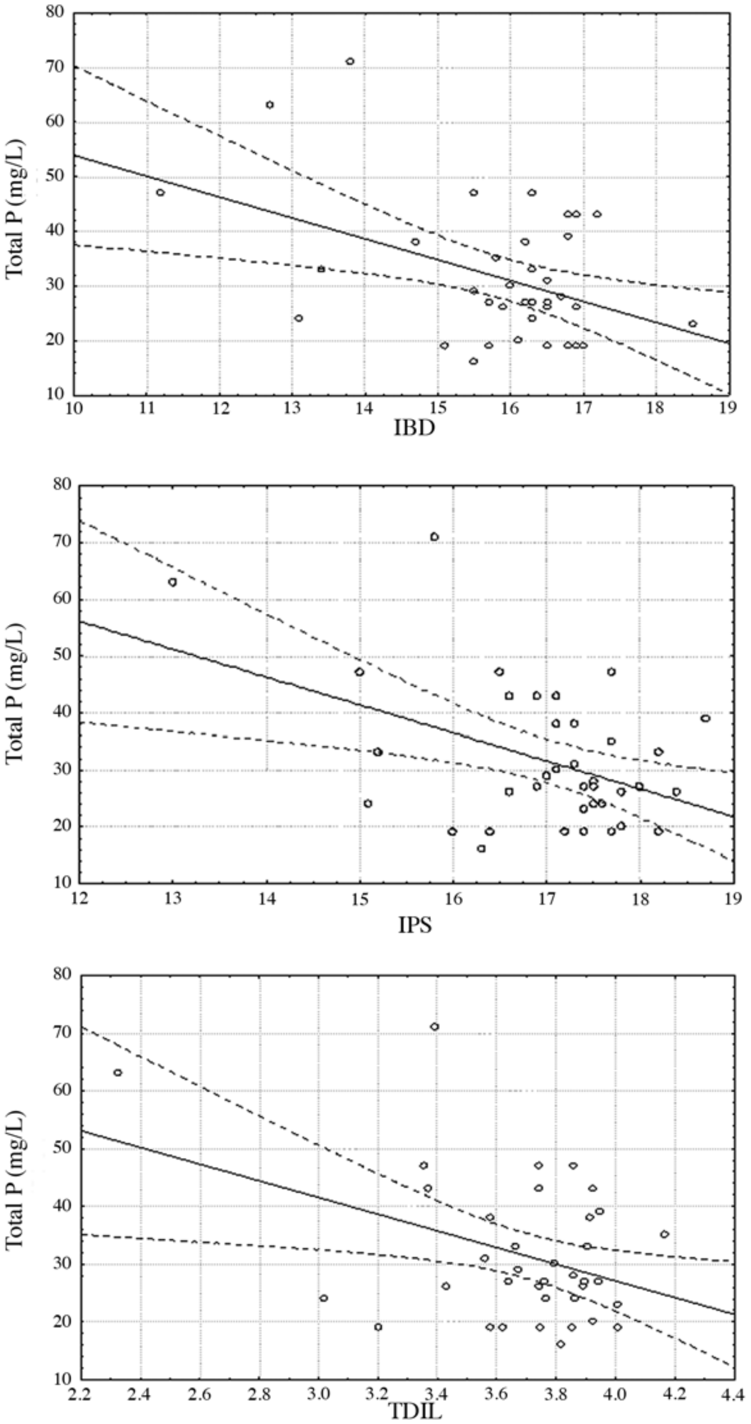

Fig. 8. – Coherency between TP values in Lake Balaton and IBD, IPS and TDIL values based on samples collected from reed in 2006 and 2007.

TDIL classified samples into a higher category, and the same can be stated about IBD in 6 % of the cases. In accordance with trophic gradients decreasing from west to east, values of IBD and TDIL increased the same direction, indicating better water quality as nutrient supply diminished (Fig. 10). Since both IBD and TDIL have proved to be suitable for qualification purposes, we have developed a multimetric index from the two (Multimetric Index for Lake Balaton, MIB = (IBD+TDIL)/2) and we used this index for the evaluation of ecological status, which was good in the investigated period (average was 15.2 on reed, detailed data not shown. The boundaries of this index are in Table II). The effect of the nutrient-load of River Zala was well seen in MIB index values for the western part of the lake. In this region we often experienced only moderate water quality. Values of MIB index increased from the western part to the eastern part of the lake (data not shown). The correlation between the MIB and phytoplankton chlorophyll *a* content was -0.4 and between MIB and TN was -0.52.

DISCUSSION

Differences between basins

The SOM is an increasingly popular tool in diatom ecology and has been used to describe algal assemblages (e.g., Gosselain *et al.* 2005, Rimet & Ector 2005, Rimet *et al.* 2005). We also used this method for the evaluation of different diatom compositions of samples collected from different sites of Lake Balaton. We demonstrated that the composition of periphyton is not homogeneous in the lake; the basins have differing benthic diatom biota, depending on the environmental circumstances. At the same time, the distinction of the southern and the northern part of the lake based on diatom data is more conspicuous. Vörös *et al.* (2006) has also shown that higher biomass values can be measured in the periphyton of the southern parts of the lake. The southern shore is shallower: the water body heats up more easily, besides this shore is more exposed to wind. Since this part of the lake is more turbid, species that can adapt to these circumstances are expected to be abundant here. This assumption was confirmed by our results, since a great number of species (with higher abundances) belonging to the *Fragilaria sensu lato* group were found in our samples originating from the southern shore. These taxa are known to be well-adapted to lower light intensity and disturbances as well (King *et al.* 2006).

The composition of diatom communities in the two sampling periods (summer and autumn) was different. However, recently Pados *et al.* (2009) presented evidence that epiphyton of Lake Balaton became light-limited after mid-summer, and this phenomenon can influence the

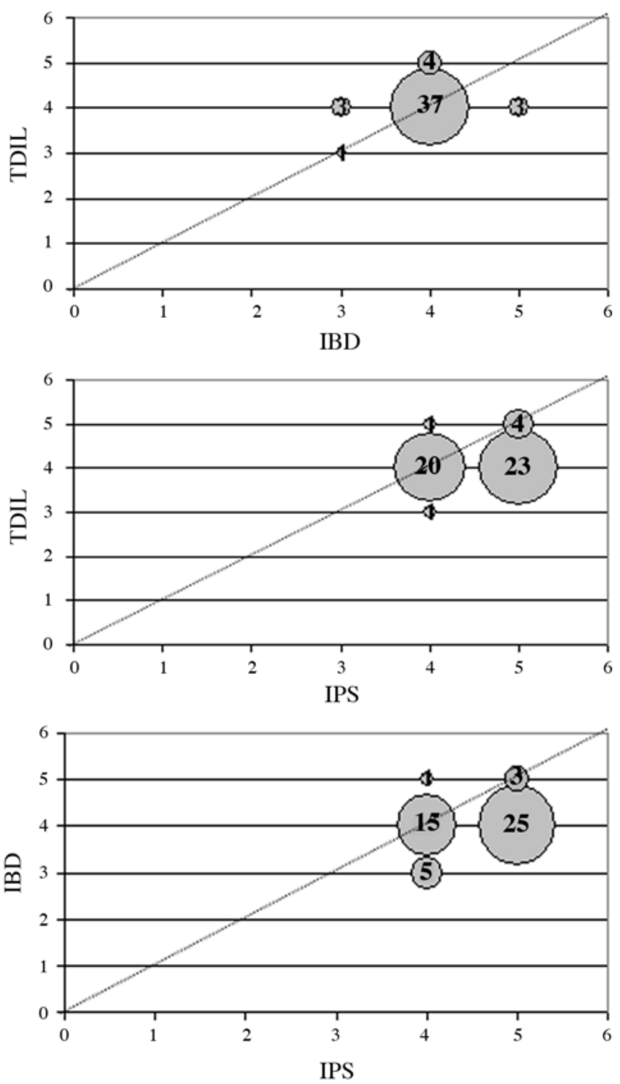

Fig. 9. – Categorization of periphyton samples collected in 2006, 2007 and 2008 by IPS, IBD and TDIL. Numbers in the circles refer to the number of samples which belong to the given qualification category.

results of sampling. Considering these findings our suggested sampling month is June in case of Lake Balaton. Since reed starts to grow at the end of April, and at least 6 weeks is needed for colonization (Ács & Buczkó 1994), it is questionable to collect mature periphyton before this month.

Diatom composition on different substrates

There were some differences in species composition on the investigated substrates. Sampling of sediment proved to be a useful tool in ecological qualification. This method is prevalent also in river and deep lake investigations (Stoermer & Smol 1999). However, several studies pointed out that it is less suitable for shallow lake studies. Epipelon is affected by numerous environmental factors, such as species settled down from the plankton. Poulíčková *et al*. (2004) investigated perialpine lakes in Austria. They found that the proportion of planktonic species reached a maximum of 60 % in the epipelon in some cases. Planktonic taxa were also notable in our epipelon samples. Epipelon is a special mixture of living cells from real epipelic species, dead frustules and species drifted from the plankton or other substrates due to wind disturbance (Poulíčková *et al*. 2004), which may also affect the accuracy of assessment. King *et al*. (2006) pointed out that diatom composition of sediments is mainly determined by chemical properties of interstitial water and not by water body. In our study the variability of samples was highest in the case of epipelon and water quality calculated on the basis of sediment samples was generally worse with one category than values calculated on the basis of other substrates. Therefore, we do not recommend using epipelon for ecological status assessment of Lake Balaton.

Stones have been found by some authors (e.g., Round 1991, Schaumburg *et al*. 2005) to be a useful and effective substrate for monitoring purposes. Stones are considered to be inert substrates the benthic diatom community of which is mainly determined by nutrient-concentration of the water body (Burkholder 1996). However stones accumulate dead cells of previous years' biota. Therefore they reflect not only the water quality in the investigated period but the situation in earlier years as well. The same applies to epipelon. In addition diatoms from the plankton or the metaphyton drift to those substrates and can reach a high proportion. Poulíčková *et al*. (2003) found that epilithon samples were contaminated with planktonic Centrales taxa, and high variability between samples and water quality was calculated from them. The same was found in epilithon samples of Lake Balaton: species composition varied strongly between samples and diatom indices calculated from epilithic data were in weak correlation with chemical results. In our study epilithon was more similar to epipelon than to artificial substrate, which is a neutral surface for benthic diatoms. Barbiero (2000) compared benthic diatom assemblages of artificial substrates (glasses and tiles) and stones and found these communities to be different. Uncertainty of samples collected from stones is compounded by disturbances to which stones are exposed such as grazing effects. For example King *et al*. (2006) often found stones entirely overgrown by mussels and sponges in the proximity of sampled rocks.

Several studies focused on the metabolic relationship between macrophytes and periphyton. Since microorganisms that live on plants gain part of their nutrients from host macrophytes (Wetzel 1996), the host plant may influence species composition that grows on it. Eminson & Moss (1980) examined how the type of host macrophyte affects species composition of periphyton. They found that diatom communities were host-specific in lakes with low trophy while this could not be declared in the case of eutrophic lakes. Blindow (1987) claimed that submerged macrophytes are not neutral substrates for benthic dia-

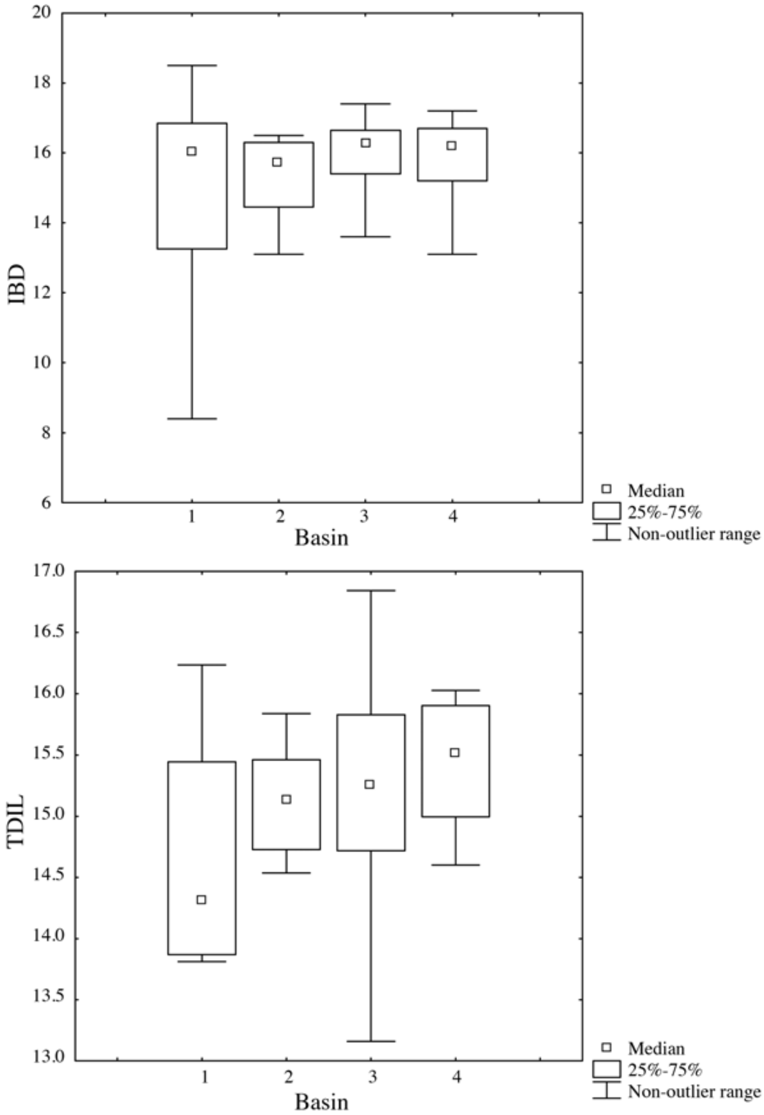

Fig. 10. – Values of IBD and TDIL in four basins of Lake Balaton (1 = Keszthely basin, 2 = Szigliget basin, 3 = Szemes basin, 4 = Siófok basin).

toms. In contrast, Cattaneo (1987) found that macrophytes do not influence considerably periphyton communities. To classify the situation in Lake Balaton, we examined the periphyton of artificial substrate from this point of view. Comparing species composition of periphyton from artificial substrate and reed epiphyton, high similarity was observed. This result suggests that metabolic relationships do not have a great importance in the case of Lake Balaton. This assumption is confirmed by relatively high correlations between chemical variables and diatom indices calculated from epiphytic data. Besse-Lototskaya *et al.* (2006) and Poulíčková *et al.* (2003, 2004) have presented similar results. The latter emphasized that macrophytes have further advantages: they are less contaminated by species drifted from other habitats and periphyton is renewed every year as new shoots grow, thus periphyton is expected to be a good indicator of actual chemical states.

Studies on artificial substrates are more and more prevalent, since these can be considered as real neutral surfaces for benthic diatoms. Lane *et al.* (2003) compared two types of artificial substrates (glass microslides and clay tiles) with natural substrates (stone, reed and sediment). On the whole artificial substrates represented the natural communities quite well, however highest similarity was observed between the two types of artificial substrates. They highlighted that artificial substrates are easy to handle and provide fresh periphyton with living diatom cells. They suggest one month for the incubation time. Ács *et al.* (2007) compared benthic diatom communities of a soda lake (Lake Velencei) on six different substrates (granite, andesite, old and green reed stems, Plexiglas, polycarbonate). They found that biofilms of artificial substrates deviated to a great extent from those of the reed and stone. Cattaneo and Amireault (1992) stated in their review that main proportion of literature data supports the theory that natural communities are similar to those of artificial substrates. Aloi (1990) reached a contradictory conclusion.

In our investigation epipelon and epilithon proved to be less susceptible because of several elements of uncertainty and week correlation with chemical data. Epiphyton and periphyton of matted glass were very similar, variance between samples was lower and indices calculated based on these substrates showed significant correlations with chemical variables. Since reed is characteristic for the littoral region of Lake Balaton and can be gathered easily, we recommend using green reed stems as natural substrate for further monitoring studies.

Diatom indices and water quality assessment

Diatom based qualification systems are widely used

Table II. – The proposed boundaries for the 16th lake type.

No. of type in Hungarian typology	Index	Index H/G	Index G/M	Index M/P	Index P/B	EQR H/G	EQR G/M	EQR M/P	EQR P/B
16	MIB	16.2	12.4	8.6	3.8	0.81	0.62	0.43	0.19

in numerous countries of Europe. Most of diatom indices were developed for riverine ecosystems, but some authors have found that several indices can be appropriate for ecological status assessment of lakes (e.g., Blanco *et al.* 2007, Cejudo-Figueiras *et al.* 2010). Since EU accession, investigations according to WFD became more intensive in Hungary as well and a new trophic diatom index for lakes (TDIL) has been developed by Stenger-Kovács *et al.* (2007). Species optima and tolerance have been determined for TP concentrations. Vilbaste (2001) noticed that consideration of the hydrological and physicochemical characteristics of the given water body and preliminary analysis of adequate indices are indispensable in choosing the best evaluation methodology.

Another important aspect of the suitable index is that it should take into consideration as many species as possible, especially dominant ones (King *et al.* 2006, Werner & Dreβler 2007). Otherwise indices are not sufficiently reliable for qualification purposes (Blanco *et al.* 2007). Based on our results, there are three indices that met these requirements: IPS, IBD and TDIL. As IBD show closer relation with the amount of *Achnanthidium minutissimum* (Stenger-Kovács *et al.* 2006) where this taxon is abundant in the periphyton (such as Lake Balaton), it is suggested that IBD be applied rather than IPS. Presumably, the high dominance of this species in our samples can explain why IBD showed higher variability between the different sampling sites of the lake. TDIL has this advantage: being a trophic index especially developed for Hungarian lakes, indicator and sensibility values of species can be considered reliable in reference to TP. Nevertheless, one disadvantage of TDIL was that we could assign indicator and sensibility values only to 90 species from the total 289, thus this index took only 40 % of the species found into consideration. This value can be improved if the database of the index will be expanded. Based on our results we suggest applying a multimetric index (MIB = Multimetric Index for Balaton) constituted as the mean of TDIL and IBD to assess the ecological status of Lake Balaton. Its border is given in Table II, EQR can be calculated with the following formula: EQR = MIB/MIB max. Balaton has a good ecological status according to our three-year study based on epiphytic diatoms.

Our study has clearly shown that Lake Balaton has an exceptionally diverse benthic diatom biota, the composition of which is influenced by both chemical background patterns and substrate. Based on our results the following recommendation was made for the Lake Balaton, which can be useful for other similar lakes:

(1) Spatial arrangement of benthic diatom communities is in accordance with the heterogeneity of chemical characteristics. Dissimilarity of the southern and northern shore caused by prevailing wind and different light conditions plays a major role in the formation of spatial patterns. Longitudinal chemical gradients of nutrients also have an important effect on species composition. For further monitoring, we suggest sampling at a minimum of six points of the littoral region (3-3 on southern and northern shore of the Siófok- , Szigliget-, and Keszthely- basins, the exact points are shown in Table I.), in accordance with the geological feature of the lake.

(2) Species composition of periphyton have changed from summer to autumn but since the epiphyton becomes light-limited by mid-summer, which can influence final results, sampling in early summer (June) is suggested

(3) Significant differences have been found in species composition on different substrates. We have found sediment less suitable than stones. The artificial substrate (matted glass bottles) is suitable, based on our study, but its periphyton was almost the same as reed epiphyton. However, it is expensive. Therefore green reed stems are suggested as substrate for further monitoring of the Lake Balaton.

(4) We advocate using the Multimetric Index for Balaton (MIB) for the ecological status assessment of Lake Balaton.

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