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Do benthic diatom assemblages reflect abiotic typology: a case study of Croatian streams and rivers

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Abstract – Benthic diatoms are widely used in Europe and worldwide to access ecological status of running waters. One of key goals of Water Framework Directive is to classify rivers and streams using biological quality elements and type specific reference conditions. According to system B which incorporates additional abiotic descriptors, there are 24 water types in Croatia. For biological analyses 92 rivers and streams with 140 sampling points were chosen and sampled for benthic diatoms and water chemistry simultaneously. Self organizing map (SOM) analysis was used to define biotypes from species composition and abundance of benthic diatoms. Grouping of samples in SOM resulted in 10 distinctive groups. Based on their geographical position and site characteristics, groups represent sites with similar properties (as waterbed, catchment size, altitude, size of stream) belonging to different ecoregions in Croatia. Analysis of variance revealed statistically significant differences ($p<0.05$) among SOM groups concerning ammonia, nitrates and total phosphorus. Indicator species analysis (IndVal) singled out species that were significantly characteristic ($p<0.05$) for SOM and abiotic types. Compared to abiotic groups, in which 7 out of 24 have no indicator species, all SOM groups have one or several characteristic diatom species, thus indicating diatom assemblages as valuable site descriptors. Canonical analysis of principal coordinates analysis also indicated that SOM grouping of samples is statistically reliable. Grouping of similar sites, although placed into different abiotic types, makes SOM groups with its corresponding representative species an easy tool for water quality assessment and description of reference assemblage.

Keywords: benthic diatoms, self organizing map, Water Framework Directive, water typology.

Abbreviations: BMU – best matching unit, SOM – self organizing map,

Introduction

According to Water Framework Directive (WFD), European Union member states have to achieve a good ecological status for all streams whose catchment area exceeds 10 km^2. Since the assessment is to be performed by observing deviations from the reference conditions, one of the key goals of the Water Framework Directive was to classify rivers and streams using biological quality parameters and to describe hydromorphological, physicochemical and biological type specific reference conditions (EC 2000). That should be determined using five biological quality elements: phytoplankton, phytobenthos, macrophytes, benthic invertebrates and fish. As commonly dominant phytobenthic representatives, benthic diatoms are used worldwide to assess the ecological status of running waters (Reid et al. 1995, Ács et al. 2005, Kireta et al. 2012, Martin and Reyes Fernandez 2012, Kahlert et al. 2016). They are widespread and can be found in almost any type of running water which, combined with short generation time and ability to clearly define nutrient status of their habitat, makes them great indicators of water quality. Also, they are relatively easy to sample and their ecological valences and habitats have been known for more than 100 years (Kolkwitz and Marsson 1908). During the past decades numerous indices, mostly based on trophic and saprobic status, have been used specifically for that purpose (Sládeček 1986, Kelly and Whitton 1995). Unlike the previously set index class boundaries, water quality assessment according to WFD is performed by comparison to reference conditions. As different habitats

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in their undisturbed stages support different assemblages, it is essential to describe reference condition for every type of habitat (Bennion et al. 2014).

As a starting point, all water bodies should be classified according to WFD hierarchical water body typology. It is organized by firstly placing the surface water bodies into the broad categories such as rivers, lakes, transitional/coastal bodies, etc. Two typologies were acknowledged within those categories: "System A" fixed typology, and "System B" alternative typology, which comprises a mixture of obligatory and optional factors (like catchment size, elevation, flow velocity, granulometric properties of the waterbed, tufa forming conditions, permanence of flow, etc.). Abiotic typology is then used as a base for describing type specific reference conditions. Eventually, water quality is determined by a comparison of the actual conditions to the ones that have been referenced.

Using water type system B, 24 different water types were discerned in Croatia (Mihaljević et al. 2011). Types were first separated by region (continental, pannonian and coastal), then by catchment size, type of waterbed, altitude of stream flow and stream flow discharge which is determined from the size, altitude and slope (Tab. 1). Such a high number of types is a reflection of geographical position and shape of the country of which two thirds belong to karst influenced by high mountains separating the coastal area from the continent, along with a rather flat Pannonian area which is extensively used for agriculture.

Croatia has scarce and non-continuous data on attached diatoms (Plenković-Moraj 1995) with parts of the country, especially poorly populated areas, that have never been explored. This study yielded the first comprehensive diatom dataset on most waterbodies in Croatia. The aim of this paper is to investigate relationship between previously determined 24 types of rivers and streams in Croatia and potential biotypes that are derived from benthic diatom assemblage data. Considering the number of types in neighbouring countries and previous data, it is expected that diatom assemblages will show regional differences along with some differences from other factors, but will altogether group certain abiotic types and thus reduce them.

Materials and methods

Sampling design

Data on diatom species composition, in the form of proportion of total diatom valves in each sample, has been gathered within the two projects for nation-wide survey covering all relevant important types of running waters along with concurrent and relevant environmental variables. The final goal of those projects was to describe water quality elements for determination of water class category ac-

Tab. 1. Abiotic water types (codes from AB1-AB24) in Croatia according to System B with factor values.

Abiotic type	Altitude / m	Catchment / km^2	Discharge (Q / m^3 s^{-1})	Region	Waterbed
AB1	>600	10–100	Q<2	Pannonian	silicate
AB2	200–600	10–100	Q<2	Pannonian	carbonate-silicate
AB3	<200	10–100	Q<2	Pannonian	silicate
AB4	<200	100–1000	2<Q<20	Pannonian	silicate
AB5	<200	1000–10000	Q>20	Pannonian	silicate
AB6	<200	1000–10000	Q>20	Pannonian	silicate
AB7	<200	1000–10000	Q>20	Pannonian	carbonate
AB8	<200	1000–10000	Q>20	Pannonian	silicate
AB9	>600	10–100	Q<2	Continental	carbonate
AB10	>600	10–100	Q<2	Continental	carbonate
AB11	200–600	10–100	Q<2	Continental	carbonate
AB12	200–600	100–1000	2<Q<20	Continental	carbonate
AB13	200–600	1000–10000	2<Q<20	Continental	carbonate
AB14	<200	100–1000	2<Q<20	Continental	carbonate
AB15	<200	1000–10000	Q>20	Continental	carbonate-silicate
AB16	200–600	10–100	Q<2	Coastal	carbonate
AB17	<200	10–100	Q<2	Coastal	carbonate
AB18	200–600	10–100	2<Q<20	Coastal	carbonate
AB19	<200	100–1000	2<Q<20	Coastal	carbonate
AB20	200–600	1000–10000	Q>20	Coastal	carbonate
AB21	<200	1000–10000	Q>20	Coastal	carbonate-silicate
AB22	200–600	100–1000	2<Q<20	Coastal	carbonate
AB23	200–600	10–100	Q<2	Coastal	carbonate-silicate
AB24	<200	100–1000	2<Q<20	Coastal	carbonate

cording to the Water Framework Directive. Investigations were performed during spring and summer of 2006, 2007 and 2009. Altogether, samples were collected on 140 sampling points from 92 different rivers and streams in Croatia. Diatom sampling, preparation of slides and counting followed CEN standards (European Committee for Standardization 2003, 2004). At least 5 stones were randomly chosen at each site, where diatoms were scraped from a surface area of approximately 10 cm^2 using a toothbrush or a scalpel (depending on substrate type). Diatoms were acid cleaned (sulphuric acid and sodium nitrate) and mounted in Naphrax (Brunel Microscopes, UK). On each slide at least 400 valves were counted using random transects under light microscopy at a magnification of 1000x. Identification of diatoms was performed using Krammer and Lange-Bertalot (1991a, b, 1997a, b) and Lange-Bertalot (2001). As supportive data, a dataset with physical and chemical variables of the investigated sites was also used. Values of water temperature, pH, conductivity, oxygen concentration, and oxygen saturation were measured in situ using a WTW Multi 340i handheld multimeter. Concentrations of nitrate, nitrite, ammonia, orthophosphate and total phosphorus were determined from the water samples that were simultaneously collected and measured in the laboratory using standard methods (APHA 1995).

Self organizing map

In order to analyze the pattern of diatom assemblages self organizing map (SOM) module was applied according to Kohonen (2001). The SOM is a neural network analysis tool which converts complex statistical relationships between high-dimensional data into a simple, low-dimension by compressing information while preserving the important topological and metric relationships in the original data (Kohonen 2001). The SOM analytical method has been used in diatom ecology describing benthic algal assemblages in France, Luxembourg and Hungary (Rimet et al. 2004, Gosselain et al. 2005, Varbiro et al. 2007). The data matrix consisted of 141 samples and 182 variables as species, with additional 4 environmental variables. Environmental variables used for describing abiotic types were used as nominal variables, and coded as follows: region categories – continental, pannonian and coastal regions as 1, 2 and 3 respectively; catchment size of 10–100 km^2 – creeks and small streams (1), 100–1000 km^2 – medium sized rivers (2), 1000–10000 km^2 – large rivers (3) and >10000 km^2 – very large rivers (4); type of waterbed with silicates (1), carbonates (2), organic (3) and mixed (4); altitude of stream's flow – lowland streams <200 m (1), submountain streams 200–600 m (2) and mountain streams >600 m (3); stream flow discharge (determined from the size, altitude and slope) as 3 groups: (1) low ($Q<2$ m^3 s^{-1}), (2) medium (2 m^3 $s^{-1}<Q<20$ m^3 s^{-1}) and high ($Q>20$ m^3 s^{-1}). The assemblage data for each taxon were normalized before the rescaling process by logarithmic transformation [log (x=1)]. The SOM consists of two layers: an input layer containing the samples and its variables, and an output layer containing so-called virtual units (VU). A VU can be considered a virtual sample unit with its virtual set of variables. In selection phase, weights of output layer were randomly assigned. Then a sample unit was chosen randomly and a best matching unit (BMU) was selected by calculating the Euclidean distance between the weights of the input layer and the weights of the output layer. It is important to note that the selection of the BMUs was based exclusively on diatom species abundances and environmental variables were masked out. This was done by applying a mask function that assigns a weight of 1 to the biological variables used for the selection of the SOMs VU and a weight of 0 to environmental variables. In the learning phase, the BMU weights (variables) in the output layer defined in the selection phase were updated with the weights of the input unit. The update process included both biological and environmental weights. The BMU was not the only grid unit updated: a neighborhood was defined around the BMU and all units within this neighborhood were also updated. In this way the values of environmental variables can also be visualized on an SOM that was previously trained, but only with the biological variables (Céréghino and Park 2009). The resulting hexagon map with its weights was visualized using the SOM toolbox as component planes. Each component plane represents variables that the SOM algorithm has learned. The SOM toolbox (http://www.cis.hut.fi/projects/somtoolbox) was used to implement the SOM in a MATLAB™ environment. The detailed algorithm of the SOM can be found in Kohonen (2001) and Lek and Guégan (1999) for ecological applications. To divide the output SOM into different diatoms groups according to their similarity, a hierarchical cluster analysis of the Ward linkage method with Euclidean distances was used (Park et al. 2004, 2006).

After clustering the SOM the BMUs of the original data matrix were calculated, thus we could obtain in which cluster a given sampling event would occur. Species which were important and stable for a given cluster were obtained using the indicator value index (IndVal) according to Dufrene and Legendre (1997) based on the original data matrix.

Statistical analysis

To test the differences among assigned groups (both biotic and abiotic), one-way analyses of variance (ANOVA) with abiotic and SOM groups as grouping factors, followed by post-hoc Tukey honest significant difference (HSD) tests were employed with a statistically significant value of $p<0.05$. ANOVAs were performed using statistical program Statistica 9.0. Primer 6 (Clarke and Warwick 2001) was used to allocate physical and chemical parameters that had the greatest impact on the assemblage. Firstly, the Bio-Env procedure which allocates the combination of environmental variables explaining the highest proportion of variance within the assemblage was used. For the analysis both Bray-Curtis similarity matrix of biotic data and of normalized environmental data were used. Afterwards, canonical analysis of principal coordinates (CAP) was performed. Its purpose was to find axes through multivariate cloud of points that either: (i) are the best at discriminating among *a priori* groups or (ii) have the strongest correlation with some other set of variables. Discriminant analysis of CAP

procedure was used to identify proportion of the samples that were correctly allocated to both SOM and abiotic types used as nominal factors of initial matrix. The other way of using CAP is to analyze how well multivariate data predict positions of samples along a continuous or quantitative gradient (Anderson et al. 2008). For discriminant analysis only Bray-Curtis similarity matrix of biotic data was used, and for canonical analysis, a matrix with normalized environmental data was used additionally.

Results

Typology

Grouping of samples in SOM (Online suppl. Fig. 1) resulted in 10 distinctive biotypes. Based on their geographical position and site characteristics, groups represent sites with similar properties (such as waterbed, catchment size, altitude, size of stream) belonging to different ecoregions of Croatia (Fig. 1). Most of the samples of group D1 are small to medium sized streams on higher altitudes on carbonate waterbed in continental region. Groups D2, D3 and D4 combine small to medium streams on lower (group D3) and higher altitudes (groups D2 and D4) on silicate (group D2) or carbonate waterbed (groups D3 and D4) mostly in coastal region. Groups D5, D6, D8 and D10 combine streams on silica waterbed in continental and pannonian region (group D5, D6, D8, D10) – they differ in size of stream and its altitude. Sites from groups D7 and D9 belong to coastal region and combine medium to large lowland rivers (group D7) or small streams in higher altitudes (group D9) on karstic carbonate waterbed (Online Suppl. Tab. 1).

Environmental variables

One-way ANOVA revealed that SOM groups statistically significantly differ ($F=2.16$, $p<0.001$) concerning combination of all environmental variables. To single out variables that contributed mostly towards statistical significance, post-hoc Tukey HSD test was performed. It revealed that group 6 is statistically different ($p<0.05$) from all other groups concerning concentrations of ammonia, nitrates, orthophosphates and total phosphorus. Influence of nominal variables that were the base for abiotic typology can be seen in component planes of the SOM for the typological attribute categories (Fig. 2).

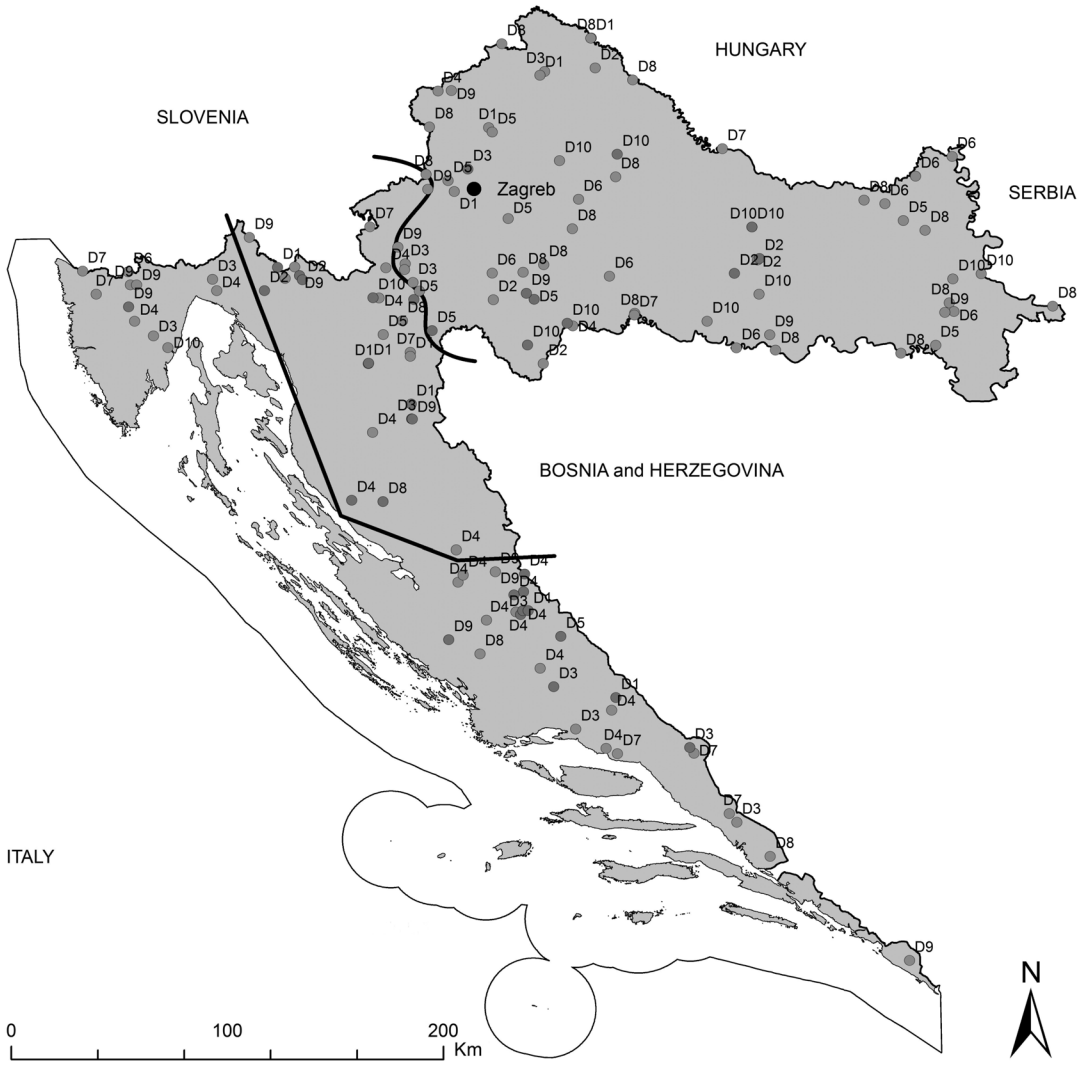

Fig. 1. Map of Croatian investigation sites divided into diatom-based self organizing map (SOM) groups. SOM cluster groups are assigned with codes from D1–D10. Lines denote different ecoregions of Croatia.

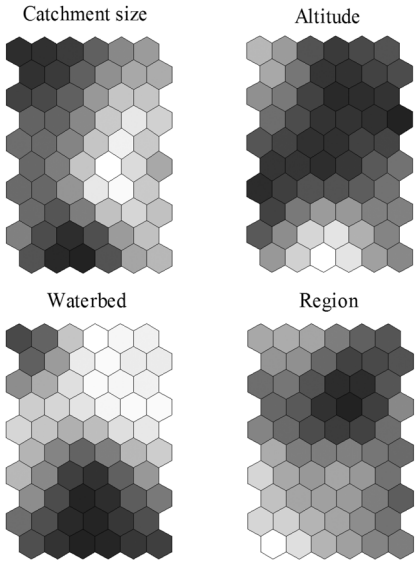

Fig. 2. Component planes of the self organizing map (SOM) for the typological attribute categories. Darker cells mean higher values of the given attributes.

Among abiotic groups ANOVA also revealed statistically significant differences ($F=1.59$, $p<0.001$), but post-hoc Tukey HSD test did not single out any particular parameter for any of the groups to be statistically significant (threshold: $p<0.05$).

Biotic data

One-way ANOVA on diatom abundance data set revealed that for both typologies their corresponding groups statistically significantly differ one from another ($F=138.08$, $p<0.001$ and $F=2.4$, $p<0.001$, for SOM and abiotic groups, respectively). IndVal analysis was employed to discover which of the variables species have significant indicator value for each of the groups. The analysis confirmed species with highest abundance and/or frequency within each SOM group as significant representatives of each group (Tab. 2), which can also be seen from SOM component planes (Fig. 3). Unlike SOM groups, some of the abiotic groups (groups AB4, AB8, AB9, AB12, AB15, AB18 and AB19) were not represented by any of the species (Tab. 3).

Tab. 2. Self organizing map (SOM) groups with indicator species according to indicator species analysis (IndVal). Abundance is represented as average percentage (%) of total number of valves of particular species for each site belonging to that group ± standard deviation.

Cluster	CODE	Species name	Abundance	IndVal	p-value
D1	ADBI	*Achnanthidium biasolettianum* (Grunow) Bukhtiyarova	74.42±4.09	0.64	<0.001
D2	ACAF	*Achnanthidium affine* (Grunow) Czarnecki	7.36±3.18	0.41	<0.001
	CPLA	*Cocconeis placentula* Ehrenberg	23.51±5.37	0.34	<0.001
D3	CAFF	*Cymbella affinis* Kützing	3.55±0.99	0.23	0.01
	CALS	*Caloneis* sp.	0.09±0.06	0.13	0.05
	CYMS	*Cymbella* sp.	1.32±0.65	0.27	0.01
	NPAL	*Nitzschia palea* (Kützing) W.Smith	2.45±0.71	0.23	0.02
	NRHY	*Navicula rhynchocephala* Kützing	0.22±0.12	0.19	0.01
	SPHO	*Stauroneis phoenicenteron* (Nitzsch) Ehrenberg	0.04±0.03	0.13	0.05
	SRMA	*Staurosira martyi* (Heribaud) Lange-Bertalot	0.56±0.31	0.25	0.01
	SSMU	*Staurosira mutabilis* (W.Smith) Pfitzer	1.67±1.02	0.25	0.01
D4	ENCM	*Encyonopsis microcephala* (Grunow) Krammer	6.8±1.93	0.42	<0.001
D5	CMTS	*Cymatopleura* sp.	0.04±0.03	0.20	<0.001
	GYAC	*Gyrosigma acuminatum* (Kützing) Rabenhorst	1.54±0.78	0.23	<0.001
	GYRS	*Gyrosigma* sp.	0.17±0.11	0.19	<0.001
	NAMP	*Nitzschia amphibia* Grunow f. *amphibia* Grunow	0.36±0.27	0.19	<0.001
	OROE	*Orthoseira roeseana* (Rabenhorst) O'Meara	2.19±1.99	0.20	<0.001
	PTDS	*Planothidium* sp.	0.76±0.46	0.20	<0.001
D6	AUGR	*Aulacoseira granulata* (Ehrenberg) Simonsen	2.75±1.19	0.30	<0.001
	CYLS	*Cyclotella* sp.	7.68±4.58	0.51	<0.001
	FPYG	*Fallacia pygmaea* (Kützing) Stickle & Mann ssp. *pygmaea* Lange-Bertalot	7.04±2.69	0.56	<0.001
	GYAT	*Gyrosigma attenuatum* (Kützing) Rabenhorst	1.02±0.61	0.40	<0.001
	HHUN	*Hippodonta hungarica* (Grunow) Lange-Bertalot, Metzeltin & Witkowski	0.56±0.39	0.34	<0.001
	NACI	*Nitzschia acicularis* (Kützing) W.Smith	15.23±4.60	0.53	<0.001
	NVLC	*Nitzschia valdecostata* Lange-Bertalot et Simonsen	2.14±1.05	0.40	<0.001
	SHAN	*Stephanodiscus hantzschii* Grunow	10.7±6.59	0.62	<0.001
D7	ADMI	*Achnanthidium minutissimum* (Kütz.) Czarnecki	59.95±7.32	0.31	<0.001
	ECAE	*Encyonema caespitosum* Kützing	4.82±2.57	0.53	<0.001
	ESLE	*Encyonema silesiacum* (Bleisch) D.G.Mann	2.55±1.58	0.30	<0.001

Tab. 2. – continued

Cluster	CODE	Species name	Abundance	IndVal	p-value
D7	FRAS	*Fragilaria* sp.	0.29±0.15	0.13	0.05
	FSAP	*Fistulifera saprophila* (Lange-Bertalot & Bonik) Lange-Bertalot	0.37±0.17	0.43	<0.001
	GTRU	*Gomphonema truncatum* Ehrenberg	1.13±0.96	0.37	<0.001
D8	AFOR	*Asterionella formosa* Hassall	14.19±4.61	0.69	<0.001
	APEL	*Amphipleura pelucida* Kützing	1.06±0.32	0.29	<0.001
	ATRI	*Achnanthes trinodis* (W.Smith) Grunow	1.68±0.74	0.32	<0.001
	CCMP	*Cymbella compacta* Østrup	0.82±0.44	0.27	<0.001
	CLBE	*Cymbella lange-bertalotii* Krammer	3.05±1.15	0.38	<0.001
	DOBL	*Diploneis oblongella* (Nägeli ex Kützing) Cleve-Euler	7.74±2.53	0.65	<0.001
	DVCA	*Diatoma vulgaris* var. *capitulatum* Grunow	14.07±3.91	0.63	<0.001
	EPRO	*Encyonema prostratum* (Berkeley) Kützing	5±1.55	0.53	<0.001
	ETUR	*Epithemia turgida* Kützing	0.68±0.31	0.21	<0.001
	FARC	*Fragilaria arcus* (Ehrenberg) Cleve	2.23±1.81	0.34	<0.001
	GDEC	*Geissleria decussis* (Østrup) Lange-Bert. & Metzeltin	1.49±0.57	0.43	<0.001
	SSPE	*Staurosira* sp.	0.7±0.27	0.35	<0.001
D9	CPED	*Cocconeis pediculus* Ehrenberg	6.41±1.82	0.21	0.04
	ENVE	*Encyonema ventricosum* (C.Agardh) Grunow	1.45±0.42	0.19	0.03
D10	ADSU	*Achnanthidium subatomus* (Hustedt) Lange-Bertalot	11.01±5.38	0.19	0.01
	CRBU	*Craticula buderi* (Hustedt) Lange-Bertalot	3.88±2.30	0.22	0.03
	FVUL	*Frustulia vulgaris* (Thwaites) De Toni	0.12±0.09	0.15	0.03
	NMEN	*Navicula menisculus* Schumann var. *menisculus*	5.98±2.52	0.25	<0.001
	NUMB	*Nitzschia umbonata* (Ehrenberg) Lange-Bertalot	1.27±0.45	0.38	<0.001
	SBRE	*Surirella brebissonii* Krammer & Lange-Bertalot var. *brebissonii*	1.81±0.96	0.20	0.02
	STAN	*Stauroneis anceps* Ehrenberg	0.05±0.04	0.15	0.03

Tab. 3. Abiotic groups with indicator species according to indicator species analysis (IndVal). Average represents percentage (%) of total number of valves of particular species for each site belonging to that group. STD – standard deviation.

Abiotic group	Species	Average	STD	p-value
AB1	*Achnanthidium affine* (Grunow) Czarnecki	8.4	0.101	<0.01
	Cocconeis placentula Ehrenberg	30.0	0.202	<0.05
	Craticula cuspidata (Kützing) Mann	5.8	0.115	<0.05
	Cymbella tumida (Brébisson) Van Heurck	3.6	0.072	<0.05
	Gomphonema gracile Ehrenberg	3.0	0.060	<0.05
AB2	*Diatoma tenuis* Agardh	0.5	0.012	<0.05
	Navicula trophicatrix Lange-Bertalot	<0.1	0.001	<0.05
	Sellaphora pupula (Kützing) Mereschkowksy	4.8	0.081	<0.05
AB3	*Achnanthidium subatomus* (Hustedt) Lange-Bertalot	18.5	0.227	<0.05
AB5	*Aulacoseira granulata* (Ehrenberg) Simonsen	2.3	0.033	<0.05
	Gyrosigma sp.	0.2	0.003	<0.05
AB6	*Asterionella formosa* Hassall	35.3	0.483	<0.05
	Cymbella stuxbergii (Cleve) Cleve	1.2	0.017	<0.01
	Fallacia pygmaea (Kützing) Stickle & Mann	12.3	0.173	<0.05
AB7	*Asterionella formosa* Hassall	32.7	0.463	<0.01
AB10	*Encyonema ventricosum* (Agardh) Grunow	5.9		<0.05
	Nitzschia dissipata (Kützing) Grunow	4.8		<0.05
	Nitzschia sp.	1.2		<0.01
AB11	*Achnanthidium biasolettianum* (Grunow) Bukhtiyarova	62.2	0.400	<0.05
	Aulacoseira ambigua (Grunow) Simonsen	0.2	0.004	<0.05
	Cymbella subhelvetica Krammer	0.1	0.003	<0.05
	Nitzschia linearis W.Smith	0.2	0.004	<0.05

Tab. 3. – continued

Abiotic group	Species	Average	STD	p-value
AB13	*Didymosphenia geminata* (Lyngbye) Mart.Schmidt	0.1	0.002	<0.05
	Encyonema ventricosum (Agardh) Grunow	2.7	0.028	<0.05
	Gomphonema olivaceum (Hornemann) Brébisson	3.9	0.059	<0.05
	Navicula slesvicensis Grunow	0.5	0.010	<0.05
	Nitzschia palea (Kützing) W.Smith	7.5	0.110	<0.05
	Surirella ovalis Brébisson	0.1	0.001	<0.05
AB14	*Aulacoseira italica* (Ehrenberg) Simonsen	7.4	0.127	<0.01
	Campylodiscus noricus Ehrenberg	0.1	0.003	<0.01
	Fragilaria crotonensis Kitton	6.1	0.106	<0.01
	Ulnaria ulna var. *acus* (Kützing) Lange-Bertalot	7.9	0.137	<0.01
AB16	*Gomphonema acuminatum* Ehrenberg	0.2	0.005	<0.05
AB17	*Cymbella compacta* Østrup	2.6	0.046	<0.01
	Denticula tenuis Kützing	8.4	0.135	<0.05
	Epithemia argus (Ehrenberg) Kützing	0.1	0.001	<0.01
	Gomphonema pala Reichardt	0.2	0.003	<0.01
	Gomphonema subclavatum Grunow	0.2	0.004	<0.05
	Navicula hofmanniae Lange-Bertalot	6.0	0.056	<0.05
AB20	*Cyclotella ocellata* Pantocsek	5.6	0.083	<0.05
	Navicula cryptotenelloides Lange-Bertalot	2.9	0.051	<0.01
AB21	*Cyclotella ocellata* Pantocsek	4.0	0.080	<0.05
	Encyonema caespitosum Kützing	5.9	0.119	<0.05
	Melosira arenaria Moore ex Ralfs	12.5	0.250	<0.05
	Urosolenia eriensis (H.L.Smith) Round & R.M.Crawford	0.2	0.004	<0.05
AB22	*Encyonopsis microcephala* (Grunow) Krammer	27.8		<0.01
	Sellaphora minima (Grunow) Mann	1.2		<0.01
AB23	*Epithemia* sp.	0.6	0.012	<0.05
	Frustulia creuzburgensis (Krasske) Hustedt	1.7	0.034	<0.05
	Nitzschia palea (Kützing) W.Smith	5.4	0.063	<0.05
	Stauroneis smithii Grunow	0.3	0.007	<0.05
AB24	*Navicula antonii* Lange-Bertalot	2.4	0.041	<0.01
	Navicula weinzierlii Schimanski	1.0	0.018	<0.05
	Reimeria sinuata (Gregory) Kociolek & Stoermer	0.4	0.008	<0.05
	Surirella angusta Kützing	0.3	0.005	<0.05
	Surirella linearis W.Smith	0.1	0.001	<0.05
	Surirella ovalis Brébisson	0.1	0.001	<0.05
	Surirella sp.	4.1	0.071	<0.05

Bio-Env procedure showed that the main environmental variables describing the data set were nitrogen fractions NH_4^+ and NO_3^- and total P (Rho=0.235; significance level of sample statistic=0.1%, number of permutations performed 999).

CAP procedure in Primer that corresponds to canonical analysis of principal coordinates (to test correlation to environmental variables) did not completely confirm those results (Fig. 4). The results show strong and significant correlations between the diatom abundance and the environmental variables (p=0.001). First canonical correlation was 0.74 and the second one was 0.67. CAP axis 1 was correlated with oxygen saturation (r=–0.924) and CAP axis 2 with total P (r=0.571) and NO_3^- (r=0.490).

Discriminant CAP analysis showed that 10 SOM groups are indeed distinguishable one from another. First two canonical correlations are quite high (δ_1=0.90 and δ_2=0.76). Diagnostic showed that the choice of 3 PCA axes includes 34.29% of total variation, i.e. that many samples were correctly classified, with values of Q^0_m'HQ^0_m=3.17 (p=0.001) and δ_1^2=0.81 (p=0.001).

Discriminant CAP on abiotic groups showed that abiotic typology is also relevant – with first two canonical correlations being 0.91 and 0.74 (δ_1 and δ_2, respectively). Although permutation test revealed high statistical significance (Q^0_m'HQ^0_m=4.85 (p=0.001) and δ_1^2=0.84 (p=0.001), abiotic grouping correctly allocated only 19.29% of samples.

Tab. 4. Relationship among benthic diatom-based SOM (self organizing map) groups and abiotic typology for Croatian rivers and streams. Sum represents a number of sites belonging to the group/site.

		Croatian abiotic type																								
		AB 1	AB 2	AB 3	AB 4	AB 5	AB 6	AB 7	AB 8	AB 9	AB 10	AB 11	AB 12	AB 13	AB 14	AB 15	AB 16	AB 17	AB 18	AB 19	AB 20	AB 21	AB 22	AB 23	AB 24	sum
SOM type	D1		1	1				1	1	2		3	1	1			1				1					13
	D2	3	1		2	1				2																9
	D3	1		1						1				1	1	4	1		2	3					1	16
	D4		1			1				2			2			2	2	1	5	1	2	1	1			21
	D5			2	1	2			1			1			1	1			1							10
	D6			1	1	3	1		3																	9
	D7					1			1					1		1			1	1		1		1	1	9
	D8				3	2	1	1	9	1						1		1				1				20
	D9		1	1	1	1			1	2	1	1		1		2		1	2	1		1		2	1	20
	D10		2	6	2				1						1									1		13
sum		4	6	12	10	11	2	2	17	10	1	5	3	4	3	11	4	3	11	6	3	4	1	4	3	140

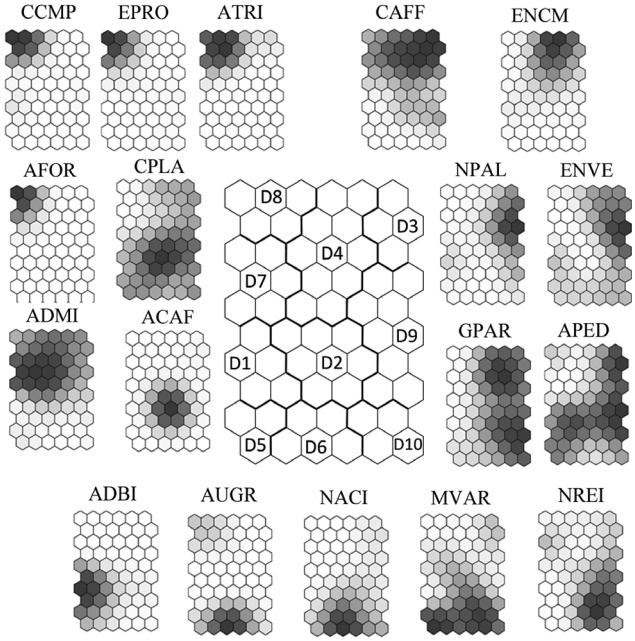

Fig. 3. Component planes of the self organizing map (SOM) for characteristic diatom species. SOM cluster groups are assigned with codes from D1–D10. Darker cells mean relative higher abundance of the given diatom species. Abbreviations of diatom names are the same as in Tables 2 and 3.

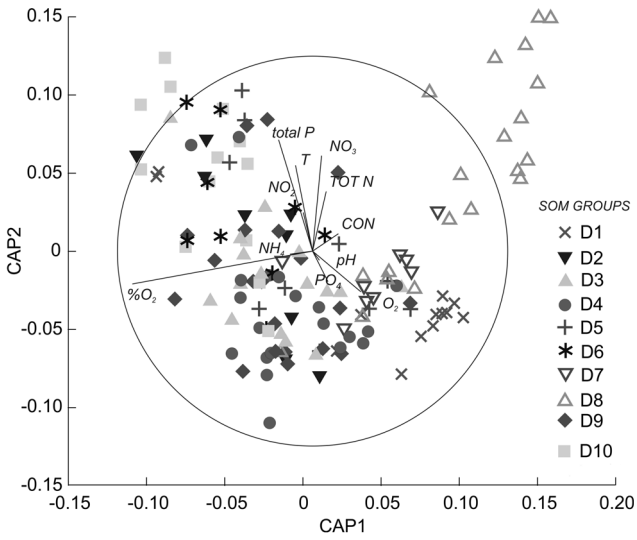

Fig. 4. Canonical analysis of principal coordinates (CAP) ordination plot relating benthic diatom assemblages to environmental variables.

Finally, all investigated sites plotted according to environmental variables and biotic data were efficiently associated with both biotic (SOM) and abiotic groups (Tab. 4).

Discussion

Determination of water quality according to WFD greatly depends on reliable typification of waterbodies, which includes entire range of hydrological, geographical, physical, chemical and biological characteristics of sampling sites. Since the status itself is derived by comparison to reference conditions, it is essential to have all sites and their corresponding reference assemblages described and grouped into usable groups. As abiotic typology in Croatia resulted in 24 different types, which in relation to surface area seem rather high, especially in comparison to Germany (20 types, Schmedtje et al. 2001), Hungary (26 types, Van Dam et al. 2007), Great Britain (14 types, Davy-Bowker et al. 2006) or Sweden (11 types, Davy-Bowker et al. 2006), it was expected for some types to group together. SOM analysis revealed 10 groups according to diatom assemblages, which is a number of types comparable to other countries.

As shown in SOM component planes, type of waterbed, altitude, size of catchment area and type of stream (its size) were variables influencing the development of diatom assemblage, as also shown in the other studies (Biggs 1995, Pan et al. 2000, Potapova and Charles 2003). All of those variables are in a direct relation to measured physical and chemical parameters of an investigated site, although there

are other variables, such as type of vegetation, size of substrate or exposure, that also greatly influence the nutrient availability. Altitude is usually related to flow velocity which also greatly influences the development of the assemblage (Primc-Habdija et al. 2001). Size of the catchment area and size of the stream, in a way, reflect the number of tributaries which bring additional nutrients to the stream. Subsequently, it also extends the possible presence of settlements such as industrial zones and agricultural estates which greatly influence nutrient inflow. Increasing phosphorus levels can be mainly related to those variables and not exclusively to the natural sources phosphorus levels. As published in many other studies (Bothwell 1989, 1988, Stanley et al. 1990) phosphorus, both as orthophosphate and total phosphorus, was shown to be an important factor in the development of benthic diatom assemblage. Phosphorus and nitrogen fractions have been generally considered to be most critical variables (Hutchinson 1957), as shown in Bio-env and CAP procedures.

Composition of diatom assemblages and the characteristic (indicator) species as *Achnanthidium*, *Cymbella*, *Encyonopsis* or *Nitzschia* species, from this set of Croatian samples closely corresponded to those observed in other geographical areas (Tison et al. 2005, Park et al. 2006, Tornés et al. 2007, Dohet et al. 2008, Tornés et al. 2012). Diatom assemblages are influenced by many variables including those that are site specific at various temporal and special scales (DeNicola et al. 2004, Pan et al. 2004), as well as those that reflect human interventions in the environment.

Statistically, both types of typology proved to be significant, but abiotic typology resulted in several groups being without one or several discriminant species. On the other hand, SOM groups had several characteristic species for each of the groups.

Typical species for SOM group D1, small to medium, mountain, continental streams on carbonate waterbed, *Achnanthidium biasolettianum* (Grunow) Bukhtiyarova is a species reported from upstream, low human impact sites at siliceous streams (Soininen et al. 2004). Presence of *A. biasolettianum* in carbonate streams can be explained by the fact that all streams from group D1 belong to continental part of Croatia that is not karstic and carbonates are prevailing but are not exclusive in the waterbed.

Similar sized streams, but on carbonate mostly karst waterbed, belong to groups D3 and D4 whose indicative species *Cymbella affinis* Kützing and *Encyonopsis microcephala* (Grunow) Krammer were reported from Mediterranean mineralized headwaters in Spain (Tornés et al. 2007) and Pyrenean calcareous springs (Sabater and Roca 1992). Also, *Cymbella* species seem to have high tendency to inhabit such calcium-rich waters, as reported from numerous streams in USA by Potapova and Charles (2003).

Species typical for group D2 (mountain, siliceous headwater streams) are *Achnanthidium affine* (Grunow) Czarnecki and *Cocconeis placentula* Ehrenberg. Those two species are most common in the streams with higher velocities, *A. affine* as a primary colonizer and *C. placentula* as a tightly attached, flow resistant species (Hoagland et al. 1982, Plenković-Moraj and Jasprica 2000, Kralj et al. 2006).

Groups D5, D6 and D10 represent mostly lowland, larger or smaller silicate rivers and streams impacted by agriculture (predominantly in Pannonian region) or urban settlements as shown by typical planktonic species like *Aulacoseira granulata* (Ehrenberg) Simonsen and *Nitzschia acicularis* (Kützing) W.Smith with a benthic species *Navicula menisculus* Schumann, all favoring greater nutrient load (Ács and Kiss 1991, Ács et al. 2003).

Group D8 showed statistically proven differences in many aspects from all other groups. As a group it is specified by the lack of a common descriptive species and presence of species like *Achnanthes trinodis* (Ralfs) Grunow in Van Heurck, *Cymbella lange-bertalotii* Krammer, *Encyonema prostratum* (Berkeley) Kützing or planktonic *Asterionella formosa* Hassall. Group D8 proved to be specific because it encompasses sites in lower reaches of large rivers that can be considered almost as lentic sites.

Achnanthidium minutissimum (Kützing) Czarnecki as a characteristic species in group D7, (small, mountain, karstic creeks) reflects more the nature of sites than their nutrient load. The species is known as an early colonizer that favors high flow velocities (Primc-Habdija et al. 2001, Kelly 2002, Kralj et al. 2006, Stenger-Kovács et al. 2013, B-Béres et al. 2016) and is commonly reported as a dominant species of similar habitats.

Group D9 collects sites from Istra, the largest Croatian peninsula that differs from the rest of Croatian coast (Prelogović et al. 1995), consisting of upper reaches of small karstic streams and a few continental streams. Although differing significantly, they share a species *Nitzschia palea* (Kützing) W.Smith, commonly reported from sites affected by agriculture and industry (John 2002, Soininen 2002). Therefore, group D9 encompasses streams affected by high artificial nutrient load.

Since complete data set included mostly undisturbed and slightly disturbed sites, due to war in Croatia at the beginning of 1990s and consequential deterioration of entire industry, all of the data, except from extremely disturbed sites were used. That approach ignores the differences in disturbance to similar sites, but general trends which can help in further research and monitoring were observed. Benthic diatom assemblages do reflect abiotic typology by grouping similar sites and therefore reducing the number of types. That reduction implies that diatom comminutes simplify abiotic groups, as also shown in Hungary (Van Dam et al. 2007). Also, the fact that this study described typical assemblage for each SOM group, in comparison to many undefined abiotic groups, as well as practicality of considering only 10 in comparison to 24 groups, makes this approach valuable contribution towards description of biotypes and easier determination of water quality according to WFD.

Conclusions

As particular species have different ecological preferences, some with narrow and some with wide ecological valences, diatom assemblage itself does not seem to show clear preference to particular type of stream. Diatom assemblage clearly groups similar types of rivers and streams, indicating that for estimation of water quality purposes there

is a need to reduce a number of stream types that naturally occur in some area. Statistically significant grouping of diatoms into SOM groups with several characteristic species in this study has shown that diatom assemblages can be used as valuable site descriptors. Grouping of similar sites, although they initially, according to abiotic typology, belong to different types, makes SOM groups with its corresponding representative species an easy tool for determination of water quality and description of reference assemblage.

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References

Ács, É., Kiss, K. T., 1991: Investigation of periphytic algae in the Danube at Göd (1669 river km, Hungary). Archiv für Hydrobiologie-Algological Studies 62, 47–67.

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 58, 545–554.

Ács, É., Reskóné, M. N., Szabó, K., Taba, G., Kiss, K. T., 2005: Application of epiphytic diatoms in water quality monitoring of Lake Velence – recommendations and assignments. Acta Botanica Hungarica 3–4, 211–223.

Anderson, M. J., Gorley, R. N., Clarke, K. R., 2008: PERMANOVA for PRIMER: Guide to software and statistical methods. PRIMER-E Ltd., Plymouth.

APHA, 1995: Standard methods, 19th edition. American Public Health Association, Washington, DC.

B-Béres, V., Lukács, Á., Török, P., Kókai, Z., Novák, Z., T-Krasznai, E., Tóthmérész, B., Bácsi, I., 2016: Combined eco-morphological functional groups are reliable indicators of colonisation processes of benthic diatom assemblages in a lowland stream. Ecological Indicators 64, 31–38.

Bennion, H., Kelly, M. G., Juggins, S., Yallop, M. L., Burgess, A., Jamieson, J., Krokowski, J., 2014: Assessment of ecological status in UK lakes using benthic diatoms. Freshwater Science 33, 639–654.

Biggs, B. J. F., 1995: The contribution of flood disturbance, catchment geology and land use to the habitat template of periphyton in stream ecosystems. Freshwater Biology 33, 419–438. doi:10.1111/j.1365-2427.1995.tb00404.x

Bothwell, M. L., 1988: Growth rate responses of lotic periphytic diatoms to experimental phosphorus enrichment: The influence of temperature and light. Canadian Journal of Fisheries and Aquatic Sciences 45, 261–270.

Bothwell, M. L., 1989: Phosphorus–limited growth dynamics of lotic periphytic diatom communities: areal biomass and cellular growth rate responses. Canadian Journal of Fisheries and Aquatic Sciences 46, 1293–1301.

Céréghino R., Park Y-S., 2009: Review of the self-organizing map (SOM) approach in water resources: commentary. Environmental Modelling and Software 24, 945–947.

Clarke, K. R., Warwick, R. M., 2001: Change in marine communities: an approach to statistical analysis and interpretation, 2nd edition. Primer-E, Plymuth.

Davy-Bowker, J., Clarke, R. T., Johnson, R. K., Kokes, J., Murphy, J. F., Zahrádková, S., 2006: A comparison of the European Water Framework Directive physical typology and RIVPACS-type models as alternative methods of establishing reference conditions for benthic macroinvertebrates. Hydrobiologia 566, 91–105.

DeNicola, D. M., Eyto, E. de, Wemaere, A., Irvine, K., 2004: Using epilithic algal communities to assess trophic status in Irish lakes. Journal of Phycology 40, 481–495. doi:10.1111/j.1529-8817.2004.03147.x

Dohet, A., Ector, L., Cauchie, H.-M., Hoffmann, L., 2008: Identification of benthic invertebrate and diatom indicator taxa that distinguish different stream types as well as degraded from reference conditions in Luxembourg. Animal Biology 58, 419–472. doi:10.1163/157075608X383719

Dufrene M., Legendre P., 1997: Species assemblages and indicator species: the need for a flexible asymmetrical approach. Ecological Monographs 67, 345–356.

EC, 2000. Directive 2000/60/EC of the European Parliament and of the Council. Official Journal of the European Communities 1–73.

European Committee for Standardization, 2003: European Standard EN 13946. Water Quality – Guidance standard for the routine sampling and pretreatment of benthic diatoms from rivers. CEN, Brussels.

European Committee for Standardization, 2004: European Standard EN 14407. Water Quality – Guidance Standard for the identification, enumeration and interpretation of benthic diatoms from running waters. CEN, Brussels.

Hoagland, K. D., Roemer, S. C., Rosowski, J. R., 1982: Colonization and community structure of two periphyton assemblages, with emphasis on the diatoms (Bacillariophyceae). American Journal of Botany 69, 188.

Hutchinson, G. E., 1957: A treatise on limnology. Volume 1: Geography, physics, and chemistry. Wiley, New York.

John, J., 2004: Diatom assemblages as indicators of wastewater discharge in a temporary stream in Western Australia. In: Poulin, M. (ed.), Proceedings of the 17th International Diatom Symposium 2002. Biopress Ltd., Ottawa, Canada.

Kahlert, M., Ács, É., Almeida, S. F. P., Blanco, S., Dreßler, M., Ector, L., Karjalainen, S. M., Liess, A., Mertens, A., Wal, J. van der, Vilbaste, S., Werner, P., 2016: Quality assurance of diatom counts in Europe: towards harmonized datasets. Hydrobiologia 772: 1–14.

Kelly, M. G., 2002: Role of benthic diatoms in the implementation of the Urban Wastewater Treatment Directive in the River Wear, North-East England. Journal of Applied Phycology 14, 9–18.

Kelly, M. G., Whitton, B. A., 1995. The Trophic Diatom Index: a new index for monitoring eutrophication in rivers. Journal of Applied Phycology 7, 433–444.

Kireta, A. R., Reavie, E. D., Sgro, G. V., Angradi, T. R., Bolgrien, D. W., Hill, B. H., Jicha, T. M., 2012: Planktonic and periphytic diatoms as indicators of stress on great rivers of the United States: Testing water quality and disturbance models. Ecological Indicators 13, 222–231.

Kohonen, T., 2001. Self-organizing maps, 3rd ed, Springer Series in Information Sciences, Vol. 30. Springer, New York.

Kolkwitz, R., Marsson, M., 1908: Ökologie der pflanzlichen Saprobien. Berichte der Deutschen Botanischen Gesellschaft 26a, Berlin, 505–519.

Kralj, K., Plenković-Moraj, A., Gligora, M., Primc-Habdija, B., Šipoš, L., 2006: Structure of periphytic community on artificial substrata: Influence of depth, slide orientation and colonization time in karstic Lake Visovačko, Croatia. Hydrobiologia 560, 249–258.

Krammer, K., Lange-Bertalot, H., 1991a: Bacillariophyceae 3. Teil: Centrales, Fragilariaceae, Eunotiaceae. In: Ettl, H., Gerloff, J., Heynig, H., Mollenhauer, D. (eds.), Süsswasserflora von Mitteleuropa. Band 2/3. Gustav Fischer Verlag, Stuttgart.

Krammer, K., Lange-Bertalot, H., 1991b. Bacillariophyceae 4. Teil: Achnanthaceae, Kritische Ergänzungen zu *Navicula* (Lineolatae) und *Gomphonema*. Gesamtliteraturverzeichnis Teil 1–4. In: Ettl, H., Gärtner, G., Gerloff, J., Heynig, H., Mollenhauer, D. (eds.), Süsswasserflora von Mitteleuropa. Band 2/4. Gustav Fischer Verlag, Stuttgart.

Krammer, K., Lange-Bertalot, H., 1997a: Bacillariophyceae 1. Teil: Naviculaceae. In: Ettl, H., Gerloff, J., Heynig, H., Mollenhauer, D. (eds.), Süsswasserflora von Mitteleuropa. Band 2/1. Gustav Fischer Verlag, Jena.

Krammer, K., Lange-Bertalot, H., 1997b: Bacillariophyceae 2. Teil: Bacillariaceae, Epithemiaceae, Surirellaceae. In: Ettl, H., Gerloff, J., Heynig, H., Mollenhauer, D. (eds.), Süsswasserflora von Mitteleuropa. Band 2/2. Gustav Fischer Verlag, Jena.

Lange-Bertalot, H., 2001: *Navicula sensu stricto*, 10 genera separated from *Navicula sensu lato*, *Frustulia*. In: Lange-Bertalot, H. (ed.), Diatoms of Europe, 2. A. R. G. Gantner Verlag K. G., Ruggell, Liechtenstein.

Lek, S., Guégan, J. F., 1999: Artificial neural networks as a tool in ecological modelling, an introduction. Ecological Modelling 120, 65–73.

Martin, G., Reyes Fernandez, M. de los, 2012: Diatoms as indicators of water quality and ecological status: sampling, analysis and some ecological remarks. In: Voudouris, K. (ed.), Ecological Water Quality – Water Treatment and Reuse. InTech, Croatia.

Mihaljević, Z., Mrakovčić, M., Mustafić, P., Kerovec, M., Primc Habdija, B., Plenković-Moraj, A., Ternjej, I., Zanella, D., Gligora Udovič, M., Ćaleta, M., Marčić, Z., Buj, I., Kralj Borojević, K., Sertić Perić, M., Dražina, T., Žutinić, P., 2011: Biological methods in assessment of the ecological status (Water Framework Directive (2000/60/EC) in the representative waterbodies of Pannonian and Adriatic ecoregion. Study of the Department of Biology, Faculty of Science, Zagreb (in Croatian).

Pan, Y., Herlihy, A., Kaufmann, P., Wigington, J., van Sickle, J., Moser, T., 2004: Linkages among land-use, water quality, physical habitat conditions and lotic diatom assemblages: A multi-spatial scale assessment. Hydrobiologia 515, 59–73.

Pan, Y., Stevenson, R. J., Hill, B. H., Herlihy, A. T., 2000: Ecoregions and benthic diatom assemblages in Mid-Atlantic Highlands streams, USA. Journal of the North American Benthological Society 19, 518–540.

Park, Y.-S., Chon, T.-S., Kwak, I.-S., Lek, S., 2004: Hierarchical community classification and assessment of aquatic ecosystems using artificial neural networks. Science of the Total Environment 327, 105–122.

Park, Y.-S., Tison, J., Lek, S., Giraudel, J.-L., Coste, M., Delmas, F., 2006: Application of a self-organizing map to select representative species in multivariate analysis: A case study determining diatom distribution patterns across France. Ecological Informatics 1, 247–257.

Plenković-Moraj, A., 1995: Diatoms (Bacillariophyceae) of the Croatian Freshwater. Acta Botanica Croatica 54, 22–33.

Plenković-Moraj, A., Jasprica, N., 2000: Microphytobenthic communities in the fresh waterof Trsteno Arboretum (southern Croatia). Acta Botanica Croatica 59, 351–359.

Potapova, M., Charles, D. F., 2003: Distribution of benthic diatoms in U.S. rivers in relation to conductivity and ionic composition. Freshwater Biology 48, 1311–1328.

Prelogović, E., Kuk, V., Jamičić, D., Aljinović, B., Marić, K., 1995: Seismotectonic activity of the Kvarner area. In: Vlahović, I., Velić, I., Šparica, M. (eds.), Book of abstracts of the 1st Croatian geological congress. Institut za geološka istraživanja, Zagreb, 487–490 (In Croatian).

Primc-Habdija, B., Habdija, I., Plenković-Moraj, A., 2001: Tufa deposition and periphyton overgrowth as factors affecting the ciliate community on travertine barriers in different current velocity conditions. Hydrobiologia 457, 87–96.

Reid, M. A., Tibby, J. C., Penny, D., Gell, P. A., 1995: The use of diatoms to assess past and present water quality. Australian Journal of Ecology 20, 57–64.

Sabater, S., Roca, J.R., 1992: Ecological and biogeographical aspects of diatom distribution in Pyrenean springs. British Phycological Journal 27, 203–213.

Schmedtje, U., Sommerhaeuser, M., Braukmann, U., Briem, E., Haase, P., Hering, D., 2001: „Top-down" – Konzept einer biozönotisch begründeten Fliessgewässertypologie Deutchlands. In: Deutsche Gesellschaft für Limnologie (ed.), Tagungsbericht 2000 (Magdeburg), Tutzing, 147–151.

Sládeček, V., 1986: Diatoms as indicators of organic pollution. Acta Hydrochimica et Hydrobiologica 14, 555–566.

Soininen, J., 2002: Responses of epilithic diatom communities to environmental gradients in some Finnish rivers. International Review of Hydrobiology 87, 11–24.

Soininen, J., Paavola, R., Muotka, T., 2004: Benthic diatom communities in boreal streams: community structure in relation to environmental and spatial gradients. Ecography 27, 330–342.

Stanley, E. H., Short, R. A., Harrison, J. W., Hall, R., Wiedenfeld, R. C., 1990: Variation in nutrient limitation of lotic and lentic algal communities in a Texas (USA) river. Hydrobiologia 206, 61–71.

Stenger-Kovács, Cs., Lengyel, E., Crossetti, L. O., Üveges, V., Padisák, J., 2013: Diatom ecological guilds as indicators of temporally changing stressors and disturbances in the small Torna-stream, Hungary. Ecological Indicators 24, 138–147.

Tison, J., Park, Y.-S., Coste, M., Wasson, J. G., Ector, L., Rimet, F., Delmas, F., 2005: Typology of diatom communities and the influence of hydro-ecoregions: a study on the French hydrosystem scale. Water Research 39, 3177–3188.

Tornés Cambra, J., Gomà, J., Leira, M., Ortiz, R., Sabater, S., 2007: Indicator taxa of benthic diatom communities: a case study in Mediterranean streams. Environmental Sciences 43, 1–11.

Tornés, E., Leira, M., Sabater, S., 2012: Is the biological classification of benthic diatom communities concordant with ecotypes? Hydrobiologia 695, 43–55.

Van Dam, H., Stenger-Kovacs, C., Ács, É., Borics, G., Buczko, K., Hajnal, E., Soroszki-Pinter, E., Varbiro, G., Tothmeresz, B., Padisák, J., 2007: Implementation of the European Water Framework Directive: development of a system for water quality assessment of Hungarian running waters with diatoms. Archiv für Hydrobiologie. Supplementband. Large rivers 17, 339–364.