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Biodiversity and seasonal distribution of benthic diatom assemblages as an indicator of water quality of small karstic river in Bosnia and Herzegovina

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Abstract – The aims of this paper were to describe seasonal changes in the qualitative and quantitative composition of diatom taxa and the potential application of benthic diatoms for ecological status evaluation. Diatom indices (IPS and TI) were calculated from data from three different locations along a longitudinal profile of the Bunica, a small karstic river in Bosnia and Herzegovina. A total of 147 taxa were recorded in 12 samples. The most common taxa were *Meridion circulare* (Greville) C.Agardh and *Ulnaria ulna* (Nitzsch) Compère. Physical and chemical analyses showed low concentrations of nutrients, good oxygenation, typical pH for carbonate bed/origin and generally oligotrophic conditions and high ecological status. All sites had similar physico-chemical conditions and there were only few seasonal differences. Ordination of the diatom data showed that samples showed neither longitudinal nor seasonal patterns. Median value for IPS (16.8) and for TI (7.3) can be possible "expected" values for ecological status assessment for small karstic rivers in the Mediterranean region. We propose the use of the *phytobenthos Intercalibration Common Metric* (pICM – an index that combines the IPS and TI) as a national metric for countries developing WFD diatom methods at a late stage. One situation is described, and a solution, which is potentially transferable to other locations in Bosnia and Herzegovina and also to other countries facing similar challenges.

Keywords: Bacillariophyceae, diatom indices, ecological status, Mediterranean river, phytobenthos, pICM

Introduction

The Water Framework Directive (Directive 2000) led to the adjustment of existing assessment systems for water quality and encouraged the design of new classification systems for the ecological status of rivers. The new systems included new approaches to the definition of reference conditions (Pardo et al. 2011, 2012, 2018), the establishment of national and European river typologies, the definition of ecological status class boundaries, and the mandatory intercalibration of boundaries between European countries (Van de Bund 2009). Ecological status assessment has to be type-specific as streams have different biological communities and reference conditions due to their physical and morphological attributes, such as stream size, altitude, catchment geology, etc.

Benthic diatoms are one group of organisms widely used as ecological indicators in water quality and ecological status assessment and monitoring and are widely used as proxies for "phytobenthos" in WFD assessments. Many indices based on species-specific sensitivities and tolerances have been developed to infer the environmental conditions in streams and rivers. The WFD requires that methods are expressed as Ecological Quality Ratios (EQRs), defined as the "observed" (O) value of a metric divided by the value "expected" (E) at reference condition (O/E). In this study two

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diatom indices; IPS (Indice de polluo-sensibilite, CEMAGREF 1982), and TI (Trophic Index, Rott et al. 1999) were used to estimate ecological status in a small karstic river in Bosnia and Herzegovina (BH). It presents the relationship between measured water quality variables in the Bunica River and values of diatom indices. Two diatom metrics (IPS and TI) were compared and then combined into the *phytobenthos Intercalibration Common Metric* (pICM, Kelly et al. 2009).

Also, various diversity indices are used to determine the distribution of benthic diatoms. Diversity index is a statistical method which is planned to evaluate the variety of a data group consisting of different types of components. Number of existing species (richness), distribution of individuals equally (evenness) and total number of existing individuals underlie the basis of diversity indices. Three diversity indices (the Shannon Diversity Index, Simpson Diversity Index, Margalef Diversity Index) and one evenness index (Pielou's Evenness Index) were used in this paper.

The purposes of this paper are: to present the list of benthic diatom assemblages; to assess the seasonal variations and ecological status of the river; to test the use of two diatom indices as tools for estimating the ecological status of small karstic rivers; and to explore the potential for using the pICM as a national metric for countries such as BH which are developing WFD-compatible phytobenthos methods at a relatively late stage.

Material and methods

Study area

The Bunica River is a limestone karstic river in the south of Bosnia and Herzegovina and is a tributary of the Buna River, itself part of the Neretva River basin (Fig. 1). In terms of geology and geomorphology the Bunica River is part of the Dinaric karst or Dinaric carbonate platform – external Dinarides (Milanović 2006). Thick layers of carbonate-formed long carbide precipitation characterize this area. The dominant clasts of the sediment are cobbles and sand. The siphon spring of the Bunica River is the underground

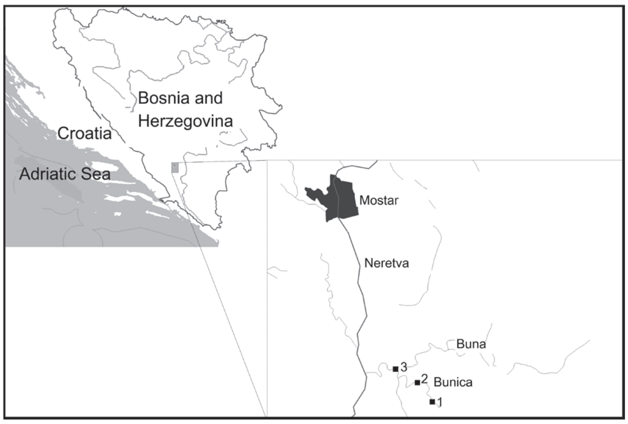

Fig. 1. Sampling sites marked with numbers 1, 2 and 3 along the studied Bunica River, tributary of the river Buna in Bosnia and Herzegovina.

extension of the flow of the Zalomka River (Fig. 1). The siphon is 73 m deep and ranks among the largest sources of the Dinaric karst (Milanović 2006). The minimum and maximum discharges registered for the Bunica Spring were 0.72 and 207 $m^3 s^{-1}$ (Milanović 2006). It is located in an area with a Mediterranean climate, characterized by long, hot summers and rainy winters with rare occurences of snow (Galić 2011). The average annual air temperature is 14.6 °C. The highest daily average temperature is 23.2 °C in July and the lowest below 5.8 °C in January (data for the city of Mostar, for the period 1971–2000, recorded by Meteorological and Hydrological Service of Bosnia and Herzegovina). The total annual precipitation is about 1515 mm. This area has approximately 2291 hours of sunshine per year. Average relative humidity is 69.

So far, diatoms were studied only in the spring area of the Bunica River, revealing high diversity of taxa on artificial (glass slides) and natural substrates (Dedić 2015, Dedić et al. 2015).

Sampling and analyses

The ecological river typology in Bosnia and Herzegovina was developed according to System B of the WFD Annex II, which allows any natural environmental parameter-influencing communities to be included. A total of 216 water bodies of surface waters have been identified in the Adriatic Sea river basin district in the Federation of BH, specifically 211 running waters, four (4) stagnant waters and one (1) coastal water body (Mrđen et al. 2018). Within this typology the Bunica River belongs to type 12, subundertype 12a, small and medium lowland rivers on limestone sediment.

For this study, a total of 12 benthic diatom samples (three sampling locations: the source of the river, the middle course, and the lower course, each each once per season: spring – May, summer – July, autumn – October in 2013, and winter in January 2014) were collected by scraping the biofilm from rock surface, applying the the standard procedure of scraping sludgy material from the rock surface while making sure that the total surface of all rocks is about 500 cm^2 (CEN 2003). The upper surface of each rock was scratched with a scalpel and carefully scrubbed with a toothbrush. The samples were fixed with 4% formaldehyde and stored in appropriately labelled bottles. In the laboratory, samples were treated with concentrated sulphuric acid, potassium-permanganate and oxalic acid (Krammer and Lange-Bertalot 1986). The cleaned suspension was used to make permanent diatom microscope slide preparations. The composition and relative abundance of diatoms were estimated at 1000× magnification, using a Carl Zeiss Jena light microscope. At least 400 valves were counted and identified. Identification and nomenclature were based on the relevant scientific literature and keys: Krammer and Lange-Bertalot (1986–1991), Lange-Bertalot (2001), Krammer (2000–2003), Krammer and Lange-Bertalot (2004). The nomenclature of taxa follows AlgaeBase (Guiry and Guiry 2020).

Physico-chemical parameters (pH, electric conductivity (EC), dissolved oxygen (DO), oxygen saturation (SA) and temperature of water (T)) were measured at the same time that diatom samples were collected using a WTW probe (Wissenschaftliche-Technische Wersättem GmbH & Co. KG-Weilheim). Water samples were also taken and analysed by standard spectrophotometric methods in in the Laboratory of Public Health Mostar to current norms. The following chemical parameters were analyzed: dissolved oxygen (DO), chemical oxygen demand (COD), nitrate (NO_3^-), ammonium (NH_4^+), total nitrogen (TN), total phosphorus (TP), orthophosphate (PO_4^{3-}) and silica (SiO_2). Diatom indices were calculated using OMNIDIA GB 5.3 software (Lecointe et al. 1993). Indices taken into account for the assessment of water quality are those with the highest proportion of species used in the calculation. Values of indices in OMNIDIA software range from 1 to 20, indicating the quality range from polluted to clean water. The following indices were used: IPS (CEMAGREF 1982) and TI (Rott et al. 1999). We estimated diatom diversity using Shannon´s (*H´*), Simpson´s (*1-lambda*), Margalef´s (*d*) diversity indices and Pielou´s Evenness Index (*J*). Indices were calculated with the PAST software (Hammer et al. 2001).

Chemical data were processed using principal component analysis (PCA) to obtain different water quality groups. The variables used in these analyses were pH, electrical conductivity, dissolved oxygen, oxygen saturation, water temperature, chemical oxygen demand, ammonium, nitrate, total nitrogen, total phosphorus, orthophosphate and silica.

Diatom data were analyzed using Primer v.6 (Clarke and Gorley, 2006): hierachical group average clustering, SIMPROF test, Principal Coordinates Analysis (PCO), to represent the distribution of the communities; SIMPER analysis (SIMilarity PERcentage) to estimate the degree of similarity within and among different groups (i.e., reference vs non-reference), and to estimate the individual contribution and the importance of each taxon to the global similarity among groups. These analyses were based on the Bray-Curtis similarity matrix which combines information on the relative abundance of species and faithfulness of the occurrence of species abundances in particular groups.

The *phytobenthos Intercalibration Common Metric* (pICM) was conceived by Kelly et al. (2009) as a means of converting national assessment metrics to a common scale. It is calculated as the average of EQRs (ecological quality ratios) computed using the Indice de Polluosensibilité Specifique (IPS, CEMAGREF 1982) and the Trophic Index (TI: Rott et al. 1999). The pICM is defined as the average of two EQRs: $ICM = (EQR_{IPS} + EQR_{TI})/2$.

The pICM metric responds along a long gradient of inorganic and organic enrichment (Kelly et al. 2009, 2012). For this study we adopted the "expected" values of 16.8 (IPS) and 7.3 (TI) used for streams similar to the karstic streams in this study during the Mediterranean phytobenthos intercalibration exercise (Almeida et al. 2014). We also used the average position of the high/good and good/moderate ecological status class boundaries (0.9 and 0.69) established during this study as a starting point for understanding the ecological condition of these streams in comparison to others in the Mediterranean basin.

Both metrics were calculated using Omnidia version 5.3 (Lecointe et al. 1993) and converted to EQRs by dividing the observed value by the 'expected' value. The pICM was then transformed against national metrics and national EQRs for High/Good and Good/Moderate status boundaries converted to equivalent values of pICM.

Results

Physico-chemical and chemical variables per seasons

The main abiotic variables measured in the Bunica River are shown in Tab. 1. Nutrient concentrations are low, the water is well-oxygenated and pH is typical for water of

Tab. 1. Main abiotic variables in the Bunica River measured in three sites along a longitudinal profile of the Bunica River once per season (spring, summer, autumn and winter in the period from 5th May 2013 to 9th January 2014). Min. – minimum value, Max. – maximum value, Avg. – average value, SD – standard deviation, T – temperature, EC –conductivity, DO – dissolved oxygen, SA – oxygen saturation, COD – chemical oxygen demand, NH_4^+ – ammonium, NO_3^- – nitrate, TN – total nitrogen, SiO_2 – silica, TP – total phosphorus, PO_4^{3-} – orthophosphate.

Parameter/ Station	Bunica 1 (n=4)				Bunica 2 (n=4)				Bunica 3 (n=4)			
	Min.	Max.	Avg.	SD	Min.	Max.	Avg.	SD	Min.	Max.	Avg.	SD
T (°C)	11	14.2	12.8	1.4	11.4	15.5	13.7	1.7	11.4	17	14.6	2.3
pH	7.6	8	7.8	0.17	7.7	7.9	7.8	0.05	7.8	7.9	7.8	0.04
EC (μS cm^{-1})	313	403	372.5	40.4	314	406	371.5	39.8	312	406	369.2	40.3
DO (mg L^{-1})	9.65	12.25	11.28	1.16	11.2	12.7	11.88	0.66	11.43	13.3	12.2	0.84
SA (%)	90.1	119.3	106.9	13.4	90.1	127.9	107.2	15.6	103.4	132	120.8	12.3
COD (mg L^{-1})	0.77	1.28	1.02	0.23	1.02	3.58	2.07	1.17	0.89	2.43	1.47	0.66
NH_4^+ (mg L^{-1})	0	0	0	0	0	0.003	0.0007	0.0015	0	0.003	0.0007	0.001
NO_3^- (mg L^{-1})	0.18	0.45	0.35	0.12	0.29	0.49	0.37	0.08	0.27	0.43	0.36	0.067
TN (mg L^{-1})	0.30	0.61	0.47	0.13	0.37	0.55	0.47	0.07	0.37	0.52	0.45	0.062
SiO_2 (mg L^{-1})	2.68	4.8	3.63	0.97	3.10	4.71	4.04	0.68	3.14	4.20	3.86	0.48
TP (mg L^{-1})	0.006	0.011	0.008	0.002	0.004	0.01	0.007	0.0009	0.006	0.008	0.0066	0.0009
PO_4^{3-} (mg L^{-1})	0.002	0.005	0.0035	0.00	0.002	0.003	0.0026	0.0007	0	0.005	0.0018	5.13

carbonate bed/origin and generally oligotrophic conditions. Water quality corresponds to natural status without anthropogenic influence, following Anonymous (2014) and should support high ecological status.

Differences between sites along the Bunica River, and fluctuations among the seasons were not pronounced. Water temperature ranged from 11–17 °C (Tab. 1) and showed relatively low values for all the seasons. The lowest seasonal fluctuations were recorded at the spring. Dissolved oxygen concentration (9.65–13.3 mg L^{-1}) and saturation (90.1–132%) (Tab. 1) were high at all sites and in all seasons. Conductivity ranged from 312 to 406 µS cm^{-1} (Tab. 1) with the lowest values in spring, and the highest in winter. All sites had slightly alkaline pH values (7.6 – 8) (Tab. 1). The most significant positive correlation was achieved among total nitrogen and silica ($r = 0.9$, $P < 0.01$), and negative correlation was found for total nitrogen and oxygen saturation ($r = 0.677$, $P < 0.01$). Multivariate analysis of the main chemical variables (PCA) in three dimensions accounted for 74.2% of the variance. The first three axes (PC1, PC2 and PC3) accounted for 45.4%, 18.9%, and 9.9% of the variance, respectively (Tab. 2). The most important parameters for the PCA axis 1 were TN and NO_3^-, with the coefficients in the linear combinations of variables of 0.400 for TN, and 0.374 for NO_3^-. The variables that weighted most for ordination for the PCA axis 2 were the COD and PO_4^{3-} (coefficients in the linear combinations of variables: –0.477 and 0.432, respectively). The first axis differentiated spring and summer samples with low scores on PC1 from autumn and winter samples with high PC1 scores (Fig. 2).

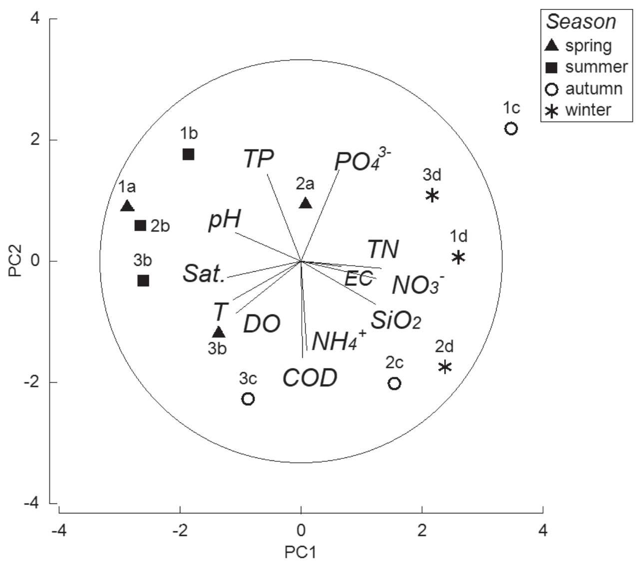

Fig. 2. Principal component analysis (PCA) ordination diagram performed on the environmental parameters for all sampling stations (1, 2, 3) in the Bunica River during four periods (a-spring, b-summer, c-autumn, d-winter) and overlapped with Pearson correlation vectors with PC1 and PC2 axes ($r > 0.4$). Abbreviations: EC – conductivity, T – temperature, NH_4^+ – ammonium, NO_3^- – nitrate, TN – total nitrogen, SiO_2 – silica, PO_4^{3-} – orthophosphate, TP – total phosphorus, COD – chemical oxygen demand, DO – dissolved oxygen, Sat. – oxygen saturation.

Tab. 2. Characteristic values of all five axes of principal component analysis (PCA) with percentage variance for a total of 12 investigated parameters.

PCA	1	2	3	4	5
Eigenvalues	5.44	2.27	1.19	1.04	0.872
% variation	45.4	18.9	9.9	8.7	7.3
Cumulative % variation	45.4	64.3	74.2	82.9	90.1

Analysis of benthic diatoms

In total, 147 diatom taxa from 43 genera were identified in the 12 samples from the Bunica River (Tab. 3). The genera with the highest number of taxa were *Gomphonema* (23 taxa), *Navicula* (18), *Cymbella* (10), *Nitzschia* (10) and *Cocconeis* (7). *Meridion circulare* (Greville) C.Agardh and *Ulnaria ulna* (Nitzsch) P.Comparé, recorded in all samples, followed by *Cocconeis placentula* Ehrenberg, *C. euglypta* (Ehrenberg) Grunow, *C. lineata* Ehrenberg, *Diatoma vulgaris* Bory de Saint-Vincent, *D. vulgaris* var. *capitulata* Grunow, *D. vulgaris* var. *producta* Grunow, *Encyonema ventricosum* (C.Agardh) Grunow, *E. silesiacum* (Bleisch) D.G. Mann, *Gomphonema olivaceum* (Hornemann) Brébisson, *Melosira varians* C.Agardh, *Navicula tripunctata* (O.F. Müller) Bory de Saint-Vincent, *Rhoicosphenia abbreviata* (C. Agardh) Lange-Bertalot and *Navicula cryptocephala* Kützing. All sampling stations had a similar species richness in all seasons. The number of taxa at Bunica 1 (spring area) ranged from 30 (in winter and summer) to 43 (in autumn), from 33 (spring) to 53 (autumn) for Bunica 2 (middle part of the river) and 40 (summer) to 49 (autumn) for Bunica 3 (the mouth of river). Values of Diversity and Evenness Indices per samples are shown in Tab. 4. Shannon's Diversity Index ranged from 1.80 to 2.75, Simpson's Diversity Index from 0.69 to 0.90, Margalef's Diversity Index from 4.50 to 8.42 and Pielou's Evenness Index from 0.20 to 0.41. The higest values of indices were recorded for the middle part of river (Bunica 2), mostly in summer (Tab. 4). The lowest values were in spring but for different locations (Tab. 4).

SIMPER analyses showed high disimilarity among the seasons. The dissimilarities among the samples in different seasons ranged from 60.83% (spring-summer) to 70.24% (autumn-winter). The spring group showed 36.8% similarity, and was characterized by *Melosira varians* (MVAR), *Diatoma vulgaris* var. *capitulata* (DVCA), *Meridion circulare* (MCIR) and *Gomphonema olivaceum* (GOLI). The summer group showed 37.77% similarity, characterized by *Encyonema ventricosum* (EVEN), *Navicula tripunctata* (NTPT), and *Cocconeis placentula* (CPLA). Autumn samples showed the lowest similarity, 35%, with *C. euglypta* (CPLE), *Achnanthes* sp. (ACSP) and *M. circulare* (MCIR) as the most typical species. Winter samples had 35.9% similarity and were characterized by *M. circulare* (MCIR), *Ellerbeckia arenaria* (EARE) and *C. placentula* (CPLA). Similar results were obtained for the analysis of longitudinal gradient/locations with dissimilarities ranging from 64.79% between the first and second sites to 68.6%, between the second and third locations.

Tab. 3. Identified diatom taxa with their maximum abundances (%) in a total of 12 samples in the Bunica River.

Taxon	Max (%)
Achnanthes inflata (Kützing) Grunow	8.33
Achnanthes sp.	83.33
Achnanthidium minutissimum (Kützing) Czarnecki	16.67
Achnanthidium pyrenaicum (Hustedt) H.Kobayasi	41.67
Amphipleura pellucida (Kützing) Kützing	8.33
Amphora ovalis (Kützing) Kützing	41.67
Amphora pediculus (Kützing) Grunow	58.33
Amphora sp.	8.33
Aneumastus sp.	8.33
Aneumastus tuscula (Ehrenberg) D.G.Mann et A.J.Stickle	25
Aulacoseira italica (Ehrenberg) Simonsen	41.67
Brachysira microcephala (Grunow) Compère	16.67
Brebissonia lanceolata (C.Agardh) Mahoney et Reimer	16.67
Caloneis bacillum (Grunow) Cleve	8.33
Caloneis placentula Ehrenberg	8.33
Caloneis silicula (Ehrenberg) Cleve	8.33
Caloneis ventricosa var. *truncatula* (Grunow) Meister	33.33
Campylodiscus noricus Ehrenberg ex Kützing	8.33
Cocconeis euglypta Ehrenberg	91.67
Cocconeis lineata Ehrenberg	91.67
Cocconeis pediculus Ehrenberg	66.67
Cocconeis placentula Ehrenberg	83.33
Cocconeis placentula var. *klinoraphis* Geitler	66.67
Cocconeis pseudolineata (Geitler) Lange-Bertalot	8.33
Cocconeis robusta A. Jurilj	25
Cyclotella meneghiniana Kützing	8.33
Cyclotella sp.	75
Cymatopleura apiculata W. Smith	8.33
Cymatopleura elliptica (Brébisson) W. Smith	25
Cymbella affinis Kützing	33.33
Cymbella excisa Kützing	8.33
Cymbella excisiformis Krammer	8.33
Cymbella hungarica (Grunow) Pantocsek	8.33
Cymbella hustedtii var. *crassipunctata* Lange-Bertalot et Krammer	8.33
Cymbella laevis Nägeli	25
Cymbella parva (W.Smith) Kirchner	16.67
Cymbella parvula Krasske	8.33
Cymbella sp.	16.67
Cymbella subleptoceros Krammer	16.67
Denticula elegans Kützing	8.33
Denticula tenuis Kützing	33.33
Denticula tenuis var. *crassula* (Nägeli) Hustedt	41.67
Diatoma vulgaris Bory	91.67
Diatoma vulgaris var. *capitulata* Grunow	83.33
Diatoma vulgaris var. *producta* Grunow	8.33
Diploneis oblongella (Nägeli ex Kützing) A. Cleve	25
Ellerbeckia arenaria (Moore ex Ralfs) R.M.Crawford	66.67
Encyonema leibleinii (C. Agardh) W.J.Silva, R.Jahn, T.A.Veiga Ludwig et M.Menezes	25
Encyonema minutum (Hilse) D.G.Mann	8.33
Encyonema silesiacum (Bleisch) D.G.Mann	66.67
Encyonema ventricosum (C. Agardh) Grunow	91.67
Encyonopsis microcephala (Grunow) Krammer	8.33
Epithemia adnata var. *saxonica* (Kützing) R.M.Patrick	8.33
Epithemia muelleri Fricke	25
Epithemia sp.	8.33
Epithemia turgida (Ehrenberg) Kützing	16.67
Eunotia arcus Ehrenberg	16.67
Eunotia valida Hustedt	8.33
*Fragilaria vaucheria*e (Kützing) J.B.Petersen	41.67
Fragilaria recapitellata Lange-Bertalot et Metzeltin	8.33
Fragilaria sp.	41.67
Gomphonema acuminatum Ehrenberg	33.33
Gomphonema angustum C. Agardh	33.33
Gomphonema apicatum Ehrenberg	8.33
Gomphonema augur Ehrenberg	41.67
Gomphonema auritum A.Braun ex Kützing	8.33
Gomphonema calcareum Cleve	25
Gomphonema capitatum Ehrenberg	8.33
Gomphonema constrictum Ehrenberg	8.33
Gomphonema gracile Ehrenberg	8.33
Gomphonema grunowii R.M.Patrick et Reimer	8.33
Gomphonema micropus Kützing	8.33
Gomphonema minutum (C.Agardh) C.Agardh	8.33
Gomphonema montanum (J. Schumann) Grunow	8.33
Gomphonema olivaceum (Hornemann) Brébisson	91.67
Gomphonema parvulum (Kützing) Kützing	16.67
Gomphonema productum (Grunow) Lange-Bertalot et Reichardt	8.33
Gomphonema pseudoaugur Lange-Bertalot	8.33
Gomphonema pumilum (Grunow) E. Reichardt et Lange-Bertalot	8.33
Gomphonema sp.	50
Gomphonema subclavatum (Grunow) Grunow	25
Gomphonema tergestinum (Grunow) Fricke	25
Gomphonema truncatum Ehrenberg	33.33
Gyrosigma acuminatum (Kützing) Rabenhorst	41.67
Gyrosigma attenuatum (Kützing) Rabenhorst	8.33
Gyrosigma sciotoense (W.S.Sullivant) Cleve	8.33
Gyrosigma sp.	16.67
Halamphora veneta (Kützing) Levkov	8.33
Hantzschia amphioxys (Ehrenberg) Grunow	25
Iconella helvetica (Brun) Ruck et Nakov	33.33
Iconella linearis (W.Smith) Ruck et Nakov	25
Iconella spiralis (Kützing) E.C.Ruck et T.Nakov	8.33
Melosira sp.	33.33
Melosira varians C.Agardh	91.67
Meridion circulare (Greville) C.Agardh	100
Navicula amphibola Cleve	8.33
Navicula capitatoradiata H.Germain ex Gasse	8.33
Navicula cari Ehrenberg	16.67

Tab. 3. Continued

Taxon	Max (%)
Navicula cincta (Ehrenberg) Ralfs	16.67
Navicula cryptocephala Kützing	75
Navicula cryptonella Lange-Bertalot	16.67
Navicula jakovljevicii Hustedt	16.67
Navicula lanceolata Ehrenberg	8.33
Navicula microdigitoradiata Lange-Bertalot	8.33
Navicula oblonga (Kützing) Kützing	25
Navicula radiosa Kützing	50
Navicula reinhardtii (Grunow) Grunow	50
Navicula tripunctata (O.F.Müller) Bory de Saint-Vincent	91.67
Navicula trivialis Lange-Bertalot	25
Navicula veneta Kützing	8.33
Navicula viridula (Kützing) Ehenberg	8.33
Navicymbula pusilla (Grunow) K.Krammer	8.33
Navigeia decussis (Østrup) Bukhtiyarova	8.33
Neidium dubium (Ehenberg) Cleve	8.33
Nitzschia acicularis (Kützing) W.Smith	16.67
Nitzschia capitellata Hustedt	8.33
Nitzschia dissipata (Kützing) Rabenhorst	8.33
Nitzschia linearis W. Smith	41.67
Nitzschia palea (Kützing) W.Smith	16.67
Nitzschia pusilla Grunow	8.33
Nitzschia recta Hantzsch ex Rabenhorst	8.33
Nitzschia sigmoidea (Nitzsch) W.Smith	33.33
Nitzschia sp.	41.67
Nitzschia sublinearis Hustedt	41.67
Odontidium mesodon (Kützing) Kützing	33.33
Pinnularia microstauron (Ehrenberg) Cleve	8.33
Pinnularia sudetica Hilse	16.67
Pinnularia sp.	8.33
Placoneis gastrum (Ehrenberg) Mereschkovsky	8.3
Planothidium hauckianum (Grunow) Round et Bukhtiyarova	8.33
Planothidium lanceolatum (Brébisson ex Kützing) Lange-Bertalot	25
Reimeria sinuata (W. Gregory) Kociolek et Stoermer	16.67
Rhoicosphenia abbreviata (C. Agardh) Lange-Bertalot	91.67
Sellaphora bacillum (Ehrenberg) D.G.Mann	41.67
Sellaphora gregoryana (Cleve et Grunow) Metzeltin et Lange-Bertalot	8.33
Staurosira construens Ehrenberg	8.33
Staurosira venter (Ehrenberg) Cleve et Moeller	16.67
Staurosirella lapponica (Grunow) D.M.Williams et Round	8.33
Surirella angustata Kützing	41.67
Surirella grunowii Kulikovskiy, Lange-Bertalot et Witkovski	16.67
Surirella librile (Ehrenberg) Ehrenberg	33.33
Surirella minuta Brébisson ex Kützing	8.33
Tryblionella angustata W.Smith	8.33
Ulnaria acus (Kützing) Aboal	8.33
Ulnaria danica (Kützing) Compère et Bukhtiyarova	41.67
Ulnaria oxyrhynchus (Kützing) M.Aboal	25
Ulnaria ulna (Nitzsch) Compère	100

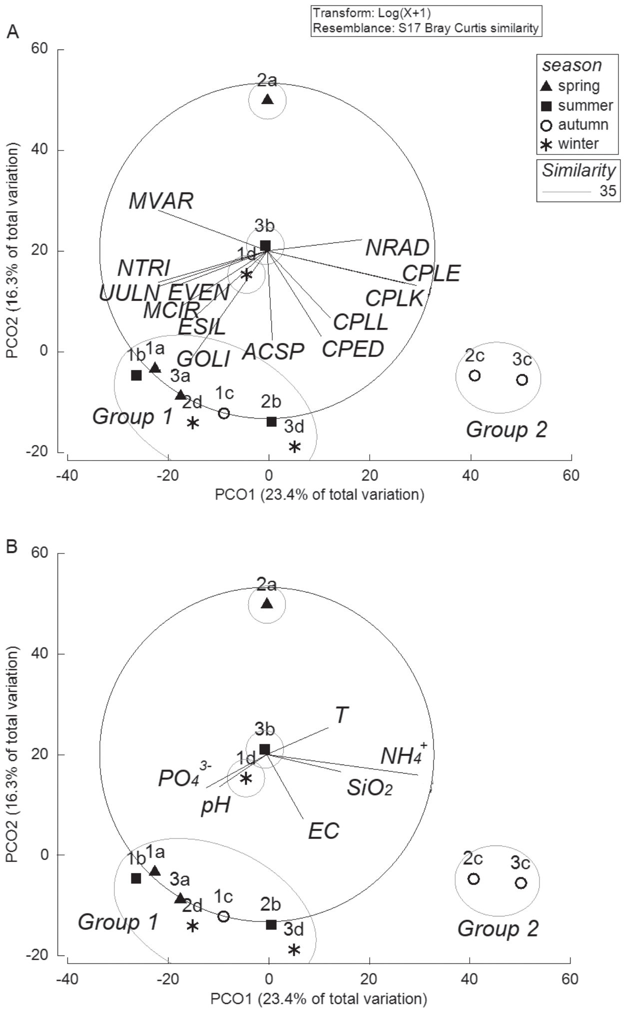

Fig. 3. Principal coordinates analysis (PCO) diagram comparing the community composition between samples in different seasons. PCO plot coded by the species vectors indicates taxa (A) and environmental vectors (B) positively correlated to PC1 and PC2 axes ($r > 0.5$; $r > 0.3$, respectively), and overlapped with the cluster groups based the 35% resemblance level of the group average clustering. Abbreviations: ACSP – *Achnanthes* sp., CPED – *Cocconeis pediculus*, CPLL – *C. lineata*, CPLK – *C. placentula* var. *klinoraphis*, CPLE – *C. euglypta*, MVAR – *Melosira varians*, EVEN – *Encyonema ventricosum*, ESIL – *E. silesiacum,* GOLI – *Gomphonema olivaceum*, MCIR – *Meridion circulare*, NRAD – *Navicula radiosa*, NTRI – *N. tridentula*, UULN – *Ulnaria ulna*; T – temperature, NH_4^+ – ammonium, SiO_2 – silica, EC –conductivity, PO_4^{3-} – orthophosphate.

However, similarity in species compostion was much higher between sampling periods at the first location of Bunica River (40.1%) than at the second (29%) or third (25.7%) locations. The most abundant taxa at Bunica 1 were: *E. ventricosum*, *E. silesiacum* and *Planothidium lanceolatum*; at Bunica 2 *Halamphora veneta, C. euglypta* and *C. placentula* and at Bunica 3 *G. olivaceum*, *Achnanthes* sp. and *C. placentula* var. *klinoraphis.* Hierarchical group average clustering and the SIMPROF test followed by SIMPER analysis identified two groups and three ungrouped samples. Figure 3 shows the results of PCO analysis conducted using a Bray Curis similarity matrix and overlain with hierarchical clustering with

Tab. 4. Values of diversity and evenness indices in 12 samples at three stations on the Bunica River. *H´* – Shannon's Diversity Index, 1-lambda – Simpson's Diversity Index, *d* – Margalef's Diversity Index, *J* – Pielou's Evenness Index.

Station	Season	Sample code	*H´*	1-lambda	*J*	*d*
BUNICA 1	spring	1a	1.94	0.71	0.24	4.50
	summer	1b	2.51	0.88	0.41	4.82
	autumn	1c	2.17	0.80	0.20	6.94
	winter	1d	1.88	0.69	0.21	4.82
BUNICA 2	spring	2a	1.85	0.69	0.20	5.40
	summer	2b	2.75	0.91	0.41	6.15
	autumn	2c	2.72	0.88	0.29	8.42
	winter	2d	2.64	0.87	0.35	6.48
BUNICA 3	spring	3a	2.44	0.85	0.25	7.27
	summer	3b	2.26	0.81	0.25	6.13
	autumn	3c	2.40	0.81	0.22	8.09
	winter	3d	2.11	0.77	0.20	6.45

Tab. 5. Values of *phytobenthos Intercalibration Common Metric* (pICM) and water quality classes according to diatom indices (IPS and TI) and chemical conditions at three stations on the Bunica River. IPS – Specific Pollution-sensitivity Index (CEMAGREF 1982), TI –Trophic Index (Rott et al. 1999), NH_4^+ – ammonium, NO_3^-– nitrate, TN – total nitrogen, TP – total phosphorus.

Station	Season	Sample code	IPS	TI	pICM	NH_4^+	NO_3^-	TN	TP
BUNICA 1	spring	1a	II	V	0.57	I	I	I	I
	summer	1b	I	V	0.64	I	I	I	I
	autumn	1c	I	V	0.77	I	I	I	I
	winter	1d	II	V	0.47	I	I	I	I
BUNICA 2	spring	2a	IV	V	0.21	I	I	I	I
	summer	2b	II	V	0.61	I	I	I	I
	autumn	2c	I	III	1	I	I	I	I
	winter	2d	II	V	0.61	I	I	I	I
BUNICA 3	spring	3a	I	V	0.63	I	I	I	I
	summer	3b	II	IV	0.74	I	I	I	I
	autumn	3c	I	IV	0.76	I	I	I	I
	winter	3d	I	V	0.70	I	I	I	I

vectors given by the Pearson correlation coefficient with environmental data and most significant taxa. The first group on the plot included all samples from location 1, except winter samples, summer and winter samples from location 2, and spring and winter samples from location 3. This group was characterized by *G. olivaceum* (GOLI), *E. ventricosum* (EVEN), and *M. circulare* (MCIR) up to 35% cumulative contribution and is associated with lower values of PO_4^{3-}, consumption of chemical oxygen demand (COD) and electrical conductivity (EC). The second group is composed of autumn samples from the second and third location and was characterized by *C. euglypta* (CPLE) and *C. placentula* var. *klinoraphis* (CPLK) with up to 50% cumulative contribution; these samples are associated with higher values of SiO_2 and NO_3^-. The ungrouped samples were 1d (dominated by P. *lanceolata (*ALAN) and *C. placentula* (CPLA), 2a with high abundance of *Ulnaria oxyrhynchus* (UOXY) and *H. veneta* (AVEN) and 3b, dominated by *Cymbella laevis* (CLAE) and *Nitzschia pusilla* (NIPU).

Diatom indices

The data were used to calculate values of IPS and TI for all samples. IPS calculations used 95–100% of diatoms per sample whilst TI calculations used 65–95%. The values of indices for IPS ranged from 8.9 to 18.5 and for TI from 4.9 to 12.6. The two indices were not significantly correlated with each other or with measured physical and chemical parameters ($P > 0.05$). The pICM values ranged from 0.21 (2a) to 1 (2c) (Tab. 5), with mean values suggesting good status at Bunica 3 but not at the other two sites (based on an intercalibrated boundary value of 0.69). The concentrations of physical and chemical parameters should mean that the Bunica River can support high ecological status (Tab. 5), so the reason for this mismatch needs to be explored. Biotic index classes assigned by Omnidia showed variations in results from high to low ecological status. IPS values mostly in autumn season on all sites referred to the first category, while TI values mostly varied from fourth to fifth category, indicating extremely high trophic status (Tab. 5). However, these classes are over-simplistic and do not give nuanced insights into conditions in any particular river. The percentage of pollution tolerant taxa for site 1 were 0.3% in spring, 1.8% in summer, while for autumn and winter they were not registered. For site 2 there were 39.7 pollution tolerant taxa registered and for site 3 those taxa were not recorded.

Discussion

In this paper the benthic diatom assemblages in karstic river in BH were studied in order to estimate ecological status and to test the future applicability of diatom indices. Benthic diatoms have been recommended in recent decades as appropriate tools for pollution assessment in rivers (e.g., Coste et al. 1991, Whitton and Kelly 1995), and have been used worldwide (Porter et al. 2008, Mangadze et al. 2015). Although our results indicate that the nutrient concentrations of the water do not determine the composition and structure of the benthic diatom assemblage in the Bunica River, this is likely to be due to the short gradient encountered during this study. All sites in all seasons had low nutrient concentrations and good oxygenation. The high oxygen saturation and water transparency, as well as low nutrient concentrations, indicate the oligotrophic character of the river. For the investigated stations in different seasons, no major seasonal fluctuations and differences in physical and chemical parameters were identified, which is probably due to the proximity and impact of springs along this short river (just 5.8 km in length). Water temperature (11–17 °C) showed relatively low values for all seasons. The smallest fluctuations were closest to the spring due to the temperature stability of spring water. High values of dissolved oxygen (9.65–13.3 mg L^{-1}) and saturation (90.1–132%) are due to lower water temperature, porosity of the rock aquifer and contact between groundwater and the atmosphere (Cantonati 1998). Higher conductivity values indicate higher mineralization and our results suggest that the Bunica River has moderate mineralization. Physical and chemical values in this study are in accordance with values for limestone substrate (Mogna et al. 2015).

The analysis of the diatom assemblages revealed high species diversity. The investigated sites in the Bunica River were characterized by similar numbers of diatom taxa, and high species diversity indicates that there were favorable conditions for development. A similar number of diatom taxa (117) was recorded in the Mura River (Krivograd-Klemenčić and Balabanič 2010), while in the Bunica spring 104 (Dedić et al. 2015) and 87 (Hafner and Jasprica 2010) taxa were recorded. Although this is not universally applicable, similar correspondence between low levels of pollution and high species diversity were also observed by Noga et al. (2014). The Bunica River has a low level of organic pollution and it is not impacted by many anthropogenic stressors (high density of population, livestock farming, developed industry, etc.). This was also supported by diversity and evenness indices. Although values of indices varied for longitudinal and temporal patterns they indicated that the structure of habitats were stabile and balanced. Their variations reflect the heterogeneity of the diatom population. Those genera that were well represented in this study (*Gomphonema*, *Navicula* and *Nitzchia)* were also well represented in other studies of karstic rivers (Wojtal 2009, Dedić et al. 2015). All three genera are found across the whole range of the Bunica River, with particular species favouring different levels of ions and nutrients. *Meridion circulare* and *Ulnaria ulna* were recorded in all samples. *Meridion circulare* is a widespread, mesosaprobous and oligo to eutraphentic alkaliphilous diatom (Van Dam et al. 1994). *Ulnaria ulna* occurs in oligo- to polytrophic and oligo-saprobic to α-mesosaprobic waters (Hofmann et al. 2013) and is also classified as alkaliphilous (Van Dam et al. 1994). The occurence of large numbers *Halamphora veneta* in a spring at the middle location is, however, very unusual. It occurs in fresh and slightly brackish water. According to Van Dam et al. (1994) it is an alpha/polisaprobic and eutrophic taxa.

Taxa of the genera *Amphora*, *Cocconeis* and *Diatoma,* frequently found in this research, have a high indicator values for pH, organic nitrogen, oxygen, saprobity and trophic state (Van Dam et al. 1994). Diatoms are influenced by many factors including those that are site-specific on various temporal and species scales (DeNicola et al. 2004) as well as those that reflect human interventions in the environment. Those factors include chemical properties of the water (Potapova 1996), nutrient load (Rott et al. 1997) and also flow velocity and substratum type (Rimet 2009). The results of this study showed that physical and chemical variables and seasons did not have a notable effect on the diversity of benthic diatoms in the Bunica River. It is possible that hydromorphological and other water conditions such as flow velocity, type of substrates, discharge of water, marginal vegetation in karstic river also influenced the structure and composition of benthic diatom assemblages more than physical and chemical variables.

IPS and TI did not always give consistent results along the entire length of the Bunica River. One important reason is that they were developed for different purposes – IPS measures "general degradation" whilst TI was developed, specifically, to measure the impact of inorganic nutrients. IPS index showed better water quality along the entire length of the Bunica river (mostly moderate, II and III class) compared with the TI trophic index, which indicated a poor or bad ecological status of the river (IV-V class). This variation has also been noted by others, Kitner and Poulícková (2003), Poulíčková et al. (2004), and Stenger-Kovács et al. (2007), Szczepocka and Szulc (2009), Kalyoncu and Şerbetci (2013). Because the indices were developed for different purposes, naive division of the scale into equal-sized segments, though an easy option (e.g., Eloranta and Soininen 2002), is unlikely to give meaningful results or consistency between indices. Others have noted that indices developed in certain regions of Europe were not effective in others (Pipp 2002, Rott et al. 2003).

Our study also noted a mismatch between status assessment using the average pICM boundary set during the Mediterranean intercalibration exercise (Almeida et al. 2014) and observations in this study. The nature of the Bunica River is such that we should estimate high or good status based on diatom assemblages, whereas our results indicate less than good status at two of the sites. Kamberović et al. (2019 a) used TI index in a study of springs on Konjuh Mountain (BH) and reached similar conclusions about the suitability of existing indices, recommending some modifications to the TI. Our study has also identified potential mismatches between ecological status boundaries used elsewhere in the Mediterranean basin and those used in BH, suggesting a need to recalibrate the pICM, if it is to be used successfully here. Based on this study, we recommend "expected" values for pICM calculations of 16.8 and 7.3 for IPS and TI, respectively.

Diatom indices have been shown to be one of the most effective tools for evaluating ecological status in European rivers (Eloranta and Soininen 2002, Kelly et al. 2008); however, further testing of IPS and TI diatom indices was needed to ensure their utility in small karstic rivers in BH. The suitability of indices depends, to some extent, on the percentage of taxa used for index calculation. In this study 95–100% of taxa were used for the IPS calculation, suggesting that it is highly suitable. For the TI, a smaller percentage was used (60–90%). In the study of epiphytic diatoms in Lake Modrac in northeastern BH, Kamberović et al. (2019b) also concluded that the IPS and TI were most suitable. However, consideration should also be given to how sensitivity values are assigned to taxa and, in this respect, the IPS does not have a transparent process for determining such scores whilst the TI has never been updated since first published (Rott et al. 1999). On the other hand, both are widely used around Europe (Poikane et al. 2016) and, therefore provide a means by which results from BH can be compared with data from other countries. Rather than develop a new index, therefore, we have adopted the "*phytobenthos Intercalibration Common Metric*" (pICM, Kelly et al. 2009), based on these two metrics, as an interim tool for evaluating ecological status using phytobenthos in BH. These values are similar to those proposed for Mediterranean streams by Kelly et al. (2012), and higher similarities were recorded in IPS than in TI, but have the benefit for being more closely tuned to local conditions within BH.

Conclusions

The Bunica River has a high diversity of benthic diatom taxa (147). Physical and chemical analyses indicated oligotrophic conditions in all locations and high ecological status. Measured physical and chemical parameters and seasons did not have a notable effect on the biological diversity of benthic diatoms. It is possible that hydromorphological and other water conditions such as flow velocity, type of substrates, discharge of water, marginal vegetation had a greater influence on structure and composition of benthic diatom taxa than physical and chemical variables. The diatom indices IPS and TI were used in our study and we concluded that combining them into the "*phytobenthos Intercalibration Common Metric*" (pICM) gave us more results that were more easily compared with those from elsewhere in Europe than would have been the case if only a single index had been used. We therefore recommend pICM as a good starting point for ecological status assessment in BH and propose it as a potential national metric for phytobenthos assessment in the future. However, testing on a larger number of sites and along a longer gradient of ecological quality is necessary before this can be confirmed.

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