MACROZOOBENTHIC INVERTEBRATE ASSEMBLAGES IN THE COZLA AREA, IRON GATES NATURAL PARK, ROMANIA

CUPȘA Diana, PASCAR Bianca, COVACIU-MARCOV Severus Daniel, FERENȚI Sára, TELCEAN Ilie Cătălin

Abstract. On 20 June 2020, we sampled eight aquatic habitats from the Iron Gates Natural Park near Cozla to determine the structure of the macrozoobenthic invertebrate assemblages. We measured some of the most important physicochemical parameters of the water in each sampling site (dissolved oxygen, temperature, conductivity, pH). We also determined the number of taxa, number of individuals, Shannon-Wiever index, and evenness in all sample sites. The invertebrate assemblages from the anthropogenic habitats differ compared to the natural habitats. In some of the investigated sites, the values of the water physicochemical parameters were below the tolerance limit of most invertebrate groups, as the assemblages contained a very small number of taxa. The Jaccard and Bray Curtis similarity indexes showed which sites had the most similar communities. The Mann-Whitney test showed significant differences between some of the sites. The correspondence between the environmental factors and the sample sites was highlighted by the CCA analysis. We did not find invasive species in the sample sites, even if they are present in the Danube. The structure of the assemblages and the abundance of the individuals indicate the crucial role of the habitat type in how the species associate to form the macrozoobenthic assemblages. In the same habitat types, local factors (e.g., the producers' assemblage and the quantity of the debris in the substrate) determine the differences between the communities.

Keywords: macrozoobenthos, water quality, physico-chemical parameters, biodiversity.

Rezumat. Comunități de nevertebrate macrozoobentice din zona Cozla, Parcul Natural Porțile de Fier, România. În 20 iunie 2020 am colectat probe din habitate acvatice de pe teritoriul Parcului Natural Porțile de Fier din apropierea localității Cozla, pentru a determina asociațiile de nevertebrate macrozoobentice care trăiesc în acestea. Am măsurat cei mai importanți parametri fizico-chimici ai apelor în fiecare stație (oxigenul dizolvat, temperatura apei, conductivitatea și pH-ul). Am determinat numărul de taxoni, numărul de indivizi, indicele Shannon Wiever și echitabilitatea pentru fiecare comunitate macrozoobentică din cele 8 stații de prelevare a probelor. Am observat că asociațiile macrozoobentice diferă în special în habitatele artificiale. În unele dintre stațiile de prelevare a probelor valorile parametrilor fizico-chimici ai apelor au fost sub limita de toleranță a majorității grupelor de nevertebrate macrozoobentice, aici comunitatea era alcătuită dintr-un număr foarte mic de specii. Indicii Jaccard și Bray-Curtis au permis stabilirea stațiilor care au avut comunitățile cele mai asemănătoare între ele. Testul Mann-Whitney a evidențiat diferențele semnificative între unele dintre stațiile investigate. Analiza CCA a evidențiat corespondența între parametri mediului de viață și comunitățile macrozoobentice din stațiile analizate. Nu am identificat specii invazive în stații, chiar dacă acestea sunt prezente în Dunăre. Structura comunității și abundența indivizilor arată faptul că tipul habitatului joacă un rol fundamental în asocierea speciilor macrozoobentice. În cadrul habitatelor de același tip, influențele locale (ex. asociația de producători, cantitatea de detritus din substrat) determină diferențierea comunităților.

Cuvinte cheie: macrozoobentos, calitatea apei, parametri fizico-chimici, biodiversitate.

INTRODUCTION

Macrozoobenthic invertebrates are considered indicators of water quality in aquatic ecosystems (HARAHAP et al., 2018; KEROVEC & MIHALJEVIĆ, 2010; WIDIASTUTI et al., 2023), a fact already established also in Romania (CUPȘA et al. 2010). An important area in Romania because of its high level of biodiversity expressed by numerous protected species is the Iron Gates Natural Park (IGNP) (ROZYLOWICZ et al., 2019). Several invertebrates and vertebrates of high conservative or biogeographic value are present in this area (e.g., COVACIU-MARCOV et al., 2009; TĂUȘAN & TEODORESCU, 2017; TEODOR et al., 2019, RUICĂNESCU & DUMBRAVĂ, 2020). The Danube Gorge, which overlaps with IGNP, is an area with high touristic potential (BÂC & ROSCA, 2017; OGARLACI, 2016), attracting an increased number of tourists each year, as it is considered an area of international touristic importance. Their presence may impact the aquatic communities if the waste of touristic activities is not managed adequately. The watercourses can be polluted by domestic wastes or chemical substances, e.g., fuels from boats (HAQUE et al., 2020). However, despite the high biodiversity, several forms of anthropogenic impact exist in the area and have existed in the past. Among these, we can mention numerous mines which left behind affected areas and tailings (NICULAE et al., 2014). One of these mining sites was located in the Cozla area, which nowadays is considered a zone with the potential of becoming a tourist attraction (BOENGIU, 2012). In the Cozla area, coal formed in the inferior Jurassic era was exploited (POPA, 2003). The mines were closed after the 2000s, like many other southern Carpathian mines (POPA & PREDEANU, 2018). After the mining activities, several traces were left behind, such as closed mine galleries, water settling basins, abandoned and ruined buildings, empty blocks of flats, and even port facilities at the bank of the Danube.

The Danube is located on Europe's southern aquatic invasion corridor (MUNJIU & SHUBERNETSKI, 2010). Several invasive species are already present in this area (see: GOIA et al., 2014), such as *Corbicula fluminea* (MARKOVIĆ et al., 2012) and *Faxonius limosus* (PÂRVULESCU et al., 2009). These species can represent a threat to the native fauna due to their better capacity to use food resources (the case of *Corbicula fluminea* (LUCY et al., 2012;

LI et al., 2023)) or by their pathogens which can determine the extinction of the native species (the case of *Faxonius limosus*, as this fact probably already happened in the region (GROZA et al., 2021)).

Macrozoobenthic invertebrates are considered indicators of water quality (WIDIASTUTI et al., 2023) and are an essential part of food for fish and some amphibians (BĂCESCU, 1954; HOWE et al., 2014; SHARIFIAN FARD et al., 2014; CICORT-LUCACIU et al., 2004, 2005; BOGDAN et al., 2011, 2013; SUCEA et al., 2014). Although IGNP includes studies on macrozoobenthic invertebrate assemblages in some Danube tributaries, these targeted only large watercourses (CURTEAN-BĂNĂDUC, 2014). Unlike this, we approached, on a much smaller surface, various types of aquatic habitats from the perimeter of the former mining site from Cozla. Due to their past anthropogenic disturbance, we hypothesized that there would be differences between the macrozoobenthic assemblages from these waters of various characteristics. The aims of our study were: 1. To determine the assemblage of the macrozoobenthic invertebrates from different types of water bodies from the Cozla region (rivulets, artificial basins, and the Danube near the shore). 2. To determine the differences in the structure of the assemblages due to the habitat type or physicochemical parameters of the waters. 3. To determine the similitudes between different assemblages.

MATERIALS AND METHODS

During the study, we collected eight macrozoobenthic samples from different aquatic habitats. The samples were collected on 20 June 2020. The sample sites were the following:

Cozla 1 (C1) is an artificial basin made of concrete with relatively shallow stagnant water invaded by vegetation represented by *Typha* sp. And *Phragmites* sp. Although the walls of the basin are made out of concrete, these were broken, making the habitat easily accessible; the water contains much debris resulting from the nearby ruins.

Cozla 2 (C2) is an artificial basin like the first one but with deeper water; macrophytes were present only at the banks; the water is greenish due to heavy eutrophication of unknown origin. This basin has vertical concrete walls, as it was probably a former settling pond, just like the previous one.

Cozla 3 (C3) is a big pond in the former river port, with stagnant water, aquatic vegetation along the bank, and natant macrophytes on most of the water's surface.

The Cozla 4 (C4) sampling site is situated at the opposite end of the previous pond, with deeper and less eutrophicated water.

Cozla 5 (C5) is an approximately 1 m wide rivulet that flows through boulders and pebbles in a forested area (oaks and hornbeams). The riverbed lacks aquatic vegetation, and the water flows fast but also contains fire salamander larvae.

Cozla 6 (C6) is situated near a road on the same rivulet as C5, downstream with approximately 100 meters. The rivulet bed here is 1 m wide, and the water flows slower; the riverbed is covered by sand and pebbles and crosses the same forest. There is a sealed mine entrance upstream of the sampling site, from which, however, we did not notice any residual water flowing into the stream.

Cozla 7 (C7) is situated on the Danube. The river is embanked in this area, the height of the banks is around 2 m, pebbles cover the riverbed, and the aquatic vegetation is absent.

Cozla 8 (C8) is also on the Danube. The banks are lower at this site; the water is shallow near the banks, with submerged vegetation close to the banks. Pebbles and sandy beaches cover the riverbed.

For sampling, we used a Surber sampler with an area of 0.1 m^2, equipped with a mesh size of 250 μm. The samples were preserved on the field in 4% formalin, labelled, and taken to the lab. At each sampling site, we measured some of the most important physical and chemical parameters of the water (dissolved oxygen, temperature, conductivity, pH). In the laboratory, the macrozoobenthic samples were sorted under a 100X - 400X magnifying stereomicroscope, transferred in 80% ethyl alcohol, and determined to the lowest taxonomic level possible, using specific keys to different groups (AUBERT, 1959; UJHELYI, 1959; STEINMANN, 1968; ELLIOTT et al., 1988; SOLEM & GULLEFORS, 1996; BOUCHARD, 2004).

We calculated the following indices, primarily used in these kinds of studies: the number of individuals (N), the number of taxa (S), and diversity indexes (Shannon –Wiever and evenness). The degree of similarity between the assemblages was tested with the Jaccard and Bray-Curtis similarity indexes; the Kruskal Wallis test and the Mann-Whitney test were conducted to test significant differences between sites and CCA (canonical correspondence analysis) to test correspondence with the environmental parameters; all tests were performed using the Past software (HAMMER et al. 2001).

RESULTS

The values of the physical and chemical parameters varied between narrow limits, with a few exceptions in some of the sample sites (Table 1). The most constant was the temperature, and the most variable was the conductivity. The dissolved oxygen values varied between 1.09 mg/l (C3) and 5.08 mg/l (C2). The water temperature values ranged between 16.80 (C5) and 27.30°C (C2). In C6 and C8, the water temperature was above 20°C. The conductivity values were between 41 (C7) and 352 (C2), and the pH values were between 3.25 (C2) and 8.29 (C7). In the eight sample sites, we identified 48 macrozoobenthic invertebrate taxa. The number of taxa per sample site varied between 2 (C2) and 24 (C1). The variation in the number of specimens was pronounced; it varied between 112 (C6) and 1825 (C8) (Table 1).

Table 1. The percentage abundance of taxa, physicochemical parameters, number of individuals (N), number of taxa (S), Shannon-Wiever diversity (H) and evenness (e) in the eight sampling sites (C1-C8).

	C1	C2	C3	C4	C5	C6	C7	C8
Hydrozoa	0.13	-	-	-	-	-	-	-
Turbellaria	-	-	-	0.12	-	-	-	-
Oligochaeta	0.26	-	3.14	1.27	0.38	2.67	7.98	3.34
Hirudinea	-	-	12.99	13.12	0.38	0.89	0.25	-
Gastropoda	0.13	-	11.41	2.42	-	0.89	11.42	0.71
Bivalvia	-	-	-	-	-	-	0.34	0.21
Hydrachnidia	-	-	-	-	0.19	-	0.08	-
Mysidacea	-	-	-	30.70	-	-	49.74	77.53
Gammaridae	-	-	2.75	0.25	53.80	3.57	2.14	0.93
Isopoda	-	-	12.20	4.96	-	-	-	-
Collembola	-	-	0.39	-	-	-	-	-
Ephemeroptera larvae - Baetidae	2.36	-	7.48	3.05	2.47	13.39	0.08	-
Ephemeroptera larvae - Caenidae	-	-	5.51	2.80	-	-	-	-
Ephemeroptera larvae - Ephemerellidae	-	-	0.39	-	0.19	-	0.08	-
Total Ephemeroptera larvae	**2.36**	**-**	**13.38**	**5.85**	**2.66**	**13.39**	**0.17**	**-**
Odonata larvae - Lestidae	5.39	-	-	-	-	-	-	-
Odonata larvae - Calopterygidae	-	-	1.18	-	-	-	-	-
Odonata larvae - Coenagrionidae	4.73	-	6.69	6.49	-	-	-	0.05
Odonata larvae - Cordulegastridae	0.39	-	-	-	-	-	-	-
Odonata larvae - Libellulidae	2.76	-	-	-	-	-	-	-
Odonata larvae - Aeshnidae	0.39	-	-	-	-	-	-	-
Total Odonata larvae	**13.68**	**-**	**7.87**	**6.49**	**-**	**-**	**-**	**0.05**
Plecoptera larvae - Chloroperlidae	-	-	-	-	-	1.78	-	-
Plecoptera larvae - Nemouridae	-	-	-	-	4.75	0.89	-	-
Total Plecoptera larvae	**-**	**-**	**-**	**-**	**4.75**	**2.67**	**-**	**-**
Heteroptera - Pleidae	0.26	-	-	-	-	-	-	-
Heteroptera - Corixidae	-	-	12.20	9.80	-	-	-	-
Heteroptera - Naucoridae	0.52	-	-	2.03	-	-	-	-
Heteroptera - Nepidae	-	-	-	0.12	-	-	-	-
Total Heteroptera	**0.78**	**-**	**12.20**	**11.97**	**-**	**-**	**-**	**-**
Coleoptera - Dytiscidae - adults	0.26	-	0.78	-	-	-	-	-
Coleoptera - Dytiscidae - larvae	1.05	-	1.96	1.65	-	1.78	-	-
Coleoptera - Haliplidae - adults	0.13	0.22	1.18	0.12	0.19	-	-	-
Coleoptera - Haliplidae - larvae	-	-	0.39	-	-	-	-	-
Coleoptera - Hydrophilidae - adults	-	-	0.39	-	-	-	-	-
Total Coleoptera	**1.44**	**0.22**	**4.72**	**1.78**	**0.19**	**1.78**	**-**	**-**
Trichoptera larvae - Hydroptilidae	-	-	5.11	-	-	-	-	-
Trichoptera larvae - Rhyacophilidae	-	-	-	-	0.38	-	-	-
Trichoptera larvae - Leptoceridae	0.26	-	-	0.12	-	-	-	-
Trichoptera larvae - Sericostomatidae	0.26	-	-	-	-	-	-	-
Trichoptera larvae - Polycentropidae	-	-	-	-	1.52	-	-	-
Total Trichoptera larvae	**0.52**	**-**	**5.11**	**0.12**	**1.90**	**-**	**-**	**-**
Lepidoptera larvae	-	-	5.51	0.12	-	-	-	0.32
Diptera larvae - Tipulidae	5.78	-	-	-	0.19	-	-	-
Diptera larvae - Chaoboridae	10.65	-	-	-	-	-	-	-
Diptera larvae - Chironomidae	61.97	99.77	7.48	20.50	19.96	26.78	27.83	16.87
Diptera larvae - Simuliidae	-	-	-	-	15.39	45.53	-	-
Diptera larvae - Stratiomyidae	0.52	-	-	0.12	0.19	-	-	-
Diptera larvae - Culicidae	1.05	-	0.78	0.12	-	-	-	-
Diptera larvae - Tabanidae	0.39	-	-	-	-	-	-	-
Diptera larvae - Ephydridae	0.13	-	-	-	-	-	-	-
Diptera larvae - Dixidae	0.13	-	-	-	-	-	-	-
Diptera larvae - Psychodidae	-	-	-	-	-	0.89	-	-
Diptera larvae - Sciomyzidae	-	-	-	-	-	0.89	-	-
O_2 mg/l	2.87	5.08	1.09	2.39	3.15	1.35	3.87	2.80
Temperature (°C)	23.50	27.30	21.90	22.00	16.80	17.00	23.00	21.00
Conductivity	150	352	106	114	63	65	41	44
pH	7.35	3.25	7.45	8.08	8.04	8.02	8.29	7.95
S	24	2	21	20	14	12	10	8
N	760	442	254	785	526	112	1164	1825
H	1.54	0.01	2.66	2.09	1.38	1.06	1.29	0.73
e	0.19	0.51	0.68	0.41	0.28	0.41	0.36	0.26

The abundance of macrozoobenthic invertebrates in the eight sample sites encountered high amplitude variations. In C1, the most abundant group was Chironomidae larvae, followed by Chaoboridae larvae and Tipulidae larvae. Here, we also found a few Hydrozoans. In C2, the most abundant group was Chironomidae larvae. In C3, high abundance values were encountered in the case of Hirudinea, Isopoda, and Heteroptera Corixidae. In C4, Mysidacea was the most abundant, followed by Chironomida. In C5, Gammaridae has a value of their abundance over 50%; in C6, the most abundant were de Simuliidae larvae; in C7, Mysidacea made up almost half of the invertebrates, and in C8 the Mysidacea are the most abundant (Table 1). The values of the Shannon-Wiever index varied between 0.01 in C2 and

2.66 in C3; most of the values were between 1 and 2.09 (Table 1). The values of the evenness ranged between 0.19 (C1) and 0.68 (C3) (Table 1).

The Jaccard index indicated the highest similarity between sites C3 and C4 (0.51); very close to this value was the similarity between C7 and C8 but on another branch of the cladogram. The sites C5 and C6 are grouped closer to C7 and C8. The lowest values were encountered between C2, situated on a separate branch of the cladogram, and the rest of the sample sites (Fig. 1A). According to the values of the Bray-Curtis similarity index, the most similar are sample sites C1 and C2 (0.73), and they are situated in a distinct branch of the cladogram. On the other branch, C7 and C8 also have a high degree of similarity (0.65). A distinctive branch from these contains sample sites C5 and C6 (0.31), grouped with C3 (Fig. 1B).

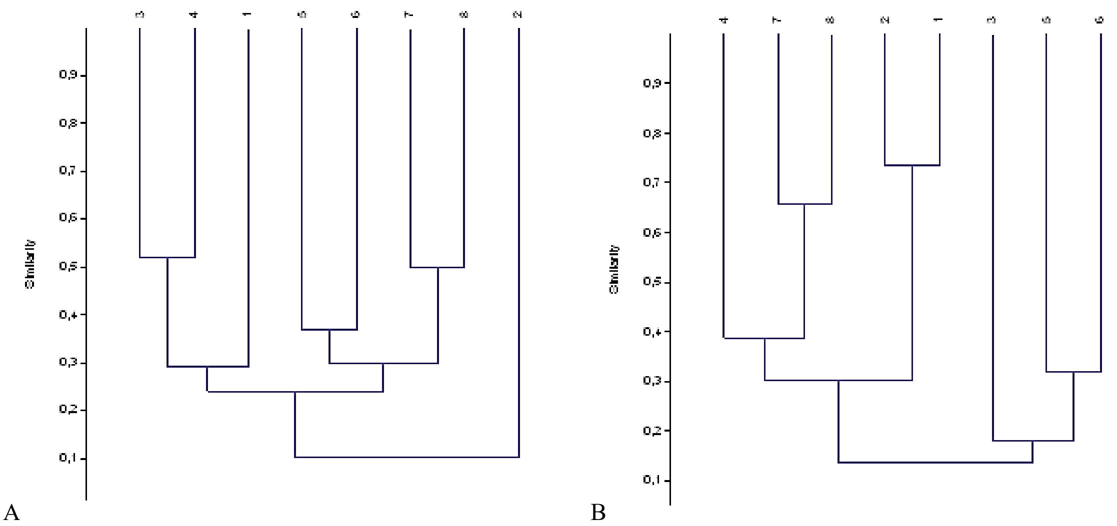

Figure 1. Values of the (A) Jaccard and (B) Bray Curtis similarity indexes between the sampling sites.

The Kruskal-Wallis test for equal medians shows significant differences between the investigated sample sites ($p<0.0001$). The Mann Whitney test shows significant differences between sites C1 and C2, C5-C8, site C2 and all other sites, C3 and C2, C6-C8, site C4 and C7-C8, site C5 and C1-C2, C6 and C1-C3 (Table 2). The canonical correspondence analysis (CCA) indicated that the most important environmental factors which influence the structure of the macrozoobenthic invertebrate assemblages are conductivity, temperature, and the amount of dissolved oxygen. The oxygen content and the water temperature strongly correlate with the assemblages of C3 and C4, a weak correlation with C1, C2, C7, and C8, and a negative correlation with C5 and C6 (Fig. 2). The conductivity values strongly correlate with C1-C4, weakly with C7 and C8, and negatively with C5 and C6. The pH has a weaker correlation with the assemblages from the sample sites. The number of taxa is strongly correlated with C1-C4, weakly with C7-C8, and the number of specimens is highly correlated with C4, C7, and C8 and weakly with C3. The diversity indexes (Shannon-Wiever and evenness) strongly correlate with C3 and C4, moderately with C7 and C8, and weakly with C1 and C2 (Fig. 2). Between the sampling sites, CCA shows a strong correlation between C1 and C2, C3 and C4, and C7 and C8.

Table 2. Differences between the assemblages from the sampling sites according to the Mann-Whitney test (p values).

	Cozla 1	Cozla 2	Cozla 3	Cozla 4	Cozla 5	Cozla 6	Cozla 7	Cozla 8
Cozla 1								
Cozla 2	<0.0001							
Cozla 3	0.693	<0.0001						
Cozla 4	0.572	<0.0001	0.987					
Cozla 5	0.036	0.001	0.110	0.177				
Cozla 6	0.006	0.004	0.028	0.050	0.586			
Cozla 7	0.007	0.014	0.029	0.045	0.449	0.795		
Cozla 8	0.002	0.046	0.009	0.013	0.222	0.479	0.647	

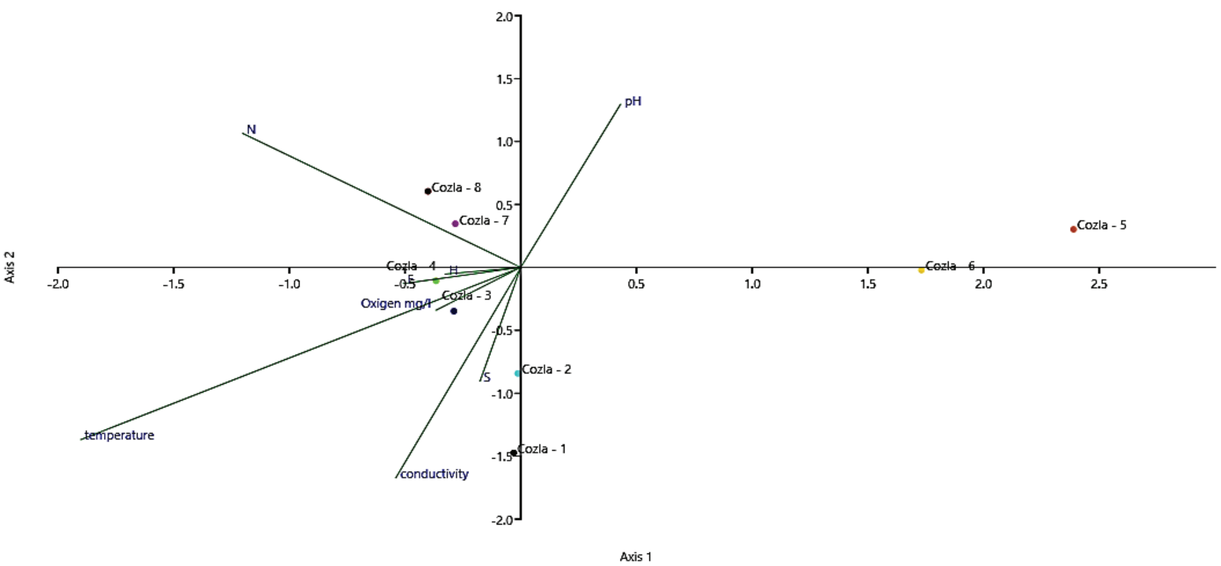

Figure 2. Canonical correspondence analysis (CCA) based on the diversity index values with respect to environmental variables (green lines = environmental variables, N = number of individuals, S= number of taxa, H= Shannon-Wiever index, E= evenness, coloured circles = sample sites).

DISCUSSION

The dissolved oxygen content varies depending on the type of the investigated habitats. Low values were measured in C3, which is a highly eutrophicated basin. In this habitat, eutrophication induced an excessive algal mass, causing the greenish colour of the water. Also, it is well known that algae consume the oxygen from the water, causing hypoxia (WATSON et al., 2016). Another sample site with a low oxygen content is C6, situated on the rivulet, which crosses a forest. The substrate in this sector is sandy, with an important organic content resulting from the fallen leaves of the trees nearby. The vegetal debris undergoes a decomposition process during the warm summer period. The microbial activity during this process consumes the oxygen from the water, creating hypoxia even in flowing water as it is observed frequently in lakes (WEINKE & BIDDANDA, 2018).

The lowest water temperature values were measured in the two sites along the rivulet (C5 and C6). The stream flows through a shaded area as it is situated in a forest, so the water is not warmed during the day by the sun, a phenomenon registered in the scientific literature (KALNY et al., 2017). The highest temperature value was measured in the artificial basin with stagnant water. The concrete from which the basin is made gets warm during the day when the sun shines over the basin.

The conductivity has the highest measured value in C2. This is the artificial basin with eutrophicated water, probably used in the past as a settling pond of the mine. The increased conductivity value in this basin results from the high electrolytic content. These can come from external sources such as domestic waste waters or agricultural fertilizers or result from the microbial mineralization process of the organic compounds from the water (CHISLOCK et al., 2013), but in this case, they probably remained from when the mine was still functional. Low conductivity values were measured in C1, C4, and C5; these are all artificial habitats with stagnant water but less eutrophicated than site C2. The sample sites C5 and C6 situated on the rivulet have much lower conductivity; these are higher quality waters than those from the basin. The water at sample sites C7 and C8 along the Danube River registered a lower conductivity than the basins. This is due to the river's flowing character, the water's high volume, and the lower organic content in the riverbed.

The measured pH values are between limits that can be tolerated by most groups of macrozoobenthic invertebrates, characteristic of the lowland water courses (GANONG et al., 2021). Only in C2 the pH value was extremely acidic (3.25); this value is much lower than the tolerance limit of most invertebrate groups, so we registered a minimum of the taxa number in this habitat. Even climate changes are considered responsible for increasing the incidence of pH decrease, a fact that can determine the disappearance of some taxa (GANONG et al., 2021). Nevertheless, the pH correlated with the high conductivity of the water clearly shows that this artificial habitat is extremely unfavourable for aquatic biodiversity, most likely due to past anthropic activities related to mining.

The 48 taxa identified in the eight sample sites reveal a great aquatic invertebrate biodiversity, expectable in IGNP, known to have outstanding biodiversity and numerous protected species (ROZYLOWICZ et al., 2019). The high number of taxa is not found in each investigated sample site because these differ greatly regarding physicochemical and hydrological parameters. The richest sites in taxa are C1, C3, and C4, represented by the artificial basins. These are artificial habitats, but their aquatic vegetation offers a substrate for various groups of invertebrates which find shelter and food resources between the plants. Also, the artificial basins, except for C1, have a substantial volume of water, as water depth and aquatic vegetation are considered indicators of water health (DE et al., 2019).

At sample site C1, the high abundance of the Chironomidae larvae indicates a high degree of organic pollution. The shallow water at this site and the abundant aquatic vegetation stimulate the activity of the microorganisms, which decompose the organic matter. These conditions are limitations for some sensitive species but are favourable to the resistant ones found with high abundance in this habitat. At sample site C2 we only encountered two taxa: Chironomidae larvae and adults of Coleoptera Haliplidae. The strong eutrophication and the acidity of the water were the main environmental factors that have limited the development of most aquatic invertebrate groups. Only Chironomidae larvae known to be highly tolerant can install here and aquatic Coleoptera, which are protected from the acidity of the water by their thick chitinous cuticle, as they were found in acidic peat pools (BUCZYŃSKA & BUCZYŃSKI, 2019), but not so acidic as in our case. At C3, the groups with the highest abundances are Hirudinea, Gasteropoda, Isopoda, Heteroptera, and Corixidae. Each of these groups, resistant to hypoxia or breathing oxygen from the air, has a small number of individuals in this habitat. These groups are adapted to a high organic content; they are tolerant and even indicators for low-quality water. At C4, we have found a high abundance of Mysidaceae. At this site, the oxygen content of the water is higher than at C3, so representatives of the Mysidaceae group can develop in such conditions (BĂCESCU, 1940, 1954). The origin of this group is in the Danube, where these were frequently reported, even more upstream (WITTMANN, 2007; BÓDIS et al., 2012); C3 and C4 have permanent communication with the Danube so that the Mysidaceae can enter the basin from the river. Mysidaceae were absent from C3, probably due to the hypoxia in this part of the basin. C5 is situated on the rivulet, where we encountered a high abundance of Gammaridae. Their presence is associated with organic debris in the water originating, in this case, from the fallen leaves of the trees nearby. The assemblage in this sample site is highly different from the previous ones. It is characteristic of a rivulet with flowing water with a smaller volume of water and lower temperature. The invertebrate community at this sample site is characterized by the presence of several sensitive groups as Plecoptera larvae (LOCK & GOETHALS, 2014). The assemblage from C6 has fewer taxa (12) and individuals (112) than C5. This is the second sampling site along the rivulet with a higher organic content in the riverbed and lower oxygen content, creating more limited conditions for invertebrates. Even in the case of a large watercourse from the area, the differences between the discharge area in the Danube and upstream zones were obvious (CURTEAN-BĂNĂDUC, 2014). At C7, we encountered only ten taxa but with a high number of individuals (1164). The sample site is situated in the Danube River. The small number of taxa results from the fact that we only collected right near the riverbank, so we could only sample macrozoobenthic invertebrates located in this region of the riverbed. The high number of specimens suggests that the water is good quality and offers an adequate trophic base to sustain abundant populations. In the second sampling site from the Danube (C8), we identified eight taxa and 1825 individuals. As in the previous sampling site, the most abundant were the Mysidacea. The assemblage structure is very similar to that from C7, as the two areas are close, and there are no differences in the hydric regime, physicochemical parameters, or riverbed.

In samples C1 and C2, the most abundant taxa were the Chironomida larvae. Their high abundance indicates organic water pollution associated with hypoxia (HERCUȚ et al., 2008). This fact is undeniable in C2, where the abundance of the Chironomidae larvae was very high. A high abundance of Gammarida was found only in one sample site (C5). Their presence is usual in water courses containing high amounts of nutrients (MEDUPIN, 2020). Isopoda was the most abundant in C3; they were scarce or absent in the rest of the sample sites. At least one species from this group is considered extremely tolerant to poor water conditions (O'CALLAGHAN et al., 2019). Gastropoda forms relatively dense populations; they are present in almost all sample sites. They were most abundant in C3 and C7. Mysidacea was very abundant in the sample sites from the Danube C7, C8, and also in C4. Their presence is associated with high oxygen content in the water (BĂCESCU, 1940, 1954). Plecoptera larvae were encountered in C5, but their abundance was small. They are sensitive species (LOCK & GOETHALS, 2014), associated with high oxygen content, low temperature, and high water velocity, and they are usually found in mountainous streams. In the IGNP, several species characteristic of mountainous regions were found at low altitudes (PAŞCOVSCHI, 1956; COVACIU-MARCOV et al., 2009; TEODOR et al., 2019). Plecoptera are not an exception; they will also be present if they find the proper environmental conditions in a low-altitude rivulet. Oligochaeta were found in almost all sample sites, but their abundance was low. Ephemeroptera larvae had a low abundance (0.08% S7 and 0.39% S3). They are characteristic of good quality waters with high oxygen content and low organic load, and an important number of species are typical of mountainous sectors of the rivers; therefore, their presence could be explained similarly to the Plecoptera mentioned before.

The highest values of the Shannon-Wiever index were registered at C3 (2.66) and C4 (2.09). Here we found an assemblage with an increased number of species of invertebrates. These two sample sites are situated in the same basin, connected to the Danube, so the eutrophication is lower than in other sample sites. The connectivity with the Danube allows the species to enter the basin, which causes a high similarity between these two habitats. Still, there are some differences between the assemblages caused by environmental factors. The lowest value of the Shannon-Wiever diversity index was calculated for the C2 sample site (0.016), represented by the eutrophicated basin.

According to the Jaccard similarity index, the most different sample site is C2. This is a strongly eutrophicated artificial basin with only two groups of invertebrates: Chironomidae and Coleoptera Haliplidae. Also, the water in this sample site is very acidic. C1, C3, and C4 form a cluster in which the most similar are C3 and C4 (0.51), as more than half of the species found in these two sites are in common because the two sites are situated in two different regions of the same basin. C1 is located in the same cluster as C3 and C4, but the latter are more similar. This moderate similarity of C1 is given by the main characteristics of this habitat, as it is a smaller basin with aquatic vegetation and some organic load, so the macrozoobenthic assemblage is not totally different here. Another cluster of the cladogram contains sample sites C7 and C8 with a similarity index of 0.5 and S5 and S6 sample sites with a similarity of 0.4, on the other hand. C7 and C8 are situated on the Danube, they have almost similar habitat conditions, so it was expected that the assemblage would have some degree of similarity. C5 and C6 are situated on the rivulet; they have a distinctive assemblage of macrozoobenthic invertebrates, which is more like those from the Danube than from the other sample sites. So even if there is a big difference between the size of the Danube and the investigated rivulet, the structure of the macrozoobenthic assemblages looks much alike compared to the assemblages from the stagnant waters.

The Bray-Curtis index shows two clusters. Sample sites C3, C5, and C6 are located on one branch. Sample site C3 has some similarities with C5 and C6 because of its diverse assemblage of invertebrates. The other cluster contains sites C1 and C2 on one hand and C4, C7, and C8 on the other. Sample sites C1 and C2 have a similarity of 0.74, which is the greatest among all sample sites. These are the two artificial basins with similar sizes, more or less eutrophicated, and with much aquatic vegetation. Sample sites C7 and C8 have a similarity of 0.66. C4 is situated in one region of the big basin, connected to the Danube. Here the species from the Danube can enter the basin (e.g., Mysidacea), so the assemblage will look much like that from the Danube.

The Mann-Whitney test has shown significant differences between most sample site assemblages. These differences result from the characteristics of the investigated habitats, which are different in water volume, their direct connectivity, the values of the physicochemical parameters, their stagnant or flowing character, and their natural or anthropic origin (Table 2). We found no significant differences between C1, C3, and C4. These three sites represent artificial habitats. Their assemblages are significantly different from those in C2, which has the same construction type but has different physicochemical parameter values, especially pH and dissolved oxygen. The limitation values of these two physicochemical parameters are restrictive for most groups of macrozoobenthic invertebrates (GANONG et al., 2021). There were no significant differences between C4 and C5 or C6 on the rivulet. Although C4 is a site with stagnant water with similar physicochemical parameters, the small distance to the rivulet and the connectivity of the three sample sites with the Danube have determined the presence of the assemblages, which are not very different from each other (Table 2). Sample sites C5 and C6 on the rivulet also have non-significant differences with C7 and C8 in the Danube. These sample sites are situated close to each other; the rivulet flows into the Danube, in the Danube samples were taken from near the banks where the water is not very deep, and the riverbed is sandy with pebbles similar to the riverbed of the rivulet, the water flow is slower so the assemblages will be alike those in the rivulet.

CCA analyses showed that the dissolved oxygen content and the water temperature are determinant factors for the assemblage structure in C3 and C4. The conductivity strongly correlates with the assemblage in C1, C2, C3, C4. In these sample sites, the values of conductivity had much higher values than in the other sites due to the eutrophication processes from these sites (Fig. 2). The similarity pattern between the sample sites shown by the CCA analysis was like the one calculated by the Jaccard index; C3 and C4 on one hand, and C7 and C8 on the other hand, have the most similar assemblages. The sites from the rivulet C5 and C6 are grouped together but at a significant distance from all other sites, mainly because the rivulet has the lowest water volume and flows through a shaded forest area. C1 and C2 are also grouped separately but closer to the other two sample sites with stagnant water and those in the Danube.

Analysing the macrozoobenthic invertebrate assemblages from the eight investigated sample sites, we can ascertain the importance of the habitat type in structuring the invertebrate communities. We found assemblages in artificial habitats with a distinctive structure from natural habitats. The habitats connected to the Danube River have highly similar assemblages to those from the Danube. They shelter species that enter from the Danube and find here food resources and better protection from predators than in their natural habitat. In these conditions, they reproduce in mass, as we have seen in the case of Mysidacea. The great abundance of Mysidacea ensures a substantial trophic base for the fishes in the habitats where they occur in mass as they are eaten by most fish species (BĂCESCU, 1954). The basins C1 and C2 will probably undergo a warping process because they are already invaded by aquatic vegetation and do not have a connection with the Danube or other water bodies.

ACKNOWLEDGEMENTS

This study was realized in collaboration with the Iron Gates Natural Park administration, to whom we would like to thank in this way for their support.

REFERENCES

AUBERT J. 1959. *Plecoptera. Insecta Helvetica Fauna, Volume 1*. Swiss Entomological Society. 140 pp.

BĂCESCU M. 1940. Les mysidacés des eauxroumaines: etude taxonomique, morphologique, biogeographique et biologique. *Annales scientifiques de l'Université de Jassy*. Universitatea din Iași. **26**: 454-804.

BĂCESCU M. 1954. *Crustacea: Mysidacea. Fauna R. P. R.* Edit. Academiei R. P. R. Bucuresti. **4**(1). 124 pp.

BÂC D. & ROȘCA P. 2017. A short analysis of the forms of sustainable tourism present in selected central and eastern european countries. *Annals of Faculty of Economics, University of Oradea, Faculty of Economics.* University of Oradea Publishing House. **1**(2): 43-50.

BÓDIS E., BORZA P., POTYÓ I., PUKY M., WEIPERTH A., GUTI G. 2012. Invasive mollusk, crustacean, fish and reptile species along the Hungarian stretch of the rover Danube and some connected waters. *Acta Zoologica Academiae Scientiarium Hungaricae*. Hungarian Natural History Museum, Biological Section of the Hungarian Academy of Sciences. **58**(supplement): 29-45.

BOGDAN H. V., IANC R. M., POP A. N., SÖLLÖSI R. Ș., POPOVICI A. M., POP I.F. 2011. Food composition of an *Ichthyosauraalpestris* (Amphibia) population from the Poiana Ruscă Mountains, Romania. *Herpetologica Romanica*. University of Oradea Publishing House. **5**: 7-25.

BOGDAN H. V., COVACIU-MARCOV S.-D., GACEU O., CICORT-LUCACIU A.-S., FERENȚI SÁRA, SAS-KOVÁCS I. 2013. How do we share food? Feeding of four amphibian species from an aquatic habitat in south-western Romania. *Animal Biodiversity and Conservation*. Museu de Ciencies Naturals de Barcelona. **36**(1): 89-99.

BOENGIU V. 2012. Evaluation of tourism resources in the Iron Gates Natural Park in order to identify the potential of tourism development. *AnaleleUniversității din Oradea – SeriaGeografie*. University of Oradea Publishing House. **22**(2): 234-240.

BOUCHARD R. W. JR. 2004. *Guide to aquatic invertebrates of the upper midwest.* University of Minnesota. 203 pp.

BUCZYŃSKA E. & BUCZYŃSKI P. 2019. Aquatic insects of man-made habitats: environmental factors determining the distribution of caddisflies (Trichoptera), dragonflies (Odonata), and beetles (Coleoptera) in acidic peat pools. *Journal of Insect Science*. Oxford University Press. **19**(1): 17.

CHISLOCK M. F., DOSTER E., ZITOMER R. A., WILSON A. E. 2013. Eutrophication: causes, consequences, and controls in aquatic ecosystems. *Nature Education Knowledge*. Springer. **4**(4): 10.

CICORT-LUCACIU A.-Ș., COVACIU-MARCOV S.-D., CUPȘA DIANA, PURGEA I., ROMOCEA M. 2004. Research upon trophic spectrum of a *Triturus vulgaris* (Linnaeus, 1758) populations of the Beiuș Depression area (România). *Universitatea din Bacău, Studii și Cercetări Științifice, Biologie*. Universitatea din Bacău. **9**: 201-206.

CICORT-LUCACIU A.-Ș., COVACIU-MARCOV S.-D., CUPȘA DIANA, PURGEA I., SAS I. 2005. Research upon the trophic spectrum of a *Triturus cristatus* population in the Briheni area (county of Bihor România). *Scientific Annals of the Danube Delta Institute for Research and Development*. Danube Delta National Institute for Research and Development - Tulcea. **11**:2-8.

COVACIU-MARCOV S.-D., CICORT-LUCACIU A.-Ș., GACEU O., SAS I., FERENŢI SÁRA, BOGDAN H. V. 2009. The herpetofauna of the south-western part of Mehedinţi County, Romania. *North-Western Journal of Zoology*. University of Oradea Publishing House. **5**(1): 142-164.

DE M., ROY C., MEDDA S., ROY S., DEY S. R. 2019. Diverse role of Macrophytes in aquatic ecosystems: A brief review. *International Journal of Experimental Research and Review*. **19**: 40-48.

ELLIOTT J. M., HUMPESCH U. H., MACAN T. T. 1988. *Larvae of the British Ephemeroptera: a key with ecological notes.* Freshwater Biological Association, Cumbria. 145 pp.

CUPȘA D., COVACIU-MARCOV S.-D., SUCEA F., HERCUȚ R. 2010.Using macrozoobenthic invertebrates to assess the quality of some aquatic habitats from Jiu Gorge National Park (Gorj County, Romania). *Biharean Biologist* **4**(2): 109-119.

CURTEAN-BĂNĂDUC A. 2014. Benthic macroinvertebrates communities in the northern tributaries of the “Iron Gates” Gorge (Danube River). *Transylvanian Review of Systematical and Ecological Research, special issue: The “Iron Gates” Natural Park*. DeGruyter Open. **16**: 151-164.

GANONG C., OCONITRILLO M. H., PRINGLE C. 2021. Thresholds of acidification impacts on macroinvertebrates adapted to seasonally acidified tropical streams: potential responses to extreme drought-driven pH declines. *PeerJ.* PeerJ Publishing. **9**: e11955.

GOIA I., CIOCANEA C. M., GAVRILIDIS A. A. 2014. Geographic origins of invasive alien species in “Iron Gates” Natural Park (Banat, Romania). *Transylvanian Review of Systematical and Ecological Research*. “Lucian Blaga” University of Sibiu, Faculty of Sciences. **16**(3): 115-130.

GROZA M. I., CUPSA DIANA, LOVRENCIC L., MAGUIRE I. 2021. First record of the stone crayfish in the Romanian lowlands. *Knowledge and Management of Aquatic Ecosystems*. Édition Diffusion Presse Sciences. **422**: 27.

HAMMER Ø., HARPER D. A. T., RYAN P. D. 2001. Past: paleontological statistics software package for education and data analysis. *Palaeontologia Electronica*. Coquina Press. **4**(1): 9.

HAQUE M. M., NILOY N. M., NAYNA O. K., FATEMA K. J., QURAISHU S. B., PARK J. H., KIM K. W., TARQ S. M. 2020. Variability of water quality and metal pollution index in the Ganges River, Bangladesh. *Environmental Science and Pollution Research.* **27**: 42582–42599.

HARAHAP A., BARUS T. A., MULYA M. B., ILYAS S. 2018. Macrozoobenthos diversity as bioindicator of water quality in the Bilah river, Rantauprapat. *Journal of Physics: Conference Series*. Institute of Physics Publishing. **1116**: 052026

HERCUȚ R., CUPȘA DIANA, PURTAN S., BALOG B. 2008. Studii privind structura comunităților de nevertebrate macrozoobentice din 3 habitate din zona localității Arginesti (Jud. Mehedinți, România). *Biharean Biologist*. University of Oradea Publishing House. **2**: 14-20.

HOWE E. R., SIMENSTAD C. A., TOFT J. D., CORDELL J. R., BOLLENS S. M. 2014. Macroinvertebrate prey availability and fish diet selectivity in relation to environmental variables in natural and restoring north San

Francisco bay tidal marsh channels. *San Francisco Estuary and Watershed Science*. Delta Stewardship Science Program, the California Digital Library's eScholarship Publishing Group, and the University of California—Davis John Muir Institute of the Environment. **12**(1): https://doi.org/10.15447/sfews.2014v12iss1art5.

KALNY G., LAAHA G., MELCHER A., TRIMMEL H., WEIHS P., RAUCH H. P. 2017. The influence of riparian vegetation shading on water temperature during low flow conditions in a medium sized river. *Knowledge & Management of Aquatic Ecosystems*. Édition Diffusion Presse Sciences. **418**: 5.

KEROVEC M. & MIHALJEVIĆ Z. 2010. Comparison of two biological methods for assessment of river water quality based on macrozoobenthos. *Croatian Journal of Fisheries*. University of Zagreb. **68**(1): 11-18.

LI Z., HE X., FENG C. 2023. A review of freshwater benthic clams (*Corbicula fluminea*): Accumulation capacity, underlying physiological mechanisms and environmental applications. *Science of the Total Environment*. Elsevier. **857**: 159431.

LOCK K. & GOETHALS P. L. 2014. Predicting the occurrence of stoneflies (Plecoptera) on the basis of water characteristics, river morphology and land use. *Journal of Hydroinformatics*. International Water Association Publishing **16**(4): 812-821.

LUCY F., KARATAYEV A., BURLAKOVA L. 2012. Predictions for the spread, population density and impacts of *Corbicula fluminea* in Ireland. *Aquatic Invasions*. International Association for Open Knowledge on Invasive Alien Species **7**(4): 465-474.

MARKOVIĆ V., ATANACKOVIĆ A., TUBIĆ B., VASILJEVIĆ B., KRAČUN-KOLAREVIĆ M., TOMOVIĆ J., PAUNOVIĆ M. 2012. Indicative status assessment of the Danube River (Iron Gate sector 849-1,077 rkm) based on the aquatic macroinvertebrates. *Water Research and Management*. Springer. **2**(2): 41-46.

MEDUPIN C. 2020. Spatial and temporal variation of benthic macroinvertebrate communities along an urban river in Greater Manchester, UK. *Environmental Monitoring and Assessment*. *Springer*. **192**(2): 84.

MUNJIU O. & SHUBERNETSKI I. 2010. First record of Asian clam *Corbicula fluminea* (Müller, 1774) in the Republic of Moldova. *Aquatic Invasions*. International Association for Open Knowledge on Invasive Alien Species. **5** (Supplement 1): S67-S70.

NICULAE M.-I., NIȚĂ M. R., VANĂU G. 2014. Analysing landscape fragmentation and classifying threats for habitats of community interest in the ”Iron Gates” Natural Park (Romania). *Transylvanian Review of Systematical and Ecological Research, special issue: The “Iron Gates” Natural Park.* DeGruyter Open. **16**: 195-208.

OGARLACI M. 2016. Tourist activities in the Mountainous Banat Villages. *Quaestus*. Edit. Eurostampa, Timișoara. **8**: 313-322.

O'CALLAGHAN I., HARRISON S., FITZPATRICK D., SULLIVAN T. 2019. The freshwater isopod *Asellus aquaticus* as a model biomonitor of environmental pollution: A review. *Chemosphere*. Elsevier. **235**: 498-509.

PAŞCOVSCHI S. 1956. CâtevaconsideraţiibiogeograficeasupraMunţilorBanatului. *Ocrotirea Naturii*. Academia Română. **2**: 111-134.

PÂRVULESCU L., PALOŞ C., MOLNAR P. 2009. First record of the spiny-cheek crayfish *Orconectes limosus* (Rafinesque, 1817) (Crustacea: Decapoda: Cambaridae) in Romania. *North-Western Journal of Zoology*. University of Oradea Publishing House. **5**(2): 424-428.

POPA M. E. & PREDEANU G. 2018. Environmental impact of coal and uranium abandoned mines in Romania. *Symposium on Environmental Pollution from abandoned mines*. Athens, Greece, 25-26 June 2018: 3-5.

POPA M. E. 2003. Geological heritage values in the Iron Gates Natural Park, Romania. *Proceedings of the First International Conference of Environmental Research and Assessment*: 742-751.

ROZYLOWICZ L., NITA A., MANOLACHE S., POPESCU V.D., HARTEL T. 2019. Navigating protected areas networks for improving diffusion of conservation practices. *Journal of Environmental Management.* Elsevier. **230**: 413-421.

RUICĂNESCU A. & DUMBRAVĂ A.-R. 2020. First record of *Kisanthobia ariasi* (Coleoptera: Buprestidae) in Romania. *Travaux du Muséum National d’Histoire Naturelle “Grigore Antipa”.* Pensoft. **63**(2): 189-194.

SHARIFIAN FARD M., PASMANS F., ADRIAENSEN C., LAING G. D., JANSSENS G. P. J., MARTEL A. 2014. Chironomidae bloodworms larvae as aquatic amphibian food. *Zoo Biology*. Wiley & Sons. **33**(3): 221-227.

SOLEM J. O. & GULLEFORS B. 1996. *Trichoptera, Caddisflies. Aquatic Insects of North Europe – A taxonomic handbook.* Edit. Anders N. Nilson: 223-255.

STEINMANN H. 1968. Alkereszek Plecoptera. *Magyarorszag Allatvilaga, Fauna Hungariae.* Pannon Enciklopedia. **92**(8): 129-186.

SUCEA F., CICORT-LUCACIU A.-Ş., COVACIU R. F., DIMANCEA N. 2014. Note on the diet of two newt species in Jiului Gorge National Park, Romania. *Herpetologica Romanica*. University of Oradea Publishing House. **8**: 11-27.

TĂUȘAN I. & TEODORESCU B. E. 2017. Contribution to the knowledge of the ant fauna (Hymenoptera: Formicidae) of the Danube Gorges (Romania). *Romanian Journal of Biology – Zoology*. Institute of Biology, Romanian Academy. **62**(1-2): 21-32.

TEODOR L. A., FERENŢI SARA, COVACIU-MARCOV S.-D. 2019. Weevils die in vain? Understanding messages from road-killed weevils (Coleoptera: Curculionoidea). *Coleopterists Bulletin*. The Coleopterists Society. **73**(2): 359-368.

UJHELYI S. 1959. Kereszek Ephemeroptera. *Magyarorszag Allatvilaga, Fauna Hungariae*. Pannon Enciklopedia. **49**(5): 71-95.

WATSON S. B., MILLER C., ARHONDITSIS G., BOYER G. L., CARMICHAEL W., CHARLTON M. N., CONFESOR R., DEPEW D. C., HÖÖK T. O., LUDSIN S. A., MATISOFF G., MCELMURRY S. P., MURRAY M. W., RICHARDS R. P., RAO Y. R., STEFFEN M. M., WILHELM S. W. 2016. The re-eutrophication of Lake Erie: Harmful algal blooms and hypoxia. *Harmful Algae*. Elsevier. **56**: 44-66.

WEINKE A. D. & BIDDANDA B. A. 2018. From bacteria to fish: ecological consequences of seasonal hypoxia in a Great Lakes estuary. *Ecosystems*. Springer. **21**: 426-442.

WIDIASTUTI A., ZAHIDAH Z., HERAWATI H., ARIEF M. C. W. 2023. Macrozoobenthos community structure as an indicator of water quality in the mangrove area of Bojong Salawe, Pangandaran, West Jawa, Indonesia. *World News of Natural Sciences.* **46**: 101-112.

WITTMANN K. J. 2007. Continued massive invasion of Mysidae in the Rhine and Danube river systems, with first records of the order Mysidacea (Crustacea: Malacostraca: Pericarida) from Switzerland. *Revue Suisse de Zoologie*. Geneva Museum, Swiss Zoological Society. **114**(1): 65-86.

Cupșa Diana, Pascar Bianca, Covaciu-Marcov Severus Daniel, Ferenți Sára, Telcean Ilie-Cătălin
University of Oradea, Faculty of Informatics and Sciences,
Department of Biology; 1, Universității Str., Oradea 410087, Romania.
E-mails: cupsa2007@yahoo.com; bpascar@yahoo.com; severcovaciu1@gmail.com; ferenti.sara@gmail.com; itelcean@gmail.com

Received: April 15, 2023
Accepted: July 28, 2023