



# **Community Response of Cladocera to Trophic Stress by Biomanipulation in a Shallow Oxbow Lake**

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Abstract: Studying contemporary and subfossil Cladocera (Crustacea) communities, we explored the effect of top-down stressors on the cladoceran communities; we are also interested in the coherence of the contemporary and subfossil communities. The studied Cibakháza oxbow lake is in E Hungary, on the left-floodplain of the River Tisza; it is a large, long, and shallow oxbow lake. Three areas of the oxbow lake were distinguished based on the strength of top-down stress: protected area with low top-down stress, biomanipulated area with high top-down stress, and recreational area with moderate top-down stress. Altogether, we identified 28 taxa in the contemporary and subfossil communities in the oxbow lake. We found that the species number of the contemporary Cladocera communities was lower (protected area: 13; biomanipulated area: 9, and recreational area: 14) than in the subfossil communities (protected area: 20; biomanipulated area: 16, and recreational area: 14). Among the environmental variables, we observed differences between the protected and biomanipulated area, while the recreational area showed a transition. Species number, abundances, and Simpson diversity also showed the effect of the fish introduction. There were no differences in beta-diversity among the contemporary and subfossil Cladocera community. The non-metric multidimensional scaling (NMDS) ordination showed that the biomanipulated area in the case of the contemporary communities was separated from the other areas, while in the case of the subfossil communities, there was no separation according to top-down stress. Our results showed that the number of species of contemporary Cladocera communities was lower in each area (3–10; 3–9; 5–9) compared to the subfossil communities (6–17; 7–12; 8–12). However, the highest abundances were found in the biomanipulated area due to the appearance of small-sized Cladocera species. Our findings suggest that the effect of a short-time fish introduction is restorable when the oxbow lake has a protected part.

**Keywords:** zooplankton; diversity; biomanipulation; nature protection; anthropogenic impacts; restoration



### 1. Introduction

Over the whole world, many aquatic ecosystems have been affected by significant transformation. Numerous studies highlight the disturbance of freshwater systems as the effect of natural and anthropogenic pressure. Disturbance or human utilization of these ecosystems is a cause of general anxiety due to their importance in providing ecosystem services [1]. Shallow lakes can be regarded as biodiversity hotspots [2] in the context of the global prevalence of water insecurity [3]. In the last decades, numerous research works have focused on zooplankton dynamics in freshwater and marine ecosystems [4], emphasizing the importance of zooplankton species in the maintenance of food webs [5], serving as a food source for different aquatic animals [6]. These oxbow lakes are endowed with many ecosystem services and have a substantial impact on the ecology, biodiversity, and social-economy of the surrounding localities, which makes them a priority for research and conservation [7,8]. These environments are ecotones between lotic [9] and lentic [10] and have regular lateral water connectivity to the main river. This connection to the river distinctively supports a high diversity of habitats for both flora and fauna [11,12]. What is more, oxbow lakes create a multiplicity of habitats and feeding grounds for various organisms such as birds, fishes, amphibians, macroinvertebrates, phytoplankton, and zooplankton [13,14].

Cladocera is one of the most important component of microcrustacean zooplankton. Moreover, they are sensitive indicators of environmental changes [15]. They usually indicate early signals of ongoing changes because of their prompt response to environmental changes. These changes seem to be intensified in small waterbodies such as ponds and oxbow lakes due to their small area and low water volume. Hence, oxbow lakes are especially vulnerable to climate change and habitat degradation.

Cladocera play an essential role in freshwater systems since they are in a middle position in the freshwater trophic structure. The Cladocera community structure is highly determined by the food web structure and not greatly determined by hydrological factors [16] compared to other organism groups such as macroinvertebrates [17] and fishes [18], especially in such peculiar ecosystems as oxbow lakes.

Generally, the stressor factors of aquatic ecosystems can be divided into biotic and abiotic ones. Nutrient enrichment (eutrophication) in lakes increases the number of levels of trophic structures and also increases the zooplankton biomass [19–21]. However, low structured food webs can be found under the highest eutrophic conditions. In addition to nutrient supply, predators also effect zooplankton. For instance, size-selective feeding of planktivorous fishes has a great impact on the dynamics and structure of the zooplankton community in temperate lakes [22–24]. High pressure from predation leads to decreases in zooplankton abundances [25]; small-sized species appear; and the size of already existing individuals of the present community also declines [26,27]. Besides the feeding type composition of the fish community, juveniles have a greater effect on the zooplankton community, since all freshwater fish feed on zooplankton during the young of the year stage [27]. In extreme situations, the predation may cause the disappearance of the prey population [28]. In the case of Cladocera, the contribution of small taxa (e.g., *Bosmina longirostris*) increases [29] due to the top-down regulation of fish [30,31].

All in all, multiple environmental stressors influence the zooplankton community. Their effects usually overlap, and very often, it is impossible to deduce which biocenotic changes were triggered by a given a factor. To track the spatial distribution of Cladocera communities under a range of pressures in small aquatic ecosystems, an oxbow lake in Hungary was studied. The Cibakháza oxbow lake is on the left-bank floodplain of the River Tisza, in East Hungary. It is one of the largest oxbow lakes in Hungary (~16.2 km long) and was created by the straightening of the meander.

We analyzed the contemporary and subfossil Cladocera communities and their abiotic environmental parameters. In the present study, three main hypotheses were assumed:

(1) The separate analyses of the contemporary and subfossil Cladocera communities are not representative enough to specify the whole of the Cladocera communities.

- (2) Due to intensive fish introduction, the structure and quantitative relationship of the Cladocera community change in the biomanipulated area.
- (3) Due to the accumulation capacity of sediment, we may find more species in the subfossil community than in the contemporary community.

### 2. Materials and Methods

The studied oxbow lake is in East Hungary (Figure 1) on the left-bank floodplain of the River Tisza (latitude N 46°57′36.21″, longitude E 20°11′16.23″). It has a surface area of 1.4 km<sup>2</sup> (A = 1.4 km<sup>2</sup>) with a maximum depth of 6.22 m ( $d_{max} = 622$  cm) and an average depth of 2.65 m.



**Figure 1.** Location of the studied oxbow lake with the markings of lakes, rivers, settlements, and the border of Hungary.

The oxbow lake was divided into three separate areas to explore the effect of biomanipulation (Figure S1). These areas are separated from each other by embankments, with small channels that allow the water to flow through to each area. The protected area of the oxbow lake was created as a nature conservation area for birds and mammals and in this study was treated as the reference area for Cladocera communities. The biomanipulated area was adopted for angling with intensive fish introduction. This area is separated by  $5 \times 5$  cm plastic nets from the other two areas to prevent the migration of fish. They are fixed by wire to the bottom. The recreational area is used for recreation purposes, but there has been no fish introduction and/or other kinds of management to create a transition area next to the protected and biomanipulated areas.

There was a fish introduction into the biomanipulated area in spring of 4000 kg of juvenile carp; approximately 0.02 kg/individual and 10,000 juvenile pike. Most of the juvenile carp became prey for Copepods [32] and for the pike [33], which were introduced into the biomanipulated area at the same time. The remainder of the carp had size-selective feeding habits and mainly fed on small rotifers [32]. Simultaneously, a small-sized species of common carp (*Cyprinus carpio*)

(~0.1–0.12 kg/individual) was also introduced. However, they were eaten by larger predators within a short period [34]. Based on this information and the feeding strategies of fish, the 2550 kg of common carp (*C. carpio*) (1.5–2 kg/individual)—which were also introduced into the biomanipulated area at the same time—probably put predation pressure on the Cladocera community [35] in this part of the oxbow lake.

There were fifteen sampling sites; samples were collected during the summer period. Seven sites were in the protected area. Five sampling points were chosen from the biomanipulated area and three from the recreational areas. At each site, first the open water and submerged coverage were registered, by our own observation. Next, the plankton samples were collected using a Schindler-Patalas plankton trap [36] with a 35-µm mesh, to follow the contemporary Cladocera community. The basic variables of the oxbow lake were also measured. For this purpose, an YSI EXO2 (Yellow Springs Instrument Company, Yellow Springs, USA) (Xylem, 599502-01) multiparameter probe was used, and the following parameters were determined: water temperature, dissolved oxygen concentration, conductivity, and pH. The next steps included sediment and water sampling. Sediment samples were taken using a gravity-corer (Jackson, USA) (WaterMark<sup>®</sup>, diameter of 0.06 m) [37]. The topmost 1 cm of soft sediment was used for further investigation of subfossil Cladocera.

The laboratory measurements of water samples included chlorophyll-a content, suspended solids,  $PO_4$ , and dissolved inorganic nitrogen analysis. The chlorophyll-a was analyzed based on the EPA Method 445.0: in vitro determination of chlorophyll-a and pheophytin a in marine and freshwater algae by fluorescence. The  $PO_4$  was analyzed based on the EPA Method 365.3: phosphorous, all forms (colorimetric, ascorbic acid, two reagent). Suspended solids were analyzed based on the EPA Method 160.2 (gravimetric dried at 103–105 °C). Dissolved inorganic nitrogen was analyzed based on the EPA Method 1687 (total Kjeldahl nitrogen in water and biosolids by automated colorimetry with preliminary distillation/digestion).

The laboratory measurements of sediment samples included  $CaCO_3$  and loss of ignition analysis. The first step was the oven drying of the sediment (1 g). The second step included the heating of the samples in a muffle furnace at 550 °C, then calculated as the loss of ignition (heating time was four hours, heat retention time four hours). The third step included the re-heating of the samples in the furnace at 950 °C, then calculating the CaCO<sub>3</sub> (heating time was four hours, heat retention time two hours) [38].

## 2.1. Processing of Cladocera Samples

The first samples were filtered using a 35-µm sieve during the preparation of planktonic samples for the analysis of the contemporary Cladocera community, and then, samples were treated with 96% Patosolv alcohol (mixture of ethyl-alcohol and isopropanol) to better preserve individual specimens. A few drops of safranin-glycerin (adding a few drops of safranin dye to a small amount of glycerin) were added to simplify identification.

Sediment samples for subfossil Cladocera analysis were prepared according to the standard methodology of Korhola and Raution [39]. One cubic centimeter of sediment was treated with 100 mL of 10% KOH solution (10 g of KOH pellets dissolved in 100 mL of deionized water) in a plastic beaker (150 mL) heated for 30 min at 70 °C in a Stuart SWB6D laboratory water bath. Then, the samples were sieved using a 35- $\mu$ m sieve. The prepared samples were also preserved with 96% Patosolv alcohol, and a safranin-glycerin solution was also used. Identification of the species was conducted using an Olympus BX53 microscope with an Olympus DP26 fidelity digital camera. In the case of the contemporary and subfossil Cladocera communities, 100  $\mu$ L of sample/slide were used, and at least 200 individuals were counted. The contemporary and subfossil Cladocera communities were identified based on the classification schemes of Frey [40] and Szeroczyńska and Sarmaja-Korjonen [41] and based on Gulyás and Forró [42], respectively.

#### 2.2. Data Analysis

We partitioned the diversity of the oxbow lake for both contemporary and subfossil Cladocera communities using "adespatial" packages [43]. In the analysis, we used the so-called SDR-simplex analysis [44]. This technique was developed to partition gamma diversity into further components. This method evaluates species replacement (RepL), richness differences (RichDiff), and similarity (Sørensen similarity). Species replacement plus richness differences represented the beta-diversity; richness differences plus similarity reflected nestedness; and similarity plus species replacement reflected species richness agreement. To visualize the result, a simplex plot was drawn using R language [45]. PAST (PAleontological STatistics) software [46] was used to evaluate the Simpson diversity for both contemporary and subfossil Cladocera communities.

Non-metric multidimensional scaling (NMDS) was used to display the similarity of Cladocera communities. The Rogers–Tanimoto index was used to measure the similarity of the species composition, and the Bray–Curtis index was used to measure the dissimilarity of the abundance distribution of communities [47]. NMDS represents the pairwise dissimilarity between objects in a low-dimensional space (usually two-dimensional) preserving the ranks of pair-wise similarities and/or dissimilarities.

# 3. Results

### 3.1. Environmental Variables

The water depth of the oxbow lake surveyed ranged between 0.36 and 6.22 m, the shallower part located mainly in the protected area (Table 1). There were no considerable spatial variabilities in pH and water transparency. In general, oxbow lakes are characterized by weakly alkaline water with a slight fluctuation in pH (7.6–8.9), while the water transparency was rather low with a maximum of 0.77 m. High variations in conductivity, chlorophyll-a, and loss of ignition (LOI) were recorded. Conductivity ranged from 398  $\mu$ S cm<sup>-1</sup>—measured in the protected area (median 424  $\mu$ S cm<sup>-1</sup>)—to 1215  $\mu$ S cm<sup>-1</sup> in the recreational area (median 1180  $\mu$ S cm<sup>-1</sup>). The highest concentrations of the living algal biomass (chlorophyll-a) were determined in the protected area, although the highest average was noted in the recreational area. LOI followed the same trend (Table 1). The value of ortho-phosphate in the protected area reached 0.06 mg L<sup>-1</sup> (maximum value). This part of the oxbow lake was characterized by the highest submerged coverage.

Table 1.	The physical	and chemical	variables	measured ii	n the stud	ied oxbow	lake.	Abbreviations:
DIN * =	dissolved inor	ganic nitroger	n, SS ** = si	uspended sc	lids, LOI	*** = loss of	f igniti	on.

	Protected Area			Biomanipulated Area			Recreational Area		
	Min.	Max.	Med.	Min.	Max.	Med.	Min.	Max.	Med.
Conductivity ( $\mu$ S cm <sup>-1</sup> )	398	788	424	709	1018	829	1142	1215	1180
Depth (m)	0.49	4.74	0.77	0.76	3.62	1.15	0.36	6.22	1.76
$DIN^* (mg L^{-1})$	0.76	3.27	1.91	0.63	1.55	1.27	0.67	1.43	0.93
Dissolved oxygen (mg L <sup>-1</sup> )	1.27	9.84	4.36	7.1	10.7	8.09	3.62	9.25	6.84
Open water surface (%)	0	100	60	15	100	100	5	100	50
Ortho-phosphate (mg $L^{-1}$ )	0	0.60	0.05	0	0	0	0	0.29	0.01
рН	7.6	8.6	7.9	8.3	8.7	8.5	7.8	8.9	8.7
$SS^{**} (mg L^{-1})$	0.6	9.6	1.8	1.8	2.9	2.3	1.8	3.1	2.7
Transparency (m)	0.39	0.77	0.59	0.46	0.64	0.54	0.36	0.73	0.73
CaCO <sub>3</sub> (%)	1.67	10.51	7.07	2.15	16.12	6.25	2.36	6.87	5.79
LOI*** (%)	7.99	78.28	9.90	1.03	25.81	15.06	2.59	30.80	15.10
Chlorophyll-a (mg m <sup>-3</sup> )	2.17	22.18	7.07	7.98	11.09	7.07	5.65	13.65	13.44
Submerged coverage (%)	0	91	0	0	5	0	0	5	0

#### 3.2. Contemporary vs. Subfossil Cladocera Communities

A total of 28 species was found, belonging to four families (Figure S2): *Bosminidae, Chydoridae, Daphniidae, Moinidae*. The contemporary community was represented by 17 species, while 23 species were identified within subfossil Cladocera communities.

There were 13 species occurring both in the water column and in sediment. Four species were found only in the contemporary community: *Moina micrura, Bosmina longispina, Camptocercus rectirostris,* and *Daphnia longispina,* while up to 10 species were noted only in the subfossil community: *Pseudochydorus globosus, Pleuroxus uncinatus, Pleuroxus trigonellus, Disparalona rostrata, Alona affinis, Alona costata, Alona rustica, Leydigia leydigi, Paralona pigra,* and *Unapertura latens.* The dominant species in the contemporary samples were *Eubosmina coregoni, B. longirostris,* and *Chydorus sphaericus,* while the majority of subfossil remains belonged to the *Chydoridae* family and were mainly represented by *Alona intermedia* and *C. sphaericus.* 

The Simpson diversity index was used to demonstrate the relationship between the contemporary and the subfossil community and the differences between each study site. The results are juxtaposed with the number of species (no dimension (ND)) and abundances (ind.  $L^{-1}$ /ind.  $cm^{-3}$ ) of both communities in Figure 2. Differences in diversity, species richness, and heterogeneity for contemporary, and subfossil communities were observed. The differences were also observed between the three parts of the oxbow lake.



**Figure 2.** Number of species (ND), abundances (individual  $L^{-1}$ /individual cm<sup>-3</sup>), and Simpson diversity (ND) of contemporary and subfossil Cladocera communities (ND: no dimension).

The number of contemporary Cladocera communities ranged between three and 10 in the protected area, while subfossil communities ranged from 6–17. In the biomanipulated area, the number of species ranged from 3–9 and from 7–12 in the contemporary and subfossil communities, respectively. Similar patterns were recorded in the recreational area where contemporary communities were made up of 5–9 species, while the species number in the subfossil community ranged between 8 and 12 (Figure 2).

Significant differences were also noticeable in abundances in the contemporary Cladocera community between the different utilization forms (protected area: ranging between 30 and 166 ind.  $L^{-1}$ ; biomanipulated area: ranging between 426 and 1209 ind.  $L^{-1}$ ; recreational area: ranging between 79 and 247 ind.  $L^{-1}$ ). Dissimilarities were also observed in the frequency of subfossil Cladocera along a stress gradient (protected area: ranging between 27 and 580 ind. cm<sup>-3</sup>; biomanipulated area: ranging between 25 and 665 ind. cm<sup>-3</sup>; recreational area: ranging between 17 and 220 ind. cm<sup>-3</sup>). Some

similarities between contemporary and subfossil communities along the different utilization forms were noticed. For instance, the highest Cladocera abundance was noted in the biomanipulated area. However, the lowest numbers of individuals of contemporary and subfossil Cladocera were observed in the protected area and the recreational area, respectively (Figure 2).

The Simpson diversity indicated that the recreational area (ranging between 0.5329 and 0.8545) was a transition between the protected area (ranging between 0.5111 and 0.8198) and the biomanipulated area (ranging between 0.4973 and 0.6150) in the case of contemporary communities. What is more, according to this index, the values of the subfossil community were higher (protected area: ranging between 0.5438 and 0.8926; biomanipulated area: ranging between 0.6100 and 0.8413; recreational area: ranging between 0.7821 and 0.8677) compared to the contemporary community (Figure 2).

Beta diversity was mostly driven by similarity in both Cladocera communities. Richness differences and replacement were very low in the contemporary and subfossil communities. Beta diversity (sum of richness differences and replacement; contemporary community: 37.2%) was lower than nestedness (sum of richness differences and similarity; contemporary community: 89.6%). This pattern was nearly the same in the case of the subfossil Cladocera communities, since its beta diversity (33.5%) was lower than its nestedness (81.5%) (Figure 3).



**Figure 3.** Simplex plots based on similarity, richness differences, and species replacement for all the pairwise comparisons of the Cladocera communities of the 15 sampling sites of the Cibakháza oxbow lake. Notations: •, contemporary Cladocera communities; •, subfossil Cladocera communities.

The results of the NMDS based on the Rogers–Tanimoto similarity (Figure 4A) showed that the species composition of contemporary Cladocera communities overlapped among the three areas. The protected area, the biomanipulated area, and the recreational area were separated from each other based on species abundances of the contemporary community (Figure 4B). The species abundances of the protected area were separated from the biomanipulated area along the first axis, while the recreational area showed similarity to the protected area (NMDS; Bray–Curtis dissimilarity). The NMDS, based on Rogers–Tanimoto similarity, also showed an overlap among the areas in the case of the species composition of subfossil communities. Nonetheless, the protected area and the recreational area displayed high similarity. These areas were separated from the biomanipulated area along the first axis (Figure 4C). The second axis separated the protected area from the recreational area (Figure 4D), while the biomanipulated area overlapped with the protected area.



**Figure 4.** Non-metric multidimensional scaling (NMDS) of the Cladocera communities based on the Rogers–Tanimoto index (binary) and Bray–Curtis index (abundance) of the Cladocera communities. Numbers/symbols represent the samples. 1–7: protected area, 8–12: biomanipulated area, 13–15: recreational area. (**A**) Contemporary Cladocera communities, Bray–Curtis dissimilarity (stress = 0.3813); (**B**) contemporary Cladocera communities, Rogers–Tanimoto similarity (stress = 0.2568); (**C**) subfossil Cladocera communities, Bray–Curtis dissimilarity (stress = 0.2732); (**D**) subfossil Cladocera communities, Bray–Curtis dissimilarity (stress = 0.1439).

1st axis

1st axis

# 4. Discussion

There is an increasing need to resolve the relationship between contemporary and subfossil Cladocera communities and to provide a reliable picture of whether the subfossil community is truly representative of the contemporary community [48–50]. Most of the published subfossil studies on Cladocera communities are specifically limited to examining deep and cold water bodies [51–53]. In contrast, we studied both contemporary and subfossil Cladocera communities in a shallow, temperate water body.

The present research confirmed the earlier findings that the contemporary Cladocera community almost always underestimated the species pool of the waterbody. Estimation of the planktonic and littoral community was regarded as satisfactory, while the number and abundances of the benthic community were underestimated. On the other hand, the study of subfossil communities seems to complement this picture and to provide representative data on benthic taxa and also on the accumulation of planktonic and littoral species. This was confirmed by our finding of 17 species in the contemporary community and 23 species in the subfossil Cladocera communities, which constituted more than 80% of the total species richness (28 species). The good accumulation ability of the sediment was also supported by the fact that mud-living *Leydigia leydigi*, which lives in association with the bottom, was only recorded from the subfossil community. What is more, concentrating only on the protected area of the oxbow lake could lead to misleading conclusions. For instance, in the protected area, there were 13 species in the contemporary community, while the subfossil community was composed of 20 species. The biomanipulated area was characterized by the presence of nine and 16

taxa in the contemporary and subfossil Cladocera communities, respectively; while in the recreational area, the contemporary and subfossil community both consisted of 14 species.

Our findings suggest that the decreasing number of species of contemporary cladoceran communities is due to fish predation. It is likely that small-sized juvenile fish can swim through the  $5 \times 5$  cm diameter net and can spread into all three areas; thus, they put similar predation pressure on the zooplankton everywhere. However, their survival rate is very low, and the main prey of juvenile fishes is rotifers [32]. The biomanipulated area was influenced by the large common carp stock, whose migration was limited to this area. This increased predation pressure can be detected in the biomanipulated area and was manifested in the decrease of the species number of cladocerans and the prevailing contribution of small-sized Cladocera species (*Bosmina, Moina*) (Figure S2). Large-sized, easily-visible Cladocera species, especially daphnids, are more vulnerable to fish predators [54], while macroinvertebrates prefer small-sized cladocerans (e.g., bosminids, small daphnids). Fish have a great influence on the size distribution of Cladocera species [55]. A decreasing biomass of fish can increase the zooplankton biomass [22,56,57].

The predation effect on benthic cladocerans has not been explored yet. Only a few studies have dealt with the effect of fish on subfossil cladocerans' remains [58–61]. Jeppesen et al. [58] found significant differences in abundance and crustacean remains in arctic lakes with and without fish and also found the effects of invertebrate predators on benthic cladocerans. Amsinck et al. [59] found that brown trout were a less important factor in shaping the cladoceran communities in Faroese lakes due to their less efficient zoo-planktivory. In spite of scarce results, we can assume that fish predation pressure on benthic species is less important than on planktonic species. In this shallow oxbow lake, we could not demonstrate a significant effect of fish on the subfossil Cladocera community since similarities among cladoceran communities were high in the different areas. Based on our results, this means that contemporary Cladocera only provide information about species richness and their abundances at sampling time, while a subfossil community includes Cladocera communities from a whole year. The results correspond well with those of recent studies [62,63]. They all show that the use of sedimentary cladoceran remains provides a more complete assessment of species richness and community structure than does conventional point-sampling in the pelagic zone. This is because the sedimentary samples include benthic communities and integrate spatial and seasonal heterogeneity and year-to-year variations.

Both contemporary and subfossil datasets had a high overall similarity (63% and 67%), i.e., the cladoceran fauna of different sites had a remarkable amount in common, so that beta diversities of contemporary and subfossil cladocerans were low, whereas nestedness was high (89.6% and 81.5%, respectively). The result of the SDR-simplex analysis on the contemporary and subfossil Cladocera communities of the oxbow lake showed that there were no significant differences in the patterns of the Cladocera communities. For both communities, the replacement of species between sites was low, and the richness differences in sampling sites were also low. Vad et al. [64] found that the beta-diversity of northern Hungarian ponds was driven by species replacement. Toth et al. [65] confirmed the importance of species replacement in the partitioning of the beta-diversity of soda pans in Hungary. One possible explanation for our results is the distinct dispersion capabilities of cladoceran species. The different dispersion capabilities of cladoceran species would lead to high species replacement. On a larger geographical scale, cladocerans can substitute each other in ecosystem functioning. In our study, distances between sampling sites were small; therefore, cladoceran heterogeneity can be responsible for the level of the nestedness element of the biodiversity of the oxbow lake. Sites 1, 4, 5, 6, 7, and 15 made the largest contribution to the diversity of the oxbow lake, while *B. longirostris*, *B. coregoni*, A. intermedia, C. sphaericus, P. laevis, and C. rectirostris abundances varied the most among sites.

These results correspond to our expectations, but if diversity values are broken down to the three areas, we get a different picture of Cladocera diversity. Considering the Simpson diversity of the contemporary Cladocera communities, it can be stated that the values of the biomanipulated area were lower than those of the protected area. This result can demonstrate the impact of introducing fish

into this part of the oxbow lake. Brucet et al. [66] examined the effects of various abiotic and biotic factors on zooplankton communities, and they also concluded that predation has the greatest impact on the zooplankton communities. This was also reinforced by the fact that fewer species made up the contemporary Cladocera communities in the biomanipulated area than in the protected area. In contrast, the average species abundance was higher in the biomanipulated area compared to the others. This can be explained by the fact that small-sized species that did not fall prey to predation were able to colonize this area efficiently. With a competitive advantage, they could colonize the area at a higher density, and with their smaller-sized bodies, they could have a higher number of individuals in the pelagic region. Reissing et al. [67] found that in the case of fish introduction, the relative density of small zooplankton species is increased. Based on our results, we can conclude that the recreational area constitutes a transition between the protected and the biomanipulated areas.

The changes in the subfossil Cladocera communities did not display the same pattern as in the contemporary community. The effect of predation on the subfossil Cladocera communities was only slightly detectable, which led us to conclude that such an impact (the introduction of fish) did not cause much transformation in the community in the short term, nor did it cause the kind of transformation that could not be restored after the biomanipulation was discontinued.

This study proved that subfossil Cladocera communities in a shallow Hungarian oxbow lake are characterized by higher diversity, species density, and heterogeneity than the contemporary Cladocera communities. This fact has been proven by several previous studies [52,68]. García-Giron et al. [69] deduced that the subfossil Cladocera community reflects the contemporary community. This finding was also true in the studied oxbow lake, but only for the protected and recreational area. In the biomanipulated area, due to the high predation pressure, the species stock of the sediment did not reflect the contemporary Cladocera community, while it might reflect the pre-biomanipulated conditions.

# 5. Conclusions

The sediment represented the Cladocera community with a larger number of species compared to the contemporary communities in all three utilization forms (protected area, biomanipulated area, and recreational area). Therefore, in order to get a representative picture of the entire Cladocera community of a shallow standing water body (oxbow lake), it is considered necessary to involve both contemporary and subfossil Cladocera communities in the research. Simultaneous implementation and use of both sampling methods (filtering of water and sediment sampling) and simultaneous evaluation of the results provided a more complex picture of the Cladocera community.

In summary, our results suggest that the protected area of an oxbow lake can ensure the recolonization of species and the maintenance of diversity. In the short term, the fish introduction does not cause any irreversible change in the Cladocera community. The original structure of the community would quickly recolonize from the protected area.

These findings suggest that it is necessary to take care of the utilization of a water body during an ecological qualification. The involvement of zooplankton species in the ecological quality assessment of the European Water Framework Directive (EU WFD) has already been promoted by Jeppesen et al. [70]. Our research pointed out that Cladocera communities are especially useful to characterize the state of a standing water body.

**Supplementary Materials:** The following are available online at http://www.mdpi.com/2073-4441/11/5/929/ s1: Figure S1: Detailed map of sampling points of Cibakháza oxbow lake with the markings of the three differently-utilized areas, Figure S2: Table of the individual density ratios of the contemporary and subfossil Cladocera communities in the examined areas (green: contemporary community; blue: subfossil community).

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