



**ECOLOGICAL CONSEQUENCES OF LAKE  
UTILIZATION ON ZOOPLANKTON: CLADOCERA  
COMMUNITY SHIFTS INDUCED BY ANTHROPOGENIC  
AND RESEARCH DRIVEN DISTURBANCES**

Thesis for the Degree of Doctor of Philosophy (PhD)

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Debrecen, 2025

Hereby I declare that I prepared this thesis within the Doctoral Council for Natural Sciences and Engineering, Doctoral School of Juhász-Nagy Pál, University of Debrecen in order to obtain a PhD Degree in Natural Sciences at Debrecen University.

The results published in the thesis are not reported in any other PhD theses.

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Hereby I confirm that Arber Hajredini candidate conducted his studies with my supervision within the Hydrobiology Doctoral Program of the Doctoral School of Juhász-Nagy Pál between 2020 and 2024. The independent studies and research work of the candidate significantly contributed to the results published in the thesis.

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DISTURBANCES**

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## List of Abbreviations

- **OL**: Oxbow lakes
- **AFL**: Abandoned fishing lakes
- **IFL**: Intensive fishing lakes
- **NMDS**: Non-metric Multi-Dimensional scaling
- **CCA**: Canonical correspondence analysis
- **L**: Represents Látóképi víztározó lake
- **KE**: Represents Kerek-erdei-tó lake
- **SZ2**: Represents Szíki-tó lake
- **S**: Represents Sáska-tó lake
- **KV**: Represents Kék Víz lake
- **TI**: Represents Tímári Holt-Tisza lake
- **SZO**: Represents Szögi Holt-Bodrog lake
- **KMT**: Represents Kis-Morotva-tó lake
- **SZ1**: Represents Szabolcsi Holt-Tisza lake
- **KBM**: Represents Keleti Holt-Bodrog lake
- **R**: Represents Rókás-tó lake
- **KTP**: Represents Kenu-pálya lake
- **VE**: Represents Vekeri-tó lake
- **V<sub>01-12</sub>**: Represents Viss oxbow lake, sampling points from 1 to 12
- **TDS**: Total dissolved solids
- **SS**: Suspended solids
- **Orto-P**: Orthophosphate
- **NO<sub>3</sub>-N**: Nitrate nitrogen
- **NO<sub>2</sub>-N**: Nitrite nitrogen
- **NH<sub>4</sub>-N**: Ammonium
- **SO<sub>4</sub>**: Sulphate
- **Cl**: Chlorine anion
- **LOI550**: Loss on ignition at 550 degrees Celsius
- **LOI950**: Loss on ignition at 950 degrees Celsius

# **1. INTRODUCTION AND OBJECTIVES**

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## **1.1. Introduction – lakes role in society**

Water is essential for the existence of life on Earth, and the availability of water directly influences human populations worldwide (Jorgensen *et al.*, 2005). Throughout history, civilizations have thrived near bodies of water due to their abundant resources and the crucial role they play in sustaining life (Maltby and Acreman, 2011). Use of water by humans - for drinking, washing and recreation - requires water free from biological, chemical and physical sources of contamination. Clean water is also necessary for animals, plants, and the ecosystems that maintain biological diversity. A specific quality of water is required for industrial operations, city power generation, and food cultivation (Mp, 2017).

Freshwater ecosystems support diverse biota and provide essential services to human societies. The availability of the water is crucial, as its quality. A growing number of water bodies in the modern world are losing their ability to support the lives of the species that inhabit and surround them. Factors influencing the quality of water are increasing over time, however anthropogenic activities driven by humans remain the primary cause. These ecosystems encompass a variety of habitats, such as rivers, lakes, wetlands, and groundwater, harboring significant biodiversity and regulating global biogeochemical cycles. Climate change, pollution, habitat degradation, and invasive species are just a few of the challenges facing lakes and the diverse

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array of species that inhabit them (Bhattarai, 2017; Weiskopf *et al.*, 2020). As the biological significance of lakes and the risks to their sustainability become more widely acknowledged, limnology research and lake knowledge have grown in importance (Ezeokonkwo and Dodson, 2004). Presently, the assessment of water quality can easily rely upon the aquatic organisms inhabiting the water body. Notably, phytoplankton and zooplankton taxa have gained considerable attention for their utility in determining the quality of a water body. Cladocera, a group of zooplankton organisms belong to phylum of Arthropoda, subphylum Crustacea, and class of Branchiopoda, inhabit a wide range of inland water bodies (Likens, 2009). These species are predominantly herbivorous-omnivorous and contribute significantly to the recycling of nutrients in aquatic environments. Their significance in the food web is unquestionable, as they serve as a preferred food source for invertebrates and planktivorous fishes (Korhola and Rautio, 2001). They are preferred subjects for experimental design due to their physiological appearance. Their life cycle, interactions with environmental conditions and physiological characteristics are well studied and understood (Likens, 2009). We used Cladocera species as well as their subfossil remains in our experiments.

### **1.2. Problem statement**

#### **1.2.1. Mediation of Cladocera species by researcher's chest-wader**

Cladocera are an order that are distributed very easily, and are found in

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almost all fresh water bodies around the world. There are more than 600 species of Cladocera recognized worldwide. They have different optimal conditions and requirements. They compete with each other for food (Threlkeld, 1988), occasionally leading to a scenario where the population of one species increases at the expense of a decrease in the population of others (Vanni, 1986). Zooplankton species have high dispersal capacities, and De Meester et al. 2002 listed several lines of evidence for this.

Cladocera exhibit good dispersal capabilities across various water bodies. In newly constructed water bodies, they can establish their own populations within a matter of weeks. There are many mechanisms facilitating the dispersal of Cladocera, including wind, birds and even human activities (Dodson *et al.*, 1997). However, the role of scientists in this dispersal process has not been extensively investigated, particularly in the context of shallow lakes where sampling involves direct entry by the scientists into the water body. A chest wader worn by scientists to remain dry during such activities may sometimes function as an unintentional vector for the intra-ecosystem transportation of Cladocera. As Cladocera produce a dormant stage, waders immersing in, any water body as well as several other factors might accidentally facilitate the movement from one aquatic environment to another (Downing *et al.*, 2006).

Oxbow lakes are mostly found along the banks of the Tisza and Danube rivers in Hungary. Our research has concentrated on shallow lakes with diverse utilization patterns, mainly in the northeastern parts of the country.

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To study the possible different effects that lake use types may have on Cladocera species mediation, we conducted investigations on protected, abandoned and actively used fishing waters.

### **1.2.2. Impact of utilization on Cladocera species composition**

Shallow lakes are widespread around the world and hold significant ecological importance (Downing *et al.*, 2006). These lakes are among the fastest ecosystems to be affected by external factors like climate change (Jeppesen *et al.*, 2009). Due to many changes in environmental conditions, shallow lakes are facing changes in their water quality which directly affects the aquatic species as well as the surrounding environment. Nutrient load and fish are the main factors influencing the condition of these water bodies. Some shallow lakes exhibit a variety of utilization patterns. This is the case with our studied shallow lake, which has three utilization patterns: a protected zone, an agriculturally influenced zone and a recreational zone. Cladocera have become widely adopted in different areas of scientific research, from monitoring aquatic system health to assessing the impacts of climate change (Jeppesen *et al.*, 2009). Today, contemporary sampling methods for Cladocera mostly record contemporaneous events or very short-term environmental change effects. By contrast, subfossil sampling is performed to obtain historical data about previous periods of water bodies (Zawisza *et al.*, 2016). Cladocerans show high preservation potential; most of them are also easily identifiable. Although specific sampling techniques offer their

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own benefits and yield intriguing data for analysis, it was very important for our study to show which of the two methods would be more suitable for particular research objectives, such as studying a shallow lake with three different types of utilization.

### 1.3. Objective of the study

The objectives of this PhD study were twofold: firstly, to enhance our understanding of the mediation capacity of Cladocera by researcher's chest wader as a vector in variously utilized water bodies during sampling activities; secondly, to investigate the disparities between contemporary and subfossil Cladocera species sampled within an oxbow lake. A key aspect of this investigation was to determine the most suitable sampling method for accurately representing the Cladocera species assemblage within the lake. During our research, we aimed to address the following questions:

#### 1. Mediation of Cladocera species by researcher's chest wader

- *What is the potential for Cladocera species to be dispersed through scientists' chest waders during sampling procedures?*
- *How do different types of lake utilization affect the extent of Cladocera dispersal facilitated by chest waders?*

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- *What impact does inadequate cleaning of chest waders prior to sampling have on the accuracy and integrity of collected data?*

## 2. **Comparative analysis of contemporary and subfossil Cladocera assemblages with respect to lake utilization and environmental factors**

- *How do contemporary and subfossil Cladocera assemblages differ in composition across distinct utilization zones within a multi-utilized lake?*

- *To what extent do different types of lake utilization influence the composition of Cladocera assemblages?*

## **2. LITERATURE REVIEW**

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### **2.1 Cladocera species**

Cladocera, a group of zooplankton species, present a compelling subject for new research, due to the fact that they are common in most inland waters (Likens, 2009). Initially described in the 1600s, Cladocera have since become the subject of extensive research, yielding detailed insights into various aspects of their physiology, community structure, birth rates, and the environmental conditions conducive to their proliferation (Likens, 2009). Cladocera inhabit a variety of aquatic environments, including freshwater, brackish water, and saline inland waters, with a very small number of species living in seawater (Smirnov, 2014). Cladocerans are preferred subjects in experimental work because of their small size and easy to culture. This allows for a deep study of the physiology, life cycle, and interactions of cladocerans with other species as well as their physical habitats (Likens, 2009). The order Cladocera are members of the subphylum Crustacea, class Branchiopoda. Cladocerans are divided into 16 families within four distantly related orders: Anomopoda, Ctenopoda, Onychopoda and Haplopoda. It is believed that there are over 700 species within the order Cladocera, with new species continuing to be described (Smirnov, 2014). Cladocera size ranges from 0.3 mm to 6 mm in many taxa, with the exception of *Leptodora* which can reach 10 mm (Smirnov, 2014). Adults and juvenile Cladocera of the same species can occasionally have very different body shapes; this is particularly evident

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in several macrothricids. Additionally, the forms of adults within the same Cladocera species can vary significantly. Notable examples include species of *Daphnia* and *Bosmina*, which exhibit a variety of morphologies (Smirnov, 2014).

### **2.1.1 Methods of investigating Cladocera species**

It is possible to gather Cladocera species from large or small bodies of water, as well as ponds, puddles, roadside ditches, fountains, or even moss (Smirnov, 2014). The physiology of Cladocera can be studied using a variety of techniques, such as closely observing living specimens or documenting the chemical and physical expressions of certain physiological processes (Smirnov, 2014). In shallow lakes, Cladocera are typically sampled from the littoral zone, open water, or benthic zones. Subfossil samples, on the other hand, are collected from the deepest part of the shallow lake, as this area represents the best accumulation site for these remains in such water bodies. Numerous research methods have been used to study Cladocera, examining various aspects such as their environmental and food preferences in living specimens. Studies have investigated food composition (Infante, 1978), while other research methods include staining internal organs (Fischel, 1908), high-speed photography (Zaret and Kerfoot, 1980), and immobilization using different techniques (Jacobs, 1980; Porter, Gerritsen and Orcutt, 1982; Peñalva-Arana *et al.*, 2007). Additionally, fluorescence analysis (Pravda, 1950), toxicological studies, and behavior recording using computer

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technology (Peñalva-Arana *et al.*, 2007), are also used in Cladocera research.

### 2.1.2 Chemical composition of Cladocera species

Cladocera species have moisture contents ranging between 80% and 90%, with calorific values spanning from 3 to 6 kcal/g dry weight (DW)(Smirnov, 2014). Their biochemical composition is characterized by fat content varying from 10% to 20%, carbohydrate content between 10% and 30%, and protein content from 30% to 40% (Smirnov, 2014). It is important to note that these values can fluctuate based on seasonal variations and dietary intake, reflecting the dynamic nature of their environmental interactions. In dry weight, Cladocera species have a nitrogen content of approximately 8.1% (Andersen and Hessen, 1991), and a phosphorus content of around 1.4% (Main, Dobberfuhl and Elser, 1997). The amino acid contents of various Cladocera species have been examined by different researchers. *Daphnia Pulex* was studied by (Malikova, 1953, 1956), while *Daphnia magna*, *Ceriodaphnia reticulata*, *Chydorus Sphaercus* by (Sadykhov, Bogatova and Filatov, 1975). In *Daphnia magna*, alanine, glutamine, glycine, and leucine were identified as the predominant amino acids (Czeczuga, 1984). The chemical composition of slime in Cladocera species, such as *Holopedium*, was investigated by (Brown, 1970). This study revealed the presence of both sulfated and carboxyl group-modified mucopolysaccharides. The nucleic acid content in some Cladocera species has also been determined. Kokova (1982) found that the nucleic acid content of *Moina* spp. ranges from 4.7% to 5.2%

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of dry weight (DW). Carbohydrates in Cladocera are found in the form of glycogen and chitin, both polysaccharides. Blazka (1966) determined the glycogen content as follows: 23% in *Bosmina longirostris*, 53% in *Ceriodaphnia reticulata*, 1-36% in *Daphnia* spp., and 33% in *Simocephalus* sp. These percentages are based on the total composition, measured as wet weight (WW). Chitin, a strong structural component of Cladocera, forms their protective exoskeleton. Upon the death of Cladocera, their chitin is deposited on the bottom of water bodies, where it remains undecomposed (Smirnov, 2014). The chitin content in some Cladocera species, such as *Daphnia*. The production rate of chitin from Cladocera is high, as determined by (Chalikov, 1951). For *Daphnia magna*, the chitin content is 11.5 g/m<sup>2</sup> (4.6 g/m<sup>3</sup>); for *Daphnia galeata*, it is 3.2 g/m<sup>2</sup> (0.16 g/m<sup>3</sup>); and for *Daphnia hyalina* and *Daphnia cucullata* combined, it ranges from 0.14 to 0.30 g/m<sup>2</sup> (0.09 to 0.2 g/m<sup>3</sup>). The lipid content in Cladocera species varies among different species. Due to their high energy value, lipids are commonly stored by Cladocera, often visible as oil droplets within their bodies. The vitamin content in *Daphnia pulex* was determined by Malikova (1956), with findings indicating the following concentrations: vitamin A at 5.19 mg WW/L, vitamin B1 at 2.55 mg WW/L, and vitamin B2 at 5.69 mg WW/L. During their metabolic processes, Cladocera produce various pigments, including red hemoglobin, orange carotenoid, green carotenoprotein, and dark ommochromes found in their eyes (Green, 1966, 1971). The mineral content of Cladocera varies depending on the environment. In *Daphnia magna*, it ranges from 12% to

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37.6% of dry weight (DW), while in *Simocephalus vetulus*, it ranges from 10% to 19.4% of DW (Stepanova, Naberezhnyi and Byzgu, 1971). Cladocera are known to accumulate various elements, including those that are nonessential to them (Smirnov, 2014). Biogenic elements (C, H, O, and N), macroelements (Ca, Na, K, P, S, Mg, Mn, Fe, Cl, and Cu), and microelements (Zn, Co, I, Se, Mo, Li, and others) are found in Cladocera tissues (Smirnov, 2014).

### 2.1.3 Cladocera species diet

Cladocera feed almost nonstop, and this process is influenced by environmental factors. In Cladocera, feeding and breathing are closely linked processes (Smirnov, 2014). Both planktonic and littoral Cladocera utilize their thoracic limbs to collect food, although their specific methods of food collection differ (Smirnov, 2014). Cladocera use their mandibles to crush their food. However, Hebert (1973) observed that in some Cladocera species, the mandibles do not cause significant damage to the food. Cladocera that collect food from the substrate exhibit species-specific specializations, as described by (Freyer, 1963, 1968, 1974, 1991). These Cladocera use their thoracic limbs to push food toward their mouths. Bottom-dwelling Cladocera have a plentiful food supply and can utilize various food sources, including organic matter in different stages of decomposition, algae, and bacteria (Smirnov, 2014). Pelagic Cladocera may experience food shortages or changes in food quality. These species are primarily filter feeders, utilizing

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the setae on their thoracic limbs as filtering fans (Smirnov, 2014). Their diet includes algae, decomposed organic matter, and bacteria. There are also carnivorous Cladocera species, such as *Pseudochydorus globosus*, which feeds on dead Cladoceran bodies, and *Anchistropus*, which preys on Hydra, as studied by Van Damme and Dumont (2007).

### 2.1.4 Respiration of Cladocera species

Cladocera species exchange gases through their body surface (Pirow, Wollinger and Paul, 1999a, 1999b). The majority of the required oxygen is absorbed via the feeding current (Pirow, Wollinger and Paul, 1999a; Seidl, Pirow and Paul, 2002) and then distributed throughout their body (Seidl, Pirow and Paul, 2002). Additionally, the rectum also serves as a site for respiration (Smirnov, 2014). Littoral Cladocera suffer more from oxygen deficiency compared to pelagic ones. Various environmental factors can influence the oxygen content in the water body. Different species have varying tolerances to oxygen concentrations: littoral Cladocera are generally more adaptable to low oxygen levels, whereas pelagic Cladocera are more sensitive to such conditions (Smirnov, 2014). Some Cladocera species, such as *Lathonura rectirostris*, *Daphnia pulex*, and *Simocephalus exspinosus*, inhabit the bottom of freshwater bodies where the oxygen content is nearly zero (Brand, 1946), there is very little information available on anaerobic metabolism in Cladocera (Smirnov, 2014).

### **2.1.5 Circulation and excretion of Cladocera species**

Cladocera possess a myogenic heart and an open circulatory system. Due to their semitransparent bodies, the flow of blood (hemolymph) can be easily observed. (Smirnov, 2014). Herrick (1884) initially described a membrane in the heart that separates venous and arterial blood. However, later research revealed that the Cladocera body is divided into three blood spaces by ventral and dorsal membranes: the ventral lacuna, dorsal lacuna, and intestinal lacuna (Hérouard, 1905; Pirow, Wollinger and Paul, 1999b). The heart and body movements enable the circulation of hemolymph in Cladocera (Smirnov, 2014). The first blood circulation scheme for a Cladocera species was described by Gruithuisen (1828), in *Simocephalus* species. It takes about 10–20 seconds for a blood cell to fully travel through the *Daphnia* circulation system (Dearborn, 1903; Maynard, 1960). Direct counting shows that the heart rates of females in both littoral and pelagic species range from 190 to 320 beats per minute at 17–18°C (Smirnov, 2014).

Even that Cladocera have a secretion organ Peters and Schlotter, (1987), noted that Cladocera species mostly excrete through body surface (Smirnov, 2014). Metabolic processes typically final products are low molecular weight compounds such as nitrogen, carbon dioxide (CO<sub>2</sub>), and water (Smirnov, 2014). In contrast, Cladocera mainly excrete ammonia, classifying them as largely ammonotelic animals (Vonk, 1960). Cladocera species absorb various substances from their surrounding environment. These substances can be beneficial, harmful, or neutral in their effects on the

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Cladocera's life (Smirnov, 2014). The elimination of these substances in Cladocera is possible through defecation or excretion. Additionally, they may transfer these substances to their eggs or eliminate them during molting (Smirnov, 2014).

### **2.1.6 Osmotic regulation and cell metabolism**

Tolerance of salinity in Cladocera species varies between species. Neck organ's role in Cladocera species is ion exchange, firstly illustrated by Potts and Durning (1980). Osmotic regulation in Cladocera maintains water and salt balance through various mechanisms, including water intake, mineral intake from food, and the removal of ions through different channels or excretion (Aladin, 1991; Smirnov, 2014).

A considerable number of enzymes are found in Cladocera species, related to various functions within their bodies. Hasler (1937); and Hebert (1978) described several enzymes in the digestive tract of *Daphnia magna*, including proteases, lipases, amylases, and cellulase. Metals or xenobiotics affect enzyme activity in Cladocera, either increasing or decreasing it, depending on the type of metal exposure (Biesinger and Christensen, 1972).

### **2.1.7 Cladocera growth and molting**

Higher temperatures and greater food concentrations increase the growth rate of Cladocera, whereas lower temperatures and limited food

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availability reduce their growth rate (Buikema, 1972). Cladocera typically grow between molts (Smirnov, 2014). Green (1956) observed that *Daphnia magna* increased in length from 1.3 mm to 1.6 mm just 10 seconds after molting. The growth rate in Cladocera is higher in juveniles and changes after they reach maturity. In some species, like chydorids, the growth rate significantly decreases after maturity. However, in daphnids, growth continues at a similar rate even after reaching maturity. (Zaffagnini, 1964; Smirnov, 1971). Different Cladocera species have varying life spans. Fritsch (1953) summarized that planktonic Cladocera can live up to 182 days. Korovchinsky (2004) noted that ctenopods have a lifespan of 19 to 74 days. Chydorids, when cultured, can live up to 3 months (Smirnov, 2014). Meijering (1958) introduced a new method to measure the lifespan of Cladocera by counting heartbeats. He studied *Daphnia magna*, which had approximately 47 million heartbeats over a lifespan of around 65 days. Meijering (1960) measured the number of heartbeats from molting to egg liberation in *Daphnia magna*, finding it to be 8,000 heartbeats. The surrounding environmental conditions also affect the lifespan of Cladocera; extreme environmental conditions can shorten their lifespan (Smirnov, 2014). The number of molts varies among different Cladocera species. Smirnov (1965) noted that some Cladocera species, such as *Acroperus*, can undergo a substantial number of molts, as many as 48. In Anomopods, embryos undergo molting immediately after leaving the brood chamber (Kotov, 1997). After this initial molt, they may undergo more prereproductive molts. For example,

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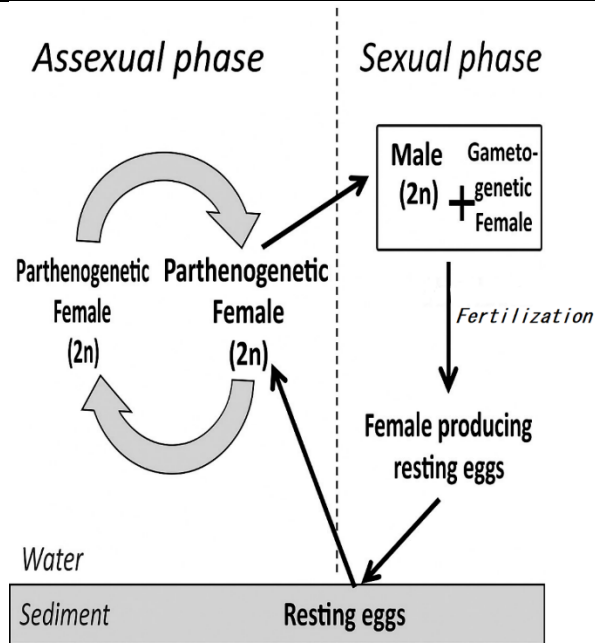
female chydorids and *Bosmina* species may have up to two molts, while daphnids and *Eurycerus* can go through up to six molts (Frey, 1982). Molting can also be considered a cleaning process, as it removes epibionts and epiparasites from the surface of Cladocera. Green (1974) demonstrated that the number of epibionts on Cladocera species increases between molts, indicating that molting helps reduce their numbers. Bosch (1969, 1972) concluded that the molting process in Cladocera is initiated and controlled by neurosecretion. There is not enough information about the senescence of Cladocera species. Meijering (1958) found increased mortality in *Daphnia magna* when the species reached 18 million heartbeats. Dudycha (2003) suggested that signs of senescence include morphological and physiological changes, such as decreased heart rate and reduced fertility.

### **2.1.8 Reproduction of Cladocera species**

Reproduction in Cladocera species can occur through both sexual and asexual means. Cladocera species have paired reproductive organs, with males possessing paired testes and females having paired ovaries (Smirnov, 2014). In the ovaries of Cladocera, there are four cell groups where one cell develops into an egg and the other three become nurse cells (Weismann, 1877), The form of spermatozoa varies between species (Wingstrand, 1978). Cladocera mainly reproduce by parthenogenesis (Smirnov, 2014). Gametogenesis usually occurs after extended periods of parthenogenesis.

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Weismann (1880) termed this prolonged parthenogenesis followed by gametogenetic reproduction as a "cycle." Depending on the number of reproductive cycles per year, populations are classified as monocyclic (one cycle per year), dicyclic (two cycles), tricyclic (three cycles), and polycyclic (more than three cycles), or they can be acyclic (no cycles). Cyclicity is affected by latitude and is differs among species (Vereshchagin, 1912). Northern species tend to undergo gametogenesis before winter, whereas species found closer to the tropics experience gametogenesis irregularly (Frey, 1982). Under laboratory conditions as observed by Banta (1925), parthenogenetic reproduction of Cladocera species continued unhindert for 13 years. In species like *Daphnia*, *Simocephalus*, and *Moina* the females can produce in the same brood: a) parthenogenetic females and gametogenetic females and males; b) parthenogenetic females and males; 3) parthenogenetic females (Papanicolau, 1910; Agar, 1920).



**Figure 1:** Reproduction cycle of Cladocera species. (according to De Meester et al. 2002.)

With the exception of *Leptodora*, none of the Cladocera have free-living larval stages (Smirnov, 2014). Cladocera are very prolific species and can produce dozens of offspring during their lifetime. According to Green (1954) the number and size of parthenogenetic eggs in Cladocera depend on environmental and seasonal conditions, as well as resource quantity. All Cladocera species produce dormant eggs, which are highly resistant to environmental conditions such as drying or freezing (Sent-Ilrt, 1860; Sars, 1886; Makrushin, Perevoznikov and Lysak, 1990).

### **2.1.9 Movement of Cladocera species**

Pelagic Cladocera and almost all littoral Cladocera are always moving; if they do not swim, they may sink because they are heavier than water (Smirnov, 2014). Cladocera mainly have individual muscles. The dilators of the esophagus and anus, the constrictors of the rectum, and the cross-striated skeletal muscles are examples of muscle fibers (Binder, 1929). The largest muscles in Cladocera species are the dorsal muscles and three longitudinal muscles. There is a difference in locomotion between Cladocera species that crawl, such as littoral species, and those that swim, like pelagic species (Smirnov, 2014). Littoral Cladocera primarily live attached to various substrates, while pelagic Cladocera inhabit open water and generally do not attach to surfaces (Smirnov, 2014). Cladocera movement depends on the viscosity of the water, which is influenced by the temperature of the water body (Freyer, 1968). Pelagic Cladocera and almost all of littoral Cladocera are always moving, if they don't swim they may sink because they are heavier than water. Littoral Cladocera make different movements; they primarily crawl and occasionally swim. Pelagic Cladocera mainly exhibit linear float and sink movements (Lochhead, 1961). Dodson et al. (1997) observed the movement of *Daphnia* species and concluded that it is affected by the chamber volume in which they were observed, as well as by light and food availability. While Cladocera species are in constant movement, investigating them requires immobilization, which can be achieved in two ways: physical or chemical immobilization (Smirnov, 2014).

### **2.1.10 Nervous system of Cladocera and sense organs**

The nervous system represents the center of animal organisms (Leydig, 1860). The nervous system of Cladocera species is cholinergic. The brain is the center of nervous system in Cladocera species and sends nerves to gut, heart and sense organs according to Fischel (1908). The primary role of the nervous system in Cladocera is to regulate neurosecretion and control the sensory organs (Smirnov, 2014). For a time, scientists believed that Cladocera possessed only two sensory organs: the eyes and the aesthetascs of the antennules. However, it is now known that they have other sensory organs, such as those that detect vibrations (Dumont and Van de Velde, 1976), tiny sensory structures on thoratic limbs (Smirnov, 1967), and a lateral frontal sense organ in the head (Gicklhorn, 1931), etc. Almost all Cladocera possess one eye and an ocellus. The size of the eye varies among different species, with some having immobile eyes and others having eyes with very limited movement (Smirnov, 2014).

### **2.1.11 Behavior of Cladocera**

Both littoral and pelagic Cladocera species exhibit species-specific behaviors and reactions. Species within the same genus can have different behaviors, and even males and females of the same species may behave differently. Their movement and behavior are highly influenced by the environments they inhabit. Due to various factors, both littoral (Szlauer, 1962,

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1963) and pelagic (Bainbridge, 1961; Wright, Collins and Van Genderen, 1980) Cladocera species perform vertical migrations without any issues related to pressure changes. For littoral Cladocera, decreases in illumination or oxygen content typically cause them to ascend in the water column. However, Szlauer (1963) demonstrated that *Daphnia magna* exhibits daily vertical migration even in the absence of changes in illumination or under dark conditions. Dependence of Cladocera populations on lunar cycles was described by Gliwicz (1986). Swarming behavior has been observed in both littoral Cladocera (Lastochkin, 1930) and pelagic Cladocera (Johnson and Chua, 1973). These swarms can vary significantly in size, ranging from a few centimeters to several meters (Young and Taylor, 1990), and can consist of a mixture of different species. Swarms may be stimulated by chemical stimulation or visual landmarks (Smirnov, 2014). Cladocera respond quickly to disturbances, often exhibiting an increased heart rate (Smirnov, 1965). Pelagic species typically react by rapidly escaping, while most littoral species may respond by becoming immobile, a behavior known as akinesis. This behavior is crucial for arthropods, as motionless species are less likely to be attacked by predators (Kerfoot, Kellog and Strickler, 1980). Pelagic species do not exhibit akinesis; instead, they demonstrate escape behavior by fleeing (Szlauer, 1964). Pijanowska and Kowalczewski (1997) tested *D. magna* with various stimuli and observed that they reacted by escaping, forming aggregates, or sinking to the bottom, concluding that this species has a greater chance of evading predators through these behaviors.

### **2.1.12 Ecophysiology and immunology of Cladocera species**

As mentioned above, Cladocera species inhabit water bodies of various sizes and salinities. The range of environmental conditions they can tolerate varies between different species. Some species are adapted to freshwater, while others can thrive in brackish or even saline inland waters. The specific limits of these environmental tolerances define the distribution and habitat preferences of different Cladocera species. Indeed, there are Cladocera species adapted to a wide range of pH environments. Some species are acidophilic, thriving in acidic conditions, while others are alkaliphilic, preferring alkaline waters (Smirnov and Timms, 1983). Cladocera species are highly sensitive to various factors in their aquatic environments. They can detect chemicals that influence their feeding behavior, escape responses, presence of competitors, and predators (Dodson, 2005). Cladocera species are significantly affected by continuously changing physical factors in their aquatic environments. Warmer temperatures are generally preferred by these species, as they enhance growth rates and reproductive success. However, each species has specific temperature limits within which they can thrive, and exceeding these limits can lead to mortality. Understanding these temperature preferences and tolerances is crucial for studying Cladocera ecology and predicting their responses to environmental changes, such as climate change. Some Cladocera species can live and reproduce at 0°C, as documented by (Rivier, 1986, 1992). However, the highest temperature range within which most Cladocera species stop normal functions is from 30 to 35°C, according

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to (Mortimer, 1936). This wide range of temperature tolerance underscores the adaptability of Cladocera to various thermal conditions, though each species has specific thresholds that influence their distribution and survival. Species like *Ceriodaphnia laticaudata* thrive in water bodies with very low oxygen concentrations. When oxygen levels increase, these species tend to disappear, often being replaced by other species better suited to higher oxygen conditions (Fox, 1945). This indicates the specialized adaptations of certain Cladocera species to low-oxygen environments and their competitive displacement by other species under changing oxygen levels. Different Cladocera species exhibit varying reactions to external stimuli, and most of the aforementioned characteristics are not absolute. According to Smirnov (2014) their responses depend on several factors, including:

- **Previous adaptations** - Historical exposure to certain environmental conditions can influence their current responses
- **Sex:** Males and females may react differently to the same stimuli.
- **Age:** Juveniles and adults might have different tolerance levels and behaviors.
- **Presence of clones with different tolerances:** Genetic diversity within a species can lead to varying reactions.
- **Other influencing factors:** These can include temperature, salinity, presence of predators or competitors, and availability of food.

These variables underscore the complexity and adaptability of Cladocera species to their environments.

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Cladocera species are susceptible to a variety of parasites and pathogens, including bacteria, fungi, amoebae, microsporidians, and nematodes. The impact of these infections varies depending on both the host and the parasite species involved (Green, 1974; Stirnadel and Ebert, 1997; Goren and Ben-Ami, 2013). Cladocera species exhibit behavioral and phenological adaptations to mitigate the risk of infectious diseases (Smirnov, 2014).

### **2.1.13 Cladocera species in water quality testing**

The main uses of Cladocera are: to measuring pollution levels, checking drinking water quality and testing human body fluids (Smirnov, 2014). Cladocera were first used as indicators of organic pollution in water bodies by Kolkwitz and Marsson (1909), who also described their ability to decrease pollution through self-purification. Hrbacek and Hrbackova (1980) proposed the use of Cladocera as indicators of eutrophication in water bodies. The first organism to be used to assess drinking water quality was *Daphnia* (Naumann, 1929). Cladocera are constantly used for testing water toxicity as test species (Lesnikov, 1967). They have been used as indicators for different environmental conditions (Walseng and Schartau, 2011), reaction to pesticides (Frear and Boyd, 1967) or sediment toxicity (Dekker *et al.*, 2006). The toxicity of human liquids, such as urine, blood serum, and different body fluids, has been attempted to be tested using Cladocera species (Billiard,

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1925; Billiard and Perrot, 1926), concluded that the lifespan of *Daphnia* in these fluids is a useful indicator: if the lifespan is 7 minutes urine is normal, the urine is considered hypotoxic if they live more than 7 minutes; and hypertoxic if they live less than 7 minutes.

### 2.2 Shallow lakes

Shallow lakes hold significant importance for various species, as they create one of the most suitable environments for a diverse array of organisms. They are quite widespread around the world, with their largest concentrations found in floodplains around major rivers (Meerhoff and Beklioglu, 2024). A substantial number of shallow lakes have been created as a result of human activities, both intentionally and unintentionally, through construction, agriculture, and mining. The prevalence of these lakes has significantly increased over the past few centuries (Oertli, 2018; Meerhoff and Beklioglu, 2024). Shallow lakes behave very differently from their deep counterparts in many ways, primarily due to the strong sediment-water interaction and the potentially significant influence of aquatic vegetation (Scheffer, 2004). These lakes are mainly polymictic, meaning the water column is mixed throughout the entire year, and stratification can occur for a short time during the summer. These ecosystems have high biodiversity with habitats and resources that support an aquatic flora and fauna range from plants, invertebrates, and fish to birds. Warm nutrient-rich waters and abundant light

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penetration create a situation conducive to high primary productivity; hence, shallow lakes are critical in maintaining ecological balance and sustaining species. A water body not deeper than 4 meters at its deepest point, with an average depth of 1 to 2 meters, which supports macrovegetation in around 80% of its total area, with a size ranging from almost a hectare to 100 km<sup>2</sup>, is considered a shallow lake (Scheffer, 2004). Many fishes, amphibians, and invertebrates find their habitat and breeding grounds through the development of a wide range of macrophyte proliferation. They draw fauna and birds as well (Scheffer, 2004). Vegetation in shallow lakes has a fundamental role in filtering pollutants and in improving water quality. Macrophytes utilize nutrients and contaminants and hence reduce the effects of eutrophication (Carpenter *et al.*, 1998). In some areas, shallow lakes are used for irrigation and as a source of water for livestock. Enhanced nutrient levels in the water due to macrophytes are favorable for agricultural practices (Bradley, 2001). Some common recreational programs include fishing, boating, swimming, and bird-watching in shallow lakes. Their million-dollar views and rich biodiversity also attract tourists, who contribute to local economies (*Ecosystems and human well-being: wetlands and water synthesis : a report of the Millennium Ecosystem Assessment*, 2005).

They play a role in carbon sequestration through the growth of aquatic plants and the accumulation of organic sediments. This helps in mitigating climate change by capturing and storing carbon (Downing *et al.*, 2006b). Shallow lakes can help in flood mitigation by acting as natural water

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reservoirs during heavy rainfall. They also contribute to groundwater recharge, maintaining water levels in surrounding areas (Bullock and Acreman, 2003). Depending on the water conditions and its relation to the nutrients shallow lakes can be found in two states: the state of clear water with plenty macrovegetation and the state of turbid water due to high phytoplankton presence (Scheffer *et al.*, 1993; Scheffer, 2004). During the last decades, the quality and quantity of these lakes have deteriorated due to extensive use and pollution from various sources (Scheffer, 2004).

### **2.2.1 Challenges that shallow lakes are facing**

Shallow lakes have serious challenges which disturb their ecological balance and health and these challenges have increased over the years as a result of several human activities as well as environmental changes. Their condition is considered pure when their water is clear and abundant with macrophytes (Scheffer, 2004). Different pollutants and nutrients discharged into these water bodies cause this state to change in a process called eutrophication. During eutrophication, the water becomes turbid, phytoplankton presence increases, and macrophytes tend to decrease, significantly altering the conditions of the water body (Moss, 1988). Once this process of deterioration begins, it almost becomes inevitable. Even though there are many ways to reduce nutrient loading, the concentration of nutrients already within the lake makes the restoration of these water bodies

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quite difficult, but different methods are used to restore the previous clear water conditions (Hanson and Butler, 1994; Moss, Madgwick and Phillips, 1997; Hansel-Welch *et al.*, 2003). Some of the challenges shallow lakes are facing are:

1. **Eutrophication:** Generally, refers to excess nutrient loading from sources such as wastewater discharges, industrial pollution, and agricultural runoff, which results in the excessive growth of algae and phytoplankton. This process reduces water quality, kills aquatic organisms, and depletes oxygen levels (Carpenter *et al.*, 1998; Smith, Joye and Howarth, 2006).

2. **Pollution:** Pollutant forms include pesticides, heavy metals, and organic contaminants, among other pollutants that may adversely affect shallow lakes. These toxins accumulate within the environment and public health is endangered as they concentrate in sediments and aquatic life (Scheffer, 2004)

3. **Sedimentation:** Increased sediment loads due to runoff and erosion fill in shallow lakes, changing habitat structure and depth in the lake. Destruction of important aquatic plants and the habitats of fish and other wildlife will probably occur (Håkanson, 1995).

4. **Loss of Biodiversity:** Pollution, invasion by alien species, and degradation of the habitat are responsible for the loss of biodiversity. Native species may be less competitive or displaced by the invading species and hence produce a less resilient ecosystem (Sala *et al.*, 2000; Dudgeon *et al.*,

2006).

5. **Climate Change:** The shallow lake hydrology and temperature structure can also be modified by changes in heat and rainfall patterns and extreme weather events. This would likely exacerbate problems related to eutrophication, decrease water levels, and increase the frequency of toxic algal blooms (Adrian *et al.*, 2009)

6. **Hydrological Alterations:** Land use changes, dam constructions and extractions of water are other anthropogenic factors that may disrupt the natural hydrological cycle of shallow lakes. Water levels, flow characteristics, and connectivity with other wetlands are all likely to be affected by these alterations (Nilsson *et al.*, 2005).

7. **Overfishing:** Unsustainable fishing practices can result in a reduction in fish populations, disruption of food webs, and alteration of the natural equilibrium of shallow lakes. Overfishing of apex predators may potentially lead to an increase in smaller, usually undesirable fish species (Post *et al.*, 2002).

8. **Recreational Pressure:** In shallow lakes, the most preferred leisure activity is boating, fishing, and swimming. Overuse may cause water pollution, environmental degradation, and even physical hindrance (Asplund and Cook, 1997).

9. **Invasive Species:** Introducing non-native organisms can disturb the natural equilibrium of shallow lakes. Invasive fishes, plants, and invertebrates tend to displace native species, alter ecosystems, and disrupt the

internal processes of nutrient cycling (Strayer, 2010).

10. **Water Withdrawal:** Water over-extraction for domestic, industrial, and agricultural activities can lower water levels and affect the thermal structure and biological integrity of smaller lakes (Gleick, 2003).

11. **Urbanization:** Urbanization in watersheds increases surface runoff, reduces groundwater recharge, and user pollutes shallow lakes. Water quality and hydrology are significantly altered by impervious surfaces and stormwater management practices (Paul and Meyer, 2008).

All these challenges can be met through multidisciplinary approaches like monitoring, intervention, rehabilitation, and sustainable approaches in addressing the environmental integrity of shallow lakes.

### **2.3 Contemporary and subfossil Cladocera sampling**

Precise sampling of Cladocera for discerning their population dynamics, biodiversity, and ecological roles. The sampling method used depends on the specific data required. Cladocera species are sampled for various purposes, including environmental monitoring, where they serve as bioindicators for water quality and environmental health (Berta *et al.*, 2019a). Additionally, they are sampled for paleolimnological research to provide insights into past environmental conditions and lake history (Korhola and Rautio, 2001b). In conservation biology, understanding their distribution and diversity is crucial. Both contemporary and subfossil Cladocera assemblages

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are used as indicators of environmental changes in water bodies. Their importance in monitoring these changes is indisputable, as their responses vary according to the degree of environmental alteration (Korhola and Rautio 2001). Cladocera play a vital role in investigating the impacts of pollution, climate change, and other stressors on freshwater ecosystems. By comparing subfossil Cladocera with contemporary specimens, researchers can evaluate the preservation capacity of various species. Studies have shown that preservation levels differ among genus; for instance, *Daphnia*, *Diaphanosoma*, *Ceriodaphnia*, *Limnosida*, and *Leptodora* are often underrepresented in sediment samples relative to their abundance in contemporary samples. By contrast, the species *Bosmina ssp.* and *Chydorus ssp.* usually over-represented themselves in the sediments (Korhola and Rautio 2001). Thereby emphasizing the need for using multiple sampling techniques for a better understanding of biodiversity and ecosystem conditions.

### **2.3.1 Contemporary sampling method**

Used for a very long time now, this method is mainly used to check the conditions of water bodies by checking the living species of Cladocera in them. Because they react quickly to changes in their environment, they serve as early indicators of ongoing changes (Berta *et al.*, 2019). In different environmental studies contemporary Cladocera are used as bioindicators to

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detect environmental stressors such as pollution, nutrient availability, or other changes in water bodies (Siciliano and Gesuele, 2013), Zawisza, Filbrandt-Czaja and Correa-Metrio, 2016a).

### 2.3.2 Subfossil sampling method

Cladocera subfossils are produced as a result of various factors after the death of the organisms, with the chemical composition of their body parts being the most significant factor (Korhola and Rautio, 2001). Subfossil Cladocera are the remnants of these organisms found in Holocene sediment layers, which are used to reconstruct historical environmental conditions, such as changes in water temperature, nutrient levels, and other ecological factors (Korhola and Rautio 2001). As Cohen (2003), said subfossils are among the most detailed sources of information among the available indications. Preservation depends on the species of Cladocera, as they have varying abilities to be preserved, depending on their chitin content (Deevey, 1964). The Chydoridae and Bosminidae families are well-preserved compared to the other nine families of Cladocera, which are very poorly preserved or not preserved at all (Hofmann, 1987; Hann, 1989).

Chydoridae, Bosminidae, and Daphniidae are the best preserved families, that can be found in lake sediments in body parts, such as head shields, carapaces and postabdomens, or as epphipia. These remains are used to track past changes in the environment, like climate (Korhola *et al.*, 2000;

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Li *et al.*, 2021), the trophic status (Brodersen, Whiteside and Lindegaard, 1998; Chen, Dalton and Taylor, 2010) etc. When the information we want to take from the sampling methods is the representation of Cladocera species during the last thousand years, we use subfossil sampling method.

### 2.4 Mediation of Cladocera species

In ecological research, community assemblages are a very important topic (Louette and De Meester, 2005), as it provides insights into the composition, structure, and dynamics of species within ecosystems. Understanding community assemblages involves studying the diversity and distribution of species, the interactions among them, and the factors that influence these patterns. The roles of various abiotic and biotic factors influencing communities have been studied previously (Caley and Schluter, 1997; Shurin, 2000), as well as the role of species' dispersal abilities (Louette and De Meester, 2005), highlighting the significance of environmental conditions on these communities.

Like many other types of zooplankton, Cladocera species are distributed worldwide. However, their distribution depends on the type of water body, its location, and other environmental conditions. Cladocera are highly dispersible species, capable of being transported through various means such as waterways, wind, birds, and human activities, both intentional and unintentional.

### **2.4.1 Different mediation ways of Cladocera species**

As mentioned above there are many different ways Cladocera species are distributed around the world. A complex array of biotic and abiotic variables mediates Cladocera species-including birds, humans, and hydrological modifications influences underlining these processes that shape these dynamics of freshwater ecosystems wherein they thrive as Cladocera. Birds participate enormously in external dispersal of Cladocera through ectozoochory that causes organisms to attach to feathers or feet of birds. This action means the transfer of Cladocera between bodies of water toward gene flow and connecting populations. Such a finding shows that waterbirds can transfer Cladocera propagules over long distances, hence influencing species distribution and community composition in fragmented habitats (Coughlan *et al.*, 2017). They act as important mediators in aspects of habitat fragmentation and climate change as it keeps up the genetic diversity and resilience within the populatio structures of Cladocera species (Coughlan *et al.*, 2017).

Besides these factors, humans also play a big role in Cladocera mediation. Nutrient loading, habitat alteration, and pollution increase the anthropogenic environmental forces that might lead to major changes in community composition and density of Cladocera. The introduction of non-native species through human-mediated pathways has been documented to effect changes in local Cladocera populations (Garibian and Kotov, 2021). Furthermore, water management practices, such as pumping and reservoir construction, change the hydrological regimes, which can affect on their

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availability of suitable habitat for Cladocera (Zawiska *et al.*, 2020). These changes mostly concern a rise in dominance within a few species, which can significantly change community structure (Zawiska *et al.*, 2020).

Hydrological changes, especially those within floodplain ecosystems, significantly determine Cladocera communities. Some effects of periodic and duration flooding on the diversity and composition of zooplankton as well as Cladocera. Research indicates that even in cases of frequent hydrological connections between floodplains and rivers, there are lower species diversity due to their homogenization of habitats (Paidere, 2009). However, with stable water levels, Cladocera communities can get established with high diversity owing to stable habitats (Paidere, 2009). Thus, hydrology-cladoceran dynamics are important for understanding the ecological function of these organisms in freshwater systems. This is a very strong statement about the mediation of Cladocera; it is biotic and includes a general range of human impacts along with hydrological changes for describing abiotic variables. These factors ultimately are necessary for water bodies to maintain their ecological integrity and would require integrated management focusing on both natural and anthropogenic drivers affecting the Cladocera populations.

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### 2.4.2 Mediation by researcher's chest wader

A little studied topic is the role of the scientists in mediation of Cladocera species, during sampling on shallow water bodies. Chest wader is the rubber clothe that scientists or fishers wear when they need to go in the water body and protect them not to get wet. While wearing it, scientist will enter the water body and the boots part will be filled with sediment from the lake, while other areas of the chest wader can be an easy attachment area for Cladocera species



**Figure 2:** Chest wader (Source: Aquavitex Online Store)

## **3. MATERIALS AND METHODS**

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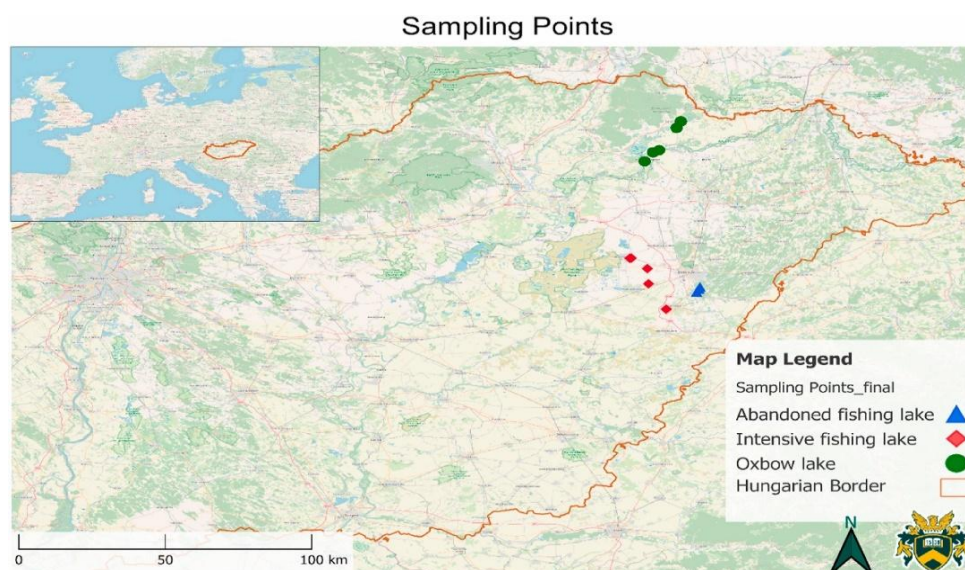
### **3.1. Mediation experiment study area**

#### **3.1.1. Studied lakes for mediation experiment**

The studied lakes are in the N and N-E Hungary (Fig. 3.) representing the micro-region of the Great Plain. During the experiment (2021 summer) we collected samples from 13 different standing waterbodies with three different types of utilization. The oxbow lakes (green marking) are functioning as a natural habitat, with naturally existing fish stocks, but without fish installation and fishing activity. Lakes with red markings are utilized as fishing lakes with intensive fish installation (and intensive fishing), while lakes with blue markings are abandoned fishing lakes. These lakes were once used intensively for fishing but dried out several times during the last decade and without a proper water supply, operators left them behind.

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**Figure 2:** This map displays the locations of the studied lakes, marked according to their utilization categories and their proximity to the Hungarian border. Abandoned fishing lakes are indicated with blue triangles, intensive fishing lakes with red squares, and oxbow lakes with green circles. The inset map highlights Hungary's geographical position within Europe. (Source: Author, 2024)

#### 3.1.2. Field work

A total of thirteen shallow lakes, exhibiting varying degrees of utilization, were selected for sampling in the northeastern region of Hungary. Fieldwork was conducted over a weekend under favorable weather conditions, ensuring optimal sampling circumstances.

##### 3.1.2.1. Sample collecting

The sampling process was performed by a single scientist, equipped

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with a chest wader. Before sampling, the researcher thoroughly cleaned their chest waders using soap and water, scrubbing with a stiff brush, and rinsing with de-ionized water. They entered the lakes up to the top edge of the chest wader, at a distance of around 10 meters from the shore. Based on prior sampling protocols, researcher remained stationary for 10 minutes to collect samples. Upon exiting the water, the scientist stepped into a plastic container, where the chest waders were carefully rinsed with filtered water to dislodge and collect any residues and sediments adhering to the gear. The samples were sieved through a 35  $\mu\text{m}$  mesh from the container and transferred into 50 mL centrifuge tubes and preserved in 96% Patosolv alcohol. Cleaning was carried out before and after sampling at each site. The same was repeated three times in order to confirm the tenderness of data.

#### **3.1.3. Laboratory work**

In the laboratory, the samples were processed using 100 mL of a 10% KOH solution following the standard protocol outlined by (Korhola and Rautio, 2001). The mixtures were placed in plastic beakers and heated for 30 minutes at 70°C in a Stuart SWB6D laboratory water bath. Post-treatment, the samples were filtered through a 35  $\mu\text{m}$  sieve to eliminate larger particles. Samples were preserved with 96% Patosolv alcohol, added with a few drops of Safranin-glycerin for the staining of easily identifiable chitinized particles. An Olympus BX53 microscope and an Olympus DP26 digital camera were

employed for species identification. For each slide, 100  $\mu$ L of the sample was used, and a total of 1 mL was examined. Cladocera species were identified according to the methods described by (Gulyás, 1974; Frey, 1986; Bledzki and Rybak, 2016).

#### **3.2.3.1. Microscopic identification and counting**

Identification of Cladocera species and their subfossil residues were made based on identification books such as: Atlas of Subfossil Cladocera from Central and Northern Europe (Szeroczyńska and Sarmaja-Korjonen, 2007).

#### **3.1.4. Data analysis**

Applying a logarithmic transformation to positively skewed abundance data makes them suitable for subsequent statistical analyses. Levene's test gave a p-value of less than 0.05 ( $p < 0.001$ ) for the present data; thus, the null hypothesis for equal variance was rejected. This indicates that there were significantly different assemblage variances. To derive diversity indices such as Shannon-Wiener and Simpson, PAST software (version 3.17c) was used. Adding Nonmetric multidimensional scaling (NMDS) further developed a relationship between the sampled lakes. It applied these relationships using Bray-Curtis similarity based on the occurrence data of each species to model

dissimilarities in low-dimensional space. Further, maps of the sampled sites were derived from QGIS software (version 3.16.10) providing spatial data of the study sites.

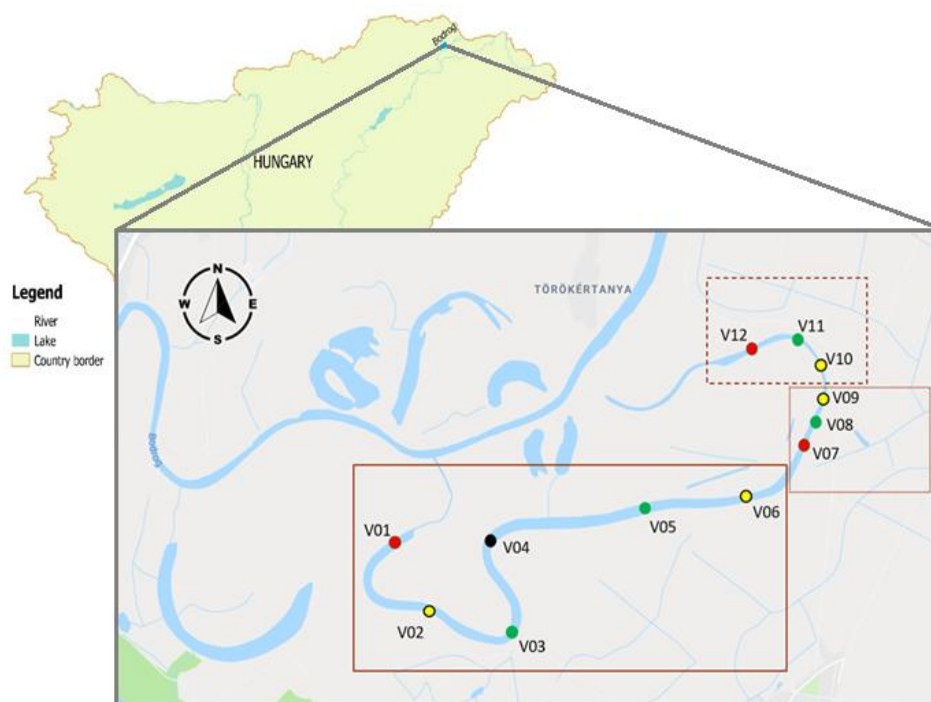
#### **3.2. Viss oxbow lake's comparative analysis of contemporary and subfossil Cladocera assemblages concerning lake utilization and environmental factors**

##### **3.2.1. Viss oxbow lake**

During this experiment we investigated the Viss oxbow lake – as a multi-utilized lake located in the northern-eastern part of Hungary. The lake is under mixed utilization, divided into three main parts (with connections to each other) which are utilized differently, as follows: The first part represents a nature protected area (dashed line), where all anthropogenic input or activity is prohibited. Only activities that enhance diversity and focus on nature conservation are allowed. The second part is heavily influenced by agricultural loads (dotted line), with an inlet channel from arable lands and water outlet for irrigation. The third part is under recreational use (solid line), where mostly fishing is the main activity, along with other recreational activities e.g. sightseeing, tourism, kayaking (see Figure 3.).

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**Figure 3:** The experimental lake with indication of the utilizations and sampling sites with the main type of vegetation coverage. Coding: red dot – submerged vegetation, black dot – emergent vegetation, yellow dot – floating-leaved vegetation, green dot – open water area. (Source: Author, 2024)

#### 3.2.2. Field work

This research's venue is the Viss oxbow lake which encompasses varied zones that face different intensities of anthropogenic pressure. In the summer vegetation period of 2019, 12 surface soft sediment and 12 filtered water samples were collected. Figure 1 represents the sampling sites with

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their types of utilization and vegetation cover. All sites were georeferenced using a GPS device (Garmin eTrex).

On-site environmental measurements were performed with a YSI EXO2 multiparameter sonde (Xylem, 599502-1). Water temperature, dissolved oxygen content, conductivity, suspended solids, as well as chlorophyll-a and pH were recorded.

Soft sediment samples were collected with an 8 cm-diameter gravity corer (WaterMark, USA). From each core, sampled was only the first surface layer which enabled collection of 1 cm sediment and storage in plastic containers in cool conditions during transfer and storage in the laboratory. Stratified sampling was also applied. Filtered water was collected using a Schindler-Patalas plankton trap with a 30µm mesh attachment with a total of 30 L of water processed per site, stored in plastic containers and preserved in the field with 96% Patosolv alcohol for further analysis.

#### **3.2.3. Laboratory work**

Filtered samples were stained with Safranin-glycerine for easier analysis, while sediment samples were prepared according to the standard method outlined by Korhola and Rautio (2001). Such prepared samples were examined through Alpha BIO-2T LED microscope. Subfossil and modern Cladocera species identification has been carried out using guides by Szeroczyńska and Sarmaja-Korjonen (2007), as well as Gulyás and Forró

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(1999). At least 200 individuals were counted from the filtered samples, whereas for sediment samples, at least 200 individuals or 350–400 remains were quantified.

The organic matter and calcium carbonate content of the sediment were assessed using the sequential loss-on-ignition method described by (Berglund and Ralska-Jasiewiczowa, 1991; Heiri, Lotter and Lemcke, 2001). Concurrently, water samples were analyzed for ortho-phosphate, nitrate-nitrogen, nitrite-nitrogen, ammonium-nitrogen, sulfate, and chloride ion concentrations. Total alkalinity and chemical oxygen demand were also measured.

#### **3.2.3.1. Microscopic identification and counting**

The inverted microscope of Olympus-IX73 was used for phytoplankton counting (ind. L<sup>-1</sup>) at a magnification of 1000X (100X) and 400X (40X) and the light microscope of Olympus-BX53 was used for identifying the phytoplankton species.



**Figure 4:** Microscopic identification of samples (Source: Author, 2022)

#### **3.2.4. Data analysis**

The data were statistically appropriate following the standardization of chemical and physical environmental factors, while a Hellinger transformation was carried out on both contemporary and subfossil Cladocera data. General diversity indices - those of Shannon, Simpson, and species richness, as well as species dominance - have been calculated with the help of the diversity function in PAST (Hammer, Harper and Ryan, 2001) ver. 3.17c. Non-Metric Multidimensional Scaling (NMDS) was employed to

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visualize patterning and test differences across species composition in a given area of study. A method that distinguishes group or separation, especially related to environmental influence and anthropogenic influences-for example, by Cladocera into types of areas that are affected by agricultural activity-has been developed. Canonical correspondence analysis was done to establish the possible relationships between species abundance and environmental variables. This analysis revealed insight into changes in species composition in Cladocera concerning chemical and physical factors like nutrients concentration, temperature, and pH. The NMDS analysis used a Bray-Curtis dissimilarity matrix to see differences in species composition. An NMDS analysis was run on a Bray-Curtis dissimilarity matrix to examine species composition. Such a measurement was captured using a Mantel test to visualize and quantify the potential correlation between assemblages modern subfossil Cladocera data across differing land uses. The results of the statistical analyses were exported and further assessed and visualized in OriginPro version 9.50 (OriginLab, 2018).

## **4. RESULTS**

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### **4.1. Mediation experiment**

#### **4.1.1. Classification and characteristics of sampled lakes**

We sampled 13 lakes, belonging to three different groups:

- a) Abandoned fishing lakes
- b) Intensive fishing lakes
- c) Oxbow lakes

Among these, five lakes were classified as abandoned fishing lakes: L (Látóképi víztározó), KE (Kerek-erdei-tó), SZ2 (Szíki-tó), S (Sáska-tó), and KV (Kék Víz). Another five identified as oxbow lakes: TI (Tímári Holt-Tisza), SZO (Szögi Holt-Bodrog), KMT (Kis-Morotva-tó), SZ1 (Szabolcsi Holt-Tisza), and KBM (Keleti Holt-Bodrog). The remaining three lakes, R (Rókás-tó), KTP (Kenu-pálya), and VE (Vekeri-tó), were classified as intensive fishing lakes.

#### **4.1.2. Cladocera taxa and individual findings**

From the 13 lakes sampled, Cladocera specimens were identified in all except lake Kék Víz. A total of 27 distinct species were recorded across the 12 lakes containing Cladocera. These specimens included whole individuals, ephippia, remains, or fragments. Oxbow lakes, which were primarily natural and protected water bodies, exhibited the highest species diversity. In

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contrast, intensively fished lakes, most of which were manmade, had the lowest species diversity.

Notably, a group of abandoned fishing lakes, unused for intensive fishing over the past 2–3 years, showed a significantly high abundance of Cladocera. However, in two oxbow lakes, species diversity was lower than expected, largely due to extended dry-out periods. Among the intensively fished lakes, no Cladocera were detected in Kék Víz, while Lake Vekeri-tó (VE) had the highest abundance, with 1,220 individuals. For oxbow lakes, Szabolcsi Holt-Tisza (SZ1) had the highest Cladocera count, with 660 individuals, whereas Kis-Morotva-tó (KMT) had the lowest, with 90 individuals (Table 1).

Of the 27 total species identified, 24 were found in oxbow lakes, 25 in abandoned fishing lakes, and 24 in intensively fished lakes. The species *Monospilus dispar* (G. O. Sars, 1861) was detected exclusively in one oxbow lake (Szögi Holt-Bodrog) and was absent in the other lake types, whereas all other species were recorded in at least two differently utilized lake types. This species typically exhibits higher densities in natural habitats (Tumurtoogoo *et al.*, 2022). So, the lakes of Kék Víz did not contain any Cladocera species and this lake will not belong to further statistical analysis.

From the 12 lakes studied, there were 27 Cladocera species identified; they belong to four families: Bosminidae, Chydoridae, Daphniidae, and Sididae. Among the lakes, Kerek-erdő (KE) and Vekeri-tó (VE) exhibited the

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highest species richness, each hosting 22 species. In contrast, Szíki-tó (SZ2) and Sáska-tó (S) had the lowest species richness, with only three species each.

**Table 1:** Presents the number of taxa and individuals of Cladocera recorded in each studied lake, along with measures of dominance, Simpson, and Shannon diversity indices. The table uses colour coding to represent lake types: red – oxbow lakes, green -abandoned fishing lakes and with blue – intensively fished lakes. (Author’s data, 2024)

	TI	SZO	KMT	SZ1	KBM	R	KTP	VE	L	KE	SZ2	S
Taxa	17	17	6	14	9	16	21	22	11	22	3	3
Individuals	310	590	90	660	140	520	760	1220	150	1060	40	40
Dominance	0.08	0.11	0.21	0.14	0.13	0.09	0.09	0.10	0.11	0.09	0.38	0.38
Simpson	0.92	0.89	0.79	0.86	0.87	0.91	0.91	0.89	0.89	0.91	0.63	0.63
Shannon	2.67	2.48	1.68	2.25	2.11	2.57	2.66	2.54	2.30	2.63	1.04	1.04

The dominant species across all samples was *Bosmina longirostris* (O. F. Müller, 1776), found in 11 of the 12 lakes. The most abundant species, however, was *Chydorus sphaericus* (O. F. Müller, 1785), which accounted for 1,030 individuals across all samples. *Monospilus dispar* was observed only once, in a single oxbow lake (Szögi Holt-Bodrog).

In terms of habitat-specific dominance:

- In oxbow lakes, *C. sphaericus* was dominant, appearing in four out of five sampled lakes with a total of 210 individuals.
- In abandoned fishing lakes, *C. sphaericus* also dominated, with 400 individuals recorded across all three sampled lakes.
- In intensively fished lakes, *C. sphaericus* maintained its dominance, found in three of the five sampled lakes with a total of 220 individuals.

The Simpson diversity index shows that abandoned fishing lakes have

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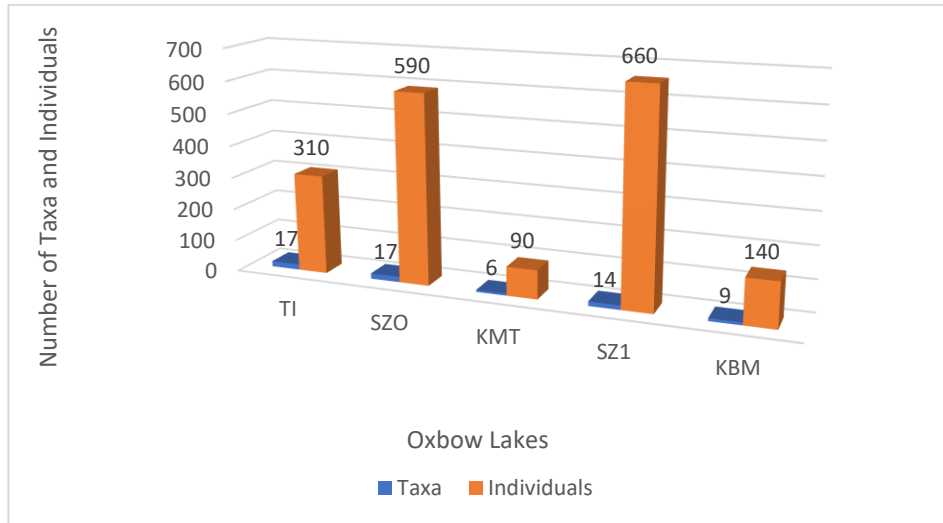
the highest mean diversity, the oxbow lakes next in line, and the least diversity in intensive fished lakes. The lowest Simpson diversity index values recorded among sampled lakes were in the intensively fished lakes S (Sáska-tó) and SZ2 (Sziki-tó), both at 0.6250. Conversely, the oxbow lake TI (Tímári Holt-Tisza) had the highest Simpson diversity index value, 0.9199 (Table 1).

Similarly, the Shannon diversity index reflects the same trend, with abandoned fishing lakes showing the highest diversity, intensively fished lakes the lowest, and oxbow lakes displaying moderate diversity. The lowest Shannon index values, 1.0400, were recorded in lakes S (Sáska-tó) and SZ2 (Sziki-tó). Meanwhile, the highest value, 2.6740, was found in lake TI (Tímári Holt-Tisza).

##### **4.1.2.1 Comparison of species between three different lake groups**

The following graphs provide a comprehensive visualization of the differences between lake groups in terms of taxa composition and abundance. They mark the differences in the number of individuals and dominance patterns, as well as in their taxa compositions. The graphs also compare Simpson and Shannon diversity indices with respect to taxa, providing some insight into the ecological diversity and distribution of the species within the lake groups.

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**Figure 5:** Comparison of Taxa and Individuals between oxbow lakes. (Author's data, 2024)

The bar graph illustrates the distribution of taxa and individuals across four oxbow lakes (TI, SZO, KMT, SZ1, and KBM). The blue bars represent the number of taxa, while the orange bars indicate the number of individuals observed in each lake.

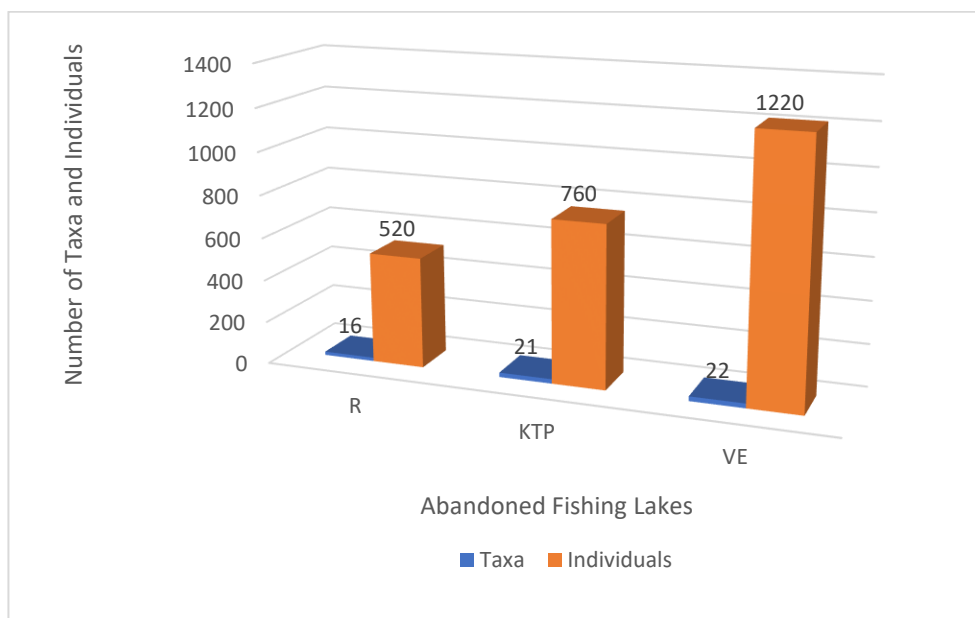
- **TI:** The lake had 17 taxa recorded, with a total of 310 individuals.
- **SZO:** Similar to TI, 17 taxa were observed, but with a higher number of individuals, reaching 590.
- **KMT:** This lake had the lowest diversity and population, with only 6 taxa and 90 individuals recorded.
- **SZ1:** Despite having slightly fewer taxa (14), this lake exhibited the highest number of individuals, totalling 660.
- **KBM:** This lake had 9 taxa and 140 individuals, reflecting moderate

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levels of both diversity and population.

In summary, SZ1 showed the largest population despite a lower number of taxa, while KMT exhibited the lowest values in both measures. This pattern suggests variability in biodiversity and population density among the sampled lakes.



**Figure 6:** Comparison of Taxa and Individuals between abandoned fishing lakes. (Author's data, 2024)

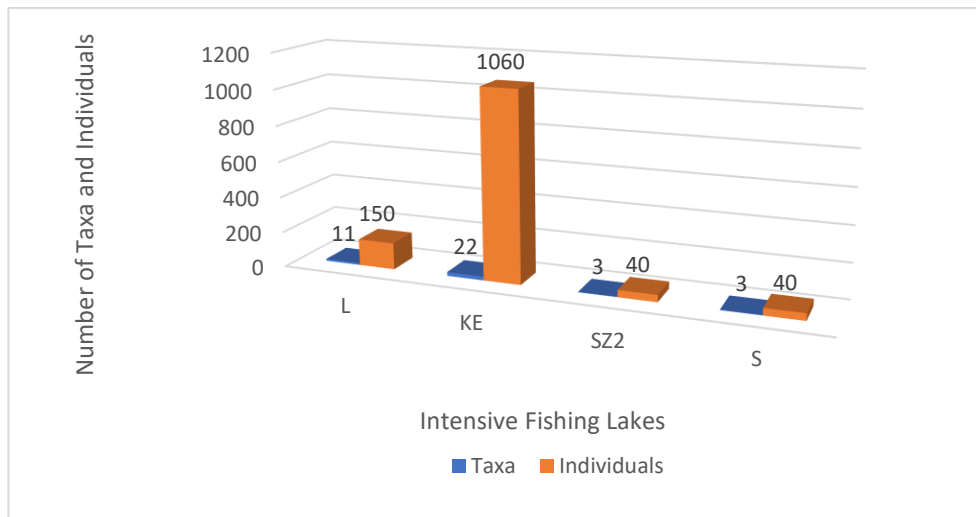
The bar graph depicts the distribution of taxa and individuals across three abandoned fishing lakes (R, KTP, and VE). The blue bars represent the number of taxa, while the orange bars indicate the number of individuals observed in each lake.

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- **R**: The lake recorded 16 taxa and a total of 520 individuals.
- **KTP**: This lake exhibited a slightly higher diversity with 21 taxa and a population of 760 individuals.
- **VE**: The highest values were observed here, with 22 taxa and 1,220 individuals, indicating the greatest diversity and population among the lakes.

In summary, the VE lake demonstrated the highest biodiversity and population density, while R exhibited the lowest. This suggests a gradient of ecological richness among the abandoned fishing lakes.



**Figure 7:** Comparison of Taxa and Individuals between intensive fishing lakes. (Author's data, 2024)

#### 4. Results

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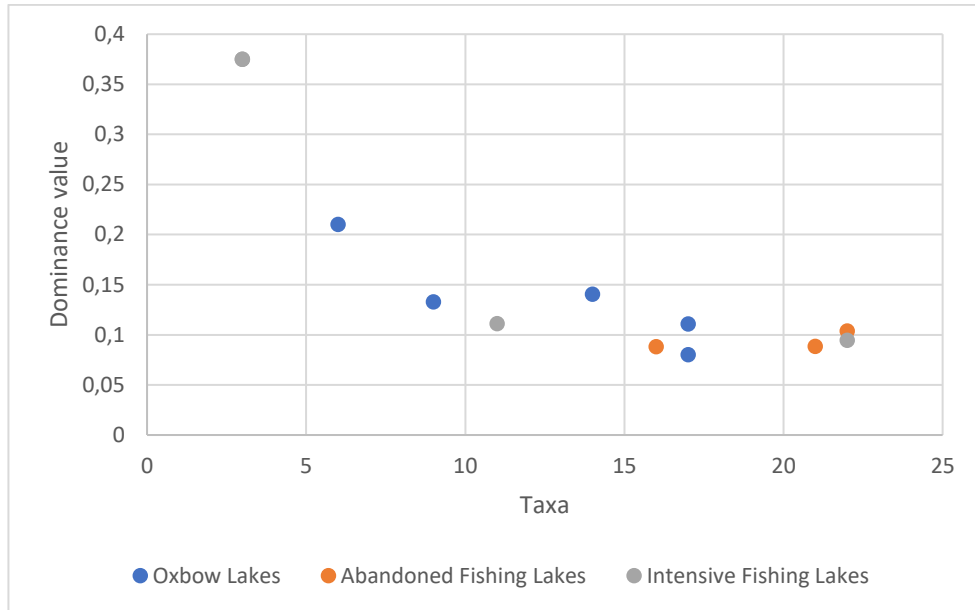
The bar graph shows the number of taxa and individuals across four intensive fishing lakes (L, KE, SZ2, and S). The blue bars represent taxa diversity, while the orange bars show the population of individuals.

- **L:** This lake recorded 11 taxa with a total of 150 individuals.
- **KE:** The highest diversity and population were observed here, with 22 taxa and 1,060 individuals.
- **SZ2:** Both taxa and individuals were comparatively low, with 4 taxa and 40 individuals.
- **S:** Like SZ2, 4 taxa and 40 individuals were observed.

In summary, KE exhibited significantly higher biodiversity and population density compared to the other lakes. L showed moderate values, while SZ2 and S demonstrated the lowest levels of both metrics. This indicates notable differences in ecological characteristics among the intensive fishing lakes.

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**Figure 8:** Comparison of Taxa from three lake groups with dominance. (Author's data, 2024)

This scatter plot illustrates the relationship between taxa (x-axis) and dominance value (y-axis) for three types of lakes: oxbow lakes, abandoned fishing lakes, and intensive fishing lakes. Here's a summary of the observations:

**Oxbow lakes** (blue points): The dominance values generally fall within a range of 0.05 to 0.15. These points are distributed across lower to mid-range taxa values (approximately 5 to 20).

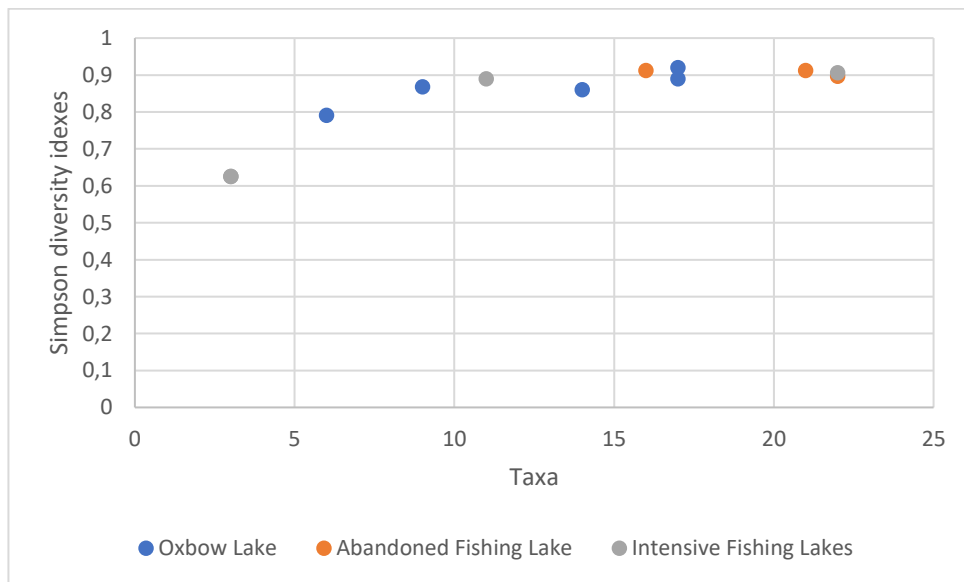
**Abandoned fishing lakes** (orange points): The dominance values are slightly higher, spanning from around 0.05 to 0.15, with taxa primarily concentrated in the mid to higher range (approximately 15 to 20).

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**Intensive fishing lakes** (gray points): These show the highest dominance values, up to 0.35, with taxa distributed across a wider range (from about 5 to 20).

**Key insights:** Oxbow lakes show relatively stable dominance values compared to the other two lake types. Abandoned fishing lakes have a similar dominance range to oxbow lakes but appear in higher taxa ranges. Intensive fishing lakes have the highest dominance values overall, indicating more pronounced species dominance. This suggests that lake type impacts dominance value and taxa distribution, with intensive fishing having the greatest effect on dominance values.



**Figure 9:** Comparison of Taxa from three lake groups with Simpson diversity index. (Author's data, 2024).

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This scatter plot illustrates the relationship between taxa (x-axis) and Simpson diversity index (y-axis) for three types of lakes: oxbow lakes, abandoned fishing lakes, and intensive fishing lakes. Here's an analysis:

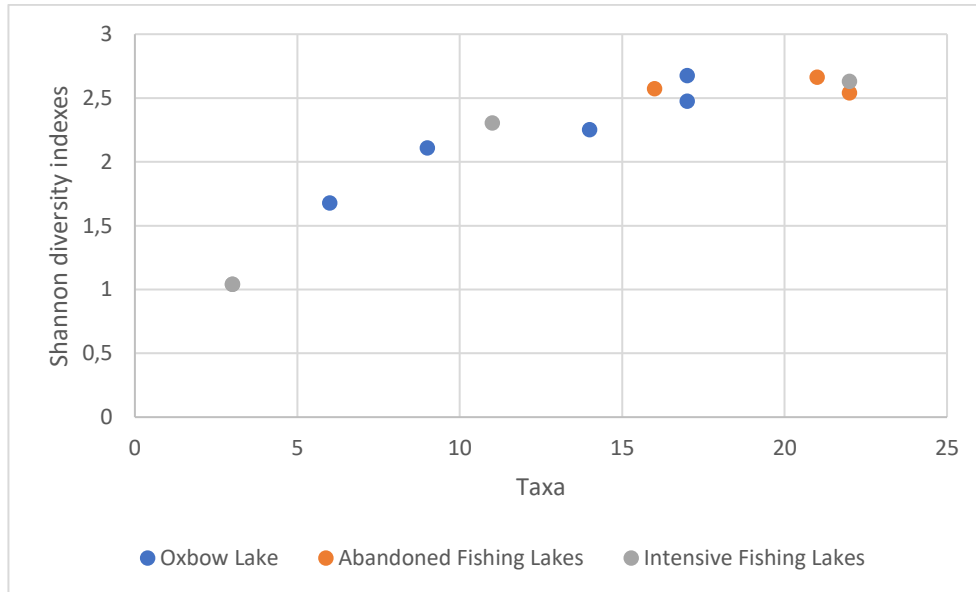
**Oxbow lakes** (blue points): The Simpson diversity index values are high, ranging from approximately 0.8 to 1. These points are distributed across low to mid-range taxa values (approximately 5 to 15).

**Abandoned fishing lakes** (orange points): The Simpson diversity index values are similarly high, clustering near 0.9 to 1, and are concentrated at the higher end of taxa values (15 to 20).

**Intensive fishing lakes** (gray points): The Simpson diversity index values vary more significantly, ranging from approximately 0.6 to 0.9. These points are scattered across the range of taxa values (5 to 20).

**Key insights:** Oxbow lakes and abandoned fishing lakes demonstrate consistently high diversity indexes, indicating well-balanced species diversity. Intensive fishing lakes show a broader variation in Simpson diversity index, suggesting less consistent species diversity and potentially more environmental pressures affecting these ecosystems. Overall, lake type influences species diversity, with oxbow and abandoned fishing lakes generally maintaining higher and more stable diversity compared to intensive fishing lakes.

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**Figure 10:** Comparison of Taxa from three lake groups with Shannon diversity index. (Author's data, 2024)

This scatter plot represents the relationship between taxa (x-axis) and Shannon diversity index (y-axis) for three types of lakes: oxbow lakes, abandoned fishing lakes, and intensive fishing lakes. Here's the analysis:

**Oxbow lakes** (blue points): Shannon diversity index range from approximately 2 to 2.5. These points are distributed across a moderate range of taxa values (around 5 to 20), suggesting stable diversity in these ecosystems.

**Abandoned fishing lakes** (orange points): Shannon diversity index are slightly higher, clustering between 2.5 and 3. These points are concentrated at higher taxa values (15 to 20), indicating greater species richness and

evenness in these lakes.

**Intensive fishing lakes** (gray points): The Shannon diversity index show more variability, spanning approximately 1 to 2.5. These points are distributed across a broad range of taxa values (around 5 to 20), suggesting uneven diversity due to potential environmental pressures or disturbances.

**Key insights:** Abandoned fishing lakes demonstrate the highest Shannon diversity index, indicating rich and evenly distributed species populations. Oxbow lakes maintain moderately high diversity, though slightly less than the abandoned lakes. Intensive fishing lakes exhibit lower and more variable diversity, likely due to intensive human activity impacting the ecosystem balance. Overall, the Shannon diversity index highlights how abandoned and less disturbed lake environments support richer and more balanced biodiversity compared to intensive fishing lakes.

### 4.1.3. Identified Cladocera species

The tables below (Table 2 and Table 3) display the Cladocera species identified in each of the sampled lakes during the study.

All the oxbow lakes were natural, protected water bodies; however, SZ1 (Szabolcsi Holt-Bodrog) and KMT (KisMorotva-to) exhibited higher water level fluctuations, with the potential to dry out, as shown in Tables 2 and 3. *C. sphaericus* was the most dominant species across these lakes, while *M. dispar* was observed only once.

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Among the abandoned and intensively fished lakes surveyed, only SZ2 (Sziki-to) and KE (Kerek-erdei-to) were natural water bodies; the remaining lakes were artificial with evident water level fluctuations. As indicated in Table 3, *C. sphaericus* remained the dominant species in both lake types. Meanwhile, *Pleuroxus trigonellus* (O.F. Müller, 1776) and *Simocephalus vetulus* (O.F. Müller, 1776) were the least frequently observed species in abandoned fishing lakes. Additionally, in intensively fished lakes, seven species were encountered only once.

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**Table 2:** Sampling sites with indication of the identified species and number of individuals (ind. L<sup>-1</sup>). Red colour represents the oxbow lakes. (Author's data, 2023)

Species	TI	SZO	KMT	SZ1	KBM
<i>Acroperus harpae</i> (Baird, 1834)	2000	3000	1000	1000	
<i>Alona guttata</i> (G. O. Sars, 1862)		1000	2000	1000	5000
<i>Alona intermedia</i> (G. O. Sars, 1862)	1000	4000	1000		2000
<i>Alona quadrangularis</i> (O. F. Müller, 1776)	1000	1000			
<i>Alona rustica</i> (Scott, 1895)	1000				1000
<i>Alonella exigua</i> (Lilljeborg, 1853)	1000				
<i>Alonella nana</i> (Baird, 1850)	1000			1000	
<i>Bosmina longirostris</i> (O. F. Müller, 1776)	4000	9000		2000	11000
<i>Camptocercus rectirostris</i> (Schoedler, 1862)		1000			
<i>Chydorus gibbus</i> (G. O. Sars, 1891)	1000	1000		1000	8000
<i>Chydorus ovalis</i> (Kurz, 1874)	4000	2000	1000		6000
<i>Chydorus sphaericus</i> (O. F. Müller, 1785)	4000	6000		3000	18000
<i>Diaphanosoma mongolianum</i> (Ueno, 1938)				1000	
<i>Eubosmina coregoni</i> (Baird, 1857)	2000			2000	3000
<i>Eubosmina longispina</i> (Leydig, 1860)		2000	1000	2000	2000
<i>Graptoleberis testudinaria</i> (Fischer, 1848)	2000	4000	3000		3000
<i>Leydigia acanthocercoides</i> (Fischer, 1854)		1000			
<i>Monospilus dispar</i> (G. O. Sars, 1861)		2000			
<i>Oxyurella tenuicaudis</i> (G. O. Sars, 1862)					1000
<i>Paralona pigra</i> (G. O. Sars, 1862)	1000	6000			1000
<i>Picripleuroxus laevis</i> (G. O. Sars, 1861)	2000	1300			3000
<i>Pleuroxus trigonellus</i> (O. F. Müller, 1776)	1000				
<i>Pleuroxus uncinatus</i> (Baird, 1850)	1000	2000			
<i>Pseudochydorus globosus</i> (Baird, 1843)	2000	1000			2000

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**Table 3:** Sampling sites with indication of the identified species and number of individuals (ind. L<sup>-1</sup>). Green colour represents the abandoned fishing lakes, while blue colour represents the intensive fishing lakes. (Author's data, 2023)

Species	R	KTP	VE	L	KE	KV	SZ2	S
<i>Acroperus harpae</i> (Baird, 1834)	2000	1000	1000		4000			
<i>Alona guttata</i> (G. O. Sars, 1862)	2000	2000	5000	1000	4000			
<i>Alona intermedia</i> (G. O. Sars, 1862)	7000	9000	9000		7000		2000	
<i>Alona quadrangularis</i> (O. F. Müller, 1776)	1000		2000		1000			
<i>Alona rectangula</i> (Sars, 1861)		2000			3000			
<i>Alona rustica</i> (Scott, 1895)	3000	1000	1000	2000	1000			
<i>Alonella excisa</i> (Fischer, 1854)			1000		1000			
<i>Alonella exigua</i> (Lilljeborg, 1853)		2000	13000		9000			
<i>Alonella nana</i> (Baird, 1850)	4000		1000		1000			
<i>Bosmina longirostris</i> (O. F. Müller, 1776)	5000	7000	11000	1000	11000		1000	1000
<i>Camptocercus rectirostris</i> (Schoedler, 1862)	2000	1000	2000					
<i>Chydorus gibbus</i> (G. O. Sars, 1891)	2000	8000	17000	2000	16000			
<i>Chydorus ovalis</i> (Kurz, 1874)	5000	5000	2000	1000	3000			
<i>Chydorus sphaericus</i> (O. F. Mueller, 1785)	6000	12000	22000	3000	18000		1000	
<i>Diaphanosoma mongolianum</i> (Ueno, 1938)				1000				
<i>Eubosmina coregoni</i> (Baird, 1857)	1000	2000	1000					2000
<i>Eubosmina longispina</i> (Leydig, 1860)		4000		1000	3000			1000
<i>Graptoleberis testudinaria</i> (Fischer, 1848)	1000	5000	2000		2000			
<i>Leydigia acanthocercoides</i> (Fischer, 1854)		1000	1000		1000			
<i>Monospilus dispar</i> (G. O. Sars, 1861)								
<i>Oxyurella tenuicaudis</i> (G. O. Sars, 1862)	3000	1000	1000	1000	1000			
<i>Paralona pigra</i> (G. O. Sars, 1862)		1000	6000	1000	6000			
<i>Picripleuroxus laevis</i> (G. O. Sars, 1861)	7000	9000	17000		11000			
<i>Pleuroxus trigonellus</i> (O. F. Müller, 1776)		1000			1000			
<i>Pleuroxus uncinatus</i> (Baird, 1850)		1000	1000					
<i>Pseudochydorus globosus</i> (Baird, 1843)	1000	1000	5000	1000	1000			
<i>Simocephalus vetulus</i> (O. F. Müller, 1776)			1000		1000			

#### **4.1.4. NMDS orientation based on Cladocera abundance across sampling lakes**

The NMDS analysis illustrates the spatial orientation of the sampled lakes based on the number of Cladocera individuals collected from each lake using chest waders after exiting the lakes (Fig. 2). Abandoned fishing lakes are closely clustered together, reflecting the similarity of the data within this group. Conversely, oxbow lakes are similarly grouped but with exceptions such as KMT and KBM, which show different orientations. Intensively fished lakes are more widely dispersed and are generally located farther from the other two groups, with the exception of KE.

All five oxbow lakes are protected water bodies; however, their orientations in the NMDS analysis varied. Lakes TI, SZO, and SZ1 exhibited similar patterns, while KMT and KBM were distinct from the other oxbow lakes. This difference is likely due to their susceptibility to drying out, with KBM additionally being used for recreational fishing.

The abandoned fishing lakes (KTP, R, and VE) were grouped together in the same area, showing a high number of Cladocera in the samples. These lakes have not been used for fishing for three years or longer.

The lakes subjected to intensive fishing exhibited the lowest number of Cladocera specimens. Lakes S, SZ, and L were located in similar positions but not in close proximity to one another. Lake KE was aligned with the abandoned fishing lakes in the NMDS plot, likely due to its high Cladocera

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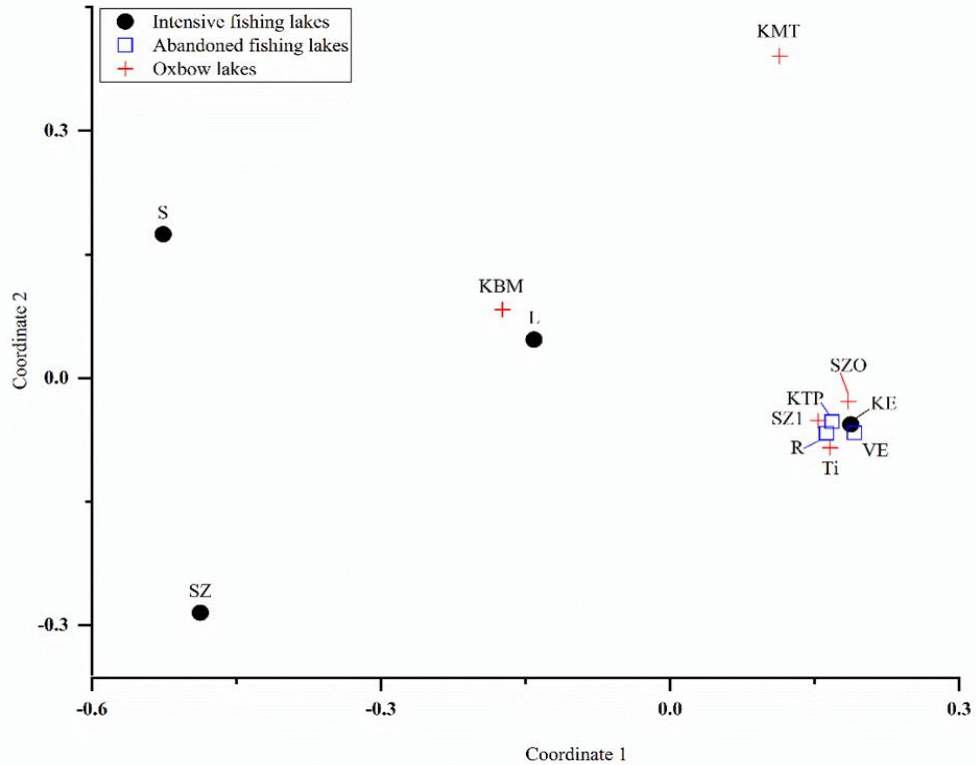


Figure 11: Non-metric multidimensional scaling (NMDS) with Bray-Curtis dissimilarity index. Analysis made on the Cladocera individuals grouped by the utilization types. Stress value is 0.09356. Red plus – oxbow lakes, blue square – abandoned fishing lakes, black dot – intensive fishing lakes. (Author's data, 2023)

numbers, which can be attributed to natural fish stocks rather than artificial fish introductions.

## **4.2. Cladocera assemblages in relation to lake utilization and environmental factors: Second study findings**

### **4.2.1. Characteristics of sampled lake**

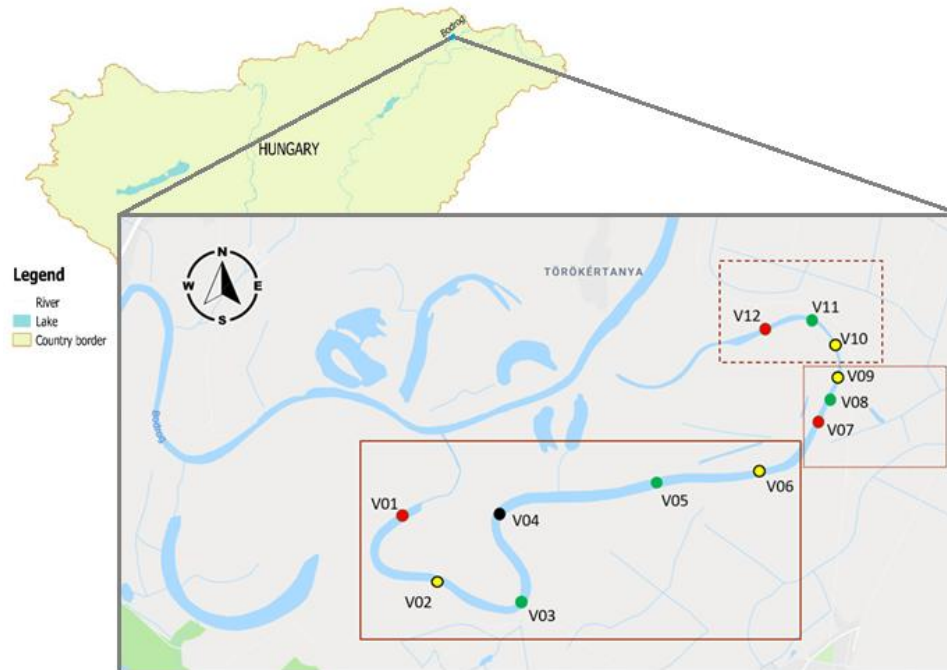
In our second study, we investigated Cladocera species in Viss oxbow Lake, a multi-purpose lake located in the northeastern part of Hungary. The lake is divided into three distinct zones based on their usage and anthropogenic impact:

- a) Protected area
- b) Agriculturally influenced area
- c) Recreational area

These three zones within the lake differ primarily in the activities permitted within them and the extent of human-induced pressures. The lake receives inflow through the agriculturally influenced area and discharges outflow through the recreational area.

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**Figure 12:** Thematic map showing the geographic location of the Viss oxbow lake in Hungary. (Created by the author, 2023)

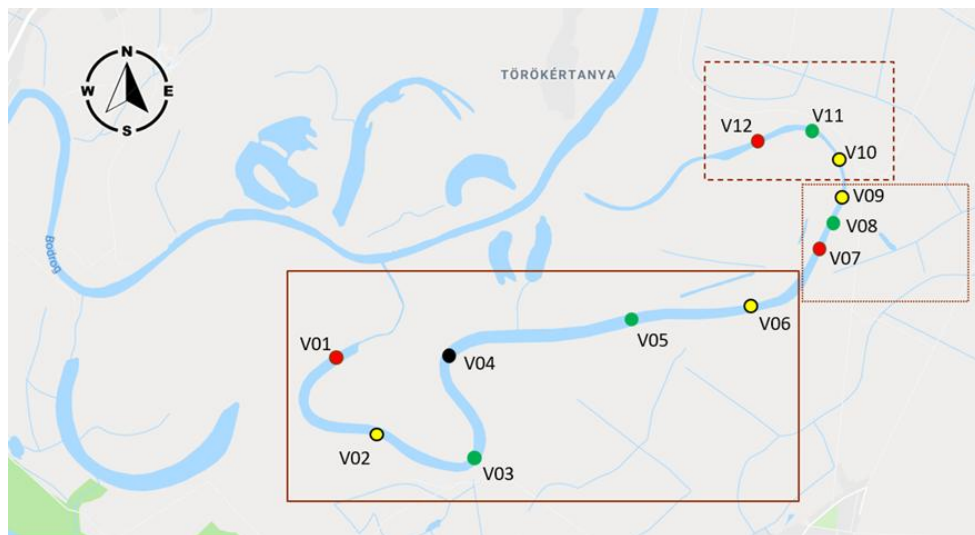
The first section is designated for recreational use, primarily focused on fishing, though other activities such as sightseeing, tourism, and kayaking also take place (represented by a solid line in Figure 1). The second section is significantly impacted by agricultural runoff (dotted line) and includes an inlet channel from surrounding arable lands as well as an outlet for irrigation purposes. The third section is a protected natural area (dashed line), where all human activities are restricted, except those aimed at biodiversity

#### 4. Results

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enhancement and conservation efforts.

Twelve soft sediment samples and twelve filtered water samples were collected during the summer vegetation period of 2019. The locations of the sampling sites, along with their respective utilization types and vegetation coverage, are shown in Figure 1. Each sampling site was georeferenced using a GPS device (Garmin eTrex).



**Figure 12:** The experimental lake with indication of the utilizations and sampling sites with the main type of vegetation coverage. Solid outer line – recreational area, dotted line – agriculturally influenced area, dashed line – protected area. (Created by the author, 2023)

During our study, we collected twelve samples each from contemporary and subfossil Cladocera assemblages across three distinct sections of the lake, reflecting different patterns of utilization. Samples V01–V06 were collected from the recreationally utilized area, which constitutes over half of the lake's

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studied region, which accounts for more than half of the lake's total studied region. Samples V07–V09 were obtained from the agriculturally impacted area, while samples V10–V12 were gathered from the protected section of the lake. The sampling strategy encompassed the variability of habitats, including open water sites, areas with submergent or emergent vegetation, and locations with floating-leaved vegetation.

##### **4.2.2. Contemporary Cladocera taxa and individual findings**

Filtered water sampling revealed ten species of Cladocera belonging to three taxonomic families: Bosminidae, Chydoridae, and Daphnidae. Among these, nine species were identified in the recreational area, seven in the agriculturally influenced area, and eight in the protected area of the lake.

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**Table 4:** Presents a comprehensive overview of the sampling sites utilized in the analysis of contemporary Cladocera populations. This table shows the attributes of each sampling site: Taxa, Individuals, Dominance, Simpson and Shannon diversity indices. Written with blue colour V01 – V06 are sampling points from recreational area, with red colour – agriculturally influenced area sampling points and with green colour – protected area sampling points. (Author’s data, 2024)

	<b>CONTEMPORARY</b>				
<i>SAMPLES</i>	Taxa	Individuals	Dominance	Simpson	Shannon
V01	5	22	0.3595	0.6405	1.271
V02	7	38	0.295	0.705	1.57
V03	4	24	0.5104	0.4896	0.8817
V04	8	42	0.2007	0.7993	1.802
V05	2	14	0.8673	0.1327	0.2573
V06	6	33	0.3811	0.6189	1.292
V07	7	63	0.3384	0.6616	1.413
V08	3	3	0.333	0.6667	1.099
V09	1	1	1	0	0
V10	5	18	0.5432	0.4568	0.9609
V11	7	53.5	0.224	0.776	1.655
V12	5	20	0.65	0.35	0.7777

Notably, *Bosmina longirostris* was present in all samples, while *Alona guttata*, *Alona intermedia*, and *Simocephalus mucronata* were found exclusively in a single sample out of the twelve collected.

The data of contemporary Cladocera species, shown in Table 4, indicate

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that the protected area of Viss oxbow lake had the highest average number of taxa and individuals in the assemblage, highlighting its suitability for sustaining healthy Cladocera populations. The agricultural-influenced area, however, exhibited the highest average dominance of Cladocera, followed by the protected and recreational zones. In terms of diversity, the recreational area recorded the highest average Simpson diversity index, with the protected area coming second, while the agricultural-influenced zone had the lowest value. Similarly, the Shannon diversity index was highest in the recreational area, followed by the protected area, and lowest in the agricultural-influenced area. These results reflect a clear difference in Cladocera dominance and diversity along the gradient of environmental zones within Viss oxbow lake.

##### **4.2.3. Subfossil Cladocera taxa and individual findings**

From the twelve subfossil Cladocera sampling sites within the Viss oxbow lake, a total of 35 distinct Cladocera species were identified, representing five taxonomic families: Bosminidae, Chydoridae, Daphnidae, Leptodoridae, and Sididae. Of these, 29 species were recorded in the recreational area, 23 in the agriculturally influenced area, and 26 in the protected area.

Three species - *Bosmina coregoni*, *Bosmina longirostris*, and *Chydorus sphaericus* - were consistently present across all twelve sampling sites. In contrast, species such as *Alonella exigua*, *Camptocercus rectirostris*,

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*Disparalona rostrata*, *Kurzia lattissima*, *Leptodora kindti*, *Oxyurella tenuicaudis*, *Paralona pigra*, *Pleuroxus truncatus*, and *Sida crystallina* were each observed in only one of the twelve collected samples.

**Table 5:** Presents a comprehensive overview of the sampling sites utilized in the analysis of subfossil Cladocera populations. This table shows the attributes of each sampling site: Taxa, Individuals, Dominance, Simpson and Shannon diversity indices. Underlined with blue colour V01 – V06 are sampling points from recreational area, with red underline – agriculturally influenced area sampling points and with green underlined – protected area sampling points. (Author’s data, 2024).

SAMPLES	SUBFOSSIL				
	Taxa	Individuals	Dominance	Simpson	Shannon
<u>V01</u>	15	1360	0.3409	0.6591	1.497
<u>V02</u>	16	683	0.2997	0.7003	1.847
<u>V03</u>	10	2617	0.4722	0.5278	1.023
<u>V04</u>	24	1244	0.1488	0.8512	2.41
<u>V05</u>	11	4574	0.5035	0.4965	0.9779
<u>V06</u>	14	5980	0.5442	0.4558	1.097
<u>V07</u>	11	4709	0.6421	0.3579	0.841
<u>V08</u>	20	2317	0.313	0.687	1.782
<u>V09</u>	15	5825	0.4266	0.5734	1.341
<u>V10</u>	16	3483	0.3352	0.6648	1.629
<u>V11</u>	21	2200	0.2534	0.7466	2.012
<u>V12</u>	14	2867	0.3337	0.6663	1.545

The data of subfossil Cladocera, presented in Table 5, reveal clear

#### 4. Results

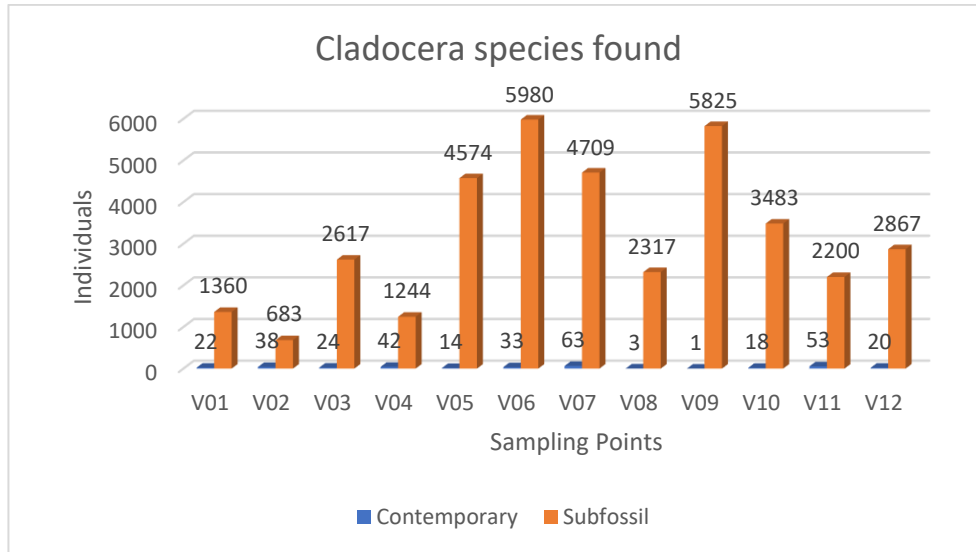
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differences in taxa richness, individual abundance, dominance, and diversity across various zones of Viss oxbow lake. The protected area had the highest mean taxa count, surpassing the agriculturally influenced zone, while the recreational area had the lowest taxa number. In terms of individual abundance, the agricultural zone showed the highest average, followed by the protected zone, with the recreational zone having the fewest individuals. The agricultural zone also exhibited the highest average dominance, while the recreational zone was intermediate, and the protected area showed the lowest dominance. In terms of diversity, the recreational zone had the highest average Simpson diversity index, followed by the protected zone, and the agricultural zone showed the lowest. Meanwhile, the Shannon diversity index was highest in the protected zone, followed by the agricultural zone, and the recreational zone had the lowest average.

#### **4.2.4. Comparison of species between contemporary and subfossil Cladocera sampling methods**

When comparing the contemporary and subfossil sampling methods, it is evident that subfossil samples consistently provided a higher number of taxa and individuals, with the difference being significantly larger than in the contemporary samples. In contrast, dominance was higher in the contemporary samples. Additionally, the subfossil samples from Viss oxbow lake exhibited higher Simpson and Shannon diversity indices.

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**Figure 4:** Comparison of individuals in subfossil and contemporary samples. (Author's data, 2024)

This bar chart shows the abundance of Cladocera species (y-axis, measured in individuals) across different sampling points (x-axis) for two datasets: Contemporary (blue bars) and subfossil (orange bars).

General observations: subfossil samples (orange) consistently exhibit a much higher number of individuals across all sampling points compared to contemporary samples (blue). Contemporary samples show relatively low and consistent abundances across all points, while subfossil samples have significant variation in abundance.

**Subfossil** (orange): The highest abundance is observed at V06 (5,980 individuals) and V09 (5,825 individuals). Other peaks include V05 (4,574),

#### 4. Results

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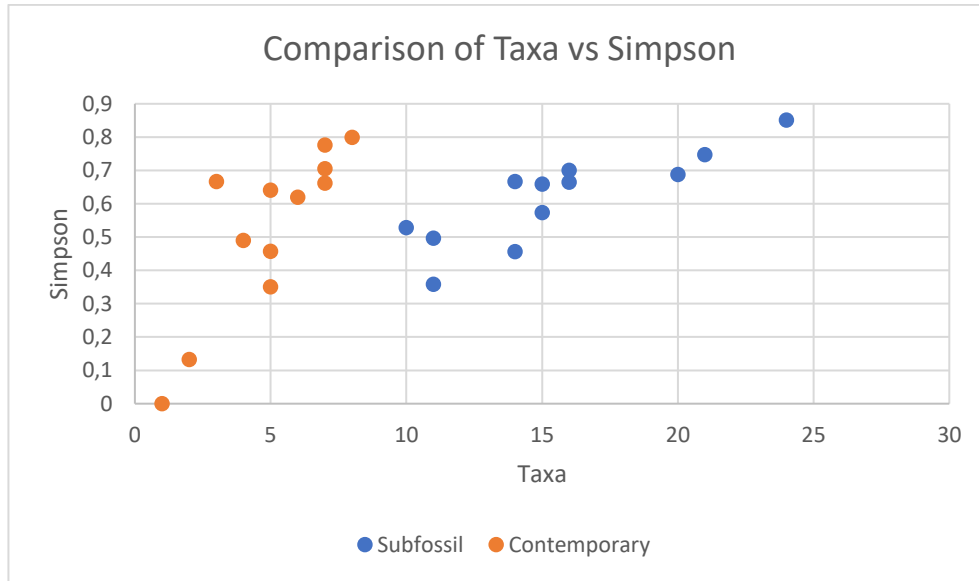
V07 (4,709), and V12 (2,867). Sampling points like V03 and V04 show lower abundances (1,244 and 14 individuals, respectively).

**Contemporary (blue):** The abundance of individuals remains very low across all sampling points, ranging from 3 to 68 individuals. The highest count is at V02 (68 individuals), but it is still far below subfossil levels. Sampling points like V03, V07, and V12 have minimal representation (24, 3, and 20 individuals, respectively).

**Subfossil vs. contemporary:** Subfossil abundances are significantly higher than contemporary abundances at all sampling points. This suggests that historical populations of Cladocera were much larger than modern populations, indicating possible environmental or ecological changes over time.

**Variability:** Subfossil data shows substantial variability across sampling points, while contemporary data is relatively uniform and consistently low.

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**Figure 13:** Comparison of taxa from contemporary and subfossil Cladocera samples with Simpson diversity index. (Author's data, 2024).

This graph compares taxa (x-axis) to Simpson (y-axis), with two datasets: subfossil (blue points) and contemporary (orange points) which represents sampling methods.

General trends: Simpson values (indicating evenness and diversity) increase with the number of taxa for both subfossil and contemporary datasets. The range of diversity values (Simpson) differs between the datasets.

**Subfossil** (blue): Taxa values range from about 10 to 25. Simpson values range from around 0.3 to almost 0.9, indicating higher diversity and evenness as taxa increase. Subfossil data demonstrates a broader spread,

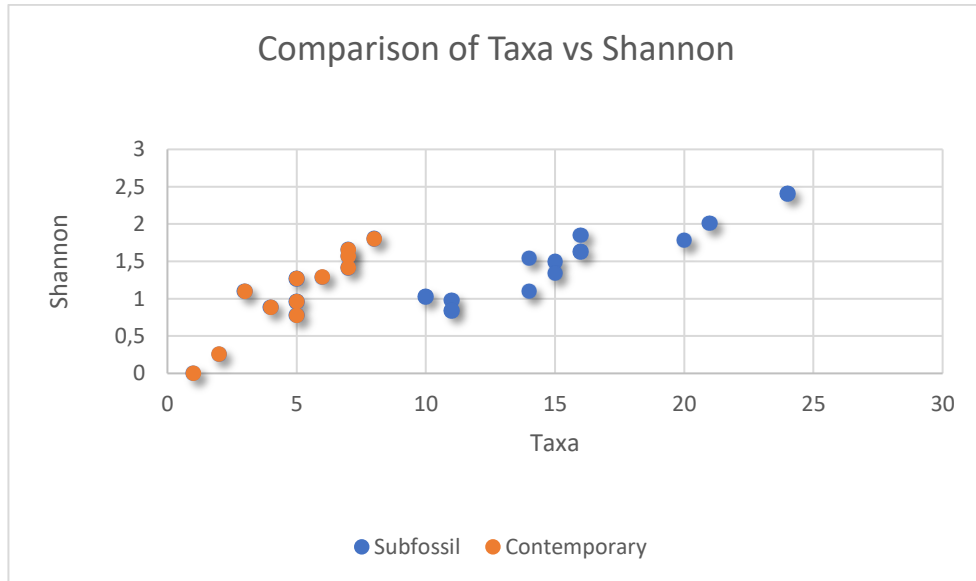
#### 4. Results

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reaching higher Simpson values as compared to contemporary data. Subfossil data shows both a wider range of taxa and higher diversity levels (Simpson) than contemporary data.

**Contemporary (orange):** Taxa values are clustered between 1 and 12, reflecting a lower range of taxa. Simpson values span from near 0.1 to about 0.8, with most values clustering below 0.7, indicating lower diversity compared to subfossil data. The diversity plateaus earlier, even at relatively low taxa levels. Contemporary samples suggest reduced diversity and evenness compared to subfossil, especially at higher taxa levels, pointing to potential changes in ecological conditions or community composition over time.

#### 4. Results



**Figure 146:** Comparison of taxa from contemporary and subfossil Cladocera samples with Shannon diversity index. (Author's data, 2024).

The graph compares taxa (x-axis) to Shannon (y-axis), showcasing two datasets: Subfossil (blue points) and contemporary (orange points) which represents sampling methods.

General trends: For both datasets, there is a positive relationship between taxa and Shannon - as the number of taxa increases, Shannon (a measure of diversity) also increases.

**Subfossil** (blue): Subfossil data spans a wider range of taxa values, from approximately 10 to 25. Shannon values range from around 0.8 to over 2.5, indicating higher diversity at higher taxa counts. Subfossil samples generally exhibit higher diversity (higher Shannon values) at comparable taxa

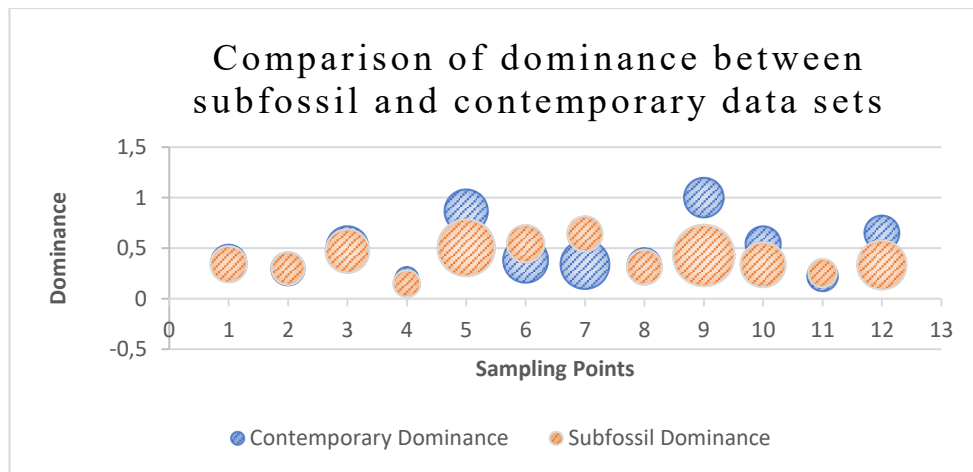
#### 4. Results

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levels.

**Contemporary** (orange): Contemporary data is concentrated in the lower range of taxa, from about 1 to 12. Shannon values range from 0 to around 2.0, suggesting lower diversity compared to subfossil data. Contemporary samples seem to cluster at lower taxa and diversity levels, indicating potential changes or reductions in diversity over time.

This may suggest a historical difference in biodiversity, with contemporary communities showing reduced diversity compared to subfossil records.



**Figure 17:** Comparison of dominance between contemporary and subfossil Cladocera samples. (Author's data, 2024).

The graph compares dominance between subfossil and contemporary datasets across different

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sampling points. Here's a description of the results:

**Axes:**

The x-axis represents sampling points (from 1 to 13).

The y-axis represents dominance values (ranging from 0 to 1.2).

**Data representation:**

Contemporary dominance: Represented by blue circles.

Subfossil dominance: Represented by orange hatched circles.

**Observations:** Dominance levels vary across sampling points for both datasets. In several instances, the blue and orange circles overlap, suggesting similar dominance values. However, there are also cases where one dataset clearly exhibits higher dominance. Sampling points like 4 and 9 show higher dominance values in the contemporary dataset compared to subfossil. Sampling points 5 and 8 display a larger spread in dominance for the subfossil dataset.

**Trends:** The dominance values fluctuate for both datasets, indicating no consistent pattern or trend across sampling points.

The bubble sizes may suggest differences in data weight or variability at each sampling point, though this requires clarification.

**Summary:** The graph highlights the differences and overlaps in dominance values between subfossil and contemporary datasets across sampling points, with variability in the extent of dominance for each dataset.

## 4. Results

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### 4.2.4.1. Other measured variables

Various variables were measured at both contemporary and subfossil sampling points, as well as within the respective samples, to obtain more precise data and gain a deeper understanding of the factors influencing species richness and the representation of different taxa at each sampling location.

**Table 6:** Measured variables on subfossil samples. Underlined with red colour V01 – V06 are sampling points from recreational area, with green underline – agriculturally influenced area sampling points and with green underlined – protected area sampling points. (Author's data, 2024).

	Dissolved oxygen saturation (% sat)	Conductivity ( $\mu\text{S}/\text{cm}^{-1}$ )	Chlorophyll-a ( $\mu\text{g. L}^{-1}$ )	Water Temperature ( $^{\circ}\text{C}$ )	LOI550 (%)	LOI950
<u>V01</u>	89.6	275.4	7.05	25.902	9.44	3.53
<u>V02</u>	87.2	275.1	7.07	25.702	10.56	3.12
<u>V03</u>	110.4	277.3	7.02	25.549	10.02	3.96
<u>V04</u>	94.2	271.8	7.81	24.925	17.26	2.45
<u>V05</u>	87.9	274.4	11.82	24.8	11.64	3.65
<u>V06</u>	67.1	275.3	9.39	24.57	14.3	2.95
<u>V07</u>	63.9	270.1	7.83	24.643	11.29	3.87
<u>V08</u>	47.3	257.4	9.73	21.488	12.89	3.95
<u>V09</u>	34.5	265.6	20.16	21.456	14.32	4.21
<u>V10</u>	35.4	263.8	5.87	21.789	15.51	3.71
<u>V11</u>	33.7	263.3	17.74	21.782	19.97	5.23
<u>V12</u>	36.4	262	5.43	21.852	11.09	4

Different variables were measured. In the subfossil samples, the highest average dissolved oxygen was recorded in the recreational area, where the

#### 4. Results

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highest average conductivity and temperature were also measured. The highest average concentration of chlorophyll-a was observed in the agriculturally influenced area, while the highest percentages of LOI550 and LOI950 were found in the protected area. In the contemporary samples, the highest averages of suspended solids and  $\text{NO}_3\text{-N}$  were measured in the protected area, whereas the highest total dissolved solids and dissolved oxygen values were recorded in the recreational area. The highest average concentrations of ortho-P,  $\text{NH}_4\text{-N}$ , and chlorophyll-a were found in the agriculturally influenced area. Concentrations of  $\text{NO}_2\text{-N}$ ,  $\text{SO}_4$ ,  $\text{Cl}^-$ , and total alkalinity showed relatively similar values across all sites.

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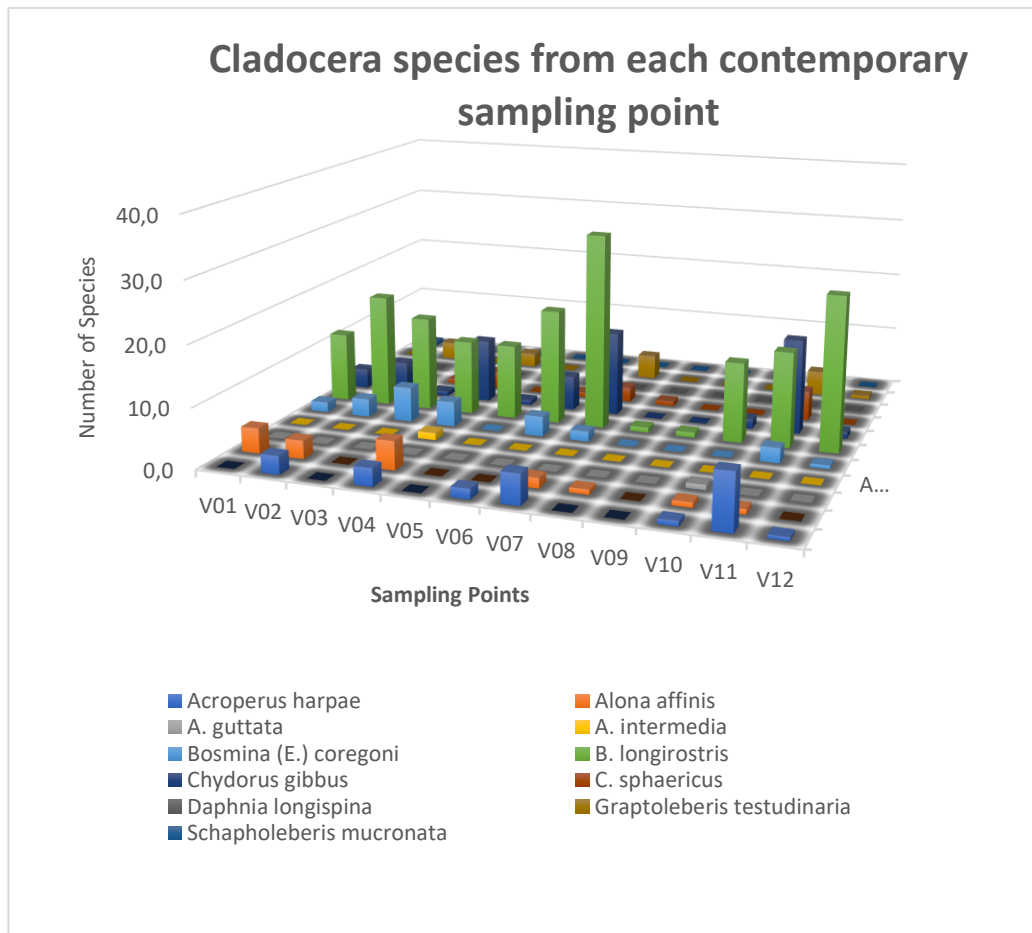
**Table 7:** Measured variables on contemporary samples. Underlined blue colour V01 – V06 are sampling points from recreational area, with red underline – agriculturally influenced area sampling points and with green underlined – protected area sampling points. (Author’s data, 2024).

	SS (suspended solids) mg. L <sup>-1</sup>	TDS (total dissolved solids) mg. L <sup>-1</sup>	Orto-P (mg. L <sup>-1</sup> )	NO <sub>3</sub> -N (mg. L <sup>-1</sup> )	NO <sub>2</sub> -N (µg. L <sup>-1</sup> )	NH <sub>4</sub> -N (µgm. L <sup>-1</sup> )	SO <sub>4</sub> (µgm. L <sup>-1</sup> )	Cl <sup>-</sup> (mg. L <sup>-1</sup> )	Chlorophyll -a (µg. L <sup>-1</sup> )	Total alkalinity	Dissolved oxygen (mg. L <sup>-1</sup> )
<u>V01</u>	8	260	0.06395	0.52313	0.01307	0.15976	10.833	2.262	7.05	2.979	6.78
<u>V02</u>	7	210	0.05938	0.53484	0.05884	0.13979	26.666	2.639	7.07	2.979	7.11
<u>V03</u>	14	225	0.05786	0.70271	0.11659	0.13180	15	3.77	7.02	2.979	9.02
<u>V04</u>	8	225	0.05786	0.59340	0.02070	0.19970	19.166	3.016	7.81	2.979	7.79
<u>V05</u>	4	145	0.04415	0.50361	0.00871	0.11715	10	3.958	11.82	2.979	7.29
<u>V06</u>	7	165	0.06852	0.37478	0.0457	0.16242	9.166	3.581	9.39	2.979	5.59
<u>V07</u>	3	115	0.09136	0.39430	0.00762	0.27159	8.333	4.335	7.83	2.979	5.32
<u>V08</u>	2	165	0.43854	0.65586	0.02288	0.18106	10	5.089	9.73	2.979	4.17
<u>V09</u>	0	255	0.18729	0.45286	0.02288	0.25029	15	2.262	20.16	2.979	3.04
<u>V10</u>	23	220	0.11724	0.73785	0.03595	0.17307	16.666	4.901	5.87	2.979	3.1
<u>V11</u>	19	215	0.23906	0.55436	0.01634	0.35280	25.833	3.393	17.74	2.979	2.96
<u>V12</u>	3	175	0.05938	0.70662	0.00980	0.11582	13.333	4.335	5.43	2.979	3.19

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### 4.2.5. Identified Cladocera species

#### 4.2.5.1. Cladocera species from contemporary sampling method



**Figure 18:** Cladocera species from each contemporary sampling point. (Author's data, 2024).

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The number of Cladocera species at each sampling point varied between the two sampling methods, with different species occurring in varying abundances across the different utilization areas. In contemporary sampling, both the number of species and their abundance were lower.

The stacked bar chart displays the relative abundance of Cladocera species across various contemporary sampling points (V01 to V12). Each bar represents a sampling point, while the different coloured segments indicate the percentage contribution of each species.

**Dominance of species:** *Bosmina longirostris* (green) is the most dominant species across all sampling points, contributing significantly to the total composition. Other species, such as *Chydorus gibbus* (blue) and *Daphnia longispina* (gray), are also prevalent but vary in abundance among sampling points.

**Sampling point variability:** Sampling points V02 and V01 show a more balanced distribution of species compared to others where *B. longirostris* is overwhelmingly dominant. Points such as V08 and V09 exhibit a more pronounced contribution from other species like *Alona affinis* (orange) and *Acroperus harpae* (dark blue).

**Species with minimal representation:** *A. guttata* (light gray) and *Graptoleberis testudinaria* (brown) have minimal representation across the sampling points.

This analysis highlights significant variations in species composition,

#### 4. Results

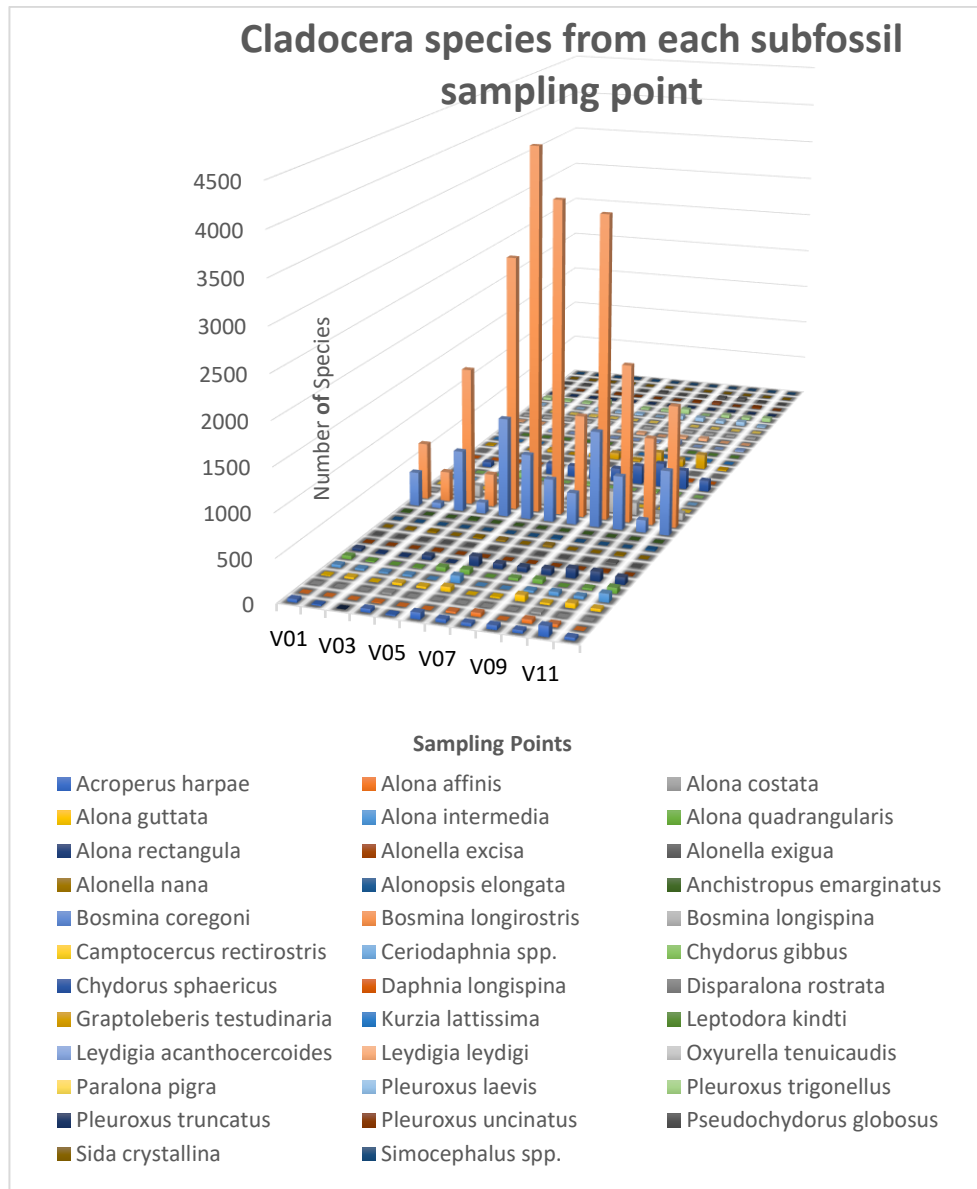
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suggesting diverse ecological conditions at each sampling point. The dominance of *Bosmina longirostris* could indicate environmental factors favouring its prevalence.

##### **4.2.5.2. Cladocera species from subfossil sampling method**

The subfossil sampling method revealed a higher number of species and greater abundance compared to the contemporary sampling method.

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**Figure 19:** Cladocera species from each subfossil sampling point. (Author's data, 2024).

#### 4. Results

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The stacked bar chart illustrates the relative abundance of Cladocera species across various subfossil sampling points (V01 to V12). Each bar represents a specific sampling point, and the coloured segments indicate the percentage contribution of different species.

**Dominant species:** *Alona affinis* (orange) consistently dominates most sampling points, contributing significantly to the overall composition.

*Bosmina longirostris* (brown) and *Chydorus sphaericus* (blue) also show substantial representation, particularly in points V04, V06, and V10.

**Species richness:** There is a notable diversity of species, with smaller contributions from taxa like *Alonella nana*, *Sida crystallina*, and *Daphnia longispina* across several points. The highest species diversity is observed in V06 and V11, where several taxa contribute to the composition.

**Sampling point variability:** Some sampling points (e.g., V02, V03, and V05) are dominated almost entirely by one or two species, suggesting less diversity. In contrast, points such as V06 and V11 exhibit more balanced distributions among several species, indicating more diverse ecological conditions.

**Rare species:** Species like *Leydigia acanthocercoides*, *Disparalona rostrata*, and *Pseudocydorus globosus* are represented in minimal percentages, indicating rare occurrences.

**Interpretation:** The dominance of *Alona affinis* across most points suggests it thrives under the prevailing environmental conditions of the

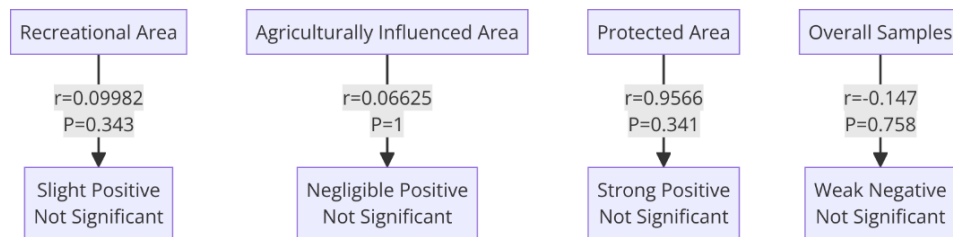
## 4. Results

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studied locations. Variations in species diversity and composition across points may reflect differences in ecological factors such as water chemistry, sediment characteristics, or historical environmental changes.

### 4.2.6. Mantel test between contemporary and subfossil Cladocera groups

The Mantel test was employed to analyse the correlation between contemporary and subfossil Cladocera groups within the Viss oxbow lake, focusing on distinct utilization zones. Separate Mantel tests were performed for the recreational, agricultural, and protected areas. Additionally, an overarching Mantel test was conducted for the entire lake to evaluate the overall relationship between the two Cladocera sampling groups.



**Figure 20:** Depicts the correlation between contemporary and subfossil Cladocera assemblages across the three distinct utilization zones of the lake—recreational, agricultural, and protected—and provides an overall comparison of the two sampling methods. (Created by the author, 2024)

The Mantel test results, depicted in the accompanying diagram, provide insights into the correlation between contemporary and subfossil Cladocera

#### 4. Results

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assemblages within each specific zone and across the entire Viss oxbow lake. These findings contribute to understanding the relationship and distribution patterns of these groups in various lake sections.

For the recreational area, the Mantel test produced an r-value of 0.099, indicating a minimal positive correlation, though this was not statistically significant, as evidenced by a p-value of 0.343. Similarly, the agriculturally influenced area demonstrated a negligible positive correlation, with an r-value of 0.066 and a p-value of 1, suggesting no statistically significant difference. Conversely, the protected area showed a strong positive relationship, with an r-value of 0.956, yet the correlation was not statistically significant due to a p-value of 0.341.

When analyzing all sampling sites collectively for both contemporary and subfossil Cladocera groups, the Mantel test revealed a weak negative correlation, with an r-value of -0.147. Nonetheless, the corresponding p-value of 0.758 indicates that this correlation is not statistically significant at the 0.05 level. These Mantel test findings provide a nuanced perspective on the relationships between contemporary and subfossil Cladocera in the Viss oxbow lake. They underscore the complexity and variability of these associations across different zones within the lake.

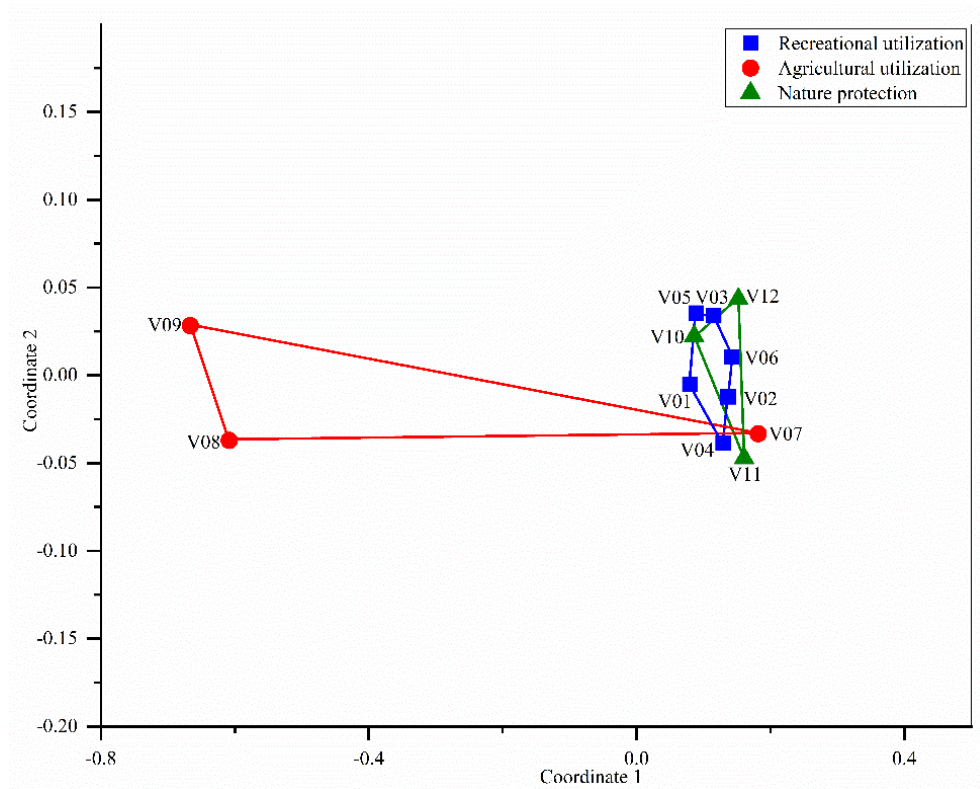
#### **4.2.7. Non-metric multidimensional scaling (NMDS) analysis of Cladocera species across different sampling methods**

An NMDS analysis was performed to visualize and interpret the ecological differences and similarities among Cladocera species sampled from three distinct zones of the Viss oxbow lake. The analysis yielded a stress value of 0.030, indicating that the NMDS plot provides a reliable representation of the species composition similarities and differences across the different utilization zones for each sampling method. Additionally, the resulting plots clearly highlight the variations in Cladocera species distribution across the lake's distinct zones.

##### **4.2.7.1. NMDS analysis for contemporary Cladocera sampling**

Analysis using non-metric multidimensional scaling (NMDS), which graphically illustrates the differences and similarities between the three different regions of Viss oxbow lake.

## 4. Results



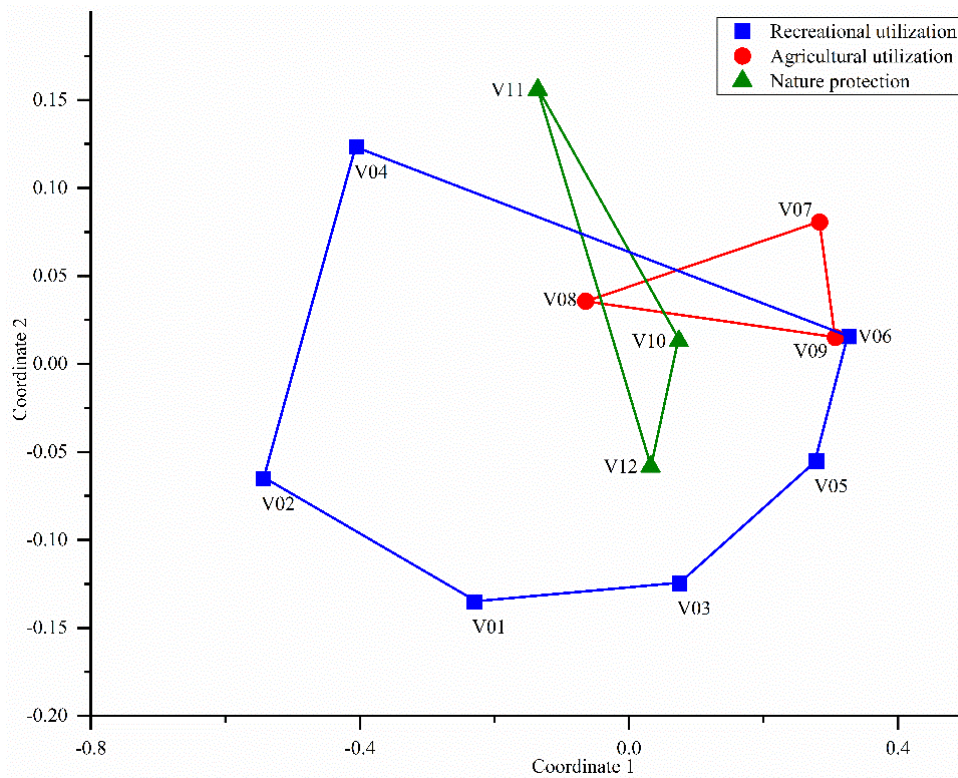
**Figure 21:** NMDS analysis, utilizing the Bray-Curtis dissimilarity index, was conducted for contemporary species of Cladocera. V01-V06 with blue colour- recreational area, V07-V09 with red colour – agriculturally influenced area, V10-V12 with green colour – protected area. (Author’s data, 2023)

In the contemporary samples, notable diversity exists across the three utilization zones of the lake. Sampling sites 8 and 9 exhibit the most significant differences, indicating distinct ecological characteristics or environmental factors influencing these areas. This variation highlights the

## 4. Results

heterogeneity of the Cladocera community across the lake's zones under current conditions (Fig.3).

### 4.2.7.2. NMDS analysis for subfossil Cladocera sampling



**Figure22:** NMDS analysis, utilizing the Bray-Curtis dissimilarity index, was conducted for subfossil species of Cladocera. V01-V06 with blue colour- recreational area, V07-V09 with red colour – agriculturally influenced area, V10-V12 with green colour – protected area. (Author’s data, 2023).

## 4. Results

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The subfossil samples display a more uniform pattern, with only minor deviations observed at sampling sites 1, 2, and 4. This indicates that historical Cladocera assemblages were more consistent across the lake or that the variations preserved in the sediment samples are less distinct compared to those in contemporary samples (Fig.4).

### **4.2.8. Canonical correspondence analysis (CCA) of Cladocera species across sampling methods in relation to measured environmental parameters**

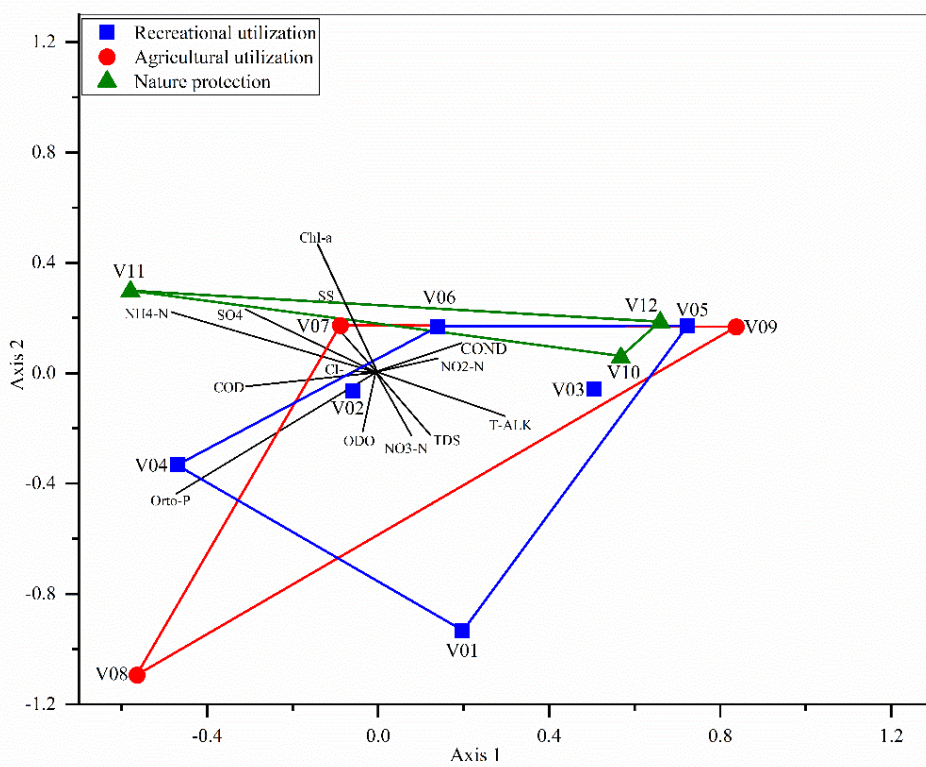
The CCA graphs illustrate the distribution of 12 sampling sites for both subfossil and contemporary Cladocera samples, alongside various measured environmental parameters. These visualizations highlight the distinctions among samples collected from the different utilization zones of Viss oxbow lake. Each point on the graph represents a specific sampling site, with its positioning reflecting the associated values of measured parameters and species abundance. The connections between points, distinguished by colours, denote the respective utilization zones of the lake.

#### **4.2.8.1. CCA analysis for contemporary sampling: Cladocera species and environmental variables**

Within the contemporary samples collected from the recreational area (V01-V06), notable variations in Cladocera communities were observed.

#### 4. Results

Sample V04, located in an emergent vegetation zone, exhibited the highest taxa richness and species count but the lowest dominance, largely influenced by elevated ortho-phosphate levels. In contrast, V02 and V06, associated with floating-leaved microvegetation, were positioned closely in the analysis.



**Figure 23:** CCA graph related to contemporary Cladocera Sampling from Viss oxbow lake, along with the measured variables: Cl-, Chl-a, COD, COND, NH<sub>4</sub>-N, NO<sub>2</sub>-N, NO<sub>3</sub>-N, ODO, Orto-P, T-ALK, TDS , SO<sub>4</sub>, SS. Areas: V01-V06 with blue colour- recreational area, V07-V09 with red colour – agriculturally influenced area, V10-V12 with green colour – protected area. (Author’s data, 2023).

#### 4. Results

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Sample V01, situated within a submergent vegetation zone, ranked third in both taxa richness and individual abundance, with slightly higher dominance values, primarily influenced by nitrate and total dissolved solids (TDS). Meanwhile, V03 and V05, collected from open water areas, were predominantly affected by nitrite-nitrogen, conductivity, and total alkalinity (Fig. 6).

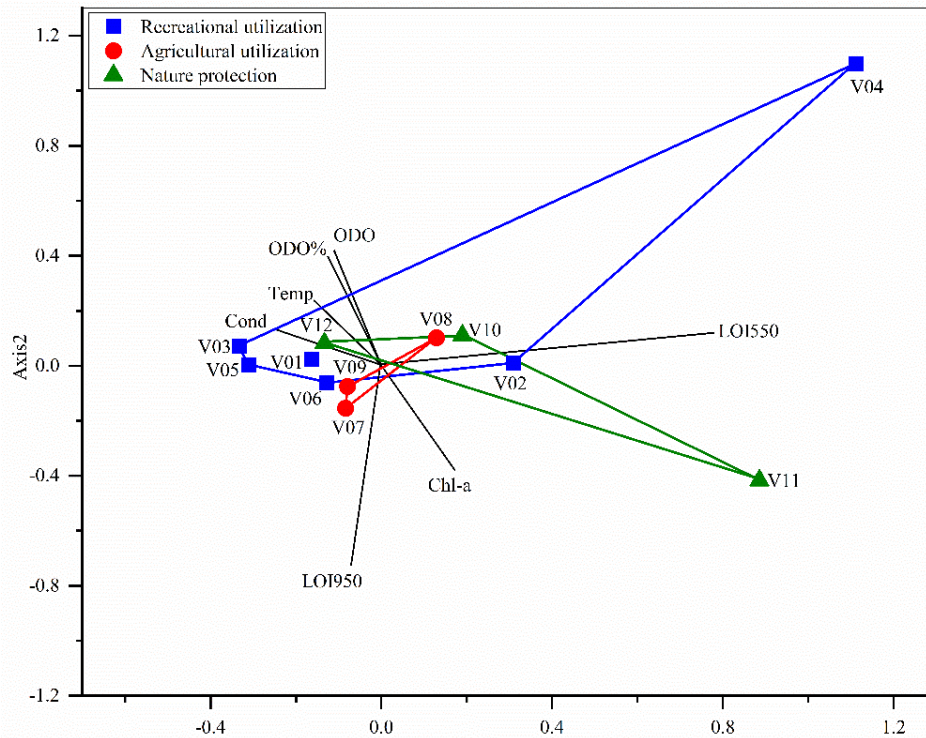
The agricultural sampling points (V07-V09) are widely dispersed on the graph, reflecting significant diversity in vegetation cover and notable variation in taxa and individual counts. Among these, V07 is distinguished by the highest taxa richness and individual abundance, coupled with minimal dominance, and is influenced by the majority of the measured variables. Following this, V08 is primarily influenced by orthophosphate levels, while V09 is characterized by the effects of nitrite-nitrogen and conductivity.

In the protected area, sample points (V10-V12) exhibit considerable spatial variation. Points V10 and V12 are closely aligned, influenced predominantly by nitrite-nitrogen and conductivity, whereas V11 occupies a distinct position on the graph, driven mainly by ammonium and sulphate levels. Each sampling point also demonstrates unique vegetation coverage, further emphasizing the ecological heterogeneity within the protected area's ecosystem (Fig. 6).

**4.2.8.2. CCA analysis for subfossil sampling: Cladocera species and environmental variables**

The investigation conducted for the recreational area V01-V06 concerning sampling sites cluster altogether except V04, which maintained its clear separation, implying demarcated contrasting environments. Among emergent vegetation, site V04 revealed the highest values of biodiversity as measured in taxa and individuals and the least dominance compared to any other sites in the recreational zone. Point V03 and V05 were sites of open water which are found very close to each other mainly influenced by conductivity.

## 4. Results



**Figure 24:** CCA graph focusing on the subfossil Cladocera sampling from Viss oxbow lake, alongside measured variables: Chl-a, Cond, LOI550, LOI950, ODO, ODO%, Temp. Areas: V01-V06 with blue colour- recreational area, V07-V09 with red colour – agriculturally influenced area, V10-V12 with green colour – protected area. (Author's data 2023)

However, contrary to that, points V02 and V06 could be associated with the same vegetation type, and however these points are far from each other in the graph. This indicates that there are various environmental influences.

When it came to the areas associated with the influence of agriculture (V07-V09), it appears that the sampling points feature high clustering; this

#### 4. Results

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may be indicative of environmental homogeneity and ecological uniformity as all the points point to the same extent being affected by the variables measured. Apart from this, within the conservation area (V10-V12), a special outlier is V11, which is located in open water: it presents the greatest diversities and dominance of taxa but the least number of individuals, whose general trend underlines the importance of habitat characteristics in biodiversity. The rest two sampling points in this protected area are not separated, despite variation in vegetation, signifying an intricate relationship between the type of habitat and ecological communities (Fig. 5).

## **5. DISCUSSION**

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In our initial study, we investigated whether chest waders might transport Cladocera species inadvertently. Thirteen lakes were sampled, representing different lake types generally found in eastern regions of Hungary. For each of the 13 lakes, three sampling repetitions were conducted to build a credible information. Altogether, across all the sampled lakes, 27 different species of Cladocera were found.

In our further research, we dealt with the different approaches for Cladocera species in a single lake with three differently utilized zones with varying degrees of anthropogenic impact.

Twelve sampling points were chosen to represent these zones based on their size. Both contemporary and subfossil Cladocera samples were collected, and different environmental variables were measured to gain a better understanding of their relationships with the distribution and diversity of Cladocera species.

### **5.1. Mediation of Cladocera species by researcher's chest wader**

Historically, the mediation of species occurred through various means, but in the past century, it has increased manifold owing to rise in human activities and transport (Carlton, 1999). Historically, the movement of species has been facilitated by factors such as the expansion of trade routes, colonial exploration, and the translocation of organisms for various purposes (Wilson

## 5. Discussion

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*et al.*, 2009). All biological invasions take place through four major stages: Transport, Introduction, Establishment, and Spread. This framework is common for human-mediated invasions (Blackburn *et al.*, 2011). This human-mediated movement of species across biogeographic boundaries, whether intentional or accidental, has dramatically reshaped the modern world (Kemp *et al.*, 2020). The mediation of Cladocera has occurred through a combination of human-mediated dispersal, biological dispersal, environmental factors, all of which have contributed to the distribution and dynamics of these small freshwater crustaceans. In limnology, sampling occasionally requires wading into the lake. This approach is necessary in situations where renting or accessing a boat is impractical, the water body is too shallow for boat use, or the lake is located in high-altitude terrain where transporting a boat is not feasible (Hajredini *et al.*, 2023). As scientists, walking through the lake to reach sampling points inevitably brings our chest waders into constant contact with various aquatic organisms, some of which may adhere to the waders and remain attached for a period. Valls *et al.* (2014) documented the attachment of various aquatic organisms to scientists' boots and their subsequent long-distance dispersal. They highlighted this phenomenon with the case of *Candona furtosae* a species native to Florida, which hatched in Spain from sediment transported by scientists attending an international biogeography meeting (Bisquert-Ribes *et al.*, 2023). In our study, the majority of the Cladocera we identified were remains, although we also observed ephippia and intact organisms. We examined three different

## 5. Discussion

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types of lake utilization: oxbow lakes, intensive fished lakes, and abandoned fishing lakes. Seven of the lakes—Tímári Holt-Tisza, Szögi Holt-Bodrog, Kis-Morotva-tó, Szabolcsi Holt-Tisza, Keleti Holt-Bodrog, Kerek-erdei-tó, and Szíki-tó—are natural water bodies, while the remaining lakes are artificial. Our objective was to determine whether the results varied depending on the lake's utilization type and to assess whether insufficient cleaning of chest waders between sampling sessions could compromise data quality. Additionally, we aimed to investigate whether scientists could inadvertently contribute to the dispersal of Cladocera between lakes. In 12 out of the 13 sampled lakes, we identified at least one species of Cladocera. However, in the single sample collected from Lake Kék-Víz, no Cladocera species were found. The primary reason for this absence is likely the lake's prolonged drying periods, combined with intensive fishing during times of abundant water. The composition and density of Cladocera populations are highly sensitive to shifts in environmental conditions and climate change (Leoni *et al.*, 2021; Liu *et al.*, 2021).

Oxbow lakes exhibited both high species diversity and a significant abundance of Cladocera. The high diversity and abundance of Cladocera observed in the oxbow lakes can be attributed to the favorable environmental conditions, such as stable water levels and low anthropogenic disturbance, which create a suitable habitat for these crustaceans to thrive (Tumurtoogoo *et al.*, 2022). Deserted fishing spots indeed exhibit a certain abundance of Cladocera. Because no fish introductions have been made and very little

## 5. Discussion

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anthropogenic activity has taken place in the last several years, the Cladocerans have been able to increase considerably. Many past studies have shown that zooplankton biomass tends to increase with fish biomass reduction (Christoffersen *et al.*, 1993; Korponai *et al.*, 1997; Sarvala *et al.*, 1997). Low abundance of Cladocera and species diversity is related to high anthropogenic activity with heavy usage in intensive fishing lakes along with constant introduction of fish. Boersma *et al.* (1991) emphasized the significant impact of fish on Cladocera distribution, while Berta *et al.* (2019) suggested that contemporary Cladocera species decline in abundance due to fish predation. However, lake KE stands out in this regard, as the lake had very high Cladocera abundance compared to the other intensive fishing lakes. Although it is an area of very intensive fishing, lake KE has had no introduction of fish into its waters, which is expected to be a contributing factor toward a higher population of Cladocera. In the 13 sampled lakes in eastern Hungary, we determined that there are 27 Cladocera species belonging to four families: Bosminidae, Chydoridae, Daphniidae, and Sididae. The majority were accounted for by the family Chydoridae with 22 species or 81.48% of the species, while Bosminidae made up 3 species or 11.11%. The families Daphniidae and Sididae were each represented by a single species (3.7%). Most of the identified species were associated with plant and vegetation habitats, followed by those linked to sediment habitats, while the smallest group consisted of Cladocera typically found in open water habitats. Hungarian water bodies are home to around 90 documented Cladocera

## 5. Discussion

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species (Hungarian Academy of Science and Experts Group, 1994), of which we identified 27 in our study, representing approximately 27.5% of the total. *Chydorus sphaericus* was the most abundant species across all three lake utilization types. This species is highly adaptable, inhabiting a wide range of water bodies and ecosystems. It is of very important quality in the fact that it survived under highly severe environmental conditions and overcame the pH range from 3.7 to 9.9 and conductivity varying between 257.4 and 277.3 mS/, including brackish waters (Langeland, 1972; Wærvågen, 1985; Walseng and Halvorsen, 2005; Jensen *et al.*, 2013).

On the contrary, the most recent recording for *Monospilus dispar* was a consistent single occurrence in one particular protected oxbow lake (Szögi Holt-Bodrog). This species occurs at elevations ranging from sea level to 663 meters above sea level and is typically absent from small ponds (less than 1 hectare) but present in 15% of lakes exceeding 1000 hectares. It is sensitive to acidic conditions and inhabits both vegetative and stony substrates. Its known habitats exhibit conductivity ranging from 1.5 to 53 mS/m<sup>-1</sup> (Wærvågen, 1985; Walseng, Halvorsen and Sloreid, 2001; Walseng and Halvorsen, 2005). If a sample containing *M. dispar* is collected from this protected lake and the same chest waders are then used in another lake with entirely different characteristics where *M. dispar* would not naturally occur, improper cleaning of the equipment could lead to the unintentional transfer of the species. This contamination could result in misleading data, as the species might be falsely recorded in an unsuitable habitat.

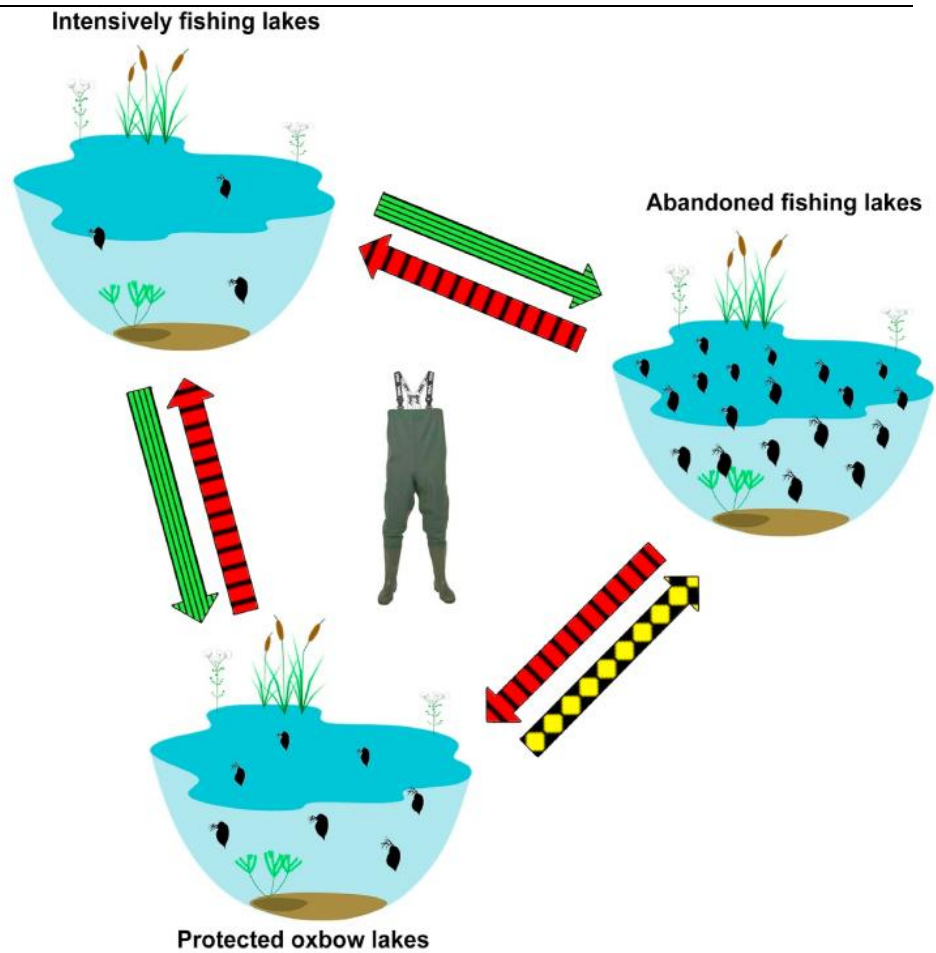
## 5. Discussion

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Thus, according to Simpson and Shannon diversity indices, the abandoned lakes have higher average diversity scores compared to oxbow lakes and the scores were the lowest for the much-fished lakes. The reason that abandoned fishing lakes are more diverse is because they have not been introduced with fish in the last several years and because of the lesseningreduced anthropogenic pressures. Predation tends to be the most critical factor affecting zooplankton communities, outweighing all other biotic and abiotic factors (Brucet *et al.*, 2010).

These results also indicate that Cladocera and other aquatic organisms can attach well to chest waders during sampling, which aligns with previous studies on aquatic invertebrate dispersal (Waterkeyn *et al.*, 2010; Valls *et al.*, 2016). When sampling several water bodies in the same day, each with different uses and levels of protection, large numbers of Cladocera are expected to attach onto chest wader after each lake visit. Without cleaning, the waders would therefore be a source of contaminating subsequent samples, possibly compromising data integrity and leading to different conclusions on species composition.

## 5. Discussion



**Figure 25:** Schematic graph of the investigated differently utilized lakes with indication of the possible mediations' routes. Arrows – possibility of species mediation. The red arrow - major. The green arrow - minor. The yellow arrow – moderate. (Created by the author, 2023)

**5.2. Comparative analysis of contemporary and subfossil Cladocera assemblages with respect to lake utilization and environmental factors**

Cladocera are small freshwater crustaceans, playing a very important role as indicators of aquatic ecosystem health and biodiversity (Karuthapandi and Rao, 2016). Most modern Cladocera nowadays are also considered in ecological and environmental studies as bioindicators for assessing changes in water quality, such as pollution status, nutrient availability, as well as other environmental stressors (Siciliano and Gesuele, 2013; Zawisza, Filbrandt-Czaja and Correa-Metrio, 2016b). They are essential in evaluating pollution, climate change, and other disturbances that take place within freshwater ecosystems.

On the contrary, subfossil Cladocera are those whose remains are preserved in sediment layers at the bottom, and they provide accurate information for the reconstruction of past environmental conditions. They determine changes in water temperature, nutrient levels, and other ecological parameters in the past (Korhola and Rautio, 2001). The use of modern and subfossil Cladocera assemblages is very crucial for understanding the system dynamics of freshwater ecosystems giving insights into their responses to environmental changes (Korhola and Rautio, 2001).

Researchers frequently make use of the concept of a comparison between subfossil Cladocera and their current contemporary sets to analyze preservation potential among different species. It has been found in studies,

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that there are differences in preservation levels among species. For example, *Daphnia*, *Diaphanosoma*, *Ceriodaphnia*, *Limnosida*, and *Leptodora* tend to be poorly represented in sediment samples due to their ecological abundance at present. In contrast, species like *Bosmina spp.* and *Chydorus spp.* are abundantly found in sediment deposits (Korhola and Rautio, 2001). This particular research looks at different sampling approaches between different use zones of an oxbow lake. By analyzing subfossil as well as modern-day Cladocera assemblages, this research reveals critical aspects concerning time-dependent shifts in species composition and abundance. This dual investigation, thus, enriches understanding of ecosystem alteration, making one cognizant of conservation options under anthropogenic activities and natural forces. The results thus indicate that one must apply different sampling methods in order to fully fathom biodiversity and ecosystem health. Our study showed a significant difference in the number of Cladocera species between contemporary and subfossil samples. This is consistent with the findings of Berta et al. (2019) and Tumurtogoo et al. (2022), who also reported significant differences in species numbers across different sampling methodologies. Such differences indicate the need for careful interpretation when comparing present and bygone assemblages.

Subfossil samples from the lake showed an extremely high number of Cladocera species compare with their presently existing counterparts in similar areas of the lake. In subfossil samples, species counts were seen to be about 100 times greater than those found in the contemporary samples.

## 5. Discussion

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Subfossil samples also yielded three times more species than contemporary ones, thereby making this variation in species richness and family representation between two time scales.

By this observation, very important conclusions can be drawn concerning the ecology in the past as well as the historical biodiversity of the lake's Cladocera populations. The prevalent crescent species, such as *Bosmina spp.* and *C. sphaericus*, throughout the three sites, against a relatively weak performance of larger Daphnids, suggest that predation pressure by fishes, particularly the smaller-bodied fishes, is responsible for the strongest plausible influence on the present zooplankton community composition. This is consistent with previous findings (Boersma, Van Densen and Vijverberg, 1991; Christoffersen *et al.*, 1993; Korponai *et al.*, 1997) that have recognized the effects of fish predation on zooplankton assemblages.

This is precisely the disparity that speaks volumes about species richness and family representation between the living and subfossil samples. With this representation, subfossil records, which many ecologists consider historic data, could have proved important in reconciling matters on the ecological dynamics and biodiversity changes that could have occurred in the lake ecosystem over time. The resultant data thus obtained can also make significant inputs in conservation and management efforts aimed at securing the aquatic biodiversity in this lake.

Fish predation has previously proved to be an important aspect in determining zooplankton populations, but this has also included other

## 5. Discussion

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important factors such as predators and macrophytes (Davidson *et al.*, 2007). These factors are likely to contribute to the patterns that shape zooplankton community dynamics in the lake. In addition, predation is in minds most authors as working with the strongest effect on zooplankton communities (Bruce *et al.*, 2010), a statement through which we find support with our observations. Thereby, predation by smaller fish would play critical role in inducing spatial differentiation of species in different regions of the lake with respect to composition and size distribution. The contrasting species richness and family representation between modern and subfossil samples is consistent with observations recorded in other studies, which have stressed the value of subfossils in palaeoecological research (Lotter *et al.*, 1997; Jeppesen *et al.*, 2003; Milecka, Kowalewski and Szeroczyńska, 2011). Such studies illustrate the ability of sediment layers to preserve an extensive range of species and reveal the changes that biodiversity and ecology underwent in the past. This study presents insights into the spatial distribution of Cladocera taxa richness, abundance, dominance, and diversity between several utilized zones of the Viss oxbow lake. There are serious implications for the management and conservation of aquatic ecosystems, especially regarding multiple human activities and their possible effects on water bodies (Borgwardt *et al.*, 2019).

None of the sampling methods currently can sample whole Cladocera populations (Bottrell, Duncan and Gliwicz, 1976). This reinforces the inherent difficulties in sampling Cladocera assemblages and the complex dynamics associated with aquatic ecosystems in capturing them. So, to

## 5. Discussion

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understand the populations and ecological roles of Cladocera, subfossils are great historical tools but will have to be complemented with present samples. There was a weak but negative Mantel correlation between the contemporary and subfossil sampling techniques, which was not significant. This result is further substantiated by the NMDS analysis, which showed that there is a distinct difference between contemporary and subfossil samples. NMDS also indicated variation across the three sampling areas of the lake (recreational, agriculturally influenced, and protected), which means demographic characteristics concerning the liberated environment were observed in each area. Several factors may have contributed to the differences above. Predation, one of the most influential aspects in shallow lakes such as the lake in the study, is again giving its part. It is well known, Timms and Moss (1984), that predation seems to affect the zooplankton community more than resource availability in such ecosystems, which indicates the impact of predator-prey interactions on the zooplankton community may be more important than mere presence of food sources.

Season, in addition to the previously cited factors, is a further relevant variable affecting the assemblages of Cladocera. According to Stansfield et al. (1997), the seasonal influence varies between pelagic and macrophyte-associated Cladocera populations. Seasonal changes lead to altered in the water conditions and food availability and change prey identity, thereby affecting Cladocera species distribution and abundance. The second objective of this study was to look at how different kinds of land use impact Cladocera

## 5. Discussion

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assemblages. This is critically important to the understanding of anthropogenic effects on aquatic ecosystems. The study seeks to show how recreation, agriculture, and conservation activities differ in their effects on the diversity and abundance of the Cladocera species. Identifying these impacts can inform management and conservation for the sustainability of the lake biodiversity in the face of a range of human activities. The above study shows how the composition of the contemporary and subfossil Cladocera assemblages depended on the different land uses within the water body. The specific observations with regard to the differences were in the numbers of taxa and individuals within protected sites, agriculturally impacted sites, and recreational areas. In consonance with the finding of Hajredini et al. (2023), protected oxbow lakes had higher numbers of Cladocera taxa. According to Christoffersen et al. (1993), this increase in biodiversity is attributed to minimal or complete absence of anthropogenic activities and reduced predatory fish, which leads to development of a favorable environment for Cladocera. Similar observation was made in this study in which the protected area harbored more diverse Cladocera taxa. These findings highlight the importance of considering the impacts of different land use practices on aquatic ecosystems, particularly in terms of their effects on the diversity and abundance of Cladocera communities. This information can inform management strategies and conservation efforts to maintain the ecological balance and biodiversity of the lake system.

Of all the aforementioned arguments, the most compelling is that the

## 5. Discussion

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variations in assemblage structures for Cladocera up to now between the current and subfossil samples is explained by the presence of *B. longirostris*, a well-known resilient and tolerant species to varying environment conditions (Adamczuk, 2016). Such a species, therefore, provides prominence to the area around the lake when viewed under the aspect of the very stark agricultural influences that involve the complex interplay among the ecological factors which shape community structures.

Increased nutrient loads from heavy organic inputs and intense fishing usually lead to a reduction in the number of species and individuals, as documented by Jeppesen et al. (2004) and Berta et al. (2019). This is in line with the present study for the current Cladocera community. In contrast the subfossil assemblage showed the maximum number of individuals compared to a considerably few number of species taxa was recorded. This may probably be caused by some Cladocera species such as *B. longirostris* dominating the assemblage. As Adamczuk (2016) revealed, even though small-sized species such as *B. longirostris* do not play a major role in energy transfer within the food web, they can thrive quite well in changed environmental conditions.

Gleason et al. (2003) also mention that agricultural practices influence aquatic invertebrate communities of Cladocera and others by reducing the number of ephippia (as well as other eggs of invertebrates), thereby causing a detrimental effect on overall biodiversity. This research sites the recreational area as apparently an 'interchange area' between lake areas that

## 5. Discussion

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the agriculture influences and those protected, following what has been presented by Berta et al. (2019). The intermediate status of the recreational area, observed in the contemporary Cladocera assemblage, does not hold true for the number of individuals in the subfossil Cladocera assemblage. This suggests a more complex ecological dynamic at play. The study demonstrates the differences in Cladocera assemblages between contemporary and subfossil samples. There is a pronounced disparity in both the number of taxa and the abundance of individuals found in Cladocera samples from contemporary and subfossil sources. Across the various utilization types within the lake, it was consistently observed that subfossil samples exhibited a considerably higher number of Cladocera species compared to contemporary samples, often more than doubling in species richness. This notable discrepancy highlights the critical need for careful selection of sampling methods in ecological studies, ensuring they align with the specific research objectives. Such an approach is essential for accurately interpreting the ecological status and historical changes within aquatic ecosystems.

The disparity in sampling methods emphasizes the importance of taking multiple approaches when assessing biodiversity and ecosystem health, as each method provides unique insights into the aquatic environment's condition. The differentiation between contemporary and subfossil Cladocera assemblages underscores the ongoing interactions between human activities and the ecological dynamics of Cladocera, providing valuable insights into the complex relationships that influence lacustrine ecosystems. These

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findings underscore the need for a comprehensive and multifaceted approach to understanding the ecological dynamics and historical changes within aquatic environments. By integrating contemporary and subfossil data, researchers can gain a more holistic understanding of the complex interplay between human activities, environmental factors, and the resilience of Cladocera communities over time. This knowledge can inform more effective management strategies and conservation efforts to preserve the biodiversity and ecological integrity of these valuable aquatic ecosystems.

## **6.SIGNIFICANT RESULTS OF THE STUDY**

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### **6.1. Mediation of Cladocera species by researcher's chest wader**

a) *What is the potential for Cladocera species to be dispersed through scientists' chest waders during sampling procedures?*

Species of Cladocera have dispersal potential through the researcher's chest waders when sampling. As the scientists practically go into water body with waders, these species such as Cladocera, will attach to the surface and remain there for a certain period. Long-distance dispersal of aquatic organisms by sediments transported with scientists' equipment has been documented in studies such as those of (Valls *et al.*, 2016).

Our findings also revealed a considerable number of Cladocera sticking to chest waders during sampling further substantiating the probabilities of unintentional transfer of species between waterbodies. It is worth mentioning that utilization and water body type also affect the ability and the number of Cladocera species that can and will be dispersed.

b) *How do different types of lake utilization affect the extent of Cladocera dispersal facilitated by chest waders?*

The type of lake utilization lowers or increases the chest waders Cladocera dispersal ability. Intensive fished lakes, too much anthropogenic use, would probably not have many Cladocera attach due to their number being lower from fish predation effects and environmental disturbances. Conversely, abandoned fishing lakes and oxbow lakes with much less human impact with stable environmental conditions have got high abundance and

## 6. Significant results of the study

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diversity of Cladocera making it more probable to pick species up and disperse them by means of chest waders. Hence, lakes with higher diversity and abundance of Cladocera would be more susceptible to unintentional dispersal of these species through sampling activities.

c) *What impact does inadequate cleaning of chest waders prior to sampling have on the accuracy and integrity of collected data?*

Fail to clean chest waders before sampling may compromise data accuracy and integrity. If these are not then cleaned between use at two different types of lakes, the Cladocera or some other aquatic organism can be transferred to another site, which would introduce it into the area. For example, this would false - overestimate the species richness and modified community composition in lakes sampled.

For example, the transfer of *Monospilus dispar* from a protected lake accidentally might end up in completely false data regarding the species in one locality as opposed to another. Hence, thorough cleaning before sampling proves necessary for the validity and reliability of ecological studies. Waterkeyn et al. (2010); Valls et al. (2016) present evidence regarding the roles that poor cleaning practices play in the inadvertent dispersal of aquatic invertebrates.

### **6.2. Comparative analysis of contemporary and subfossil Cladocera assemblages with respect to lake utilization and environmental factors**

a) *How do contemporary and subfossil Cladocera assemblages differ in composition across distinct utilization zones within a multi-utilized lake?*

## 6. Significant results of the study

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Cladocera composition of contemporary vs subfossil assemblages in Viss oxbow lake varied considerably within distinct utilized zones. Subfossil samples always displayed higher species richness compared to contemporary samples. For example, subfossil assemblages manifested a species count nearly 100 times more than contemporary samples having a diversity around three times higher. Such differences represent the distribution of species remains over a very long time in the sediments, preserving all the historical biodiversity since the contemporary samples do not show it due to environmental pressures and other anthropogenic stressors.

Small-sized species such as *Bosmina spp.* and *Chydorus sphaericus* dominated both contemporary and subfossil assemblages, especially in those zones experiencing increased anthropogenic activities. Fewer larger Daphnids could be found, possibly due to fish predation as the cause explored by studies such as (Boersma, Van Densen and Vijverberg, 1991). These differences between contemporary and subfossil Cladocera are informative about ecological dynamics and impacts of lake utilization during history on community structure.

b) *To what extent do different types of lake utilization influence the composition of Cladocera assemblages?*

The use of Viss oxbow lake significantly alters the diversity of Cladocera assemblages to the extent that, among its three different utilization areas, the area with little human activities is reported to be the highest in species diversity owing to stable environmental conditions and low predation pressure. However, decreasing species richness was observed from the moderate to the high-utilization zones, with the highly utilized area being greatly impacted by heavy fishing, habitat alterations, and fish predation.

## 6. Significant results of the study

Such a gradient sufficiently substantiates the far-reaching impact of lake use on Cladocera assemblages and underscores the necessity for balanced management preservation to ensure the biodiversity and integrity of ecosystems.

All in all, the different types of utilization by humans for lakes will ultimately determine the way the assemblages will be composed in Cladocera-influence factors concerning the degree of anthropogenic disturbance and exposure to predation by fishes.

## 7. NEW SCIENTIFIC FINDINGS

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### **Dispersal potential of Cladocera via chest-waders**

Scientists' chest waders can act as vector for Cladocera dispersal, especially to very biodiverse lakes with a strong call for strict cleaning protocols concerning the use of chest waders to avert cross-lake dispersal.

### **Lake utilization and Cladocera composition**

These lakes tenants are very much so intensely affected by the activities of man and the presence of fish stocking, where abandoned fishing lakes hold a greater treasure of Cladocera than over-fished lakes.

### **Contemporary vs. subfossil Cladocera discrepancy**

In contrast to contemporary samples, subfossil Cladocera assemblages were reported to be exceedingly species rich offering insight into the biodiversity that once existed in the past and the change in ecosystems over time.

### **Predation as a key driver of Cladocera distribution**

Small species of Cladocera tend to dominate highly fished lakes due to predation pressure, while large Daphnids seem to predominate in the more undisturbed environments like abandoned fishing lakes and oxbows.

## **8. SUMMARY**

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### **Introduction**

Cladocera, small freshwater crustaceans, serve as bioindicators for assessing aquatic ecosystem health and environmental changes. Two complementary studies were conducted to investigate the dispersal potential of Cladocera through scientists' chest waders during sampling and to analyze differences in contemporary and subfossil Cladocera assemblages across varying lake utilization types. These studies provide valuable insights into species dispersal dynamics, anthropogenic influences, and historical biodiversity patterns in freshwater ecosystems.

### **Study 1: Mediation of Cladocera species by researcher's chest wader**

The first study fundamentally explored the chances of Cladocera species being carried through the sampling by the chest waders of scientists. Thirteen lakes in Eastern Hungary were sampled natural and artificial water bodies, used for various types. Oxbow lakes, abandoned fishing lakes, and intensive fishing lakes were sampled with the objective of evaluating the possibility of Cladocera dispersal because of improper washing of these chest waders causing cross-contaminated samples, and consequently, false data.

Among the major findings were:

## 8. Summary

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Dispersal potential: Cladocera and other water organisms could attach to chest waders during sampling and without subsequent proper cleaning may be transferred to other lakes, introducing them into unsuitable habitats and biasing data.

Effect of lake usage: Abandoned fishing lakes and oxbow lakes were found to hold more Cladocera diversity than intensive fishing lakes. The lower diversity in intensively fished lakes is attributed to predation by fishes and anthropogenic stresses.

Case in point: The protected *Macrothrix dispar* was found in one oxbow lake but could be brought to other lakes by inadvertent movement, thereby generating erroneous data without a proper cleaning protocol.

### **Study 2: Comparative analysis of contemporary and subfossil Cladocera assemblages with respect to lake utilization and environmental factors**

The second study compared contemporary and subfossil Cladocera assemblages in Viss oxbow lake concerning various utilization zones. Areas of anthropogenic disturbance were sampled to gauge the temporal dynamics of species composition regarding environmental and anthropogenic factors affecting assemblages.

Some of the key findings included:

Subfossil assemblages showed much greater species richness: Subfossil assemblages were significantly richer in species diversity, having nearly 100

## 8. Summary

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times more individuals and 3 times greater species diverse than contemporary samples. This feature demonstrates the great value of subfossils for historical reconstructions of biodiversity and ecosystem conditions.

Dominance of some small sized species: Contemporary samples were dominated by small species such as *Bosmina spp.* and *Chydorus sphaericus*, when all sites were considered, in relation to fish predation pressure, particularly in heavily used lakes.

Temporal and spatial dynamics: The differences in species composition between contemporary and subfossil assemblages could point the finger at the effect of long- and short-term anthropogenic influences on Cladocera.

### **Broader implications**

Cleanup of sampling equipment to prevent species dispersal is as crucial as data integrity. It points to the influence of human activities on lake utilization that has been mentioned; abandoned and protected lakes have been associated with a higher diversity of Cladocera, as a result of reduced anthropogenic pressure. The divergence between contemporary and subfossil assemblage gives one important aspect of historical ecosystem changes, proving the effectiveness of the coupled method in ecologic research.

### **Conclusion**

Again, the most important observation here would relate to the human activity-sampling practice link and aquatic biodiversity. The study would show that very careful cleaning is required during sampling, and conservation practices will be much more effective if targeted at understanding and

## 8. Summary

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mitigation of anthropogenic pressure effects on freshwater ecosystems. In addition, it would be beneficial if dispersal studies could be incorporated within assemblage comparisons with a view to advancing understanding and maintaining aquatic biodiversity in the context of environmental change.

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## 11. Appendix

# 11. APPENDIX

**Appendix 1:** This table presents a detailed inventory of Cladocera species identified from contemporary samples collected at various sampling points within Viss oxbow lake. (Author's data, 2023).

Contemporary Cladocera species abundance ind. L <sup>-1</sup>	V01	V02	V03	V04	V05	V06	V07	V08	V09	V10	V11	V12
<i>Acroporus harpae</i> (Baird, 1835)	0	3	0	3	0	1.7	5	0	0	0.8	9.2	0.6
<i>Alona affinis</i> (Leydig, 1860)	4.2	3	0	4.8	0	0	1.7	0.8	0	0.8	0.8	0
<i>Alona guttata</i> (Sars, 1862)	0	0	0	0	0	0	0	0	0	0.8	0	0
<i>Alona intermedia</i> (Sars, 1862)	0	0	0	1.2	0	0	0	0	0	0	0	0
<i>Bosmina (E.) coregoni</i> (Baird, 1857)	1.7	3	5.8	4.2	0	3.3	1.7	0	0	0	2.5	0.6
<i>Bosmina longirostris</i> (O.F. Müller, 1785)	11.7	18.9	15.8	12.5	12.5	19.2	32.5	0.8	0.8	13.3	15.8	25.7
<i>Chydorus gibbus</i> (Sars, 1890)	3.3	5.3	0.8	10.7	0.8	5.8	14.2	0	0	1.7	15.8	1.1
<i>Chydorus sphaericus</i> (O.F. Müller, 1785)	0	1.5	0.8	3	0	0.8	2.5	0.8	0	0	5	0
<i>Daphnia longispina</i> (O. F. Müller, 1776)	0	0	0	0	0	0	0	0	0	0	0	0
<i>Graptoleberis testudinaria</i> (Fischer, 1848)	0	3	0	2.4	0	1.7	4.2	0	0	0	4.2	0.6
<i>Schapholeberis mucronata</i> (O.F. Müller, 1776)	0.8	0	0	0	0	0	0	0	0	0	0	0

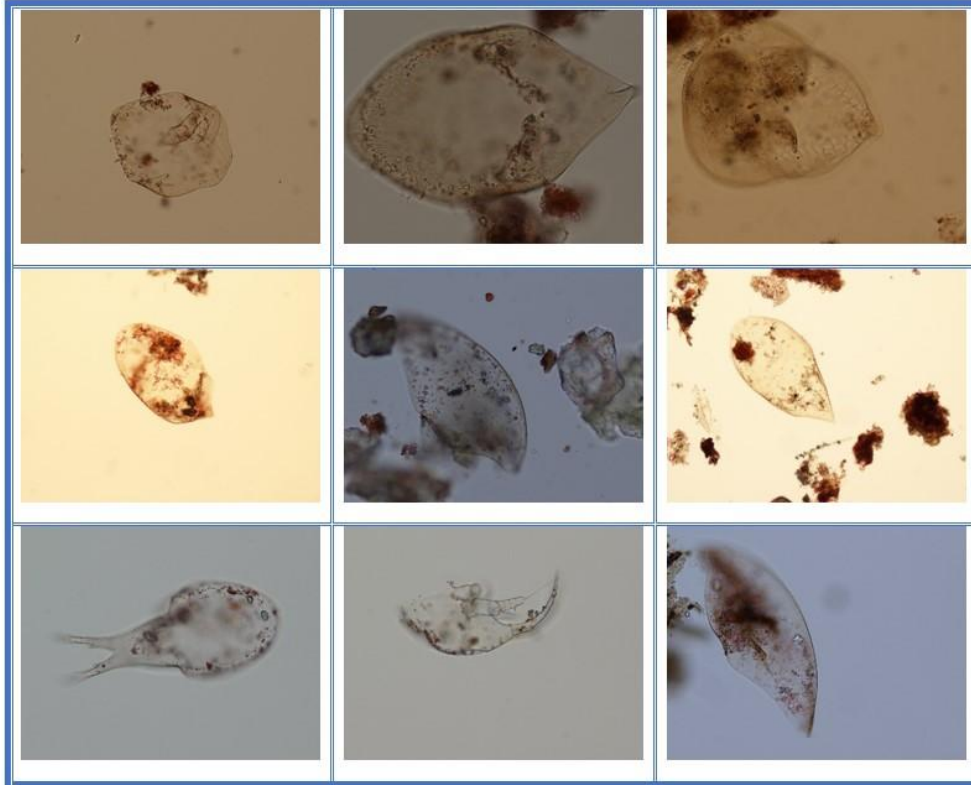
## 11. Appendix

**Appendix 2:** This table presents a detailed inventory of Cladocera species identified from subfossil samples collected at various sampling points within Viss oxbow lake. (Author's data, 2023).

Subfossil Cladocera species abundances incl. <sup>1</sup> 1000 l <sup>-1</sup>	V 01	V 02	V 03	V 04	V 05	V 06	V 07	V 08	V 09	V 10	V 11	V 12
<i>Acroporus harpae</i> (Baird, 1835)	38	14	0	42	15	77	42	38	50	36	12	33
<i>Aiona affinis</i> (Leydig, 1860)	0	0	0	7	0	0	21	45	0	45	34	0
<i>Aiona costata</i> (G.O. Sars, 1862)	0	0	0	15	0	0	0	0	0	23	0	0
<i>Aiona guttata</i> (Sars, 1862)	8	21	0	29	25	56	0	15	75	23	57	33
<i>Aiona intermedia</i> (Sars, 1862)	32	21	17	22	0	84	0	0	25	45	34	10
<i>Aiona quadrangularis</i> (O.F. Müller, 1776)	40	13	0	7	49	56	0	30	50	0	23	67
<i>Aionella excisa</i> (Fischer, 1854)	0	0	0	6	0	0	0	0	0	0	10	0
<i>Aionella exigua</i> (Lilljeborg, 1853)	0	0	0	0	0	0	0	0	0	0	20	0
<i>Aionella nama</i> (Baird, 1843)	0	7	17	0	15	19	0	25	0	0	10	0
<i>Aionopsis elongata</i> (G.O. Sars, 1862)	0	0	0	0	0	0	0	13	0	0	0	17
<i>Anchistropus emarginatus</i> (G.O. Sars, 1862)	6	0	0	12	0	0	0	0	0	0	0	0
<i>Bosmina (E.) coregoni</i> (Baird, 1857)	40	63	72	13	11	77	50	37	11	63	14	76
<i>Bosmina (E.) longispina</i> (Levins, 1860)	5	6	3	76	5	9	6	39	9	8	7	0
<i>Bosmina (E.) longispina</i> (Levins, 1860)	0	29	13	66	98	1	28	12	25	14	4	83
<i>Bosmina longirostris</i> (O.F. Müller, 1783)	67	35	16	39	30	43	37	12	36	18	10	14
<i>Camptocercus rectirostris</i> (Schödler, 1862)	6	8	39	0	20	30	33	17	08	77	39	50
<i>Ceriodanobos</i> spp. (Dana, 1853)	0	0	0	0	0	0	0	13	0	0	0	0
<i>Chydorus gibbus</i> (G.O. Sars, 1891)	0	0	0	65	0	0	21	0	0	18	10	0
<i>Chydorus sphaericus</i> (O.F. Müller, 1776)	56	61	33	6	2	5	7	3	5	6	0	3
<i>Coscinella rectangularis</i> (G.O. Sars, 1862)	32	9	8	51	0	2	63	60	75	4	11	83
<i>Daphnia longispina</i> (O.F. Müller, 1776)	0	0	8	0	0	0	0	0	0	0	10	0
<i>Diaperiona rostrata</i> (Koch, 1841)	0	0	0	0	0	0	0	13	0	0	0	0
<i>Graptoleberis testudinaria</i> (Fischer, 1851)	6	29	0	36	0	38	83	25	0	89	18	17
<i>Kozzia latissima</i> (Kurz, 1875)	0	0	0	0	0	0	0	0	0	0	10	0
<i>Leptodora kindtii</i> (Focke, 1844)	0	0	0	0	0	0	0	13	0	0	0	0
<i>Leydigia acanthocercoides</i> (Fischer, 1854)	6	4	17	0	0	0	0	13	50	0	0	17
<i>Leydigia guddigi</i> (Schödler, 1863)	6	4	0	12	0	0	21	0	25	36	0	0
<i>Orqureilla tenuicornis</i> (G.O. Sars, 1862)	0	0	0	0	15	0	0	0	0	0	0	0
<i>Paralona pigra</i> (G.O. Sars, 1862)	0	0	0	6	0	0	0	0	0	0	0	0
<i>Peucedanella truncata</i> (O.F. Müller, 1785)	13	0	0	0	0	0	0	0	0	0	0	0
<i>Picripleuoxus loxii</i> (G.O. Sars, 1861)	6	18	0	30	15	19	0	50	50	36	50	17
<i>Pleuoxus trigonellus</i> (O.F. Müller, 1776)	31	18	17	65	15	19	21	63	0	36	30	50
<i>Pleuoxus uncinatus</i> (Baird, 1850)	0	0	0	12	0	0	0	13	25	0	20	0
<i>Pseudocyclops globosus</i> (Baird, 1843)	0	14	0	12	0	19	0	13	25	36	10	0
<i>Stala costalis</i> (O.F. Müller, 1776)	0	0	0	6	0	0	0	0	0	0	0	0
<i>Simoccephalus</i> spp. (Schödler, 1858)	0	0	0	6	0	0	0	0	0	0	0	0

## 11. Appendix

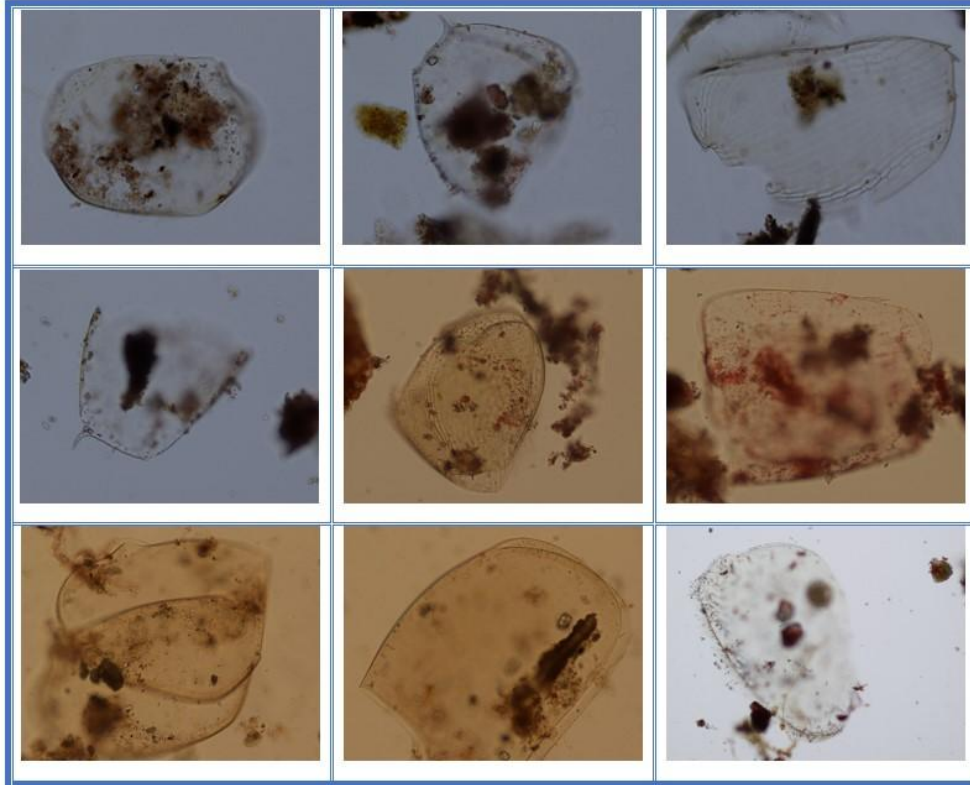
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**Appendix 3:** This appendix illustrates a selection of headshields from Cladocera species identified during the research. (Author's data, 2021); 1. *Alona guttata* (Sars, 1862); 2. *Alonella excisa* (Fischer, 1854); 3. *Graptoleberis testudinaria* (Fischer, 1851); 4. *Chydorus sphaericus* (O.F. Müller, 1776); 5. *Camptocercus rectirostris* (Schödler, 1862); 6. *Chydorus gibbus* (Sars, 1890); 7. *Bosmina longirostris* (O.F. Müller, 1785); 8. *Bosmina* (*E.*) *longispina* (Leydig, 1860); 9. *Acroperus harpae* (Baird, 1835).

## 11. Appendix

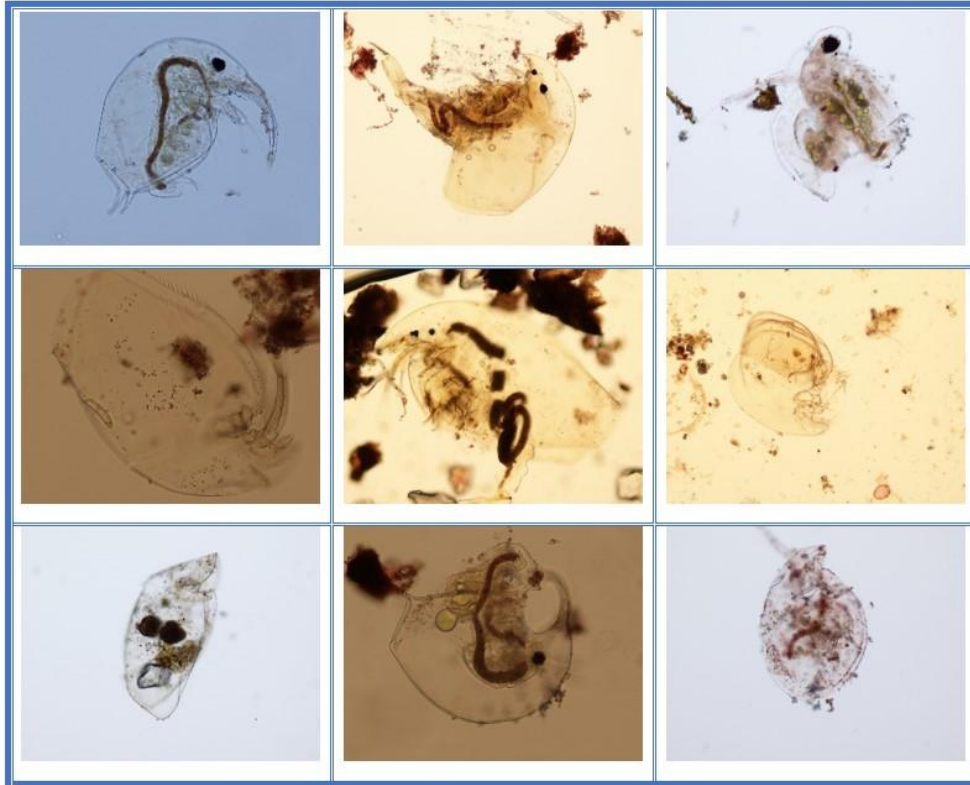
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**Appendix 4:** This appendix illustrates a selection of shells from Cladocera species identified during the research. (Author's data, 2021); 1. *Chydorus sphaericus* (O.F. Müller, 1776); 2. *Bosmina longirostris* (O.F. Müller, 1785); 3. *Graptoleberis testudinaria* (Fischer, 1851); 4. *Bosmina (E.) longispina* (Leydig, 1860); 5. *Alonella exigua* (Lilljeborg, 1853); 6. *Alona intermedia* (Sars, 1862); 7. *Chydorus gibbus* (G. O. Sars, 1891); 8. *Oxyurella tenuicaudis* (G. O. Sars, 1862); 9. *Pleuroxus uncinatus* (Baird, 1850).

## 11. Appendix

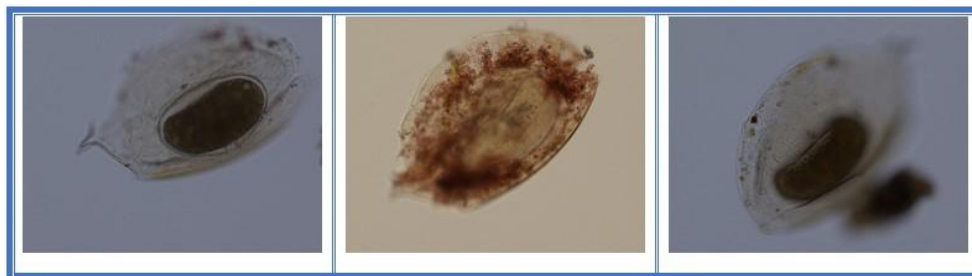
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**Appendix 5:** This appendix illustrates Cladocera species identified during the research. (Author's data, 2021); 1. *Bosmina longirostris* (O.F. Müller, 1785); 2. *Acroperus harpae* (Baird, 1835); 3. *Diaphanosoma mongolianum* (Ueno, 1938); 4. *Alona rectangula* (G.O. Sars, 1862); 5. *Camptocercus rectirostris* (Schödler, 1862); 6. *Chydorus spp.*; 7. *Graptoleberis testudinaria* (Fischer, 1851); 8. *Bosmina longirostris* (O.F. Müller, 1785); 9. *Monospilus dispar* (Sars 1862).

## 11. Appendix

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**Appendix 6:** This appendix illustrates Cladocera species ephippium identified during the research. (Author's data, 2021); 1. *Bosmina longirostris* (O.F. Müller, 1785); 2. *Bosmina (E.) coregoni* (Baird, 1857); 3. *Bosmina longirostris* (O.F. Müller, 1785).

## 12. PUBLICATIONS

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Candidate: Arber Hajredini  
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MTMT ID: 10098533

### List of publications related to the dissertation

#### Foreign language scientific articles in international journals (2)

1. Gyulai, I., **Hajredini, A.**, Varga, K., Jakab, J., Vallejo-Cuzco, G., Somlyai, I., Grigorszky, I., Berta, C.: Comparative analysis of contemporary and subfossil Cladocera assemblages with respect to lake utilisation and environmental factors.  
*Aquat. Sci.* 87 (1), 1-14, 2025. ISSN: 1015-1621.  
DOI: <http://dx.doi.org/10.1007/s00027-024-01146-y>  
IF: 2 (2023)
2. **Hajredini, A.**, Demelezi, F., Somlyai, I., Grigorszky, I., Berta, C.: Possible mediation of Cladocera species by a researcher's chest wader.  
*Heliyon*. 9 (6), 1-10, 2023. ISSN: 2405-8440.  
DOI: <http://dx.doi.org/10.1016/j.heliyon.2023.e16725>  
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#### Foreign language scientific articles in international journals (1)

3. Grigorszky, I., Kiss, K. T., Szabó, L. J., Dévai, G., Nagy, S. A., Somlyai, I., Berta, C., Gligora-Udovič, M., Borics, G., Pór, G., Yaqoob, M. M., **Hajredini, A.**, Tumurtogoo, U., Ács, É.: Drivers of the *Ceratium hirundinella* and *Microcystis aeruginosa* coexistence in a drinking water reservoir.  
*Limnetica*. 38 (1), 41-53, 2019. ISSN: 0213-8409.  
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**Total IF of journals (all publications): 6,318**

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12 June, 2025