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Same karstic substratum, different aquatic communities? Case study: three water bodies from western Romania

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SUMMARY. We investigated the phytoplankton, periphyton and microcrustacean communities developing on similar limestone substratum, in three karstic lakes: Iezerul Ighiel (Alba County), Dracului and Ochiul Beiului (Caraş-Severin County), during 2014 and 2016. Species richness was significantly higher in the lake greater in size for both algae and microcrustaceans, consistent with the species-area hypothesis. Forty algal taxa and only one microcrustacean species were common in all three lakes, even if comparable physico-chemical characteristics were recorded. Relatively similar saprobic conditions were shown by indicator species, while trophic state differed at some extent. Since current factors existing in the three environments were relatively similar (limestone substratum, physico-chemical parameters, water source etc.), the dissimilarities found in the plankton and periphytic communities were best explained by long-term factors like geographical isolation or the strength of disturbances.

Keywords: current and long-term factors, karstic lakes, microcrustaceans, planktonic and benthic algae, similarity.

Introduction

Does similar karstic substratum sustain similar assemblages of algae and crustaceans? This question was firstly asked for terrestrial habitats (McCune and Allen, 1985), but it can also be applied to aquatic communities (Jenkins and Buikema, 1998). Karstic lakes include water bodies formed through a process of chemical dissolution of rocks, most commonly composed of carbonate or sulphate minerals, but also chloride minerals (Löffler, 2004). Lakes in karst terrain are distributed all over the world, from China to Northern and Central America, from the Alps to the Balkan Peninsula, and Romania.

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The karstic lakes considered for the present study are located in two protected areas in western Romania. Lake Iezerul Ighiel, one of the largest permanent karstic lakes in Romania, is included in the Iezerul Ighiel IUCN Natural Reserve, category IV, from the Trascău Mountains, part of the Apuseni Mountains (Pop and Măhăra, 1965). The other two karstic water bodies, Lake Dracului and Ochiul Beiului are located in the Cheile Nerei – Beușnița National Park (IUCN protected area, category II), from the Aninei Mountains, south-western Romania (Pisota and Trufaș, 1971).

Algal and microcrustacean communities were the focus of the present paper. Algal communities comprise a high range of organisms with a wide spectrum of morphological, behavioral and physiological traits (Udovič *et al*., 2016). Phytoplankton represents the most important primary producer in pelagic food webs. The community composition is influenced by the physico-chemical conditions present in the aquatic ecosystem in which they develop, especially nutrient availability (Litchman and Klausmaier, 2008). Phytobenthos or periphyton represents the group of photosynthetic algae that are adapted to live on different substrates, developing a high range of morphological adaptations (Pan *et al*., 2016). Both types of algal communities have been intensively used in assessing the ecological state of the water all over the world, because their impact on the functioning of the ecosystem is high (Litchman and Klausmaier, 2008).

Microcrustaceans (Arthropoda Crustacea: Branchiopoda Cladocera; Maxillopoda Copepoda), together with protozoans and rotifers, represent zooplankton community that occupies central positions of lentic food webs in most freshwater habitats, from temporary pools to large lakes (Kobayashi *et al*., 2009). Cladocerans are mostly planktonic filtrators and detritus grazers, with only a few predator species, while copepods include herbivore, omnivore and carnivorous species (Błędzki and Rybak, 2016). Microcrustaceans play important roles in controlling the abundances of phytoplankton, nutrient recycling or lake trophic condition indicators. They are also used in water quality assessment, climatic change analyses, phylogenetic research, toxicology etc. (Błędzki and Rybak, 2016).

Previous studies conducted in Lake Iezerul Ighiel focused on geographical and abiotic parameters (Pop and Măhăra, 1965; Decei, 1981; Duma, 2009; Mihăiescu *et al.,* 2012), palaeohydrological aspects dealing with the controversial genesis of the lake (Haliuc *et al*., 2017) and biotic communities (Decei, 1981, Momeu *et al*., 2015). In Lake Dracului and Ochiul Beiului, no previous research was found.

Thus, the aim of the present study was to investigate if similar karstic substrata sustained similar algal and microcrustacean assemblages in the three lakes: Iezerul Ighiel, Dracului and Ochiul Beiului. The present study represents the first attempt to compare phytoplankton, periphyton and microcrustacean communities from different karstic lakes in Romania and to explain the dissimilarities recorded. 27 algal taxa was first cited for the Romanian flora in the study area (Cărăuş, 2017).

Material and methods

Lake Iezerul Ighiel (46°10’47.2”N; 23°21’48.4”E) is located in the central-eastern limestone plateau Ciumerna, on the Ighiu Valley, tributary to the Ampoi River (Pop and Măhăra, 1965), in a 365 ha area of strict protection of forests and meadows, representing the buffer zone for the lake. Lake Iezerul Ighiel is one of the most typical permanent karst lakes in our country (Fig. 1; Table 1). The lake is recharged during spring and precipitation events by torrents, but the water level fluctuates, with minimums in summer and winter (Pop and Măhăra, 1965; Duma, 2009).

Lake Dracului (46°51’52.3”N; 21°48’46”E) and Ochiul Beiului (44°56’09”N; 21°47’20.9”E) are two smaller water bodies formed on limestone, located in the National Park Cheile Nerei-Beușnița, in the homonymous Natural Reserve, stretching on an area of 3081.3 ha (Pișotă and Trufaș, 1971). They are both permanent systems (Fig. 1; Table 1). Ochiul Beiului is fed by several springs that refresh the water continuously. The water temperature is almost constant and it does not freeze during winter (Pișotă and Trufaș, 1971).

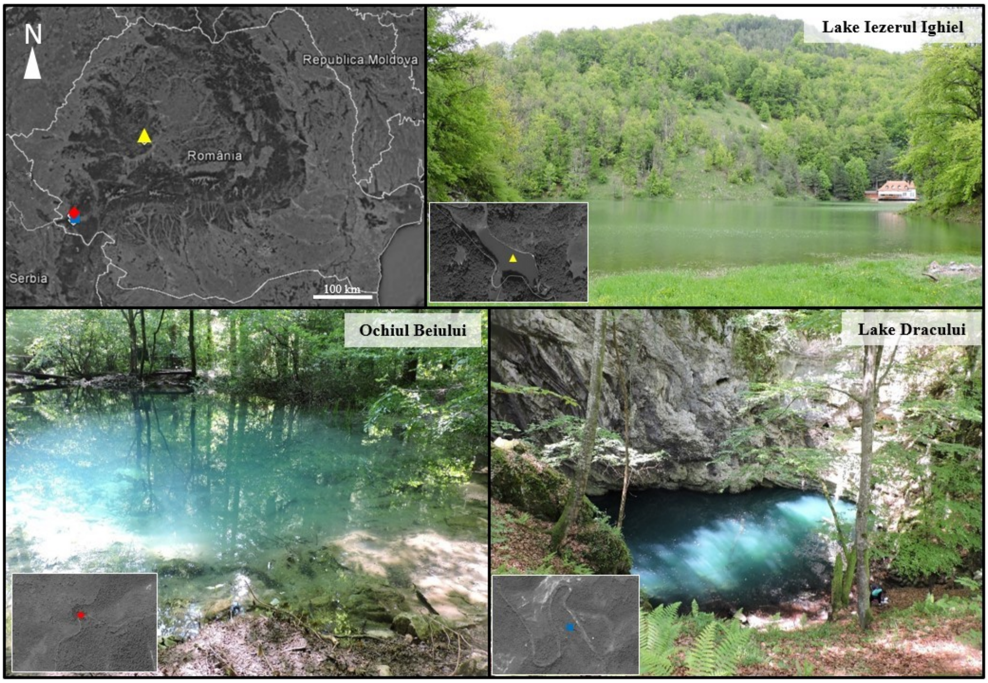

Figure 1. Location of the studied areas: Lakes Iezerul Ighiel, Dracului and Ochiul Beiului

Table 1.

Main physico-geographical characteristics of the three water bodies (depth and area according to Pop and Măhăra (1965), Pişotă and Trufaş (1971); abbreviations: SP - spring; SU - summer; AU – autumn)

Site code	Site name	Altitude (m)	Area (m^2)	Maximum depth (m)	Average depth (m)	Investigated biotopes	Substratum
IG_SP IG_SU IG_AU	Lake Iezerul Ighiel	915	52605	9	4.2	pelagial and littoral	rocks, silt, submerged macrophytes
DR_SP	Lake Dracului	215	700	9.3	4.63	littoral	rocks, logs, dead organic matter, submerged macrophytes
OB_SP	Ochiul Beiului	292	284	3.6	1.5	littoral	moss, rocks, sand, submerged macrophytes

Plankton samples from Lake Iezerul Ighiel were collected by boat from three sampling sites, in spring, summer and autumn 2014 – 2016 (May, August and November 2014; May, July and October 2015; May and July 2016). Phytoplankton data were averaged from the samples taken in 2015 and 2016, while for zooplankton, 2014 and 2016 data were considered. Only one sampling point for phytoplankton was considered for Lake Dracului, and one for Ochiul Beiului, in May 2016. Periphyton samples were collected from the banks in all three water bodies.

The physical and chemical parameters were recorded in the field: water temperature, dissolved oxygen, pH and conductivity (measured *in situ* with portable meters YSI 52 and HI98129).

Plankton samples were collected with a 20 µm mesh size net in case of phytoplankton and a 50 µm mesh size one for microcrustaceans. The benthic algae were sampled by scraping the hard substratum, by collecting the sediment using a pipette or by collecting submersed macrophytes. All samples were preserved in the field in 4% formaldehyde. Identifications were made to the species level in case of algae (Krammer and Lange-Bertalot, 1986, 1988, 1991; Ettl, 1983) and microcrustaceans (cladocerans: Negrea, 1983; adult copepods: Damian-Georgescu, 1963; 1966; 1970; Einsle, 1993; Janetzky *et al*., 1996).

Relative abundance, expressed as percentages, was calculated for algal and microcrustacean communities. For phytoplankton and periphyton roughly 400 individuals were counted in one drop of water, from every sample, at 40x magnification. In case of low number of individuals / sample, the entire surface of the slide was counted. Dominant algal taxa causing water blooms were identified. Since taxa other than Bacillariophyta dominated in some cases, counts were performed in wet mounts instead of fixed ones, specific for diatoms. This is why some diatom taxa were identified only to the genus level, in order to avoid erroneous results. In case of colonial taxa, the whole colony was considered as one during counts, except for *Dinobryon* sp., where single cells were found in most cases. Relative abundance for microcrustaceans was estimated from counts ranging from 35 to 350 individuals / sample. Only adult copepods were identified to the species level; copepodites and nauplii were assigned proportionally to the adult copepod individuals found in the samples. Frequency was also calculated.

Similarity was considered for both algal and microcrustacean communities: Jaccard and Dice indices for qualitative information; and Bray-Curtis index for quantitative data. Diversity was calculated for phyto- and zooplankton samples, based on Shannon-Wiener index and the Equitability (Washington, 1984).

Multivariate data analyses were performed in order to visualize the data. Principal Component Analysis (PCA) (Jolliffe, 1986) was used to show the aggregation of sampling locations depending on abiotic variables and the aggregation of microcrustacean species in the three water bodies. Correspondence Analysis (CA), another ordination method (Hennebert and Lees, 1991), was used to single out the most dominant phytoplankton and periphyton species in each lake, and to depict the ecological status of the lakes based on indicator species. XLSTAT Version 2017.19.03.44468 and PAST version 3.14 were used for similarity, diversity and multivariate analyses.

To assess the trophic state of the water bodies based on algal communities, the following indices were used: alpha-eutrophicity index, gamma-eutrophicity index (Oltean, 1977); Nygaard compound index (1949), the Q index of eutrophy (Järnefelt, 1951) and the diatom index (Stockner, 1972). The saprobic indicator values of certain algal species were considered, following Rott *et al.* (1997), Hindak (1978), Sládeček (1973) and Van Dam *et al*. (1994). The organic pollution index (Palmer, 1969) was also used. The indication values for water saprobity and trophic state for the most abundant microcrustaceans were considered, according to Sládeček (1973), Damian-Georgescu (1963, 1966, 1970) and Negrea (1983).

Results and discussion

Physical and chemical parameters

Several physico-chemical parameters have been measured *in situ* in order to compare the three karstic aquatic ecosystems (Fig. 2). Average values from all available data were calculated; temperature and dissolved oxygen were measured in a vertical profile, while for conductivity, pH and transparency only one value was recorded.

The values of the main physico-chemical parameters were comparable between the three karstic lakes, showing similar conditions. Higher temperature values were recorded in summer in Lake Iezerul Ighiel (18.7°C), a normal characteristic of the dimictic lakes from the northern hemisphere. In spring however, the temperature values were homogenous in all three studied areas (10.67-11.63°C).

High conductivity values were caused by the limestone bedrock characteristic to karstic lakes, due to the weathering of the substrate. Our results agree with previous studies in Lake Iezerul Ighiel (Mihăiescu *et al.*, 2012). The highest values of conductivity and dissolved oxygen were recorded in Ochiul Beiului (630 µS/cm and 10.29 mg/L, respectively), probably because of the permanent water flow through the water body, nutrient and oxygen rich (Fig. 2). Similar high water conductivity values were reported in several karst rheocrene springs in southern Poland (Wojtal and Sobezyk, 2012). Typically for karst areas, pH was circumneutral to slightly alkaline, with slightly higher values in spring and autumn in Lake Iezerul Ighiel (7.51 and 7.55, respectively). Lake Dracului and Ochiul Beiului had clear waters, with transparency values of 3 m, while the transparency of Lake Iezerul Ighiel did not exceed 1.85 m (Fig. 2), probably due to frequent algal blooms.

The range of variation for the main physico-chemical parameters measured for the present study are in accordance with the literature: the dissolved oxygen values are similar to the ones recorded in several karst lakes from Croatia (Stanković *et al.*, 2011; Udovič *et al.*, 2016); slightly alkaline pH was found in several subalpine karstic lakes from China (Pan *et al.*, 2016) etc.

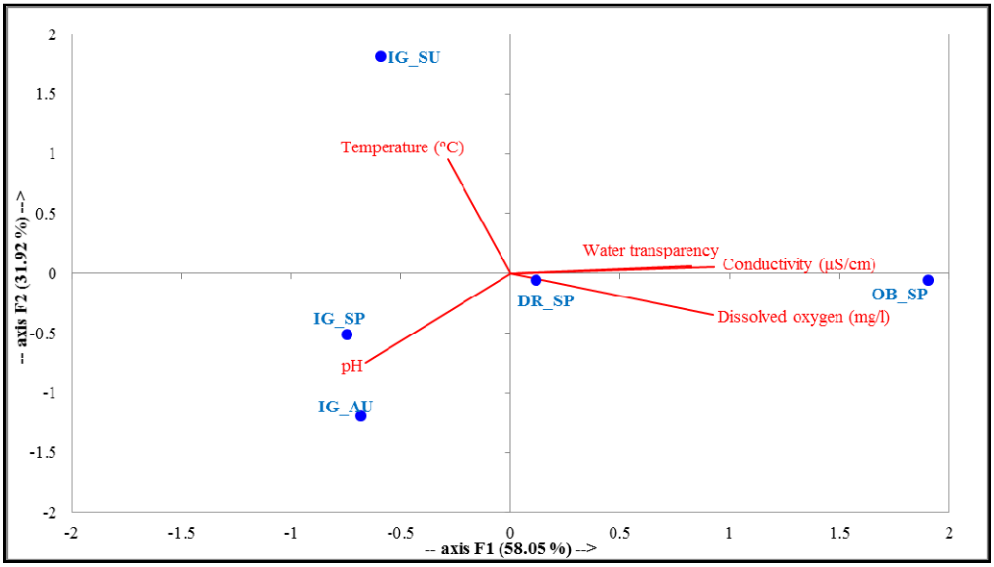

Figure 2. Principal Component Analysis (PCA) biplot (axes F1 and F2: 89.97 %) for the sampled lakes (abbreviations as in Table 1) and their aggregation based on physical and chemical parameters (average values for all available data from 2014 to 2016 were used)

Phytoplankton and periphyton

A total number of 288 taxa were identified in 2015 and 2016 from Lake Iezerul Ighiel (Bacillariophyta: 41%, Chlorophyta: 38%, Cyanophyta: 9%, Euglenophyta: 6%, Chrysophyta: 3%, Xanthophyta: 2% and Dinophyta: 1%). The total number of taxa differed with the season, from 200 taxa in spring, to 202 and 104 in summer and autumn, respectively. Only 49 taxa were identified in spring 2016 in Lake Dracului (Bacillariophyta: 88%, Cyanophyta: 8%, Chlorophyta: 2% and Xanthophyta: 2%). The same number of taxa were identified in spring 2016 in Ochiul Beiului (Bacillariophyta: 88%, Cyanophyta: 8% and Chlorophyta: 4%). Fourteen taxa were common in all three lakes, such as: *Amphora montana*, *Asterionella formosa*, *Cocconeis placentula*, *Cyclotella iris*, *Gomphonema olivaceum*, *Navicula cincta* etc.

Dominant phytoplankton and periphyton taxa were discriminated by calculating the average abundances of species in spring, summer and autumn from Lake Iezerul Ighiel and in spring from Lakes Dracului and Ochiul Beiului. Due to the low number of phytoplankton taxa in these two lakes, the average abundances were calculated only for the periphyton samples.

The Correspondence Analysis for phytoplankton from Lake Iezerul Ighiel shows the specific grouping of the samples according to their seasonal variation (Fig. 3). Water blooms were caused by the dominant taxa in spring: *Fragilaria nanana*

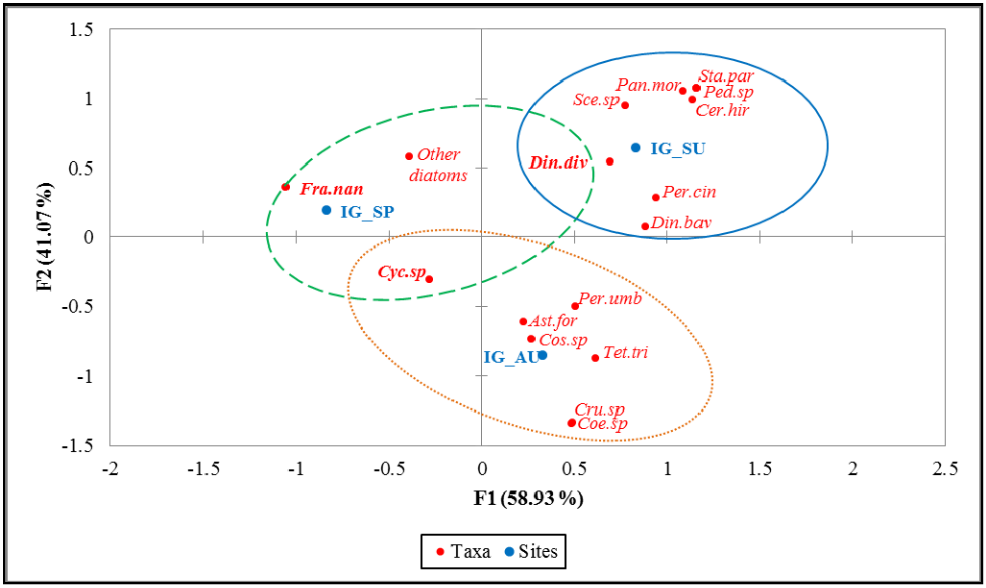

Figure 3. Correspondence Analysis (CA) plot (axes F1 and F2: 74.34%) showing the aggregation of phytoplankton samples; average abundances considered for Lake Iezerul Ighiel (abbreviations as in Table 1; *Ast.for* – *Asterionella formosa*; *Cer.hir* – *Ceratium hirundinela*; *Coe.sp* – *Coenococcus* sp.; *Cos.sp* – *Cosmarium* sp.; *Cru.sp* – *Crucigeniella* sp.; *Cyc.sp* – *Cyclotella* sp.; *Din.bav* – *Dinobryon bavaricum var medium*; *Din.div* – *Dinobryon divergens*; *Fra.nan* – *Fragilaria nanana*; *Pan.mor* – *Pandorina morum*; *Ped.sp* – *Pediastrum* sp.; *Per.cin* – *Peridinium cinctum*; *Per.umb* – *Peridinium umbonatum*; *Sce.sp* – *Scenedesmus* sp.; *Sta.par* – *Staurastrum paradoxum*; *Tet.tri* – *Tetrastrum triangulare*; Other diatoms – *Fragilaria* sp.; *Navicula* sp.; *Nitzschia* sp.

and *Cyclotella* sp. (with *Cyclotella iris* being the most abundant). *Dinobryon divergens* dominated in summer. Consistent with the typical diatom peaks of phytoplankton during spring and autumn (Willén, 2000), *Cyclotella* sp. dominated the autumn samples (again *Cyclotella iris* being the most abundant) (Fig. 3). In 2014, *Asterionella formosa* and *Cyclotella iris* were the taxa responsible for water blooms (Momeu *et al.*, 2015). This rapid shift in dominant diatom species showed the unique dynamics of Lake Iezerul Ighiel. The domination of centric diatoms in spring and autumn was also observed in deep karst lakes in Croatia (Udovič *et al*., 2016). The most abundant *Cyclotella iris*, followed by *C. ocellata*, *C. distinguenda* (Momeu *et al.,* 2015) represented the common taxa, as did *Dinobryon* sp. in summer (followed by *Peridinium cinctum*) (Udovič *et al*., 2016).

The Correspondence Analysis for periphyton from Lake Iezerul Ighiel, Lake Dracului and Ochiul Beiului shows the specific grouping of the samples according to their seasonal variation and to the specific aquatic ecosystem (Fig. 4). Diatoms dominated the periphyton communities from Lake Dracului and Ochiul Beiului, with *Gomphonema* sp. abundant in both. In Ochiul Beiului however, the dominant taxa was *Cocconeis placentula* (with a few variations like: *C. plancentula* var. *euglypta* and var. *linearis*).

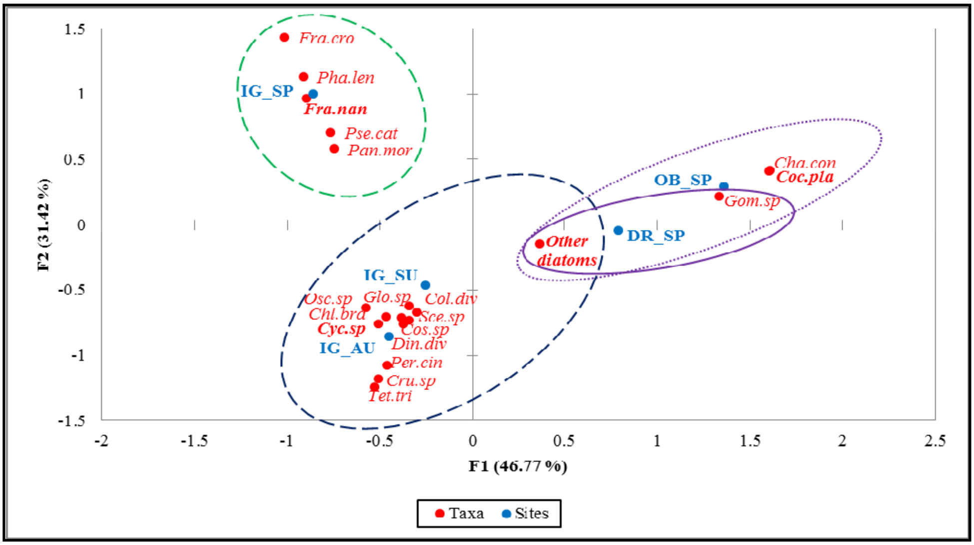

Figure 4. Correspondence Analysis (CA) plot (axes F1 and F2: 77.93 %) showing the aggregation of periphyton samples average abundances considered from the three waterbodies; abbreviations as in Table 1; *Cha.con* - *Chamaesiphon confervicolus*; *Chl.bra* - *Chlamydomonas braunii*.; *Coc.pla* - *Cocconeis placentula*; *Col.div* - *Coleochaete divergens*; *Cos.sp* - *Cosmarium* sp; *Cru.sp* - *Crucigeniella* sp; *Cyc.sp* - *Cyclotella* sp.; *Din.div* - *Dinobryon divergens*; *Fra.nan* – *Fragilaria nanana*; *Fra.cro* - *Fragilaria crotonensis*; *Glo.sp* - *Gloeotila* sp.; *Gom.sp* - *Gomphonema* sp.; *Osc.sp* - *Oscillatoria* sp.; *Pan.mor* - *Pandorina morum*; *Per.cin* – *Peridinium cinctum*; *Pha.len* - *Phacotus lenticularis*; *Pse.cat* - *Pseudanabaena catenata*; *Sce.sp* - *Scenedesmus* sp; *Tet.tri* – *Tetrastrum triangulare*; Other diatoms – *Achnanthes* sp., *Fragilaria* sp., *Navicula* sp., *Nitzschia* sp.

Diatoms, chlorophyceae and cyanophyceae were found in Lake Iezerul Ighiel periphyton. The spring samples separate themselves from the other two seasons (Fig. 4), with *Fragilaria nanana, F. crotonensis*, *Phacotus lenticularis* and *Pandorina morum* among the most abundant taxa. In the other two seasons, *Cyclotella* sp., *Dinobryon divergens*, *Tetrastrum triangulare*, *Gloeotila* sp. and *Coleochaete divergens* dominated.

Consistent with the data from nine karstic lakes in Jiuzhaigou Nature Reserve (Pan *et al*., 2016), taxa like: *Pseudanabaena* sp., *Leptolyngbya* sp., *Oscillatoria* sp., *Achnanthes minutissima* and *Denticula tenuis* were also frequent in the periphyton samples from Lake Iezerul Ighiel.

From the total number of taxa identified in the three studied areas, 130 taxa are known to have a cosmopolitan distribution: *Cocconeis placentula, Pandorina morum, Navicula cincta* etc. However, 25 taxa are described from mountain areas: *Anomoeoneis vitrea, Chroococcus subnudus, Cymbella affinis, Diatoma mesodon, Gonatozygon brebissonii, Neidium binodeforme, Leptolyngbya fontana* etc. A number of 41 true planktonic taxa were also identified: *Ankyra lanceolata, Asterionella formosa*, *Peridinium cinctum*, *Trachelomonas hispida* f. *minor* etc. Several other species have different physico-chemical preferences, like neutral to alkaline pH: *Cosmarium regnellii*, *Fragilaria crotonensis*, *Navicula oblonga* etc.; low water temperature: *Nitzschia hantzschiana*, *Gomphonema acuminatum* etc.

Consistent with the nature of the karst substrate, 22 calciphile taxa were found: *Aphanocapsa parietina*, *Achnantes flexella*, *Aulacoseira crenulata*, *Chamaesiphon confervicolus*, *Cymbella tumidula*, *Eunotia arcus*, *Gomphonema clavatum*, *Ophiocytium arbusculum*, *Phormidium foveolarum* etc.

According to Cărăuş (2017), 27 algal taxa from all the sampling sites are first cited for Romania: *Aphanocapsa hyalina*, *Astasia hypolimnica* (Fig. 5), *Bitrichia longispina*, *Characium strictum*, *Characium substrictum*, *Chlamydomonas braunii*, *Chlamydomonas*

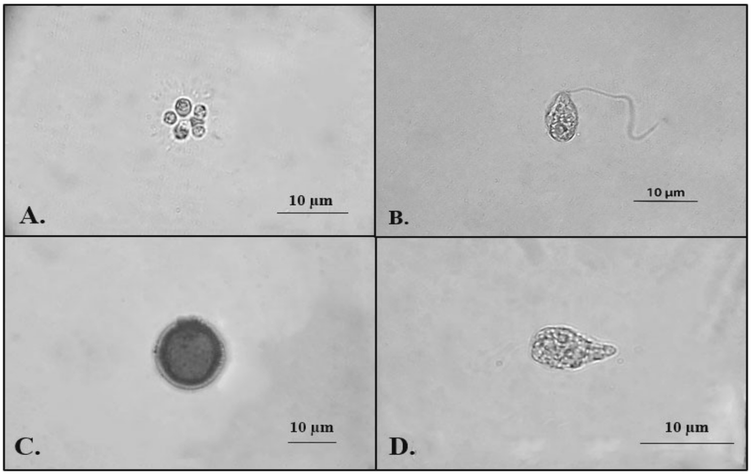

Figure 5. New taxa cited for Romania: A. *Radiococcus wildemannii*, B. *Astasia hypolimnica*, C. *Trachelomonas lomnickii*, D. *Pyramimonas fasciata*

pseudagloë, *Chlamydonephris impressa*, *Cosmarium taxichondriforme* var. *nudum*, *Diplostauron angulosum*, *Dysmorphococcus pseudovariabilis*, *Euglena chlamydophora*, *Geminella planctonica*, *Leptolyngbya fontana*, *Navicula laevissima* var. *perhibita*, *Pyramimonas fasciata* (Fig. 5), *Radiococcus wildemannii* (Fig. 5), *Radiosphaera minuta*, *Scherffelia bichlora*, *Sphaerellopsis ignava*, *Sporotetras pyriformis*, *Stichococcus minutissimus*, *Stokesiella acuminata*, *Thorakochloris nygaardii*, *Trachelomonas hispida* f. *minor*, *Trachelomonas lomnickii* (Fig. 5), *Tribonema taeniatum*.

Microcrustaceans

Twelve cladoceran species and five copepod species were identified in Lake Iezerul Ighiel in 2014 and 2016 (Fig. 6). With 38% from the microcrustacean community in terms of average relative abundances from all available data, *Cyclops vicinus* (Ulianine 1875) represented the most abundant taxa, followed by *Acanthodiaptomus denticornis* Kiefer 1932 (31%) and *Daphnia* sp. (12% for *D. galeata* and *D. rosea*, considered as one group). For copepods, adults and immature stages were considered together. On the other hand, the cladocerans *Alonella nana (Baird 1843)* and *Bosmina longirostris (O. F. Muller 1776),* species with lower relative abundances, recorded high frequency values (exceeding 67% from the total number of available samples).

Only copepods were found in Lake Dracului in spring 2016. Out of the 5 species identified in this lake, *Eudiaptomus zachariasi* (Poppe, 1886) recorded the highest relative abundance (62%), followed by *Cyclops strenuus* Fischer, 1851 (32%). Ochiul Beiului had no true plankton community, since only 2 cyclopoid copepodites were found in spring 2016 (Fig. 6).

Microcrustaceans are usually ubiquitous (Błędzki and Rybak, 2016), and similar taxa can be found in water bodies located in different karstic regions. In fact, several common taxa were found in similar studies conducted in a limestone lake from Poland: *B. longirostris, D. galeata, C. vicinus* (Ślusarczyk, 2003) or in an alpine karst lake from Austria: *Chydorus sphaericus, D. rosea, A. denticornis, Eucyclops serrulatus* (Jersabek and Schabetsberger, 1996). A few common taxa were also found in gypsum karstic lakes from the Balkans: *B. longirostris, C. vicinus* in 3 Croatian lakes (Stanković *et al*., 2011) and *Alona* sp., *B. longirostris, Ceriodaphnia pulchella* in a Greek monomictic lake (Chalkia *et al*., 2012). Moreover, very different habitats were also characterized by some common taxa, like *B. longirostris, Ceriodaphnia* sp, *Daphnia* sp. in an ephemeral karst lake from USA (Kelley *et al*., 2000) or *B. longirostris, C. strenuus* in a pseudokarstic lake formed in the depositions of a retreating glacier in North Italy (Obertegger *et al*., 2010).

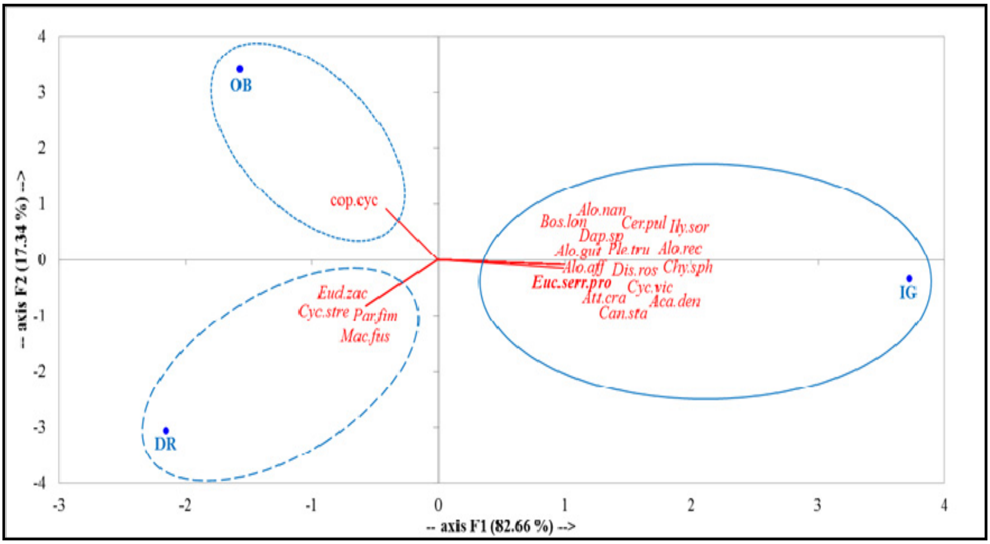

Figure 6. Principal Component Analysis (PCA) biplot (axes F1 and F2: 100 %) showing microcrustacean taxa found in Lakes Iezerul Ighiel, Dracului and Ochiul Beiului; average abundances considered from all available data; abbreviations as in Table 1; cladocerans: *Alo.aff – Alona affinis; Alo.gut – Alona guttata; Alo.rec – Alona rectangula; Alo.nan – Alonella nana; Bos.lon – Bosmina longirostris; Cer.pul – Ceriodaphnia pulchella; Chy.sph – Chydorus sphaericus; Dap.sp. – Daphnia galeata* and *D. rosea; Dis.ros – Disparalona rostrata; Ily.sor – Ilyocryptus sordidus; Ple.tru – Pleuroxus truncatus*; copepods: *Aca.den – Acanthodiaptomus denticornis; Att.cra – Attheyella crassa; Can.sta – Canthocamptus staphylinus; Cyc.stre – Cyclops strenuus; Cyc.vic – Cyclops vicinus; Euc.serr.pro - Eucyclops serrulatus proximus; Eud.zac – Eudiaptomus zachariasi; Mac.fus – Macrocyclops fuscus; Par.fim – Paracyclops fimbriatus*; cop.cyc – cyclopoid copepodites.

Diversity and assessment of ecological status of the lakes

The Shannon-Wiener index and the Equitability were calculated to assess diversity of planktonic algal and microcrustacean communities. Phytoplankton diversity was impossible to calculate in Lake Dracului and Ochiul Beiului, due to the low number of taxa. Similarly, microcrustacean diversity was null in Ochiul Beiului, since only 2 immature copepod individuals were found in the water column. The values of diversity indices were calculated from the average abundances found in each season for algae and microcrustaceans; all present taxa were considered, even if they were not included in counts.

Significant differences were recorded in species richness for the three karstic lakes, with values as different as 148 algal taxa (11 microcrustacean species) in Lake Iezerul Ighiel compared to only 49 (5 microcrustaceans) in Lake Dracului and 49 (0 microcrustaceans) in Ochiul Beiului in spring 2016. Significant positive correlation was found between the number of algal taxa and lake area (Pearson r = 0.999; p = 0.004). These findings are consistent with the species-area hypothesis formulated by MacArthur and Wilson (1967), stating that species richness results from a balance of immigration and extinction, larger areas having higher number of species.

Higher species richness could imply higher Shannon-Wiener diversity, as shown in Fig. 6 for microcrustaceans. Overall, Shannon-Wiener values were comparable to those reported in the literature from similar limestone lakes, not exceeding 1.5 (Stanković *et al.*, 2011). Direct relationships between number of taxa and lake area or lake depth were also previously found in the literature, for several karstic lakes from Spain (Armengol and Miracle, 1999).

Moreover, different diversity values were recorded for different seasons. For phytoplankton for example, summer communities had the higher diversity value, due to frequent water blooms caused by diatoms in spring and autumn that lowered the diversity score (Fig. 7). Similar results were found in the literature (Steward and Wetzel, 1986). For microcrustaceans, higher diversity was recorded in autumn, a typical situation, recorded in the literature as well (Armengol and Miracle, 1999). However, the equitability was generally low (<0.6), for algae and microcrustaceans alike, showing that only few species could thrive in these karstic environments.

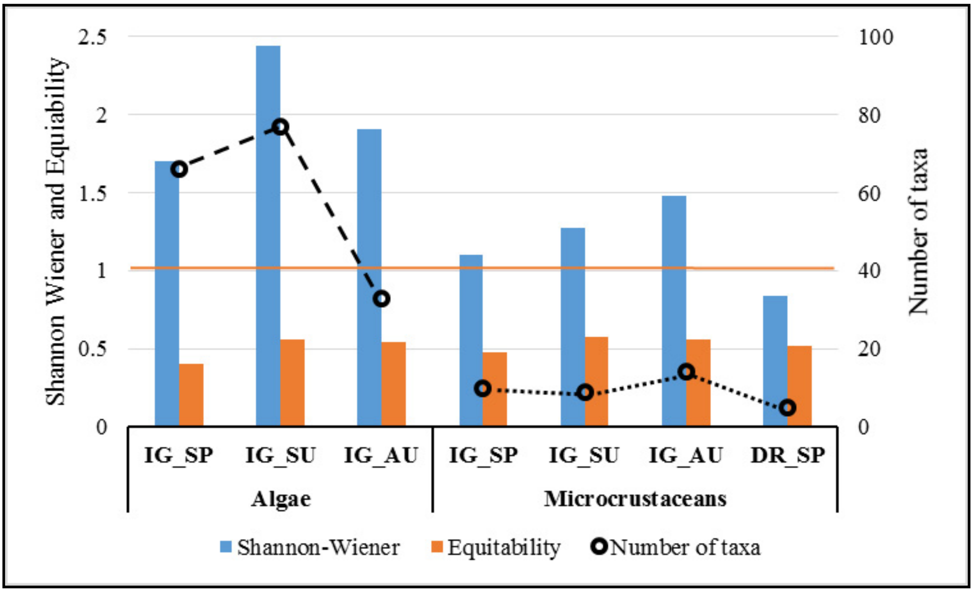

Figure 7. The diversity of the plankton communities in Lakes Iezerul Ighiel and Dracului; abbreviations as in Table 1; dotted lines: number of taxa; solid line: maximum equitability value.

Algal and microcrustacean communities used in assessing the ecological status of the karstic lakes considered for the present study gave coherent results in case of water saprobity, organic pollution and trophicity (Fig. 8). The correspondence analysis considered sums of algal and microcrustacean taxa that indicated different saprobity and trophicity levels; for Ochiul Beiului only algae were used.

Eutrophic conditions were depicted by the highest number of indicator taxa in all three karstic lakes (Fig. 8). All phytoplanktonic indices calculated for Lake Iezerul Ighiel showed eutrophic conditions: Nygaard's compound index (Willén, 2000) of 4.04 and Stockner diatom index (Stockner, 1972) of 2.14. From the total number of phytoplanktonic taxa, 18 recorded the Q indicator value (Järnefelt, 1951) higher than 1, indicating eutrophy. Trophic phytoplankton indices according to Oltean (1977) showed an on-going eutrophication process in Lake Iezerul Ighiel, since a clear transition was recorded from alpha-eutrophic conditions in spring 2014 to gamma in summer 2015 and 2016. These findings are consistent with previous results based on algae (Momeu *et al.,* 2015), or solely on total phosphorus, indicating oligotrophic conditions in 2010 (Mihăiescu *et al.,* 2012). Possible explanations could be the on the one hand the continuous tributary inputs into the lake and on the other hand the anthropic influences like camping near the lake shore, fishing and swimming.

Numerous periphyton, and not phytoplankton taxa, indicated eutrophic conditions in all karstic water bodies, probably due to the accumulation of nutrients in lake sediments. This is why most algal taxa depicted eutrophic conditions in Lake Dracului and Ochiul Beiului. But, according to Oltean (1977) phytoplankton communities had low species richness and abundances, indicating oligotrophic conditions, as did Stockner diatom indices (Stockner, 1972), which did not exceed one.

Indicator taxa, algae and microcrustaceans alike, showed relatively clean waters with respect to saprobity (oligosaprobic to β-mesosaprobic conditions) in Lake Iezerul Ighiel (Fig. 8). In terms of organic pollution, 14 out of the 20 indicator algal genera, included in the Palmer genus index (Palmer, 1969) were identified in this lake: *Ankistrodesmus, Chlamydomonas, Closterium, Cyclotella, Euglena, Gomphonema, Melosira, Navicula, Nitzschia, Oscillatoria, Pandorina, Phacus, Phormidium and Scenedesmus*. Similarly, 10 indicator taxa for organic pollution, from a total of 20, were found: *Ankistrodesmus falcatus, Cyclotella meneghiniana, Gomphonema parvulum, Navicula cryptocephala, Nitzschia acicularis, Nitzschia palea, Oscillatoria limosa, Oscillatoria tenuis, Pandorina morum* and *Scenedesmus quadricauda*. The values of the organic pollution index, both at genus and at species levels (28 and 34, respectively) indicated slightly high organic pollution in the water.

The high number of species indicating β-mesosaprobic conditions in Lake Dracului and Ochiul Beiului can be explained by the input of organic materials in form of leaves, twigs or logs from the forest surrounding the lakes.

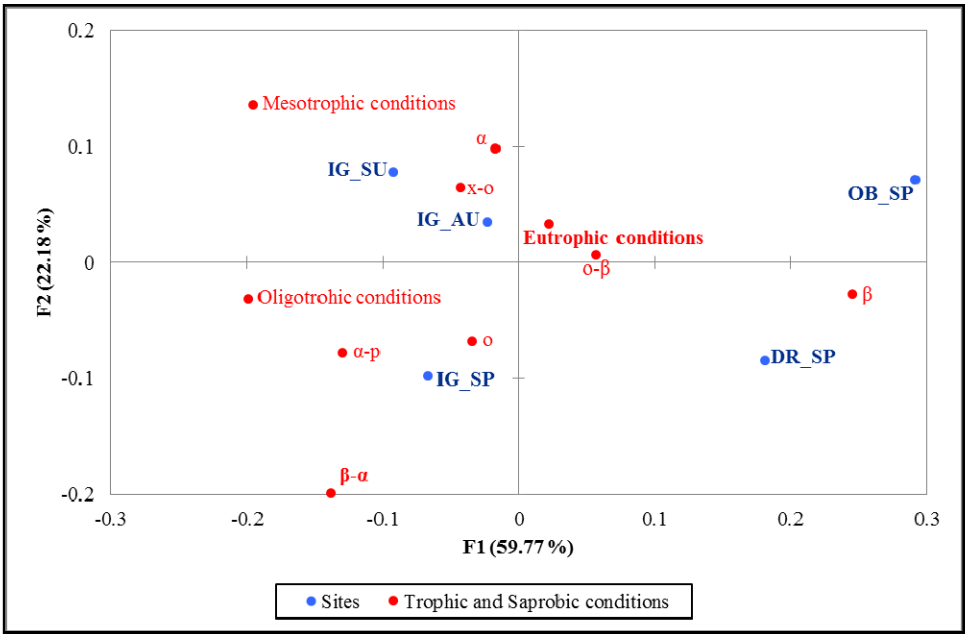

Figure 8. Correspondence Analysis (CA) plot (axes F1 and F2: 81.95 %) showing the aggregation of sampling sites with the number of algal taxa and microcrustacean species with indicator value; abbreviations as in Table 1; saprobic conditions indicated by taxa: x: xenosaprobic; o: oligosaprobic; β: β - mesosaprobic; α: α - mesosaprobic; p: polysaprobic; intermediate classes also depicted.

Similarity of aquatic communities between the three karstic lakes

Does similar karstic substratum sustain similar assemblages of algae and crustaceans? This was the initial question of the study. For freshwater microcrustaceans for example, since they consist of many ubiquitous species, capable to adapt to different habitats in various geographic locations (Błędzki and Rybak, 2016), similar conditions in limestone karstic lakes might mean similar communities. However, very different microcrustacean assemblages were identified in the three karstic lakes (Fig. 6). The similarity based on Dice index recorded just 10%, due to the one common copepod species, *Eucyclops serrulatus proximus* (Lilljeborg 1901), while the Bray-Curtis index revealed no similarity at all. This was also the case for algae, where Jaccard and Bray-Curtis indices revealed a low similarity of only 10 % between the algal community from Lake Iezerul Ighiel and the communities from the other two lakes: Lake Dracului and Ochiul Beiului, which sustain more similar assemblages (30-40%), due to the 23 common algal taxa.

These results are consistent with previous literature: Jenkins and Buikema (1998) analyzed the colonization of 12 similar experimental ponds, which sustained different zooplankton communities in terms of structure (species richness, density and biomass), but no clear differences were detected in case of community function (production, respiration, nutrient regeneration rates).

In accordance with the analyses made by McCune and Allen (1985) in coniferous forests, two types of factors influencing the qualitative and quantitative structure of aquatic communities that develop in similar sites were identified: (1) **current factors**, that characterize the site at present in terms of geo-morphology, climate, hydrology, physical-chemical properties etc.; and (2) **long-term factors**, that characterize the history of the site, long- or short-lasting processes with influences on the present status of the community (Fig. 9).

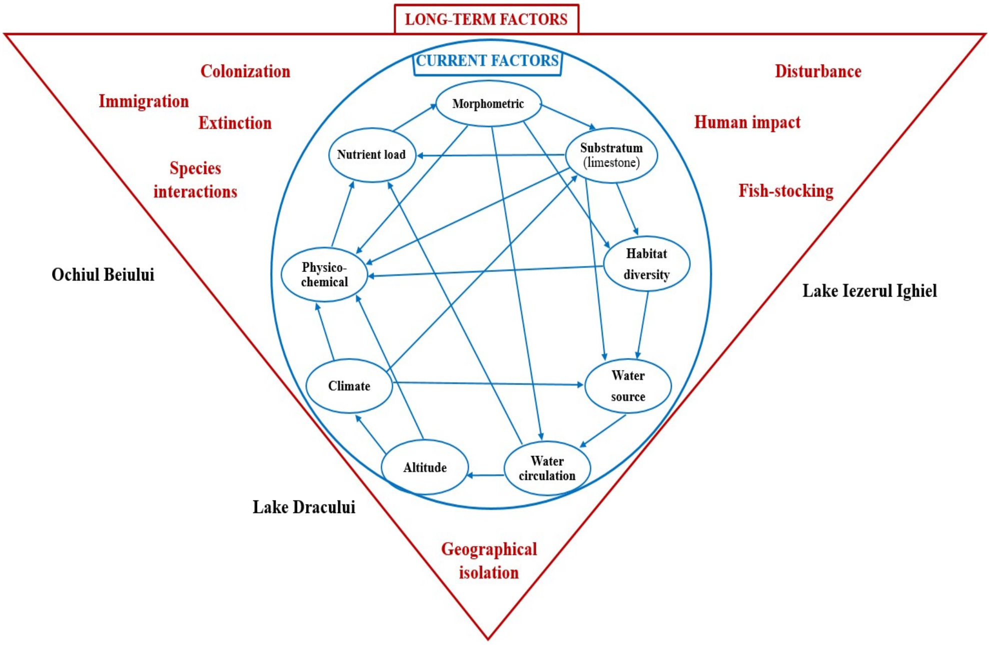

Figure 9. Schematic view of current vs. long-term factors inflencing the assemblages from the three karstic lakes considered

Most current factors characterize similar environments in the three karstic lakes considered for the present study: similar limestone substratum, high conductivity, dissolved oxygen or transparency values, relatively high habitat diversity including pelagic and littoral regions (rocks, sand, vascular and non-vascular plants, dead organic matter), water feeding from springs or surface run-off, water circulation, shading etc. There are however important differences in morphometry: lake area and volume are much higher for Lake Iezerul Ighiel. On the other hand, all long-term factors separate the three lakes. Geographical isolation that in turn influences the colonization ability and immigration/extinction rates is much higher for Lake Dracului and Ochiul Beiului, where no surface water inputs were described. The disturbances are higher for Lake Iezerul Ighiel, in form of constant tourism pressure: camping, bathing, fishing and fish-stocking. From this point of view, the higher diversity recorded here can be linked to the intermediate disturbance characteristic to the region (consistent with the intermediate disturbance hypothesis, described by Connell, 1978). Species interactions like competition and predation could also explain the disimilarities from the three karstic lakes.

Conclusions

Three karstic lakes were analysed considering the physico-chemical parameters, algal and microcrustacean communities during 2014 and 2016. Even if similar karstic conditions were recorded in Lakes Iezerul Ighiel, Dracului and Ochiul Beiului, dissimilar communities were recorded, for algae and microcrustaceans alike, in terms of species richness, average abundances and diversity.

Thus, even if most abiotic parameters like limestone substratum or physico-chemical values (the current factors) unify the three karstic environments, long-term parameters like geographical isolation and disturbance divide them, Lake Iezerul Ighiel having highly different assemplages of algae and microcrustaceans compared to Lake Dracului and Ochiul Beiului.

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