UDC: 574.587:594/595(497.75:285)"2022/2023"
Professional paper

THE STUDY OF THE MACROFAUNA OF BENTHIC INVERTEBRATES OF THE SHARR MOUNTAIN LAKES IN THE REPUBLIC OF NORTH MACEDONIA

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Abstract

Aquatic ecosystems belong to the most sensitive group of living environments, which are under the great influence of the anthropogenic factor, and precisely for this reason, research and determination of the diversity of animal species are quite important in these lentic ecosystems (lakes).In addition to abiotic indicators (physicochemical conditions), the state of each aquatic ecosystem is monitored through certain types or groups of so-called bioindicators.
The research of aquatic macroinvertebrates as bioindicators in rivers, lakes, and reservoirs is not only of scientific importance but is also for the management and conservation of aquatic ecosystems. Our research carried out in the two mountain lakes of Sharr during the summer (July 2022/23) and autumn (October 2022/23), resulted in the identification of three zoological groups (Annelida, Mollusca, Hexapoda) of benthic macroinvertebrates.
From a total of 36 individuals collected from the upper lake of Karanikolla, 6 families of benthic macroinvertebrates from the Mollusca and Hexapoda groups were identified. The family Physidae was represented by 28%, the family Gyrinidae by 22%, the family Corixidae by 17%, the family Simuliidae by 14%, the family Limnephiliidae by 11%, and the family Gerridae by 8%. From Lake Bogovina, 8 families of benthic macroinvertebrates belonging to the groups Annelida, Mollusca, and Hexapoda were identified from the 31 individuals collected. The family Glossiphoniidae was represented by 22%, the family Gyrinidae by 19%, the family Lumbricidae by 17%, the family Corixidae by 11%, the family Simuliidae by 8%, the family Nemouridae by 6% and the family Chironomidae by 3%.
The results obtained from this study add to the knowledge of the macrofauna of aquatic invertebrates of the mountain lakes of Sharr in the territory of North Macedonia and we hope that will serve as a basis for further research.

Keywords: lentic ecosystems, bioindicators, macrofauna, benthic macroinvertebrates.

1. Introduction

Glacial mountain lakes are meltwater ecosystems of high altitudes and are not endangered by pollution. Compared to lowland lakes, these lakes are generally much less affected by pesticides and sewage [1]. Mountain lakes are known as indicators of atmospheric acid deposition [2], long-distance transport of toxic and radioactive pollutants [3], as well as global climate changes [4], [5]. Altitude is considered quite important in determining changes in living conditions in remote mountainous areas. Ice caps, snow caps, as well as the water temperature in mountain lakes are affected by air temperature, and thus climate is a major factor in changes in mountain environments. Recently, mountain lakes have been studied because of their potential as indicators of climate change [6].

The composition of the macrofauna of aquatic invertebrates of the mountain lakes of Sharr, in addition to local researchers [7], has also been studied by foreign researchers [8]. The aquatic invertebrates these areas are constantly adapted to low temperatures, therefore they are quite sensitive to even smaller temperature changes in the environment [1].

Benthic macroinvertebrates are a group of organisms that inhabit the benthos of freshwater ecosystems throughout their life or during a stage of their life cycle and are considered good indicators of conditions in an aquatic environment [9], [10], [11] and with sensitivity from local and global temperature changes [12]. This group of aquatic invertebrates includes mollusks, crustaceans, snails, worms, flatworms, and insects (especially larval stages) (see **Fig. 1**).

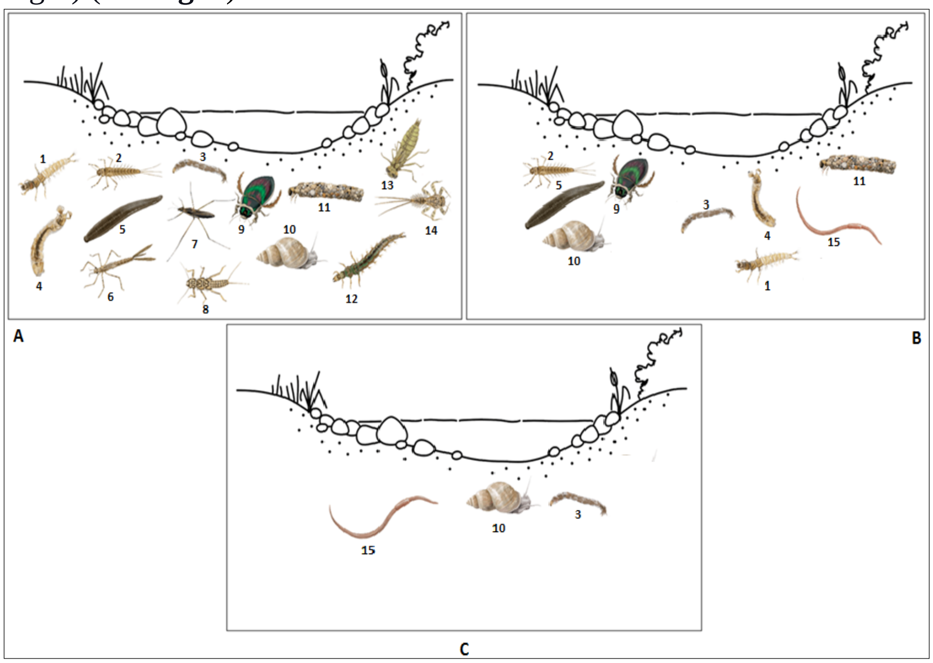

Figure 1. Benthic macroinvertebrates of mountain lakes. A. Benthic macroinvertebrates of mountain lakes with good water quality; B. Benthic macroinvertebrates of mountain lakes with average water quality; C. Benthic macroinvertebrates of mountain lakes with poor water quality.

Most of these organisms have a body size of 200-500 µm and can be collected using water nets with the same pore diameter [13]. The community of benthic macroinvertebrates belongs to the aquatic zoocenosis, along with plankton, periphyton, nekton, and neuston organisms. These organisms play an important role in energy flow and matter circulation in aquatic environments and are widely used in the biological monitoring of aquatic ecosystems [14], [15], [16]. Physico-chemical factors have a significant influence on the structure of benthic communities of aquatic macroinvertebrates. Among them, temperature, the concentration of dissolved oxygen in water, pH value, concentration of organic matter, etc. are of greater importance [17]. Of the hydromorphological parameters, the structure of the benthic communities is primarily influenced by the type of foundation, water velocity (in flowing ecosystems), water regime, etc. All these factors act synergistically in benthic macroinvertebrates, and each type has ecological valence for the environment within whose limits the particular species survives [18].

The species of benthic macroinvertebrates that inhabit fresh waters are characterized by certain adaptive features, they mostly move slowly, and some are even sessile. To make the best possible contact with the base, to hinder navigation, most species are dorso-ventrally compressed, for example, species from Plecoptera [19] and Ephemeroptera [20], species from Turbellaria secrete saliva by was reinforced even better for the foundation [21]. The species of Diptera that live in fast-flowing waters have special bracing organs or crowns with bracing hooks at the end of the body [22]. Larvae of caddisflies that live under rocks weave special nets for hunting with which they hunt prey, while some species can create portable cases made of sand particles and pebbles, they provide protection and due to their weight, the caddisflies larvae cannot be carried by water currents [23].

The amount of oxygen dissolved in water is of particular importance in the aquatic ecosystem. The adaptation of benthic macroinvertebrates to the fluctuations of this ecological factor has been done in different directions. Intensive bacteriological decomposition in waters polluted with organic matter can lead to a sudden decrease in the amount of oxygen in the water, and in such cases, species that can survive in low-oxygen water appear, such as chironomid larvae and oligochaetes from the Tubificidae family [24], [25], [26]. These species in the body fluid have a special type of hemoglobin with the help of which they can bind enough oxygen for their needs even in cases with small amounts of oxygen in the water environment. Turbellarians have no adaptations and special organs for respiration because they receive oxygen through the body's surface [21]. Crabs and insects whose body surface is hard and prevents breathing through the skin, have special organs for breathing - branchia [27].

Therefore, the purpose of this paper is to carry out research on benthic invertebrate macrofauna in different periods, of the two mountain lakes of Sharr (the upper lake of Karanikolla and the lake of Bogovina), which lie at different altitudes above sea level.

2. Materials and methods

The benthic macroinvertebrates were collected from the two mountain lakes of Sharr: from the upper lake of Karanikolla (Figure 2A), which lies at the geographical coordinates N 42°04'31,5" E 20°47'18,3", in 2163 m above sea level, with a length of 115 m, a width of 102 m, an area of 8240 m^2 and a depth of 0.5 m and from the lake of Bogovina (Figure 2B) which lies at the geographical coordinates N 41°57'03,7" E 20°47'39.0", at an altitude of 1963m, with a length of 452.2m, a width of 225m, an area of 66880m^2 and a depth of 4-5m [7].

Figure 2. A. The upper lake of Karanikolla; **B**. Bogovina Lake (Photo: G. Iseni 2022)

The determination of the geographical position (coordinates) and altitude was carried out with the help of the device for determining the position in space (GPS-Medion GoPal S3867).
The collection of benthic macroinvertebrates was carried out with the help of a net with a pore size of 500μm, during July and October 2022/23, from the surface of $0.1m^2$ of the littoral and the depth of 0.4m (Figure 3).

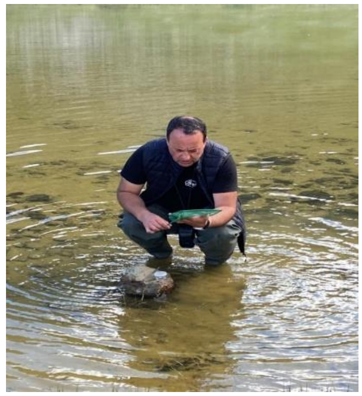

Figure 3. Moments from the collection of benthic macroinvertebrates in the field trip, in the upper lake of Karanikolla (Photo from the field trip).

The species were collected and placed in plastic containers, fixed with 75% ethanol, while the identification of species and higher taxonomic levels was carried out in laboratories with the help of a monocular stereo microscope equipped with a digital camera (Tornado) and Studio Video computer software. 7 (Figure 4).

Figure 4. StudioVideo7 computer software of the Tornado monocular stereo microscope digital camera with the help of which the macroinvertebrates of the two mountain lakes of Sharr were analyzed (Photo from the laboratory).

From each collected sample, the macroinvertebrates were divided according to the taxonomic group, in which case their number in the sample was also noted.
Separated invertebrates were fixed with 75% ethanol and the larvae of the largest insect groups, the orders Ephemeroptera, Plecoptera, Trichoptera, and the family Chironomidae, have been determined to the lowest possible taxonomic categories with the help of standard taxonomic keys [28], [29], [30], [31], [32].

The density of individuals found in the sample was expressed as the number of individuals in the test unit (1 m^2) and further analyzes were performed from the data obtained, the data were then expressed in graphic form. The frequency (%) for each family was calculated based on the number of collected individuals.
In addition to the analysis of benthic macroinvertebrates, the following abiotic water parameters were also determined: temperature, conductivity, pH, and dissolved oxygen in the water.
The determination of the water temperature was done with a Cole-Parmer digital thermometer by immersing the electrode in the glass filled with 100 cm^3 of lake water and reading the temperature value from the thermometer's display.
The conductivity (electrolytic conductivity) was realized according to the method which is based on the determination of the conductivity value [33]. The reagents that were used for this purpose were conductivity solutions with electrolytic conductivity of 1413 µS/cm and 12.88 µS/cm, while the apparatus used was the Mettler Toledo digital conductivity meter and laboratory glasses. Before reading the value, the device was initially calibrated with standard solutions with an electrolytic conductivity of 1413 µS/cm; and 12.88 µS/cm. The reading of the conductivity value is made from the display of the conductivity meter.
The analyzed water sample of 20 ml at room temperature is placed in a laboratory glass in which the electrode of the conductivity meter is then immersed, and after a time of its residence, the device is keyed and the electrolytic conductivity value is read at the moment when it doesn't change.
The pH of the water was determined according to the method which is based on the determination of the concentration of hydrogen ions and hydroxyl groups in water [34]. The reagents used to determine this parameter were buffer solutions with pH 4.0, pH 7.0, and pH 10.0. and the Mettler Toledo digital pH meter and laboratory glasses were used as apparatus.

3. Results and discussion

The results obtained from this research are presented tabularly and graphically. From a total of 36 specimens collected from the upper lake of Karanikolla, 6 families were identified (Table 1).

Table 1. List of benthic macroinvertebrate species of upper lake of Karanikolla.

Zoological groups (taxa) Phylum (subphylum)	**Classis (subclassis)**	**Order**	**Family**	**Genus**	**Species (The status)**
Mollusca	Gastropoda Pulmonata	Basommatophora	*Physidae*	Physa	Physa sp. *(T)***
	Insecta	Coleoptera	*Gyrinidae*	Dineutus	Dineutus sp.*(I)*

Hexapoda	Insecta	Diptera	Simuliidae	*Simulium*	*Simulium sp.*(T)
	Insecta	Trichoptera	Limnefilidae	-	-
	Insecta	Hemiptera	*Corixidae*	Micronecta	Micronecta sp.*(I)*
	Insecta	Hemiptera	*Notonectidae*	Notonecta	Notonecta sp.*(I)*
	Insecta	Hemiptera	*Gerridae*	Gerris	Gerris sp.*(I)*

* T- tolerant to changes
*I- intolerant to changes

The family Physidae was represented by 10 individuals, the Gyrinidae by 8 individuals, the Corixidae by 6 individuals, the Simuliidae by 5 individuals, the family Limnephilidae by 4 individuals, and the family Gerridae by 3 individuals (Figure 5).

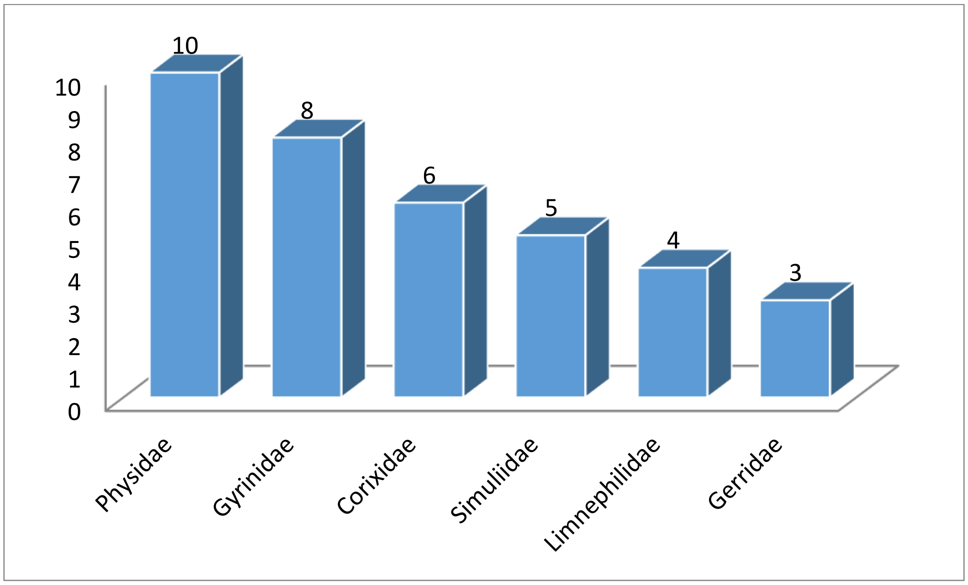

Figure 5. The number of individuals for each family of benthic macroinvertebrates of the upper lake of Karanikolla

The frequency (%) for each family of benthic macroinvertebrates of the upper lake of Karanikolla is shown in figure 6.

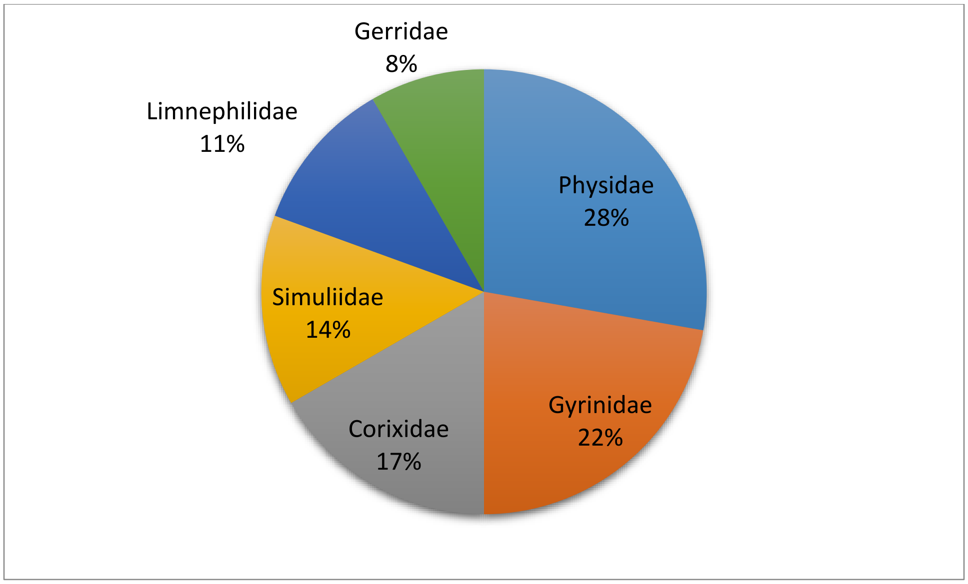

Figure 6. Frequency (%) for each family of benthic macroinvertebrates of the upper lake of Karanikolla

From the graphic presentation of the frequency of benthic macroinvertebrates of the upper lake of Karanikolla, it can be observed that the family Physidae is represented by 28%, the family Gyrinidae by 22%, the family Corixidae by 17%, the family Simuliidae by 14%, the family Limnephiliidae by 11% and the family Gerridae by 8%.

From a total of 31 individuals collected from Lake Bogovina, 8 families were identified (Table 2).

Table 2. List of benthic macroinvertebrate species of Lake Bogovina.

Zoological groups (taxa) Phylum (subphylum)	**Classis (subclassis)**	**Order**	**Family**	**Genus**	**Species (The status)**
Annelida	Clitellata (Oligocheta)	Opisthopora	Lumbricidae	Eiseniella	Eiseniella tetraedra *(T)**
	Hirudinea	Rhynchobdellida	*Glossiphoniidae*	Helobdella	Helobdella stagnalis *(T)*
Mollusca	Gastropoda	Basommatophora	*Physidae*	Physa	Physa sp. *(T)*
Hexapoda	Insecta	Coleoptera	*Gyrinidae*	Dineutus	Dineutus sp. *(I)*
	Insecta	Diptera	Simuliidae	*Simulium*	*Simulium sp.* (T)

	Insecta	Plecoptera	Perlodidae		
	Insecta	Diptera	Chironomidae	*Corynoneura*	*Corynoneura sp.* (T)
	Insecta	Hemiptera	*Corixidae*	Micronecta	Micronecta sp. *(I)*

** T- tolerant to changes*
**I- intolerant to changes*

The family Lumbricidae is represented by 6 individuals, the family Glossiphoniidae by 8 individuals, the family Physidae by 5 individuals, the family Gyrinidae by 7 individuals, the family Simuliidae by 3 individuals, the family Nemouridae by 2 individuals, the family Chironomidae by 1 individual and the family Corixidae by 4 individuals (Fig. 7)

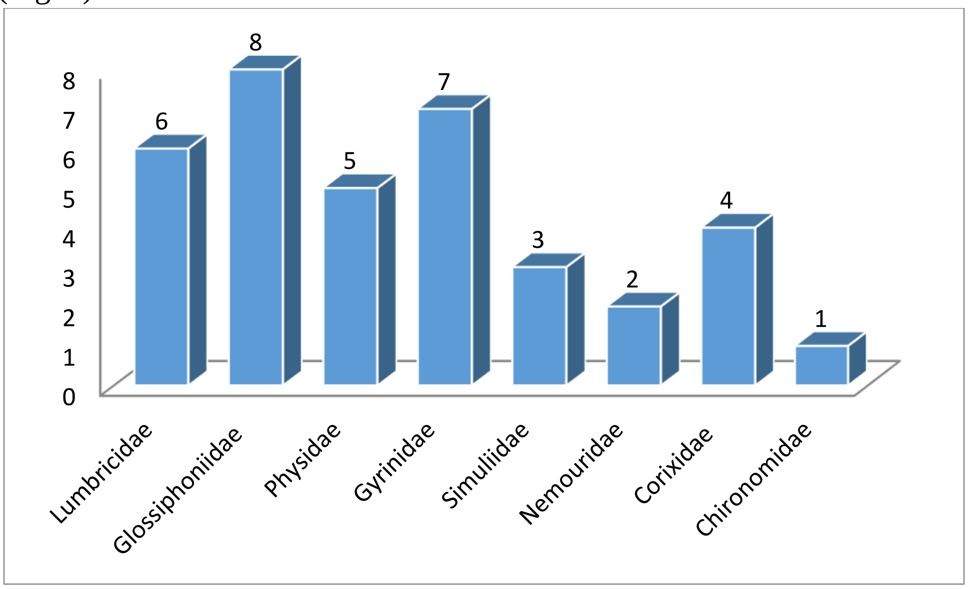

Figure 7. The number of individuals for each family of benthic macroinvertebrates of Lake Bogovina

The frequency (%) for each family of benthic macroinvertebrates of Lake Bogovina is presented in Figure 8.

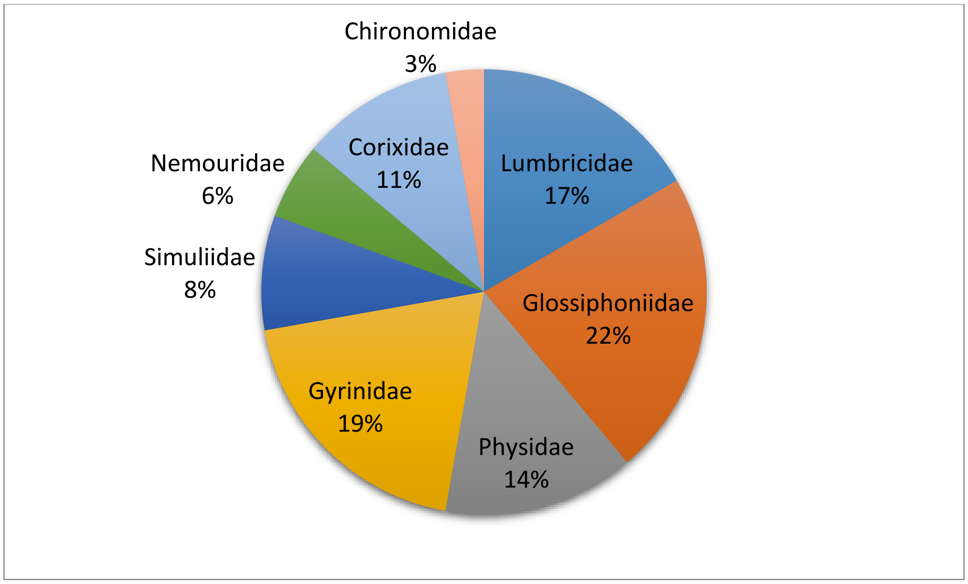

Figure 8. Frequency (%) for each family of benthic macroinvertebrates of Lake Bogovina

From the graphic presentation of the frequency of the benthic macroinvertebrates of Lake Bogovina, it can be observed that the family Glossiphoniidae was represented by 22%, the family Gyrinidae by 19%, the family Lumbricidae by 17%, the family Gyrinidae by 19%, the family Corixidae by 11%, the family Simuliidae by 8%, the family Nemouridae with 6% and the family Chironomidae with 3%.

Most of the analyzed macroinvertebrates belong to the category of species with status I (non-tolerant to changes), while some of them belong to the category of species with status T (tolerant to changes).

In addition to the quantitative and qualitative analysis of the structure of benthic macroinvertebrates, some of the abiotic parameters of the water of the mountain lakes of Sharr were also analyzed (Table 3).

Table 3. The results of the abiotic parameters of the two mountain lakes of Sharri.

The lake (period of the research)	The depth (m)	The altitude (m)	The temperature (°C)	The conductivity (mS/cm)	pH	O_2 (mg/l)
The upper lake of Karanikolla (19.07.2022)	0,5m	2163	23	0.01	9	5.2
Bogovina Lake (27.10.2022)	4m	1963	8.4	0.02	7	7.2

The results of the analysis of biological indicators and abiotic water parameters showed that these ecosystems are in relatively stable conditions.

The benthic macroinvertebrates of the upper lake of Karanikola and the lake of Bogovina are shown in the following photographs, obtained by a digital camera of a monocular stereomicroscope.

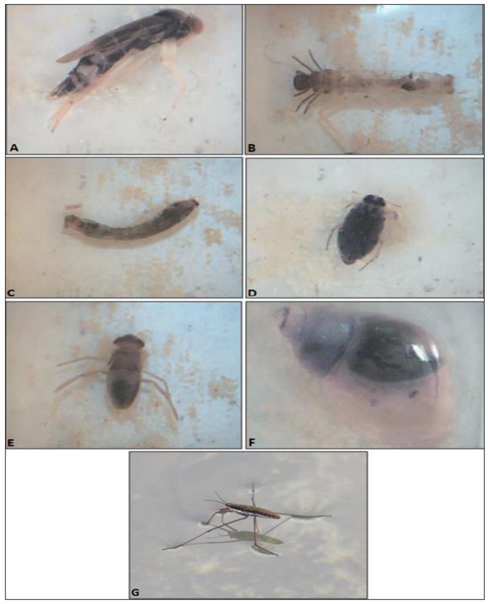

Figure 9. Benthic macroinvertebrates of the small Karanikolla lake. A. Adult stage of limnephilids, B. Larval stage of limnephilids. C. Larval stage of simulids, D. Adult stage of gyrinids, E. Adult stage of corixids, F. Adult stage of physids, G. Adult stage of gerids (Photographs acquired by Tornado digital camera and monocular stereomicroscope).

Figure 10. The benthic macroinvertebrates of Bogovina lake. F. Adult stage of Nemouridae, H. Larval stage of chironomids, J. Larval stage of simulids, K. Adult stage of girinids, L. Adult stage of notonectids, M. Larval stage of oligochaetes, N. Adult stage of erpobdelids, P Adult stage of physids (Photographs acquired by Tornado digital camera and monocular stereomicroscope).

According to previous studies, it has been established that depth, conductivity, oxygen dissolved in water and nutrients are quite important and influential in the composition of zoocenosis of macroinvertebrates of mountain lakes [35], [36], [37].

In extreme climatic conditions, the chemical composition of water and food can be tolerated only by a small number of oligostenothermic species that are well-adapted to these environmental factors. Similar cases have been encountered in the alpine lakes of other countries in Europe [38], [39].

Chironomids and oligochaetes, in terms of the number and density of species and taxa, are dominant in the investigated mountain lakes in Europe (65% of the total number of species/taxa). The macroinvertebrate fauna mainly consists of species/taxa typical for high mountain lakes [39], [40], [41]. These are mostly stenotothermic species of cold countries, which are distributed in all the coldest areas of Europe [42].

Environmental temperature is critical to aquatic macroinvertebrate ecology and evolution. Almost all life stages and distribution of aquatic insects are influenced by temperature. The high sensitivity of aquatic insects to high-temperature values (except for individuals of the Coleoptera and Heteroptera groups), is expressed both in the larval (immature) and adult (mature) forms [43].

The results of our study revealed differences in the zoocenosis structure of benthic macroinvertebrates of the two lakes included in the study.

In the upper lake of Karanikolla, due to its small depth (0.4m) and the high temperature recorded during July (23oC), mainly eurythermic species were identified, while in the lake of Bogovina, which is deeper (4-5m) and where the water temperature was lower (8.4oC recorded during the October) mostly stenothermic species were identified.

From the studies of other authors on high mountain lakes, it was shown that the increase in the number of species, diversity, abundance, and biomass of the benthic fauna occurs with the improvement of living environment conditions (climatic, light, and trophic) and with the decrease of the altitude [44], [41], [45], [46], [47], [40].

Our results clearly showed the influence of altitude on the number of species. There was a clear increase in the number of species/taxa, genera, and higher taxonomic groups with decreasing altitude. In Lake Bogovina, which lies at a lower altitude (1963m), the number and faunal diversity of benthic macroinvertebrates were greater compared to the small lake of Kranikolla, which lies at a higher altitude (2163m), and which number and faunal diversity of benthic macroinvertebrates was smaller.

From the results, it can be observed that the level of altitude of the studied lakes is reflected in the change in the fauna of aquatic insects.

4. Conclusions

This study, based on the conducted research, contributes to understanding the changes caused in the fauna of benthic macroinvertebrates by environmental factors. The question arises, if the temperature in these lakes increases, then what changes can we expect in the benthic fauna of macroinvertebrates?

While the benthic fauna of macroinvertebrates will remain more or less stable, aquatic insects will be attacked by these changes. The greater number of days for development during ice-free periods (with warm water in the benthic zone) may play an important role in the possibility of more thermophilic species. The macroinvertebrate fauna will consist of species present mainly at lower altitudes (under the sub-alpine zone), thus increasing their biological diversity. At the same time, the air temperature of a high elevation will be more favorable for the survival and spread of adult forms of insects. On the other hand, the stenothermic types of cold environments will become subject to extinction. An indirect impact on benthic macroinvertebrates through food sources is also expected. Biomass of epilimnitic algae may be increased by changes in light intensity resulting from earlier ice melt.

In the upper lake of Karanikolla, due to its small depth and higher altitude, during the warm summer months, the temperature is over 20oC. This level of temperature causes the evaporation of water and as a consequence, we have the reduction of the oxygen level in the water. Therefore, among the benthic macroinvertebrates in this lake, mainly thermophilic species have been identified, which tolerate fluctuations in abiotic water parameters but not pollution. Therefore, the quantitative and qualitative analysis resulted in finding the largest number of benthic macroinvertebrates that are not tolerant of pollution.

In Lake Bogovina, although the biological diversity was greater, the qualitative analysis resulted in finding the largest number of species that are tolerant to pollution. This finding helps us to understand the process of eutrophication of the upper part of the lake, as a result of the development of macrophyte plant populations. The bloom of macrophytes may be the result of the penetration of nutrients containing nitrogenous compounds, which are released by the washing of fertilizers from atmospheric precipitation, and are located near the lake, but to prove this, the analysis of the level of nitrites in the water is needed.

The research of macrofauna of aquatic invertebrates, in particular in mountain lakes, is not only of scientific importance but is also important for the management and preservation of aquatic ecosystems in general.

The results obtained from this study increase the knowledge of the macrofauna of aquatic invertebrates of the mountain lakes of Sharr in the territory of North Macedonia and will serve as a basis for further research in this direction.

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