COMPARATIVE ANALYSIS OF EPILITHIC DIATOM ASSEMBLAGES OF SPRINGS AND STREAMS IN THE KONJUH MOUNTAIN (BOSNIA AND HERZEGOVINA)
Komparativna analiza epilitskih dijatomejskih zajednica u izvorima i potocima planine Konjuh (Bosna i Hercegovina)

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Abstract
Springs are recognized as hotspots for freshwater biodiversity conservation. The study objective was to analyze and compare the biodiversity of epilithic diatom communities of spring and stream habitats in the Konjuh Mountain (B&H). The measuring of basic water physical and chemical parameters and diatom sampling were carried out during summer and autumn period in 2013.
Diatom assemblages were collected from 10 locations: (i) five locations were placed in small mountain springs that flow into headwater streams, where another 5 localities were placed. Diatoms were collected by scrubbing the rocks using standard methodology. Relative diatom cell abundances were established by counting up to 400 frustules on permanent slides prepared in Naphrax.
Spring habitats were more diverse in diatoms ($H^\prime_{\log_{e}}=1.67$ average) in comparison with headwater stream habitats ($H^\prime_{\log_{e}}=1.15$ average), which was confirmed by t-test ($t=2.79; p=0.013$). The similarities of community structure from different habitats were investigated by non-metric multidimensional scaling, on the Bray-Curtis similarity matrix in software package Primer 6.
Using Permutational Manova test, a different diatom composition was proved between spring and stream communities (Pseudo $F=2.66$, $p=0.012$), while the seasonal difference in the community structure was not confirmed ($F=0.586$, $p=0.876$). The most abundant and frequent species in spring habitats were: Achnamthidium minutissimum (Kützing) Czarnecki, Cocconeis pseudolineata (Geitler) Lange-Bertalot, Gomphonema pumilum var. elegans E.Reichardt & Lange-Bertalot, Meridion circulare (Greville) C.Agardh and Planothidium lanceolatum (Brébisson ex Kützing) Lange-Bertalot. The most frequent and abundant species in headwater stream habitats were: Achnamthidium minutissimum (Kützing) Czarnecki, Cocconeis placentula var. euglypta (Ehrenberg) Grunow, Cocconeis placentula var. lineata

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(Ehrenberg) van Heurck and *Cymbella tridentina* Lange-Bertalot, M.Cantonati & A.Scalfi.

In comparison with streams habitats, the springs are heterogeneous with specific diatom composition due to more stable water flow. Although the springs cover very small areas, the diversity of species that inhabit them must not be neglected in planning the use of these natural resources.

**Key words**: diversity, epilithic diatoms, springs, streams, habitats, Konjuh Mountain (*B&H*)

**INTRODUCTION - Uvod**

Benthic or periphytic algae are very important primary producers in lotic ecosystems, and they are also very important biological indicators of water quality due to their possibility of rapid reproduction and quick response to environmental changes (Lowe and Pan 1996).

Depending on the colonized substrate type, periphytic algal communities are divided into: epilithic, epiphytic, epipsamic, and epipelic algal stream communities. According to Stevenson (1996) epilithic algae are defined as those which grow on hard, relatively inert substrata, such as gravel, pebble, cobble, and boulder.

Recently, spring areas have been identified as important refuge for conservation of algal biodiversity (Cantonati et al. 2012a), and investigations of these habitats have been intensified since the mid of the nineties (Sabater and Rocca 1990; Cantonati 1998; Aboal et al. 1998; Werum and Lange-Bertalot 2004; Poulíčková et al. 2005; Woutal and Sobczuk 2006; Ózvööck and Preisig 2007; Frankova et al. 2009; Cantonati et al. 2012b, 2012c).

Several studies have focused on the research of the algal community structure in the spring - fed stream complex, such are those in Canada (Sherwood et al. 2000), in Texas in the USA (Sherwood and Sheath 1998), in Majorca (Delgado et al. 2013), in Adamelo Brenta region in Italy (Cantonati and Pip 2000), in Dolomiti Bellunesi National Park in Italy (Cantonati and Spitale 2009). Crenal communities of algae and macrophytes are studied in spring habitats of the Konjuh Mountain in Bosnia and Herzegovina (Kamberović 2015). However, diatom assemblages in stream epilithic habitats have not been compared with diatom assemblages in springs before. In general, researches of spring algal communities in Bosnia and Herzegovina are scarce and rare (Blagojević 1974, 1976a, 1976b; Kapetanova and Hafner 2007; Kapetanova et al. 2011; Đedić et al. 2015).

The objective of this study was to analyze and compare the biodiversity of epilithic diatom communities of five spring and five stream habitats in the Konjuh Mountain in northeastern Bosnia and Herzegovina.
METHODS - Metode

Diatom sampling was carried out in summer (July and August) and in autumn (October) by scrubbing the rocks (epilithon) from five to six cobbles in 2013 (Kelly et al. 1998). Samples were collected in the place where groundwater comes to the surface (5 spring localities), and in the places after the spring fed-streams flow into streams (5 stream localities). Samples were treated by concentrated sulfuric acid (Hustedt 1930), and cleaned valves were embedded in Naphrax. Light microscopes (Motic and Olympus BX41) and immersion objective (magnification 1000x) for the identification and quantification of algae were used. The determination of algae species was performed using the following sources: Krammer and Lange-Bertalot (1986; 1988; 1991a; 1991b), Krammer (2000; 2001; 2002), Hofmann et al. (2011). Nomenclature of species is in accordance with AlgaeBase (Guiry and Guiry 2016), and the base was accessed on 9 December 2016. Assemblages diversity was quantified with the Shannon – Wiener Diversity Index (logarithms to the base ln; Shannon and Wiener 1949). Temperature, pH and conductivity were measured in situ using a Multimeter Hanna HI 9828. For statistical analysis, the method of non-metric multidimensional scaling (n-MDS) for spring ordering was used. The ordination was conducted on the Bray-Curtis similarity matrix of species data (Legendre and Legendre 1998). The data were transformed by square root to restrict the influence of abundant taxa on the results. Permutational Manova test (Anderson 2001) was used for the comparison of spring and stream diatom assemblages and IndVal analysis for detecting indicator species for spring and stream habitats (Dufrene and Legendre 1997). Statistical analyses were performed in Primer 6.0 (Clarke and Warwick 2001) and PC ORD 5 (Grandin 2006).

STUDY AREA – Područje istraživanja

Konjuh Mountain is a part of the central Dinarides on the Balkan Peninsula, with altitude to 1326 m a.s.l. It belongs to the moderate continental climate zone. The mean annual temperature varies from 9.2 °C – 10.0 °C, and the annual precipitation is from 900 to 1250 mm (according to the closest meteorological station in Kladanj). The investigation was performed on five spring and five stream localities: Tarevo (TA), Miljevica (MI), Gluha Bukovica (GB), Katranica (KA) and Kesovača (KE).

RESULTS - Rezultati

Ecomorphological characteristics of springs and streams

Investigated springs and streams (Table 1) are located on altitudes ranging from 536 to 875 m. a. s. l. The most of the studied springs are small rheocrene. As expected, water temperature was slightly higher in streams than in springs, and in the summer compared with autumn period. Moreover, pH of the water was weakly
alkaline, and it had similar values compared investigated localities. Conductivity was moderately high (ranged between 123 -387 $\mu$Scm$^{-1}$).

**Table 1. Ecomorphological, physical and chemical characteristics of water and species diversity ranging from summer to autumn, R – rheocrenic springs, H-P – hygropetric springs**

| Locality | KE spring | KE stream | MI spring | MI stream | TA spring | TA stream | GB spring | GB stream | KA spring | KA stream |
|----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|
| Coordinates | 44°18'43.52"N 44°19'6.67"N | 44°16'26.98"N 44°19'6.67"N | 44°12'48.91"N 44°14'56.84"N | 44°18'43.52"N 44°19'6.67"N | 44°16'26.98"N 44°19'6.67"N | 44°12'48.91"N 44°14'56.84"N | 44°18'43.52"N 44°19'6.67"N | 44°16'26.98"N 44°19'6.67"N | 44°12'48.91"N 44°14'56.84"N |
| Flow condition | R | R | H-P | R | R | R | H-P | R | R | R |
| Flow type | episodic | steady | steady | steady | steady | steady | episodic | steady | steady | steady |
| Altitude m a.s.l. | 694 | 694 | 815 | 813 | 536 | 536 | 692 | 692 | 875 | 875 |
| Temperature °C | 11.8 | 12.07 | 11.0 | 18.5 | 9.0 | 12.85 | 8.5 | 11.24 | 14.22 | 14.2 |
| pH | 8.7 | 8.3-8.8 | 8.7 | 8.74 | 8.4 | 7.3 | 7.77 | 7.52 | 9.3 | 8.81 |
| Conductivity $\mu$Scm$^{-1}$ | 181 | 190 | 265 | 251 | 401 | 313 | 312 | 312 | 249 | 237 |
| Number of species (N) | 6 - 7 | 4 - 6 | 9 - 3 | 6 | 15-14 | 6 - 4 | 7 - 8 | 8 | 11 - 9 | 5 - 7 |
| Shannon-Wiener Diversity Index (H'ln) | 1.11 | 1.15 | 1.56 | 1.12 | 1.92 | 1.32 | 1.77 | 1.77 | 0.34 |

**Diatoms**

A total of 50 diatom taxa were identified in 19 samples. The summer sample of stream GB was diatom empty, and it was excluded from statistical analysis. The total of 45 taxa in the samples of spring habitats, and 17 taxa in the stream samples were found. In spring habitats following taxa had abundance in average above 5%, and frequency more than 50%: *Achnanthidium minutissimum*, *Cocconeis placenta* var. *lineata*, *Cocconeis pseudolineata*, *Gomphonema pumilum* var. *elegans* and *Planothidium lanceolatum*. In stream habitats taxa with frequency more than 25%, and abundance in average above 5% were: *A. minutissimum*, *Cocconeis placenta* var. *euglypta*, *Cocconeis pseudolineata*, *Cymbella tridentina*, *Gomphonema pumilum* and *Meridion circulare*. The following species have been found exclusively in springs habitats: *Amphora indistincta*, *Achnanthidium affine*, *Achnanthidium straubianum*, *Adlafia minuscula*, *Amphora copulata*, *Amphora ovalis*, *Brachysira* sp., *Cymatopleura solea*, *Humidophilla perpusilla*, *Diploneis krammeri*, *Encyonopsis cesatti*, *Eolimna minima*, *Navicula cryptotenella*, *Navicula antonii*, *Navicula tripunctata*, *Pinnularia subrupestris*, *Planothidium dubium*, *Planothidium*
frequentissimum and Sellaphora pupula. The following species have been found exclusively in stream habitats: Cocconeis placentula var. euglypta, Cocconeis pediculus, Cymbella tridentina, Encyonopsis krammeri and Epithemia frickei. The list of identified taxa and comparison of frequency and abundance of species in springs and streams is given in the Table 2.

Table 2. List of identified diatom taxa in springs and streams, species relative frequency, average abundance, and statistical significance ($p$) of indicator value (IV) given by IndVal analysis for diatoms in the springs and streams, taxa with names in bold were more abundant or had tendency for one of the two researched habitats

Tabela 2. Popis identificiranih dijatomeja u izvorima i potocima, relativna frekvencija vrsta, prosječna abundanca i statistička značajnost indikatorske vrijednosti dobijene IndVal analizom za dijatomeje izvorskih i potočnih staništa, taksoni sa boldiranim nazivima su češće zastupljeni ili imaju afinitet pojavljivanja za jedan od dva istraživana staništa
| Taxa | Relative frequency (%) | Average abundance (%) | IV | p |
|------|------------------------|-----------------------|----|---|
|      | springs                | streams               | Stream habitats summer samples | Stream habitats summer samples | Stream habitats autumn samples |        |    |
| Achnanthidium affine (Grunow) Czarnecki | 10 | 11.1 | 10 | 1 |
| Achnanthidium lineareoides (Lange-Bertalot) | 20 | 3.6 | 3.7 | 20 | 0.48 |
| Achnanthidium minutissimum (Kützing) Czarnecki | 61 | 25 | 17.1 | 26.8 | 29.5 | 8 | 68.8 | 0.16 |
| Adlafia minuscula (Grunow) Lange-Bertalot | 10 | 3.7 | 10 | 1 |
| Amphora copulata (Kützing) Schoeman & R.E.M.Archibald | 10 | 2.5 | 10 | 1 |
| Amphora pediculus (Kützing) Grunow ex A.Schmidt | 50 | 13.6 | 3.2 | 50 | 0.84 |
| Brachysira sp. | 10 | 0.8 | 10 | 1 |
| Cocconeis placentula var. euglypta Ehrenberg | 36 | 27 | 30.9 | 32.2 | 11.6 | 10.4 | 36.2 | 0.78 |
| Diploneis krammeri Lange-Bertalot & E.Reichardt | 10 | 0.8 | 10 | 1 |
| Encyonopsis cesatii (Rabenhorst) Krammer | 11 | 2.0 | 11.1 | 0.48 |
| Encyonopsis fonticola (Hustedt) Krammer | 10 | 0.6 | 10 | 1 |
| Epithemia frickei Reichardt in Lange-Bertalot & Krammer 1987 | 22 | 2.3 | 2.5 | 22.2 | 0.22 |
| Frustulia vulgaris (Thwaites) De Toni | 10 | 0.6 | 10 | 1 |
| Gomphonema pumilum var. elegans | 59 | 23.0 | 1 | 13.6 | 1 | 58.7 | 0.05 |
| Gomphonema tergestinum (Grunow) Fricke | 10 | 1.1 | 10 | 1 |
| Navicula tripunctata (O.F.Müller) Bory de Saint-Vincent | 10 | 0.6 | 10 | 1 |
| Nitzschia dissipata (Kützing) Rabenhorst | 10 | 0.6 | 10 | 1 |
| Nitzschia palea (Kützing) W.Smith | 18 | 1 | 30.8 | 4.3 | 3.3 | 18.1 | 0.59 |
| Nitzschia sigmoidea (Níkrač) W.Smith | 10 | 0.7 | 10 | 1 |
| Phaeothrix abbreviata C.Agardh | 10 | 0.7 | 10 | 1 |
| Planodiscus doshinum (Grunow) Round & B.Bukhtiyarova | 30 | 10.5 | 1.1 | 30 | 0.22 |
| Planodiscus frequentissimus (Lange-Bertalot) Round & L.B.Bukhtiyarova | 20 | 0.8 | 0.3 | 20 | 0.47 |
| Planothidium dubium (Grunow) Czarnecki | 10 | 3.6 | 3.7 | 20 | 0.48 |
| Planodiscus lincolnanus (Brebisson ex Kützing) Lange-Bertalot | 74 | 2 | 19.8 | 0.9 | 12.9 | 8 | 74.4 | 0 |
| Pleurochrysis rotula (C.Agardh) Lange-Bertalot | 10 | 1.5 | 10 | 1 |
| Rhizosolenia abbreviata (C.Agardh) Lange-Bertalot | 13 | 19 | 5.7 | 9.8 | 1.0 | 2.1 | 18.5 | 0.84 |
| Stauroneis smithii (Grunow) Czarnecki | 10 | 0.6 | 10 | 1 |
| Ulnaria ulna (Níkrač) P.Compeyre | 1 | 40 | 1.2 | 0.5 | 0.9 | 39.8 | 0.11 |
The number of taxa per sample varied from 2 to 27, and Shannon - Wiener Diversity Index varied between 0.34 and 2.52, and it indicated low to moderately high species diversity.

Statistically important differences, using t test ($t=2.79$; $p=0.013$), were established between values of Shannon - Wiener Diversity Index for spring habitats (in average $H'=1.67$) and Diversity Index values for stream habitats (in average $H'=1.15$). Based on 996 permutations, using Permutation of residuals under a reduced model (Permutational Manova test), it was proven that diatom assemblages in spring habitats differ from diatom communities in stream habitats ($Pseudo F=2.668$; $p=0.012$). The differences between summer and autumn samples were not statistical significant ($Pseudo F=0.586$; $p=0.879$). Plot of nonmetric multidimensional scaling (nDMS) is shown on the Figure 1. According to IndVal analysis, spring samples were clearly different from stream samples with higher abundance of *Planothidium lanceolatum*, *Amphora indistincta*, *Cocconeis placentula* var. *lineata* and *Gomphonema pumilum* var. *elegans* ($p<0.05$). Seasonal variations on nDMS plot were indistinct. The only exception was spring GB, which is characterized by seasonal variation of discharge during the year. On this locality, 27 taxa were determined in the autumn, while in the summer only seven taxa were found with domination of species *Nitzschia palea*.

Stream habitats are quite heterogeneous, and they unclear grouped on the plot. Typical characteristics of stream samples were lower number of taxa and high values of abundance of several dominant taxa, such as *Achnanthidium minutissimum* (streams MI and TA), *Cymbella tridentina* (stream MI), *Gomphonema pumilum* (stream TA), *Cocconeis placentula* var. *euglypta* (streams TA and KA), *Cocconeis placentula* var. *lineata* (stream KE).
Figure 1. Nonmetric multidimensional scaling (nDMS) ordination of diatoms in 19 samples in 5 spring and 5 stream locality during investigation period. Acronym of the species with higher abundance or indicator species given by IndVal analysis: COCNPLEu - Cocconeis placentula var. euglypta, RHSPABBR - Rhoicosphenia abbreviata, CYMBTRID - Cymbella tridentina, COCNPLi - Cocconeis placentula var. lineata, AMPHPEDI - Amphora pediculus, ACHDMINU - Achnanthidium minutissimum, GOMPPUel - Gomphonema pumilum var. elegans, PLANLANC - Planothidium lanceolatum, AMPHINDS - Amphora indistincta

DISCUSSION - Diskusija

The performed investigation confirms the higher values of diatom species diversity in spring than in stream habitats, which is in accordance with the results of CANTONATI and Pip (2000) and SPITALE et al. (2012). These authors have compared longitudinal and seasonal differences in diatom assemblages in springs on contrasting lithology. Similar to their study results, high domination of rheobiont taxa downstream, such as A. minutissimum or Gomphonema pumilum sensu lato, was established during the investigated period. However, similarities of the identified diatom indicator species for spring habitats and downstream localities in this research were not found in comparison to the previous similar studies. This can be explained by
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heterogeneity and mosaicism of spring habitats, which had previously accentuated as very important characteristics of springs (CANTONATI et al. 2006). In opposite to conducted research and the results of above-mentioned authors' studies, SHERWOOD et al. (2000) report an increase in species richness of the algal and higher plant communities in a downstream direction. However, seasonal differences were indistinctive in their or other previous studies between summer and autumn sampling. Analyzing the taxonomic composition, most of the determined species have been typically found in spring habitats of Europe (CANTONATI 1998; ABOAL et al. 1998; FRANKOVÁ et al. 2009; WERUM and LANGE – BERTALOT 2004).

Very abundant species in the studied springs, for example epiphytic taxa _Planolithidium lanceolatum_, _Cocconeis placentula_ var. _lineata_ were not so abundant in streams in this research. That can be caused by higher flow velocity and absence of mosses in stream habitats.

Most of the identified diatom species are widespread in the freshwater ecosystems, such as _Achnanthisium minutissimum_, _Gomphonema pumilum_ and _Nitzschia palea_. Species _Achnanthisium minutissimum_ is one of the most common diatoms in freshwater phytobenthic samples (KRAMMER and LANGE-BERTALOT 1991), and it occurs in the alkaline and acidic, oligotrophic and hypertrophic waters (ROUND 2004). Some very abundant species that we have found in studied springs in the Konjuh Mountain (_Planolithidium lanceolatum_, _Cocconeis pseudolineata_, _Cocconeis placentula_ var. _lineata_, _Nitzschia palea_) are indicators of mesotrophic and eutrophic water quality guided by VAN DAM et al. (1994). On the other hand, simultaneously in the same samples, typical crenophilous species such as _Diatoma mesodon_ and _Meridion circulare_ were identified. Comparing these two habitats, higher frequency of the species typical for small rheohelocrene springs such as _Humidophila perpusilla_, _Diploneis krammeri_, _Encyonopsis cesatii_ (CANTONATI et al. 2012a) and many _Navicula_ species were noted in the studied springs of Konjuh Mountain.

Comparing diatom assemblages from headwaters that inflow to the River Drinjača in this study with results for Drinjača lower course (ŠALKIĆ et al. 2012), the same four common species (_Cocconeis placentula_, _Cymbella_ sp., _Meridion circulare_ and _Nitzschia sigmoida_) were identified.

Higher values of the Shannon-Wiener Diversity Index and a greater number of diatom species in springs in comparison with stream habitats indicate the purpose to preserve these rare and extremely endangered ecosystems, which can serve as refuge for endangered species of algae, especially diatoms.

Despite their small size, spring ecosystems are important refuge for preserving and conservation freshwater diatoms. Uncontrolled management and capturing of mountain springs, and modification of spring source into the fountain can result in the reduction of the diversity of rare taxa in the future.
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Comparative analysis of epilithic diatom assemblages of springs and streams in the Konjukh Mountain (Bosnia and Herzegovina)

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Izvori su prepoznati kao značajni refugiji važni za očuvanje biodiverziteta slatkovodnih biotopa. U radu je analiziran biodiverzitet i upoređivana je struktura epilitskih zajednica silikatnih algi između izvorišta i pripadajućih potoka na planini Konjuh (BiH). Uzorkovanje algi kremenjašica (silikatnih) i mjerenje osnovnih fizičko-hemijskih parametara na terenu provedeno je na pet manjih planinskih tekućica, pri čemu su uzorkovana izvorišna područja i njima pripadajući gornji tokovi tekućica u ljetnoj i jesenjoj sezoni tokom 2013. godine. Epiliton je uzorkovan standardnom metodologijom prema Kelly i sar. (1998). U laboratoriju su izrađeni i pregledavani trajni preparati silikatnih algi uklopljeni u Naphrax, pri čemu je određivana relativna abundancna vrsta brojanjem do 400 frustula. Utvrđeno je da su izvorska staništa brojnija i raznovrsnija vrstama (srednja vrijednost $H' \log_e = 1.67$), a t testom je potvrđeno da se vrijednosti Shannon-Wienerovog indeksa diverziteta statistički značajno razlikuju u poređenju sa staništima gornjih tokova potoka (srednja vrijednost $H' \log_e =1.15; t=2.79; p=0.013$). Sličnosti u strukturi zajednica s različitim lokalitetima istraživane su metodom nemetričnog multidimenzionalnog skaliranja, baziranom na Bray-Curtis indeksu sličnosti. Upotrebom permutacijskog Manova testa, dokazano je da postoji razlika u strukturi zajednica izvora i potoka (Pseudo F=2.66, $p = 0.012$), dok razlika u strukturi zajednica između ljetne i jesenje sezone statistički nije potvrđena (Pseudo F = 0.586, $p = 0.876$). Najfrekventnije i najabundantnije vrste u uzorcima izvora bile su: *Achnanthidium minutissimum* (Kützing) Czarnecki, *Cocconeis pseudolineata* (Geitler) Lange-Bertalot, *Gomphonema pumilum* var. *elegans* E.Reichardt & Lange-Bertalot, *Meridion circulare* (Greville) C.Agardh i *Planothidium lanceolatum* (Brébisson ex Kützing) Lange-Bertalot. Najfrekventnije i najdominantnije u staništima potoka su bile: *Achnanthidium minutissimum* (Kützing) Czarnecki, *Cocconeis placentula* var. *euglypta* (Ehrenberg) Grunow, *Cocconeis placenta* var. *lineata* (Ehrenberg) van Heurck i *Cymbella tridentina* Lange-Bertalot, M.Cantonati & A.Scalfi. Izvori su heterogena staništa sa specifičnom florom dijatomeja i imaju stabilniji tok vode u odnosu na potoke. Iako su površinom jako mala područja, diverzitet vrsta koji ih nastanjuje ne smije biti zanemarivan prilikom planiranja upotrebe ovih prirodnih resursa.

Ključne riječi: diverzitet, epiliton, dijatomeje, izvorišna područja, Konjuh planina (BiH)