"OVIDIUS" UNIVERSITY OF CONSTANȚA
DOCTORAL SCHOOL OF APPLIED SCIENCES
DOCTORAL FIELD: BIOLOGY

DOCTORAL THESIS ABSTRACT

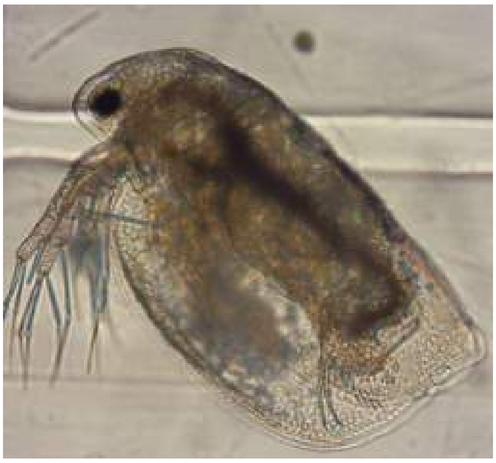
Doctoral Supervisor:
Prof. Univ. Dr. TOFAN Lucica

Doctoral student:
SALI (Suliman) Iasemin

Constanța, 2023

"OVIDIUS" UNIVERSITY OF CONSTANȚA
DOCTORAL SCHOOL OF APPLIED SCIENCES
DOCTORAL FIELD: BIOLOGY

Research on zooplankton communities in the ecological reconstruction areas of Carasuhat (natural flood regime) and Zaghen (controlled flood regime)



Doctoral Supervisor:

Prof. Univ. Dr. TOFAN Lucica

Doctoral student:

SALI (Suliman) Iasemin

Work carried out within the project "Program for increasing performance and innovation in excellence doctoral and postdoctoral research – PROINVENT" Contract no. 62487/06.03.2022, POCU/993/6/13 - SMIS Code: 153299

Constanța, 2023

Lider: Parteneri:

TABLE OF CONTENT

Note: The content has been preserved in the same form as in the doctoral thesis.

ACKNOWLEDGEMENTS

The development of this doctoral thesis was made possible due to the support and guidance of exceptional people who, with a high degree of professionalism and dedication, have contributed to my formation, instilling in me the courage to move forward.

First of all, I wish to express my gratitude to my academic advisor, Professor Univ. Dr. Lucica Tofan, for the idea behind this doctoral thesis, meticulous coordination, patience, understanding, and unconditional support, as well as for her model of perseverance and professionalism.

I thank Mr. CS I. Dr. Marian Tudor, the General Director of the National Institute for Research and Development in the Danube Delta, Tulcea, for recommending the development of the thesis topic and for his unwavering support throughout.

I am deeply grateful to Professor Univ. Dr. Geta Rîșnoveanu for her openness, support and constructive advice generously given, for which I express my gratitude, and also for the honour of being part of the Public Defence Committee and of reviewing this thesis.

I also thank to the members of my guidance committee: Mrs. CS I. Dr. Iuliana – Mihaela Tudor, Professor. Univ. Dr. Dan Cogălniceanu, and Assoc. Prof. Dr. Marius Skolka, for their valuable recommendations that have contributed to the improvement of the results and the work.

My gratitude goes to my colleagues from the Hydrobiology Laboratory of the National Institute for Research and Development Danube Delta, Tulcea, Phd. Orhan Ibram and Angelica Bănescu, who have helped, guided, and supported me during this phase.

The research would not have been possible without the assistance of colleagues from the Chemistry Laboratory of the National Institute for Research and Development in the Danube Delta, Tulcea, Dr. Adrian Burada, Dr. Cristina Despina, Daniela Seceleanu – Odor, and Mihaela Țigănuș, to whom I am thankful and grateful. I want to thank to Dr. Marian Tudor for the assistance provided in conducting statistical analysis and to Dr. Marian Mierlă for creating the distribution maps. I thank all colleagues who have supported and assisted me in conducting field research.

I would like to address my warmest gratitude to my family, and especially to my husband Levent, and my daughter Alya, who have motivated me and have always been by my side with love and much patience. I thank my dear mother who gave me the strength to follow my dreams and to approach everything with passion, desire, and determination.

I thank Prof. Univ. Dr. Monica Vlad and Prof. Univ. Dr. Ciugureanu Carmen-Adina for their guidance within the project *"Program for enhancing performance and innovation in doctoral and postdoctoral excellence research (PROINVENT)",* through which I benefited from a research scholarship that proved valuable in my research activities and in completing the doctoral thesis. Since any work needs financial support, the PhD thesis is carried out as a result of the research carried out within the project *"Assessment of the ecological status of aquatic ecosystems on the territory of the Danube Delta Biosphere Reserve", Core Program "Danube Delta" 2022, code PN 19 12 01 02, Contract 41N/2019,* developed by the Danube Delta National Institute for Research and Development, Tulcea, which provided the general framework and financial support for the development of the individual research program, as well as the achievement of the proposed objectives.

INTRODUCTION

Human intervention on the Danube Delta has caused major changes in the structure and functioning of the whole area. The creation of navigation rectification channels and the damming of large areas for agriculture, fish farming and forestry have had serious negative effects on aquatic ecosystems, such as the Carasuhat and Zaghen wetlands, which were heavily affected by human activity through the damming works that took place between 1960 and 1989, mainly for agricultural purposes.

The Carasuhat and Zaghen areas have recently been renaturated through ecological reconstruction activities aimed at restoring natural conditions and restoring their original ecological functions. Thus, the research activity is based on the investigation of zooplankton communities, as well as the assessment of water quality within the two aquatic ecosystems.

It aims to understand how the ecological parameters of these restored ecosystems approach those of a natural lake, specifically Lake Uzlina, providing valuable insights into the success of the restoration processes.

By comparing the collected data, similarities or differences in the structure and dynamics of zooplankton communities can be identified. Furthermore, valuable information about the state of aquatic ecosystems can be provided, serving as a basis for the development and implementation of management and conservation measures.

Using interdisciplinary methods such as sample collection, optical microscopy, spectroscopy, ecotoxicology, and statistical analysis, a comprehensive dataset has been obtained. This dataset provides information about the structure and composition of zooplankton communities, abundance and biomass, physico-chemical water quality parameters, nutrient and heavy metal concentrations, as well as toxicity.

The doctoral thesis comprises:

- 209 pages, including 22 pages in the General Part
- 97 figures
- 30 tables
- 292 bibliographic references

The structure consists of four main chapters:

The first chapter presents information from the specialized literature about anthropogenic changes and ecological reconstruction in the Danube Delta, the history of zooplankton knowledge in the Danube Delta, the main studied zooplankton groups, the importance of zooplankton in aquatic ecosystems, the distribution and toxicity of heavy metals in the aquatic environment. **The second chapter** presents the materials and methods used throughout the research activity, including the methods for collecting physico-chemical and biological samples, analyzing zooplankton, physico-chemical parameters, heavy metals, and nutrients. It also describes the data analysis methods and the application of Toxkit microbiotests for assessing the toxicity of water samples. **The third chapter** encompasses the presentation of results and discussions based on the conducted analyses. **The final chapter** outlines the conclusions drawn from the obtained results. The work concludes with a bibliography and the dissemination of research results.

PART I. GENERAL PART

CHAPTER 1. CURRENT STATE OF KNOWLEDGE

1.1. Anthropogenic changes and ecological reconstruction in the Danube Delta

Taking into account the policies promoted and implemented over time in the Danube Delta, two distinct periods can be identified: one characterized by economic development policies between 1860 and 1989, and another marked by conservation and ecological restoration policies starting from 1990 until the present (Giosan *et al.,* 2013; Bondar, 1990).

The large-scale interventions that occurred in the Danube Delta since 1860 have had a significant impact on the structure and functions of the ecosystems (Romanescu, 1999; Brețcan et al., 2008; Romanescu and Stoleriu, 2014).

Economic activities such as intensive agriculture and fish farming, sand exploitation, and navigation have transformed the natural landscape of the Danube Delta into an artificial one, dominated by agricultural polders and fish farms (Dumitrescu, 2003). Additionally, the development of river-maritime navigation and the intensive use of natural resources have led to significant changes in the hydrological system (Niculescu *et al.,* 2017).

According to Coleman et al. (2008), starting in 1987, the wetland area of the Danube Delta decreased by 62% due to human interventions. By the early 1990s, the embanked area in the Danube Delta had reached 977 km2, leading to the deterioration or loss of many ecosystem services provided to local communities (Uhel *et al.,* 2010).

According to Gomez-Baggethun et al. (2019), approximately two-thirds of ecosystem services have experienced a decline since 1960, particularly those related to natural resources, nutrient cycling, erosion control, sediment balance, flood protection, habitat for flora and fauna species, spiritual values, tourism, and recreation. The construction of polders, especially in the northwestern part of the Danube Delta, has had a strong impact on aquatic ecosystems (Staraș and Baboianu, 2005).

In 1990, the Danube Delta, along with the floodplain, the Razim-Sinoe lagoon complex, and the Black Sea coastal area, were designated as a Biosphere Reserve. This international recognition of the Danube Delta as a Ramsar site and its inclusion in UNESCO's Man and the Biosphere (MAB) program led to the establishment of the Danube Delta Biosphere Reserve Administration and the initiation of conservation and restoration policies.

After the fall of the communist regime in 1990, the policy of intensive economic development was replaced with the principles of sustainable development. As a result, studies and projects were initiated for the ecological reconstruction of degraded and economically inefficient agricultural lands, forest areas, and fish farms (Schneider, 2010).

In 1993, the first ecological reconstruction program was launched, focusing on degraded and inefficiently utilized agricultural lands, forested polders, and fish enclosures (Staraș, 1994). The pilot project areas were the Babina agricultural enclosure (2,100 ha) and the Cernovca enclosure (1,560 ha). The reconnection of the Babina polder, which was previously used for agriculture, took place in 1994, followed by the reconnection of the Cernovca enclosure in 1996. Other ecological restoration projects were implemented at Furtuna (2,115 ha), Popina II (3,600 ha), Holbina I and II (4,370 ha), and Dunavăț II (1,260 ha) (Gomoiu, 1996).

1.3. Role of zooplankton in aquatic ecosystems

Zooplankton is a diverse group of small-sized heterotrophic organisms that includes a wide variety of taxa, ranging from unicellular organisms such as ciliates and flagellates to multicellular animals that float freely or are suspended in the water column of aquatic ecosystems. (Scheffer, 1998; Carpenter and Kitchell, 1996).

Zooplankton is of particular importance in the functioning of aquatic ecosystems, as it consumes phytoplankton, bacteria, including other zooplankton, while being consumed by a range of invertebrates and vertebrates. Therefore, zooplankton plays an important role in the transfer of matter and energy in the trophic networks of aquatic ecosystems, influencing water quality and supporting life at higher trophic levels.

Zooplankton plays a significant role in the energy transfer between primary producers and consumers, contributing significantly to nutrient recycling (Lampert and Sommer, 1997).

Due to its essential position in aquatic ecosystems, is closely linked to higher levels of the trophic network. It can be affected by algal blooms during bottom-up processes (Jeppensen *et al.,* 2011; Stamou *et al.,* 2019) and can rapidly respond or exert pressure in top-down control, thereby determining the composition and abundance of phytoplankton (Naselli-Flores and Rossetti, 2010).

Abiotic environmental factors limit the abundance and distribution of zooplankton organisms, and biotic factors (such as phytoplankton) and intra- and interspecific competition for environmental resources also play a role. Physico-chemical parameters such as temperature, dissolved oxygen, pH, conductivity, and turbidity can determine species associations in the water column. (Lampert 1997; Spoljar *et al.,* 2018). Additionally, zooplankton can serve as a good indicator of environmental conditions and trophic status (Anas *et al.,* 2013; Kuczynska-Kippen *et al.,* 2020).

Due to their short life cycles, zooplankton species respond rapidly to changes in water quality, which is why their specific composition, numerical abundance, and biomass can provide important information about the quality of the aquatic ecosystem.

Since the specific composition of zooplankton remains relatively constant over a period of time, the appearance or disappearance of certain species can indicate changes in water quality.

Through its qualitative and quantitative components, zooplankton provides useful elements for defining the ecological status of the aquatic ecosystem, offering information regarding the stage of evolution of the ecosystem in terms of trophic conditions (Sladecek, 1983). In recent decades, there has been a growing trend in the use of efficient methods for environmental monitoring based on biotic indicators (Duggan *et al.,* 2001; Carpenter *et al.,* 2006; Haberman and Haldna, 2014), and zooplankton can be considered a highly important indicator in assessing the ecological and trophic status of the ecosystem. In the past, numerous studies have highlighted the indicator role of zooplankton (Gulati, 1983; Sladecek, 1983; Berzins and Pejler, 1989).

PART II. PERSONAL CONTRIBUTIONS

CHAPTER 2. ORGANIZATION OF THE INDIVIDUAL RESEARCH PROGRAM

2.2. Aims and Objectives

The aim of this study is to assess the efficacy of the ecological reconstruction process in the Carasuhat and Zaghen areas, comparing them with the natural Lake Uzlina. Additionally, the research aims to enhance our understanding of zooplankton fauna composition, ecosystem conditions in the Danube Delta Biosphere Reserve, and the practical application of European Union directives and international agreements related to aquatic ecosystem conservation and sustainability, such as the Water Framework Directive (WFD).

The objectives and specific activities that formed the basis of the individual research program are as follows:

1. Characterizing the spatial and temporal dynamics of zooplankton communities within the aquatic ecosystems restored through ecological reconstruction efforts.
2. Identifying key controlling factors that shape the dynamics of zooplankton communities in the restored areas.
3. Evaluating the ecological state of the studied aquatic ecosystems in relation to the presence of nutrients and heavy metals
4. Testing the water's toxicity using Toxkit microbiotests.

2.3. Spatial and temporal organization of the individual research program

The research program was designed spatially - at the scale of aquatic ecosystems represented by the ecological reconstruction areas of Carasuhat, Zaghen, and the natural Lake Uzlina - and temporally, spanning a two-year period between March 2021 and October 2022.

The Carasuhat study area, situated in the western part of the Danube Delta, bordered by the Litcov Canal to the north and the Sfântu Gheorghe Arm to the south, west, and east. It underwent agricultural development from 1985 to 1989, covering 3436 hectares, but was discontinued in 1990, resulting in the transformation of natural ecosystems into anthropogenic ones. From March 1, 2012, to August 30, 2015, a collaborative project led by the Danube Delta

Biosphere Reserve Administration (ARBDD), WWF Romania, and Mahmudia Local Council aimed at ecologically reconstructing the Carasuhat Agricultural Enclosure. This effort revitalized the Carasuhat wetland by restoring 924 hectares of the former enclosure to its natural hydrological regime through the Sfântu Gheorghe Arm.

The Zaghen study area in the eastern part of the municipality of Tulcea, in the adjacent floodplain of the city's terrace. It is bounded to the north by the Tulcea Arm of the Danube River and by the DJ222C Tulcea-Malcoci road to the south. Between April 5, 2012, and April 7, 2015, the project *"Ecological Reconstruction in the Zaghen Polder within the Romania/Ukraine Transboundary Danube Delta Biosphere Reserve"* was implemented. As a result of this project, an area of approximately 200 hectares was inundated.

Lake Uzlina, where no ecological reconstruction works have been carried out, is part of the Gorgova – Isac, lake complex. It is a natural lake with active water exchange and covers an area of 749 hectares (Oosterberg *et al.,* 2000).

Five stations were chosen for Carasuhat (Figure 2.1), four for Zaghen (Figure 2.2), and five for Lake Uzlina (Figure 2.3). Sampling campaigns occurred in March, July, and October of 2021 and 2022 for Carasuhat and Lake Uzlina. For Zaghen, sampling took place in March 2021, July 2021, October 2021, March 2022, October 2022, and March 2023. However, challenges like inaccessibility due to hydrological conditions were faced, such as the inability to sample the Zaghen area in July 2022 due to low water levels and aquatic vegetation growth.

Figure 2.1. The location of the sampling stations, Carasuhat study area (Google Earth)

Figure 2.2. The location of the sampling stations, Zaghen study area (Google Earth)

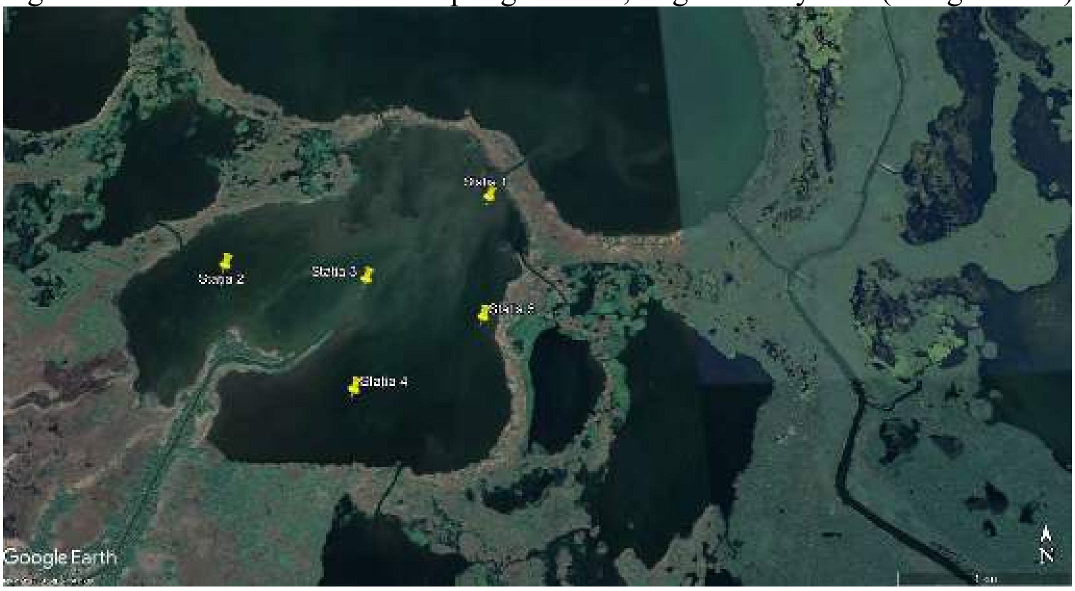


Figure 2.3. The location of the sampling stations, Uzlina study area (Google Earth)

2.5. Material and methods

2.5.1. Methods for determining biological parameters

2.5.1.1. Collection and preservation of biological samples

The zooplankton samples were collected from the surface layer, at a depth of 0.5 - 1 m, using a 10-liter plastic container. The collected water was then filtered through a 55 µm mesh size zooplankton net. The quantity of water collected for each sample, in order to represent a significant sample, was 30 liters (Tudor *et al.,* 2015). The collected samples were carefully transferred into plastic containers with a volume of 100 ml and properly labeled. Immediately after collection, each sample was preserved using 96% ethyl alcohol. At the end of the expedition, the samples were transported to the laboratory and left to settle for two weeks.

2.5.1.2 Qualitative and quantitative analysis of biological samples

After sedimentation, the supernatant from the samples is removed through repeated siphoning. Depending on the abundance of organisms in the sample, the final sample volume will be 30-50 ml. From the concentrated sample, 1 ml is transferred into a Sedgwick-Rafter counting chamber and left to settle for a few minutes. For qualitative and quantitative analysis, 2 ml of the sample is examined under the Zeiss Axio Lab 1 optical microscope, using a magnification factor of 10X. Organisms are counted and identified following the specialized literature: Rudescu (1960), Damian – Georgescu (1963), Negrea (1983), and Błędzki and Rybak (2016).

2.5.1.3. Data processing

After analyzing the zooplankton samples, the data were input into a database and the calculation of numerical density, biomass, relative abundance and frequency of occurance was performed using Microsoft Office Excel 2021. Diversity Indices calculation was performed using the Margalef (D), Shannon (H̀), and Pielou (J̀) indices, calculated using the PAST 4.03 software (Hammer *et al.,* 2001). Descriptive and inferential statistical analysis was conducted using IBM SPSS software (version 27) (Morgan *et al.,* 2019). Univariate statistical analysis (Pearson correlations) and multivariate statistical analysis based on non-metric Multi-Dimensional Scaling (nMDS), Principal Component Analysis (PCA), were performed using the PAST 4.03 software. Redundancy Analysis, and Variance Partitioning were performed with R software (version 4.2.3). For this, the data was logarithmically transformed and standardized using the decostand function in R. Correlated variables ($p \leq 0.05$) and those with VIF values >4 were removed from the analysis.

2.5.2. Sample collection, preservation, and analysis of physico-chemical parameters

Water temperature (ºC), dissolved oxygen concentration (mg/L), pH (pH units), electrical conductivity (µS/cm), and chlorophyll "a" concentration (µg/L), measurements were carried out using a portable multiparameter probe, YSI EXO2 (Xylem, USA). Depth and water transparency were measured using a Secchi disk. Water samples were collected from the surface layer, following the SR ISO 5667-1/2007 standard, in polyethylene containers, fixed with specific reagents and transported to the Chemistry Laboratory within the Danube Delta National Institute for Research and Development, Tulcea. Nutrients concentration measurements were conducted using molecular absorption spectrophotometry, with the Perkin Elmer UV-VIS Lambda 10 spectrophotometer employed as the analytical instrument. For he analysis of heavy metal contents was used the ICP-MS mass spectrometer ELAN® DRC-e.

2.5.3. Application of aquatic ecotoxicology techniques based on Toxkit microbiotests

The thesis's final part assessed Zaghen study area water toxicity using Toxkit microbiotest technology, an article accepted for publication. Thamnotoxkit FTM and Daphtoxkit FTM were applied to *Daphnia magna* and *Thamnocephalus platyurus* in March 2023, with 5 water samples collected. The microbiotests followed standard procedures from the kits (Tofan *et al.,* 2023), done at the Applied Ecology Laboratory of "Ovidius" University of Constanța. Test organisms (*Daphnia magna*, *Thamnocephalus platyurus*) were supplied in latent form within the kits, hatched and exposed for 24 to 48 hours. Dead or immobilized organisms were recorded for LC50 and EC50 calculation (probit analysis). Heavy metal analysis (Cd, Zn, Cu, Pb, Ni, Cr) was done at the Danube Delta National Institute for Research and Development Tulcea Chemistry Laboratory.

CHAPTER 3. RESULTS AND DISCUSSION

3.1. Characterization of hydrogeomorphological units

3.1.1. Dynamics of physico-chemical parameters in studied aquatic ecosystems during the period 2021-2022

Water temperature displayed typical seasonal fluctuations, ranging from 6.23 to 31.31°C in Carasuhat, 8.62 to 29.6°C in Zaghen, and 5.61 to 28.69°C in Uzlina. Dissolved oxygen levels varied from 5.59 to 21.9 mg O2/L in Carasuhat, 4.19 to 11.98 mg O2/L in Zaghen, and 5.69 to 16.93 mg O2/L in Uzlina. Water pH generally remained alkaline, spanning 7.56 to 8.67 in Carasuhat, 7.20 to 8.81 in Zaghen, and 7.62 to 8.85 in Uzlina. Electrical conductivity ranged from 303 to 439 µS/cm in Carasuhat, 1886 to 2470 µS/cm in Zaghen, and 271 to 454 µS/cm in Uzlina, with higher values in the Zaghen area. Chlorophyll-a concentrations fluctuated between 3.25 to 31.89 µg/L in Carasuhat, 22.39 to 85.46 µg/L in Zaghen, and 2.13 to 24.13 µg/L in Uzlina. Carasuhat showed minimum (5.75 µg/L) and maximum (15.56 µg/L) values in spring and summer 2021. Zaghen had notably higher averages, with a spring low (32.15 µg/L) and autumn peak (71.38 µg/L) in 2021. Uzlina displayed mean values reaching spring low (2.94 µg/L) and summer high (16.11 µg/L) in 2021. Chlorophyll-a indicated eutrophic conditions in Carasuhat and Uzlina and hypereutrophic conditions in Zaghen.

3.2. Structure of the zooplankton community in the studied ecological systems during 2021-2022

3.2.1. Taxonomic composition and frequency of occurrence

The analysis of the taxonomic composition of zooplankton fauna during the period March 2021 to October 2022, within the aquatic ecosystems represented by the ecological reconstruction areas of Carasuhat and Zaghen, as well as the natural lake Uzlina, revealed the presence of 66 zooplankton taxa, belonging to the three major functional groups of freshwater zooplankton ecosystems, namely cladocerans, copepods, and rotifers (Table 3.2). The analysis of frequency occurrence in the samples allows for the differentiation of the following findings: species meeting the criteria of consistency (F > 50), expressed in terms of frequency, include the following: *Bosmina longirostris, Chydorus sphaericus, Moina brachiata, Asplanchna priodonta, Megacyclops viridis, Anuraeopsis fissa, Brachionus angularis, Brachionus calyciflorus, Brachionus diversicornis, Brachionus falcatus, Brachionus leydigi, Brachionus quadridentatus, Brachionus urceolaris, Keratella cochlearis, Keratella quadrata, Keratella serrulata, Keratella valga, Notholca acuminata, Ascomorpha ovalis, Polyarthra vulgaris, Pompholyx sulcata, Trichocerca longiseta, Filinia longiseta.* Most of these species exhibit high ecological adaptability and are common in the permanent aquatic ecosystems of the Danube Delta, indicating high trophic conditions. In Carasuhat, a diverse zooplankton assemblage with 55 species (40 rotifers, 10 cladocerans, and 5 copepods) was observed. In the Zaghen, characterized by controlled flooding, lower diversity was recorded with 27 species (20 rotifers, 3 cladocerans, and 4 copepods). In Uzlina, a total of 46 species (39 rotifers, 4 cladocerans, and 3 copepods) were identified. Rotifers dominated the zooplankton composition across all areas, comprising the majority of the species in each ecosystem (Figure 3.37).

Table 3.2. The list of zooplankton species identified in the studied aquatic ecosystems during March 2021 to October 2022

Nr. crt.	Taxonomic Group / Family	Species	Taxonomic Component	Carasuhat					Zaghen				Uzlina					*Frequency %
				Station														
				1	2	3	4	5	1	2	3	4	1	2	3	4	5	
CLADOCERA	**Bosminidae**	*Bosmina coregoni* (Baird, 1857)	*Primary consumers*					*x*										*C1*
		*Bosmina longirostris (*O. F. Muller, 1776)	*Primary consumers*	*x*	*x*	*x*	*x*	*x*					*x*	*x*	*x*	*x*	*x*	*C3*
	Daphniidae	*Ceriodaphnia reticulata (*Jurine, 1820)	*Primary consumers*	*x*	*x*			*x*										*C1*
		*Simocephalus vetulus (*O. F. Muller, 1776)	*Primary consumers*	*x*	*x*			*x*				*x*						*C1*
	Eurycercidae	*Alona quadrangularis (*O. F. Muller, 1776)	*Primary consumers*														*x*	*C1*
		*Alona rectangula (*Sars, 1862)	*Primary consumers*				*x*											*C1*
		*Alonella nana (*Baird, 1843)	*Primary consumers*				*x*											*C1*
		*Chydorus sphaericus (*O. F. Muller, 1776)	*Primary consumers*	*x*	*x*	*x*	*x*	*x*		*x*	*x*	*x*	*x*		*x*	*x*	*x*	*C3*
		Pleuroxus aduncus (Jurine, 1820)	*Primary consumers*			*x*												*C1*
	Moinidae	*Moina brachiata (*Jurine, 1820)	*Primary consumers*	*x*	*x*		*x*	*x*	*x*	*x*	*x*	*x*	*x*				*x*	*C3*
	Sididae	*Diaphanosoma brachiurum (*Lievin, 1848)	*Primary consumers*	*x*	*x*	*x*	*x*	*x*										*C1*
COPEPODA	**Cyclopidae**	*Cyclops vicinus (*Ulianine, 1875)	*Secondaryconsumers*	*x*	*x*			*x*	*x*			*x*						*C1*
		*Eucyclops serrulatus (*Fischer, 1851)	*Secondary consumers*		*x*	*x*			*x*	*x*	*x*		*x*			*x*		*C1*
		Macrocyclops albidus (Jurine, 1820)	*Secondary consumers*	*x*					*x*	*x*	*x*	*x*						*C1*
		Megacyclops viridis (Jurine, 1820)	*Secondary consumers*	*x*	*x*	*x*	*x*	*x*	*x*	*x*	*x*	*x*	*x*	*x*	*x*	*x*	*x*	*C4*
	Pseudodiaptomidae	*Calanipeda aquaedulcis (*Kritschagin, 1873)	*Primary consumers*	*x*									*x*	*x*	*x*	*x*	*x*	*C2*
ROTIFERA	**Asplanchnidae**	*Asplanchna girodi (*de Guerne, 1888)	*Secondary consumers*											*x*				*C1*
		*Asplanchna sieboldii (*Leydig, 1854)	*Secondary consumers*		*x*													*C1*
		Asplanchna priodonta (Gosse, 1850)	*Secondary consumers*	*x*	*x*	*x*	*x*	*x*		*x*		*x*	*x*	*x*	*x*	*x*	*x*	*C3*
	Brachionidae	*Anuraeopsis fissa* (Gosse, 1851)	*Primary consumers*	*x*		*x*			*x*	*x*	*x*		*x*	*x*	*x*	*x*		*C3*
		*Brachionus forficula (*Wierzejski, 1891)	*Primary consumers*										*x*	*x*			*x*	*C1*
		*Brachionus plicatilis (*Müller, 1786)	*Primary consumers*					*x*										*C1*
		*Brachionus rubens (*Ehrenberg, 1838)	*Primary consumers*			*x*						*x*						*C1*
		*Brachionus angularis (*Gosse, 1851)	*Primary consumers*	*x*	*x*	*x*	*x*	*x*	*x*	*x*	*x*	*x*	*x*	*x*	*x*	*x*	*x*	*C4*
		*Brachionus budapestinensis (*Daday, 1885)	*Primary consumers*					*x*					*x*			*x*		*C1*
		*Brachionus calyciflorus (*Pallas, 1776)	*Primary consumers*	*x*	*x*	*x*	*x*	*x*				*x*	*x*	*x*	*x*	*x*	*x*	*C3*
		Brachionus diversicornis (Daday, 1883)	*Primary consumers*	*x*	*x*	*x*			*x*	*x*	*x*	*x*		*x*	*x*	*x*	*x*	*C3*
		Brachionus falcatus (Zacharias, 1898)	*Primary consumers*		*x*	*x*	*x*						*x*	*x*	*x*	*x*	*x*	*C3*
		Brachionus leydigi (Cohn, 1862)	*Primary consumers*	*x*	*x*	*x*	*x*	*x*			*x*	*x*	*x*		*x*	*x*		*C3*
		*Brachionus quadridentatus (*Hermann, 1783)	*Primary consumers*	*x*	*x*	*x*	*x*	*x*		*x*		*x*	*x*	*x*			*x*	*C3*
		*Brachionus urceolaris (*O. F. Muller, 1773)	*Primary consumers*	*x*	*x*	*x*	*x*	*x*				*x*	*x*	*x*	*x*	*x*		*C3*
		*Keratella cochlearis (*Gosse, 1851)	*Primary consumers*	*x*	*x*	*x*	*x*	*x*	*x*	*x*	*x*	*x*	*x*	*x*	*x*	*x*	*x*	*C4*

		*Keratella quadrata (*O. F. Muller, 1786)	*Primary consumers*	x	x	x	x	x					x	x	x	x	x	*C3*
		Keratella serrulata (Ehrenberg, 1838)	*Primary consumers*	x	x	x	x	x	x	x	x	x	x	x	x	x	x	*C4*
		Keratella valga (Ehrenberg, 1834)	*Primary consumers*		x	x		x	x	x	x	x		x	x		x	*C3*
		Notholca acuminata (Ehrenberg, 1832)	*Primary consumers*	x	x	x	x	x			x	x	x	x	x	x	x	*C3*
		Notholca foliacea (Ehrenberg, 1838)	*Primary consumers*											x	x			*C1*
		Notholca labis (Levander, 1901)	*Primary consumers*	x		x	x	x						x	x	x	x	*C2*
		Notholca squamula (O. F. Muller, 1786)	*Primary consumers*				x	x										*C1*
		Plationus patulus (O. F. Muller, 1786)	*Primary consumers*				x											*C1*
	Epiphanidae	*Epiphanes senta* (O.F. Muller, 1773)	*Primary consumers*			x			x		x		x			x	x	*C2*
	Euchlanidae	*Euchlanis deflexa* (Gosse, 1851)	*Primary consumers*		x	x		x							x			*C2*
		Euchlanis dilatata (Ehrenberg, 1832)	*Primary consumers*		x										x			*C1*
		Euchlanis parva (Rousselet, 1832)	*Primary consumers*		x													*C1*
	Gastropodidae	*Ascomorpha ovalis* (Bergendahl, 1892)	*Primary consumers*	x	x	x	x	x					x	x	x	x	x	*C3*
	Lecanidae	*Lecane bulla (*Gosse, 1851)	*Primary consumers*										x					*C1*
		*Lecane luna (*Muller, 1776)	*Primary consumers*		x	x				x			x		x			*C2*
		*Lecane lunaris (*Ehrenberg, 1832)	*Primary consumers*								x							*C1*
		*Lecane ungulata (*Gosse, 1887)	*Primary consumers*		x	x												*C1*
	Lepadellidae	*Colurella uncinata (*O.F. Muller, 1773)	*Primary consumers*		x	x		x							x			*C2*
		*Lepadella patella (*O.F. Muller, 1786)	*Primary consumers*		x													*C1*
	Mytilinidae	*Mytilina bisulcata (*Lucks, 1912)	*Primary consumers*					x										*C1*
	Nottomatidae	*Cephalodella gibba (*Ehrenberg, 1832)	*Primary consumers*		x													*C1*
	Synchaetidae	*Polyarthra vulgaris (*Carlin, 1943)	*Primary consumers*	x	x	x	x	x	x	x	x	x	x	x	x	x	x	*C4*
		*Synchaeta oblonga (*Ehrenberg, 1831)	*Primary consumers*		x		x									x		*C1*
		*Synchaeta pectinata (*Ehrenberg, 1832)	*Primary consumers*			x	x						x	x				*C2*
		*Synchaeta stylata (*Wierzejski, 1893)	*Primary consumers*										x	x	x	x		*C2*
	Testudinellidae	*Pompholyx complanata (*Gosse, 1851)	*Primary consumers*		x										x			*C1*
		Pompholyx sulcata (Hudson, 1885)	*Primary consumers*	x	x	x	x	x		x	x	x	x	x	x	x	x	*C4*
		Testudinella patina (Hermann, 1783)	*Primary consumers*		x					x	x		x		x	x	x	*C2*
	Trichocercidae	*Trichocerca capucina* (Wierzejski & Zacharias, 1893)	*Primary consumers*											x				*C1*
		Trichocerca cylindrica (Imhof, 1891)	*Primary consumers*											x	x		x	*C1*
		Trichocerca longiseta (Schrank, 1802)	*Primary consumers*	x	x		x	x	x	x	x	x	x	x	x	x	x	*C4*
		Trichocerca rattus (Muller, 1776)	*Primary consumers*											x				*C1*
	Trichotriidae	*Trichotria pocillum* (O. F. Muller, 1776)	*Primary consumers*												x			*C1*
	Trochosphaeridae	*Filinia longiseta* (Ehrenberg, 1834)	*Primary consumers*	x	x	x	x	x					x	x	x	x	x	*C3*

***Legend:* χ -** *present*

* C1 - *Accidental species (<25.0%) C2 - Accessory species (25.1-50.0%) C3 - Constant species (50.1-75.0%) C4 - Eudominant species (75-100%)*

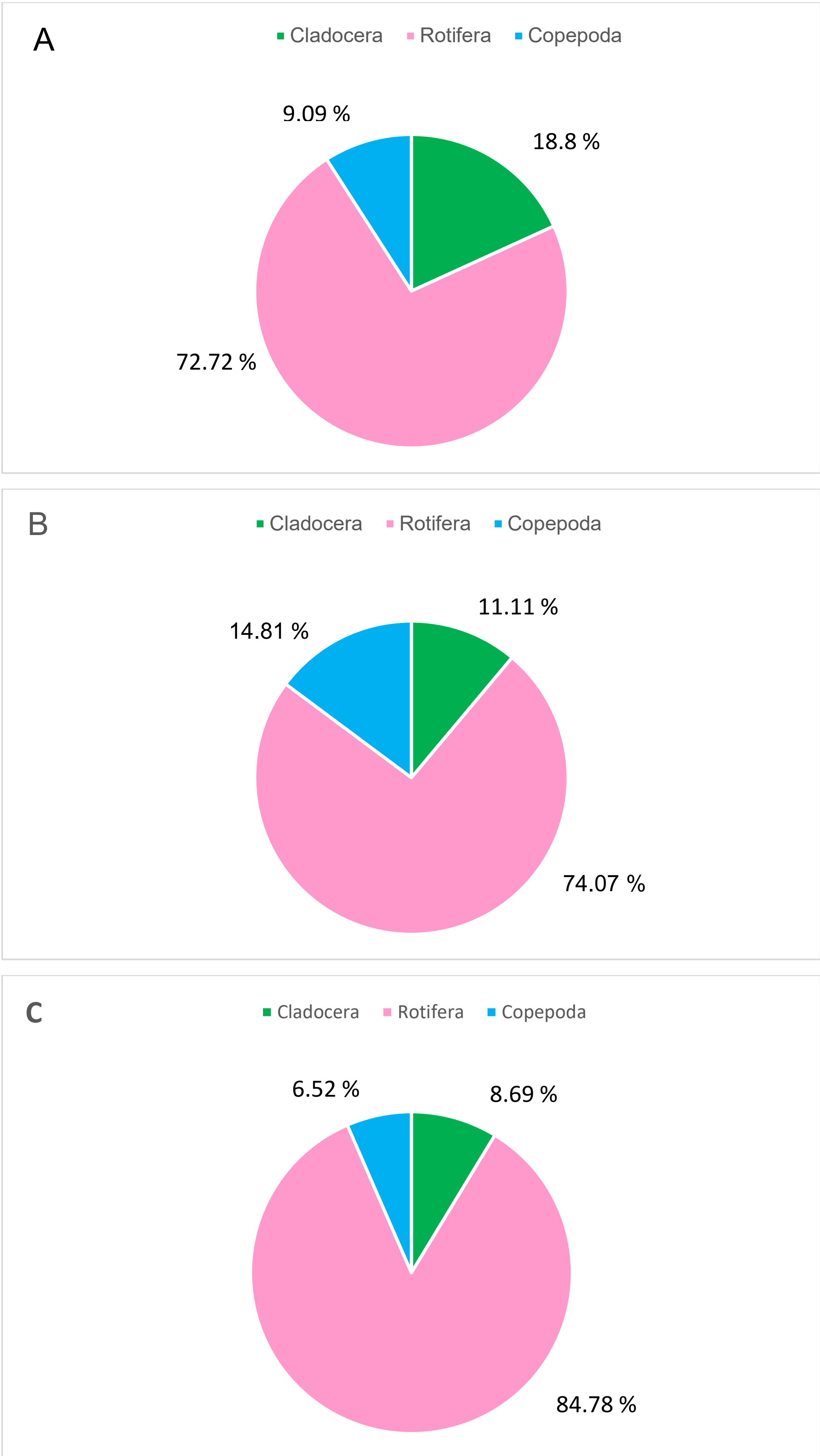


Figure 3.37. The specific percentage composition of the zooplankton community in Carasuhat (A) and Zaghen (B) and Uzlina (C), March 2021 - October 2022.

3.2.3. Diversity and evenness of zooplankton

For assessing the diversity of the studied zooplankton communities, we chose the Shannon-Wiener diversity index (H') and the Margalef index (D).

In the Carasuhat study area, it is noteworthy that the recorded diversity and richness indices for the zooplankton community varied within a wide range, with values of H' = 0.75 (October 2021) and D = 1.39 (October 2021) as lower limits, and H' = 1.8 (March 2021) up to D = 3.62 (March 2021) as upper limits of the variability range.

In Zaghen, the Shannon-Wiener diversity index (H') exhibited values ranging from 0.56 (July 2021) to 1.6 (March 2021), while the Margalef richness index (D) varied from 0.76 (March 2022) to 3.13 (March 2021).

For Lake Uzlina, the Shannon-Wiener diversity index (H') was situated within the range of 0.94 (October 2022) and 1.74 (March 2022), and the Margalef richness index (D) fluctuated from 1.12 (October 2022) to 3.11 (October 2021). No significant variations were observed between the analyzed seasons regarding the Pielou evenness index (J').

The highest diversity values of zooplankton communities were recorded in spring, correlated with a higher number of species present and a more equitable distribution of abundances among species. Among the stations in Carasuhat, S1 and S4 exhibited a less abundant composition (S=28). In Zaghen, station 1, which is situated near residential areas, had a less abundant specific composition (S=14). In Uzlina, station 5 recorded the lowest number of species (S=27).

3.2.4. Zooplankton Density Dynamics

3.2.4.1. Seasonal Distribution of Zooplankton Numerical Density

The numerical density, expressed as the count of individuals per unit volume, is a crucial parameter for quantitatively characterizing biotic communities in aquatic ecosystems. Estimating individual numbers constitutes the initial step towards comprehending the functioning of the aquatic ecosystem. When combined with biomass estimates, these data offer insights into the significance of taxa within aquatic trophic chains.

Within the Carasuhat study area, annual zooplankton densities exhibited values of 1483.583 ind/L in the first study year, and in the subsequent year, this density doubled, reaching 2434.983 ind/L. This trend could potentially be attributed to lower water levels in 2022 compared to 2021, coupled with higher water temperatures in 2022. Reduced water levels and elevated temperatures are conducive to the development of zooplankton organisms.

In 2021, rotifers exhibited the highest density at 850.71 ind/L, constituting more than 57.34% of the total zooplankton density. Copepods contributed 461.1 ind/L (31.08%), while cladocerans had the lowest density at 171.76 ind/L (11.57%). In 2022, rotifers doubled their density to 1477.55 ind/L (61% of total), copepods had a density of 628.28 ind/L (26% of total), and cladocerans exhibited the lowest density at 329.35 ind/L (13% of total).

In the Carasuhat study area, 2021 exhibited a pattern marked by a spring peak (791.78 ind/L), followed by declines in summer and autumn, with rotifers (82.85%) playing a prominent role. Conversely, 2022 displayed different trends, with summer being the peak period for zooplankton density (907.41 ind/L), predominantly driven by rotifers (60.68%). (Figure 3.38).

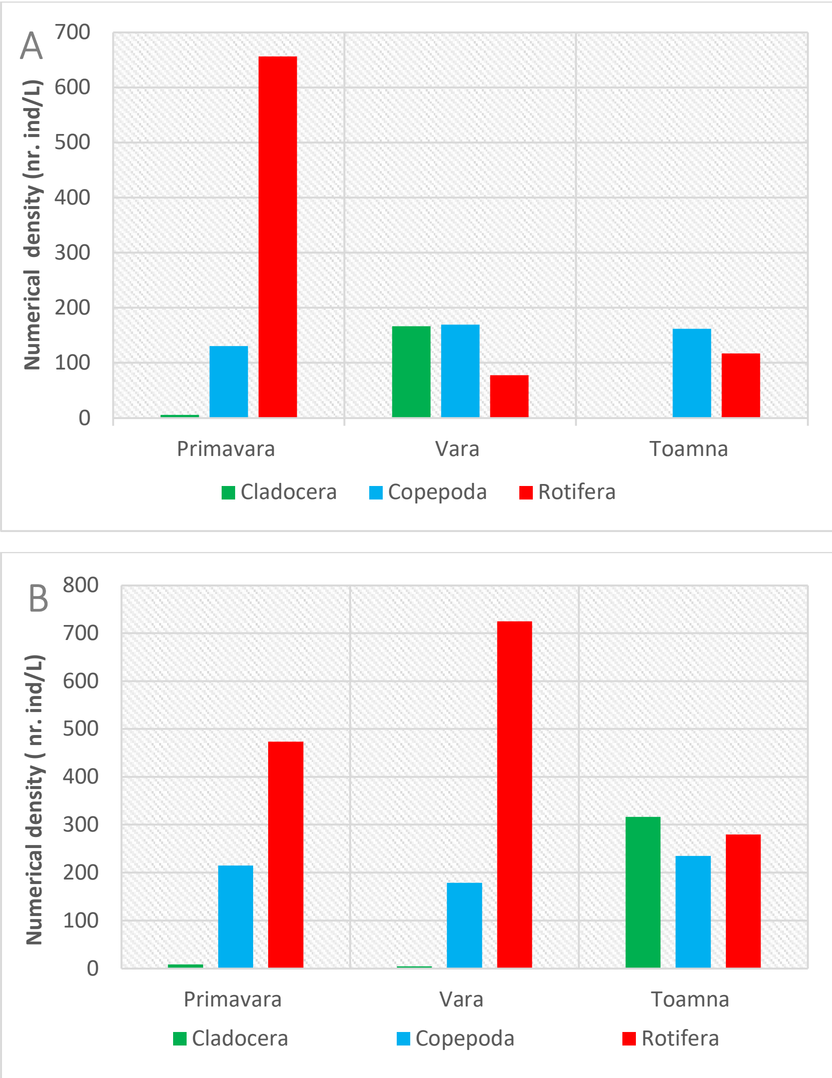


Figure. 3.38. Seasonal distribution of zooplankton density in the Carasuhat study area in 2021 (A) and 2022 (B)

In aquatic ecosystems with higher degrees of eutrophication, such as the Zaghen, higher numerical densities of zooplankton are observed (Lampert and Sommer, 1997; May and O'Hare, 2005; Garcia-Chicote *et al.,* 2018). However, this relationship, as described in literature, applied to the Zaghen area only in the year 2021, as densities varied in 2022 and were lower than those in Carasuhat. This suggests that ecosystem interactions are more complex and influenced by variable factors beyond eutrophication level. In 2021, cladocerans exhibited the highest numerical density at 1286.533 ind/L, accounting for 46.29% of the total. Rotifers had the lowest density, 539.73 ind/L, representing 19.42%. In 2022, rotifer density increased to 1070.41 ind/L (64.49%), while copepod density decreased to 588.21 ind/L (35.44%), with cladocerans having the lowest density at 0.06%; the peak zooplankton density was 1572.83 ind/L in summer 2021 and 1344.4 ind/L in autumn 2022, showing a trend of summer > autumn > spring in 2021 and autumn > spring in 2022 (Figure 3.39).

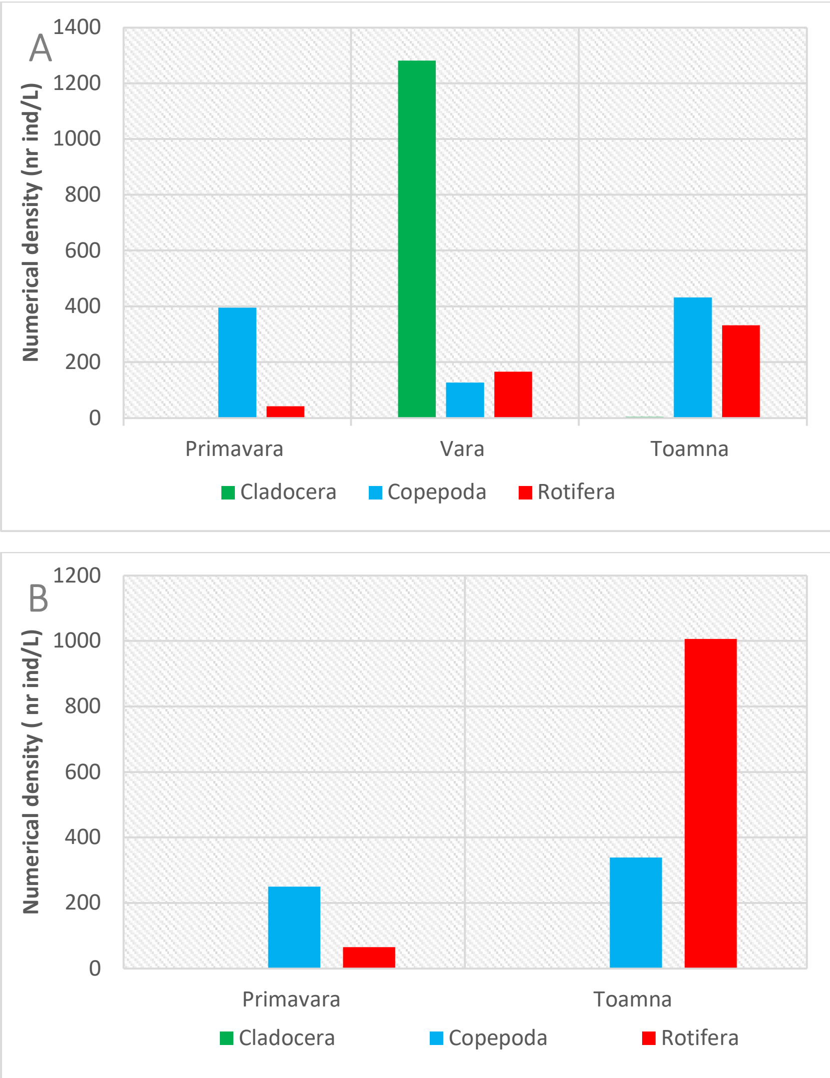


Figure. 3.39. Seasonal distribution of zooplankton density in the Zaghen study area in 2021 (A) and 2022 (B)

In Lake Uzlina, zooplankton densities were lower compared to Carasuhat and Zaghen, with values of 1128.71 ind/L in 2021 and 2460.52 ind/L in 2022. In 2021, rotifers had the highest numerical density, 843.22 ind/L (74.70% of the total), while copepod density was 279.74 ind/L (24.78% of the total), and cladoceran density was lower, 5.75 ind/L (0.50% of the total). In 2022, rotifer density doubled, reaching 1786.15 ind/L (over 70% of the total), copepod density was 644.5 ind/L (over 26% of the total), and cladocerans had the lowest density, 29.86 ind/L (1.21% of the total). The general trend observed in the literature was confirmed in 2021, with maximum zooplankton density in spring, 479.10 ind/L, and in 2022, the peak value was in summer, 1079 ind/L, with a significant contribution from rotifers, accounting for 88% in 2021 and 93% in 2022. (Figure 3.40).

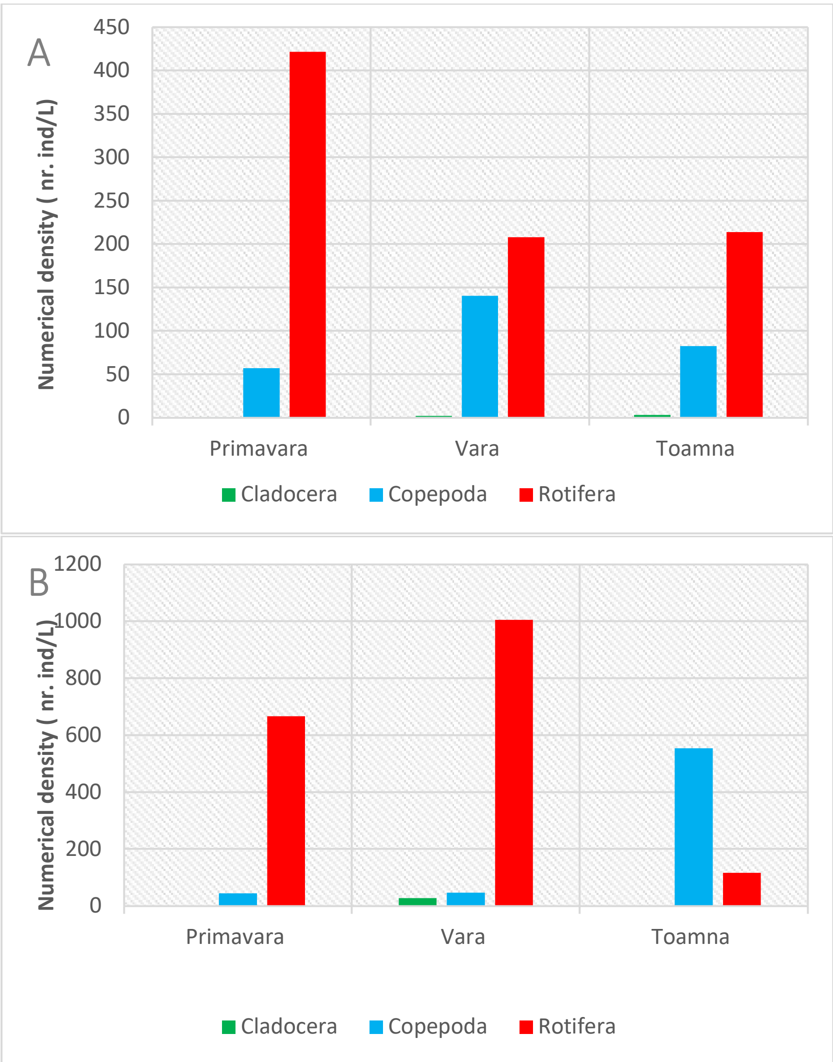


Figure. 3.40. Seasonal distribution of zooplankton density in the Uzlina study area in 2021 (A) and 2022 (B)

3.2.4.2. Seasonal distribution of density in biomass

In Carasuhat, zooplankton biomasses reached 24.14 mg ww/L in 2021 and 35.87 mg ww/L in 2022. In 2021, rotifers dominated with 8.80 mg ww/L (over 36% of total), followed by cladocerans at 8.54 mg ww/L (35.41%). Copepod biomass was 6.79 mg ww/L (28%). In 2022, cladocerans exhibited the highest biomass (17.58 mg ww/L, 49% of total), followed by rotifers at 10.14 mg ww/L (10% of total), and copepods at the lowest, 8.13 mg ww/L (22.8% of total). Zooplankton biomass peaked in summer and hit its low in autumn in 2021, following the trend summer > spring > autumn. In 2022, the pattern shifted to autumn > spring > summer (Figure 3.41).

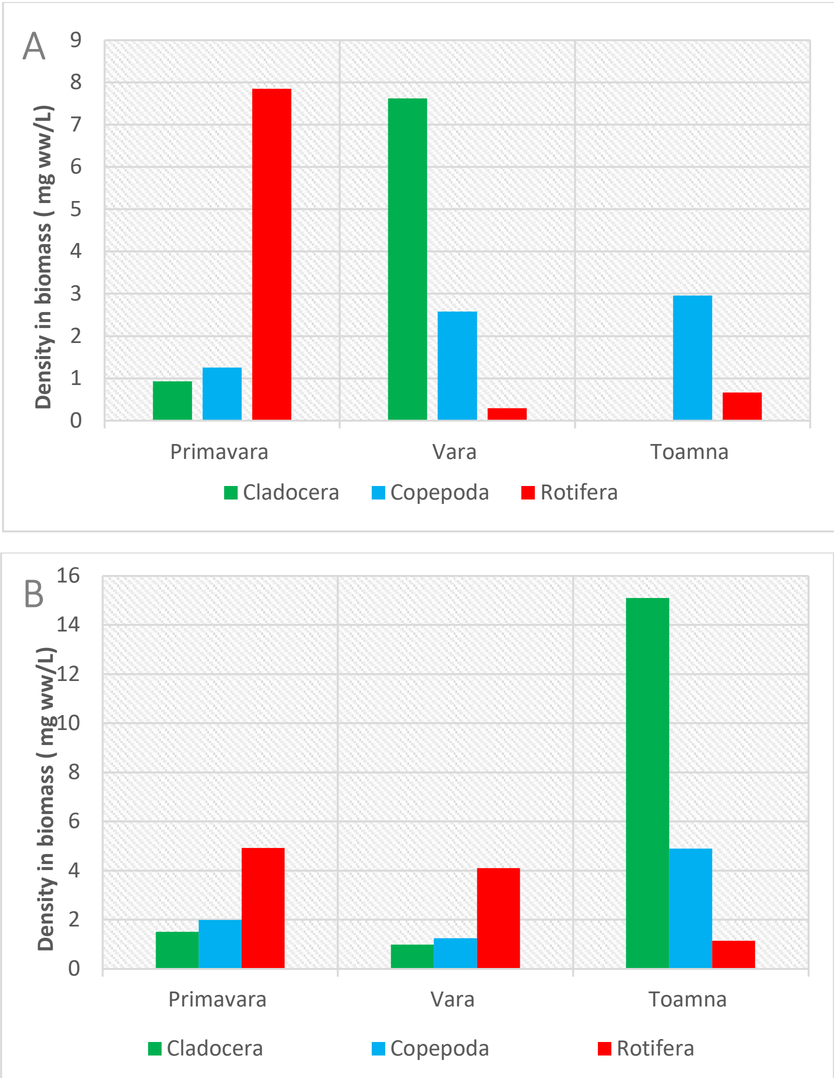


Figure. 3.41. Seasonal distribution of zooplankton density in biomass in the Carasuhat in 2021 (A) and 2022 (B)

Zooplankton biomasses in the Zaghen study area were significantly higher, reaching 319.72 mg ww/L in 2021 and 58.04 mg ww/L in 2022. Cladocerans dominated in 2021 with

288.24 mg ww/L (over 90% of total), while copepod biomass was 29.22 mg ww/L (9.13% of total); by 2022, copepods had the highest biomass at 56.96 mg ww/L (98.14% of total), followed by rotifers at 0.85 mg ww/L (1.48% of total). Zooplankton biomass peaked in 2021 summer (294.56 mg ww/L, mainly cladocerans), declining in spring, and followed summer > autumn > spring. In 2022, the highest biomass of 47.56 mg ww/L occurred in spring, primarily due to copepods (over 98%) (Figure 3.42).

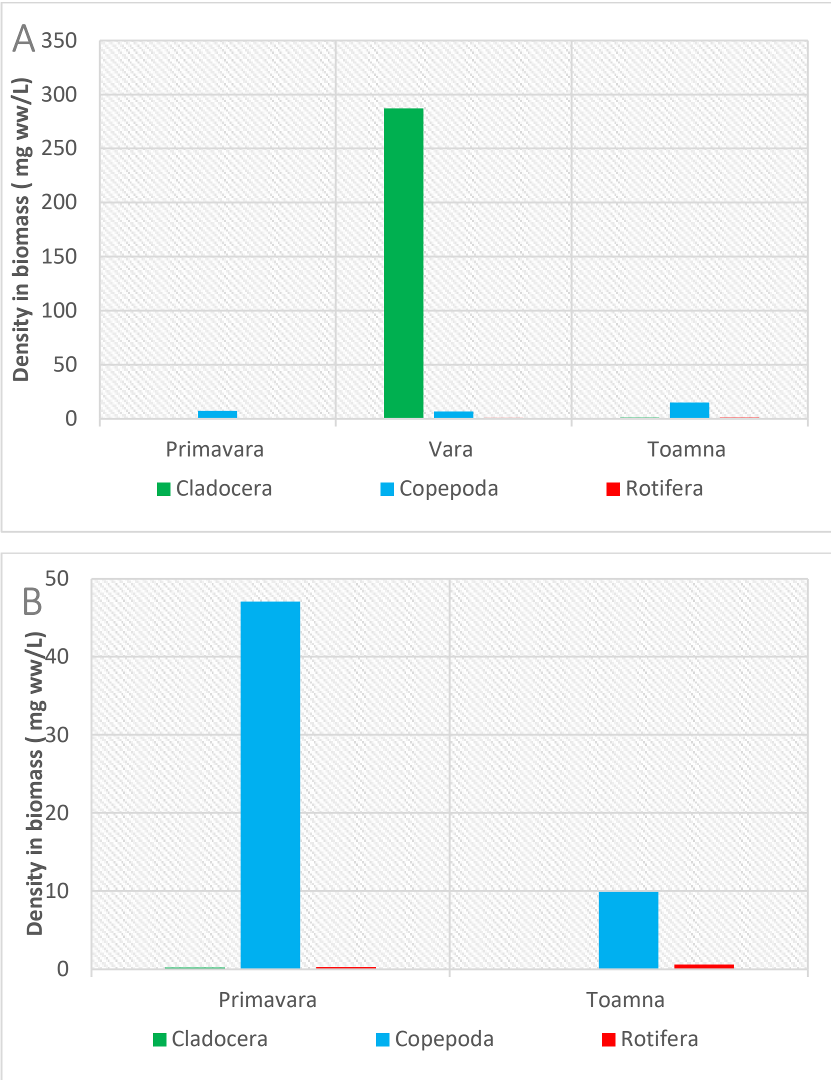


Figure. 3.42. Seasonal distribution of zooplankton density in biomass in the Zaghen in 2021 (A) and 2022 (B)

Figure 3.43 depicts zooplankton density and biomass variations in Uzlina during 2021 and 2022, with lower values than other study areas. Zooplankton biomass increased from 15.78 mg ww/L in 2021 to 24.19 mg ww/L in 2022. Rotifers dominated in 2021 (64%), while copepods (32%) and cladocerans (3%) also contributed. In 2022, rotifer biomass decreased

(50%), copepod biomass increased (44%), and cladocerans had the lowest value (less than 4%). Biomass followed autumn > spring > summer in 2021 and autumn > summer > spring in 2022.

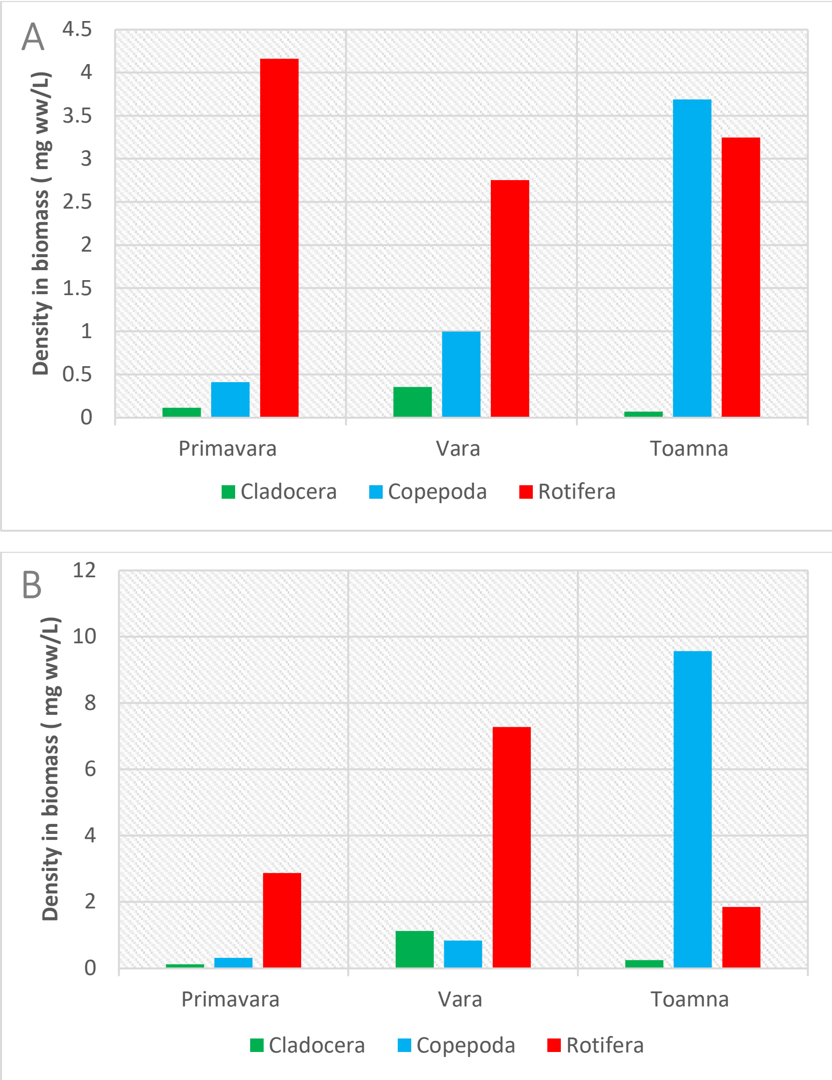


Figure. 3.43. Seasonal distribution of zooplankton density in biomass in the Uzlina in 2021 (A) and 2022 (B)

In the Zaghen area, *Moina brachiata* recorded 1281.2 ind/L in July 2021, with a corresponding biomass of 287 mg ww/L. In the same area, during autumn 2021, *Keratella valga*, exhibited 611.93 ind/L. Lake Uzlina had nauplii larvae with an average density of 364.40 ind/L in October 2022. In the Carasuhat area, *Pompholyx sulcata* had 307.43 ind/L in March 2021, while *Bosmina longirostris* had 266.42 ind/L in October 2022. Biomass density included the same species and added *Megacyclops viridis* (11.80 mg ww/L) and *Diaphanosoma brachiurum* (7.46 mg ww/L)

3.3. Identification of key drivers influencing zooplankton communities

Following the **Pearson correlation** analysis between the physical-chemical parameters in the study areas, significant differences were identified ($p > 0.05$). The Pearson correlation values that exceeded the threshold of 0.75 are as follows: chlorophyll "a" / conductivity - positive correlation with a value of r = 0.85; chlorophyll "a" / perimeter - negative correlation with a value of r = -0.8; conductivity / perimeter - negative correlation with a value of r = -0.94; area / perimeter - positive correlation with a value of r = 0.87.

The results of **non-metric Multi-Dimensional Scaling (nMDS) analysis**.graphically represented in Figure 3.51, illustrates that chlorophyll "a" and nutrient concentrations in the Zaghen area, surface and depth in the Carasuhat area, as well as transparency and heavy metals in the Uzlina area, were the parameters of greatest importance in differentiating the three clusters.

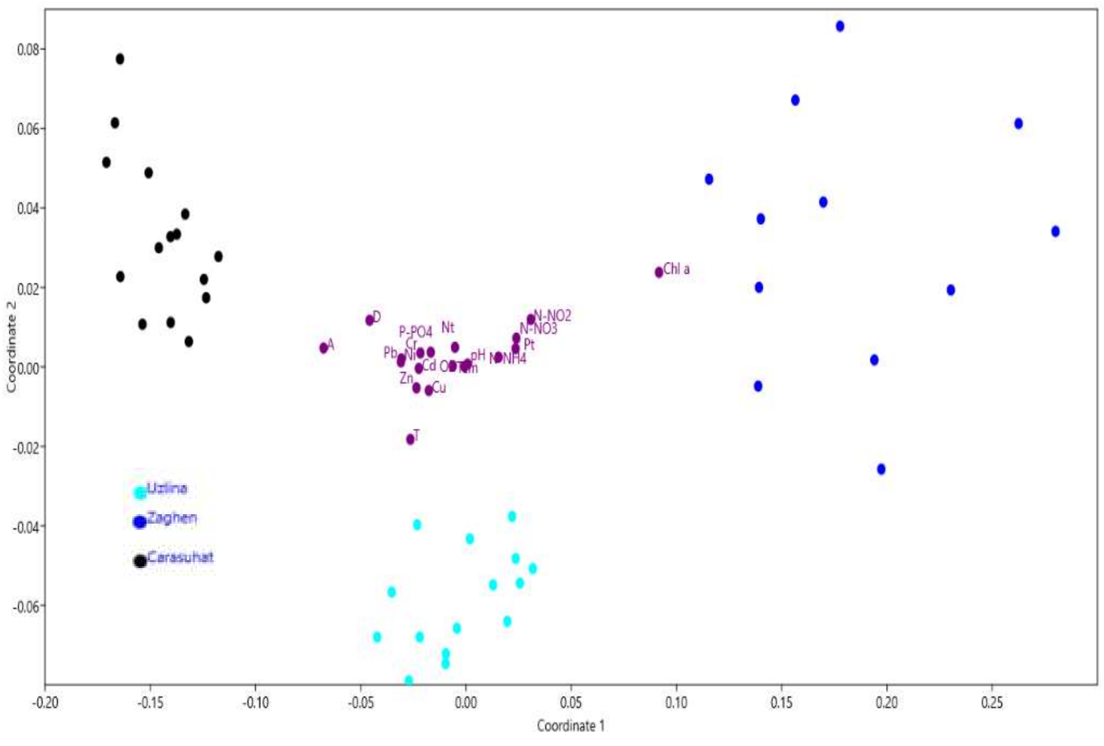


Figure 3.51. nMDS Analysis based on morphometric and physico-chemical variables within the ecological systems of Carasuhat, Zaghen, and Uzlina, during the period from March 2021 to October 2022. *The following state variables were considered in the graph: T = transparency, A = area, D = depth, Tem = temperature, Chl a = chlorophyll a, O = dissolved oxygen, pH, nutrients (Nt = total nitrogen, N-NH4 = ammonium, N-NO3 = nitrate, N-NO2 = nitrite, Pt = total phosphorus, P-PO4 = soluble orthophosphates), heavy metals (Cu = copper, Pb = lead, Cd = cadmium, Cr = chromium, Ni = nickel, Zn = zinc).*

The nMDS ordination diagram of the analyzed stations over the two years of the study, based on morphometric, physico-chemical parameters, and numerical densities of zooplankton species (Figure 3.52), highlighted the functional space occupied by species in the studied lakes.

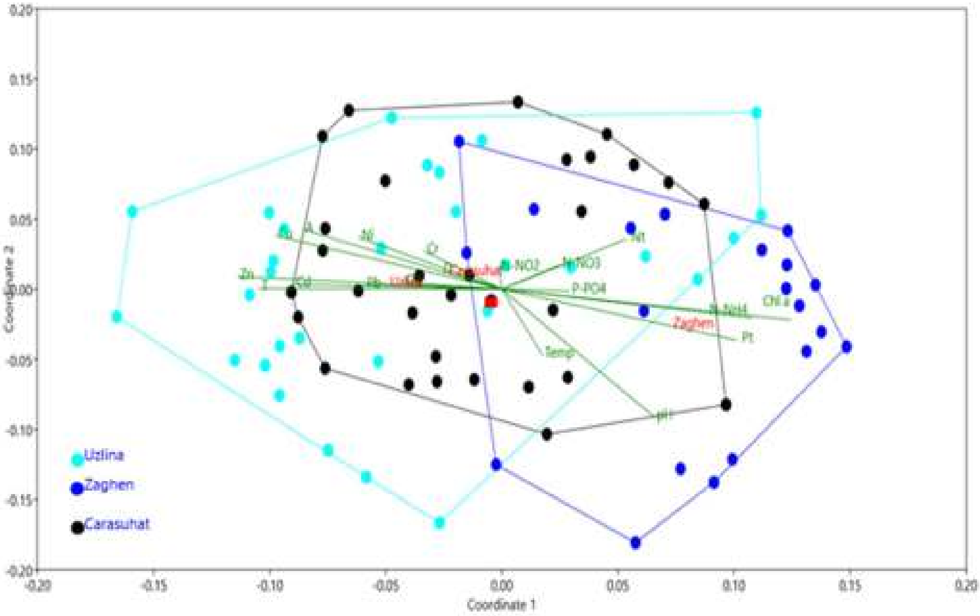


Figure 3.52. nMDS ordination diagram based on morphometric, physical and chemical parameters, and numerical densities of zooplankton species

The PCA (Principal Component Analysis) results (Figure 3.54. A and B) validate the nMDS findings. The dataset of 19 environmental factors accounted for primary variations in species composition. Species such as *Moina brachiata, Keratella valga*, nauplii larval stages, *Pompholyx sulcata*, and *Polyarthra vulgaris* emerged as key influencers, with high loadings driving significant data variation along the first axis. Likewise, *Brachionus forficula*, nauplii, *Bosmina longirostris*, *Diaphanosoma brachiurum*, and *Filinia longiseta* stood out with substantial loadings along the second axis. On the third axis, nauplii larval stages, *Asplanchna priodonta*, and *Polyarthra vulgaris* demonstrated high loadings as primary influencers.

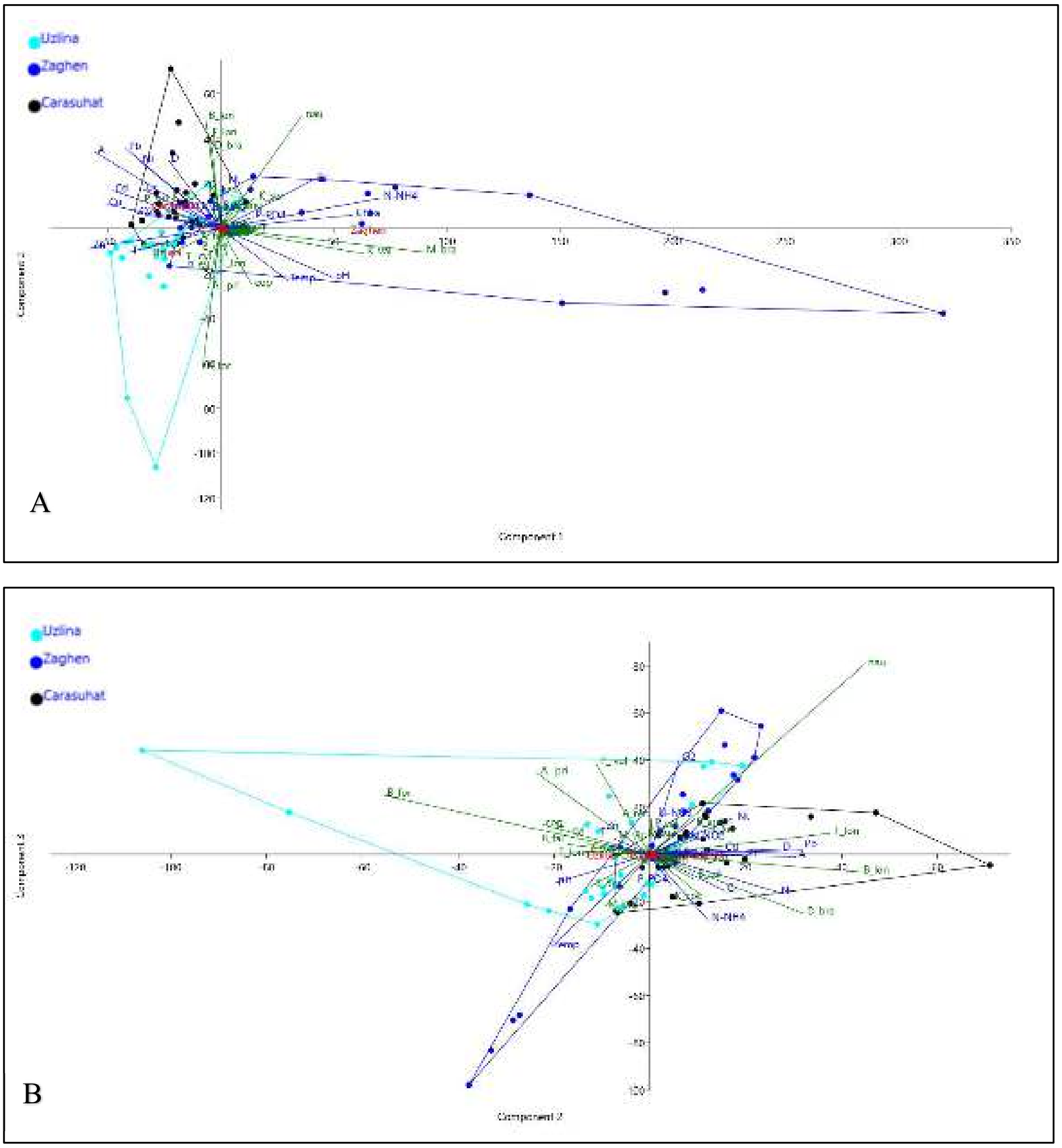


Figure 3.54. PCA Analysis in the studied aquatic systems based on numerical density and environmental variables between component 1 and component 2 (A), and between component 2 and component 3 (B).

The redundancy analysis (RDA) and variance partitioning highlight the main controlling factors that influence zooplankton communities and their relative contributions.

Along the first two RDA axes, the spatial location **PCNM (Principal Coordinates of Neighbor Matrices)** independently accounted for 3.5% of the community variation (Figure 3.56). Nutrients explained 73% of the variation (Figure 3.57), while heavy metal concentrations could account for 5.5% of the total variation (Figure 3.58). All effects were statistically significant at $\alpha = 0.05$. The species that had the highest scores in the RDA analysis included larval stages of nauplii and copepods, filter-feeding rotifers such as *Keratella valga, Keratella* *serrulata, Pompholyx sulcata, Polyarthra vulgaris, Filinia longiseta, Brachionus calyciflorus, Keratella cochlearis, Brachionus angularis*, the predatory rotifer *Asplanchna priodonta*, and filter-feeding cladocerans like *Moina brachiata* and *Diaphanosoma brachiurum*.

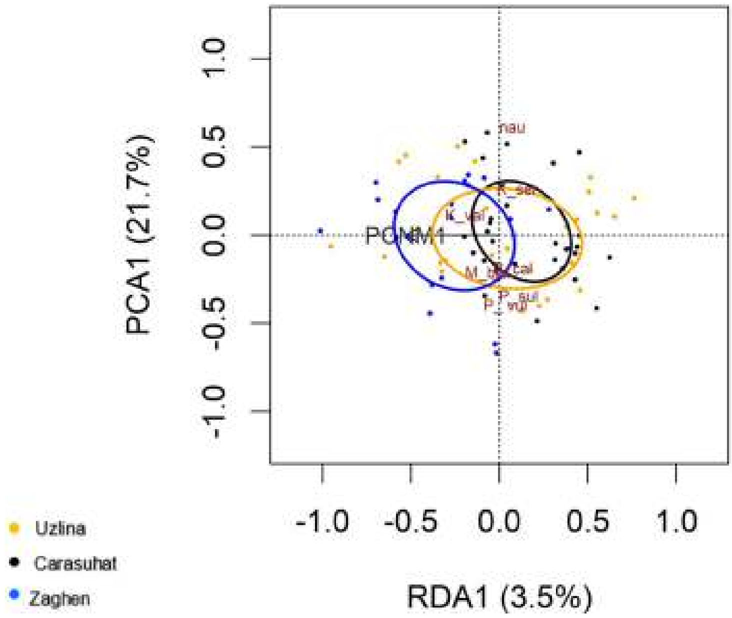


Figure 3.56. Redundancy Analysis (RDA) diagram for numeric density and the spatial variable PCNM. *Abbreviations used in the figure: nau, nauplii; cop, copepodite; K_ser, Keratella serrulata; K_val, Keratella valga; M_bra, Moina brachiata; B_cal, Brachionus calyciflorus; P_vul, Polyarthra vulgaris; P_sul, Pompholyx sulcata.*

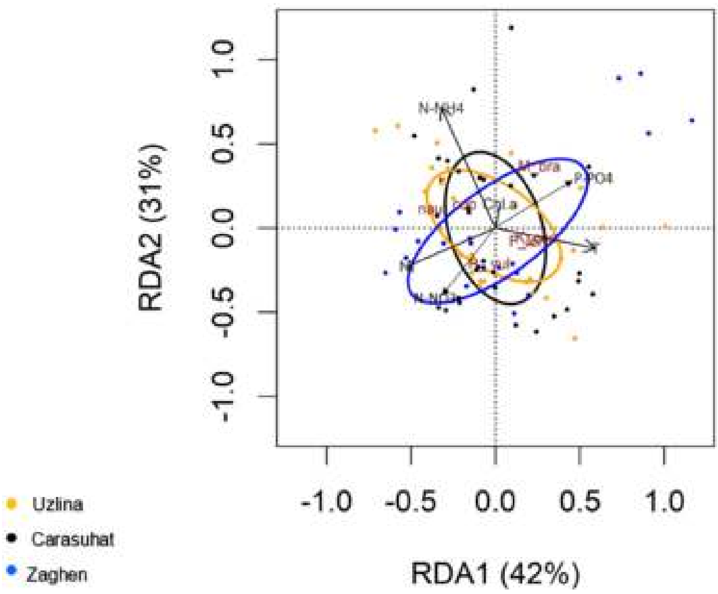


Figure 3.57. RDA Analysis of numeric density and concentrations of nutrients, chlorophyll "a," and transparency. *Abbreviations used in the figure: nau, nauplii; cop, copepodite; M_bra, Moina brachiata; K_coc, Keratella cochlearis; F_lon, Filinia longiseta; P_vul, Polyarthra vulgaris.*

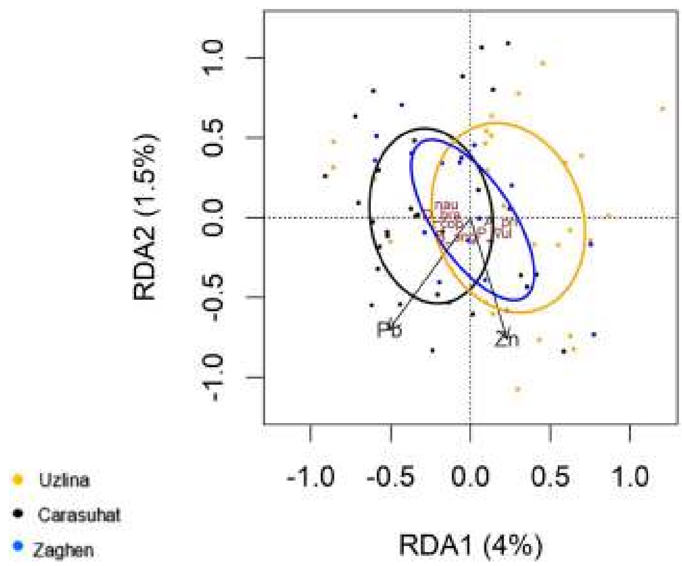


Figura 3.58 Analiza RDA a densității numerice și concentrațiile metalelor grele Abrevierile folosite în figură: nau, nauplii; cop, copepodit; *P_vul, Polyarthra vulgaris; B_ang, Brachionus angularis; A_pri, Asplanchna priodonta*; *D_bra, Diaphanosoma brachiurum*

The variance partitioning analysis showed significant spatial structuring (pcnm) of zooplankton communities (p = 0.007), explaining 2% of composition variation. Nutrient concentrations, chlorophyll "a," and transparency had a highly significant independent effect (p = 0.001), explaining 14% of community structure variation. Surprisingly, Pb and Zn concentrations contributed significantly to zooplankton structure (p = 0.006), explaining 2% of variation (Figure 3.59). Combined spatial and physico-chemical variables explained 4% of variation, while all variables combined explained 3%, indicating weak autocorrelation between nutrients and heavy metals. Unexplained residual variation was 75%.

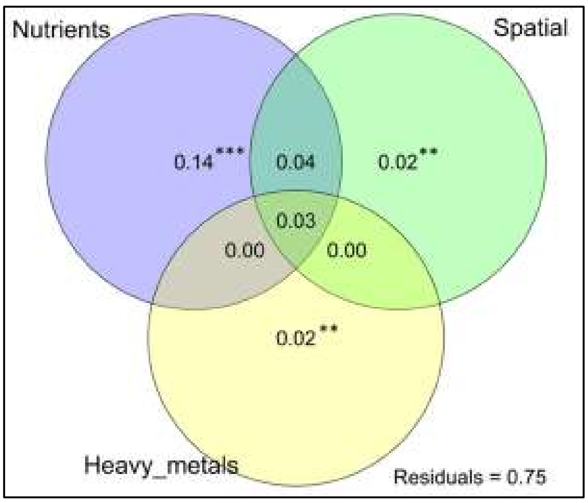


Figure 3.59. Variance partitioning analysis in zooplankton numerical density

The redundancy analysis and variance partitioning in biomass densities generally reveal the same trends as explained in the case of numeric densities, with a few peculiarities. In this case, it is observed that Cd (Cadmium) is also a parameter significantly influencing the structure of zooplankton communities, and the effect of heavy metals explains 4% of the variance in biomass densities in the studied areas (Figure 3.63). The species that had the highest scores in the RDA analysis based on biomass densities were: the rotifers *Asplanchna priodonta, Polyarthra vulgaris, Pompholyx sulcata,* the cladoceran *Chydorus sphaericus, Diaphanosoma brachiurum, Moina brachiata, Bosmina longirostris*, and the cyclopoid copepods *Macrocyclops albidus, Megacyclops viridis, Cyclops vicinus.* Figures 3.60- 3.62).

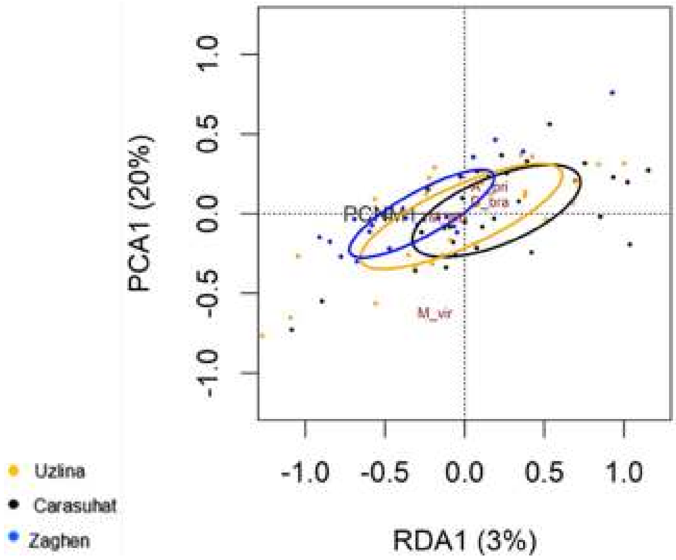


Figure 3.60. Redundancy Analysis (RDA) of biomass density and spatial variable PCNM. *Abbreviations used in the figure: A_pri, Asplanchna priodonta; D_bra, Diaphanosoma brachiurum; M_alb, Macrocyclops albidus; M_vir, Megacyclops viridis;*

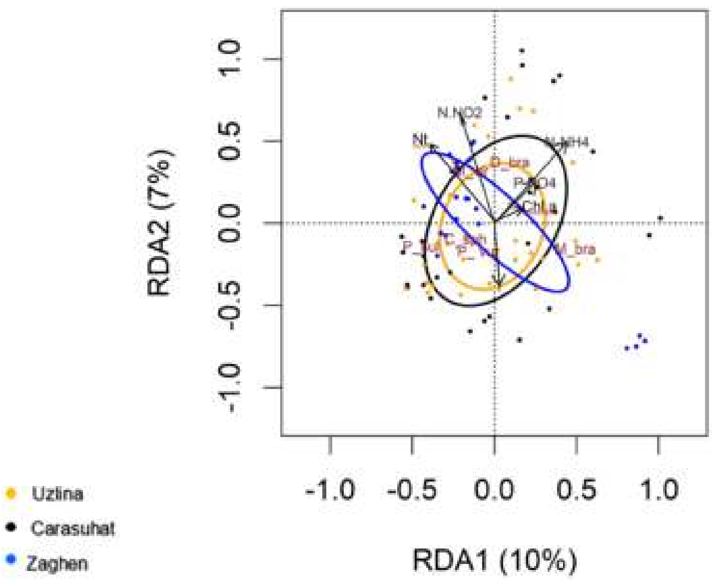


Figure 3.61. Redundancy Analysis (RDA) of biomass density, nutrient concentrations, chlorophyll "a, " and water transparency. *Abbreviations used in the figure: M_vir, Megacyclops viridis; D_bra, Diap*

hanosoma brachiurum; M_bra, Moina brachiata; P_vul, Polyarthra vulgaris; P_sul, Pompholyx sulcata; C_sph, Chydorus sphaericus.

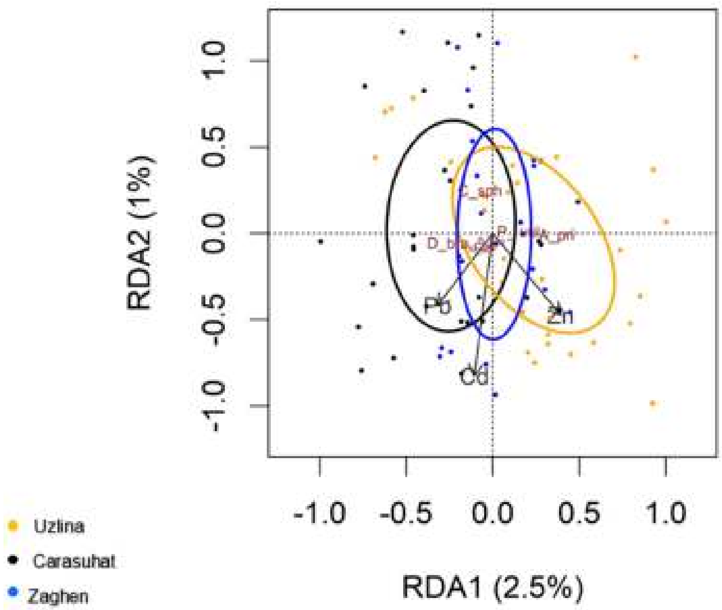


Figure 3.62. RDA analysis of biomass density and heavy metal concentrations. *Abbreviations used in the figure: C_sph, Chydorus sphaericus; D_bra, Diaphanosoma brachiurum; P_vul, Polyarthra vulgaris; A_pri, Asplanchna priodonta; B_log, Bosmina longirostris; M_alb, Macrocyclops albidus, C_vic, Cyclops vicinus.*

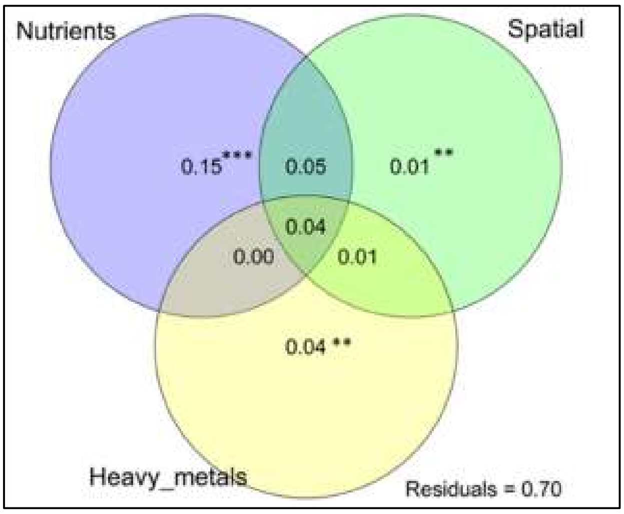


Figure 3.63. Variance partitioning analysis of zooplankton biomass density.

3.5. Assessment of water sample toxicity from the Zaghen study area using aquatic ecotoxicology techniques

In the Zaghen study area of March 2023, heavy metal concentrations were as follows: Zinc and copper aligned with the first quality class (very good ecological state). Cadmium, nickel, and chromium fell into the second quality class (good ecological state). At station 1, situated near residential areas, lead concentration fell into the third quality class (moderate ecological state), indicating potential human impact.

The toxicity test results (Table 3.19), revealed varying sensitivities between the two species. Heavy metals from stations 1 and 2 had a stronger toxic impact on *Thamnocephalus platyurus* compared to *Daphnia magna*, aligning with previous research (Persoone et al., 2003).

Significant values of low toxicity leading to mortality were recorded only for waters collected from the first two stations: *Daphnia magna* (48-hour exposure): 15% mortality, *Thamnocephalus platyurus* (24-hour exposure): 20% mortality. To assess toxicity, Toxicity Units (TUs) were calculated based on Probit-derived EC50 values and confidence limits, as per the approach of Persoone et al., 2003. The TU value for a specific compound represents the concentration at which a 50% effect (EC50 of wastewater expressed as % dilution) occurs for a specific biological endpoint. However, the TU-based toxicity score couldn't be calculated for our context due to mortality rates below 50% for both species.

Table 3.19. Determination of acute toxicity effects (EC%) in *Daphnia magna* and *Thamnocephalus platyurus* in Zaghen, March 2023.

Water samples	EC % *D. magna* 48 h	EC % *T. platyurus* 24 h
1	15	20
2	15	20
3	10*	10*
4	10*	10*
5	10*	10*

*Obs. 10% mortality is accepted for Control validation (microbiotests.com)

Despite observing low mortality rates (below 50%) and considering the water samples as highly non-toxic, particularly for the first two stations, our findings underscore the significance of ongoing biological monitoring. Additionally, to assess contamination risks in the aquatic ecosystems of the Danube Delta Biosphere Reserve, we recommend the incorporation of additional microbiotests involving algae and bacteria. These tests could aid in evaluating the risk of heavy metal contamination or other hazardous pollutants in the freshwater aquatic environment of the Danube Delta. Such supplementary tests could also contribute to assessing the effectiveness of wastewater treatment systems discharged into the entire Danube

River basin, including the city of Tulcea, as well as other direct pollution sources within the Danube Delta. This holistic approach would offer better protection for the deltaic ecosystems.

CHAPTER 4. GENERAL CONCLUSIONS AND PERSPECTIVES

In the context of this doctoral research theme, we have investigated how the ecological restoration areas Carasuhat and Zaghen have recovered following the ecological reconstruction process, and how their ecological parameters approach those of a natural lake - Lake Uzlina, within the Danube Delta Biosphere Reserve. We monitored the dynamics of the zooplankton community and assessed the ecological condition of these areas, based on 80 samples collected during 6 expeditions carried out between March 2021 and October 2022. Based on the interpretation of the obtained results, the following conclusions have been formulated:

Water temperature exhibited normal seasonal variations, with differences observed between the study areas. The highest temperatures were recorded in July 2021. The overall trend for water pH was to maintain an alkaline level. Electrical conductivity of water varied significantly, highlighting its natural variability. Higher values were measured in the Zaghen study area. Lower dissolved oxygen values observed in Zaghen could be attributed to higher nutrient loading and reduced water circulation. Chlorophyll "a", measurements highlighted the highest values in the Zaghen study area.

Nitrogen and phosphorus concentrations across the Carasuhat, Uzlina, and Zaghen study areas, evidentiated that the aquatic ecosystems studied generally exhibited ecological conditions ranging from very good to poor, based on the study zone and nutrient category.The very good and good ecological states were consistently associated with ammonium nitrogen and total phosphorus concentrations, whereas nitrite, nitrate, total nitrogen, and orthophosphates exhibited more varied values, indicating conditions spanning from very good to poor.

The analysis of cadmium, zinc, copper, lead, nickel, and chromium concentrations in water samples reveals predominantly favorable ecological conditions, falling within quality classes I and II. Occasional exceedances of limits occurred for lead, nickel, chromium, and cadmium, in a limited number of samples. Notably, elevated heavy metal concentrations were observed in Carasuhat and Lake Uzlina areas, likely influenced by the Danube River basin's metal-rich water and sediment. In contrast, the isolated Zaghen study area displayed a distinct pattern. Despite fluctuations, the overall ecological status remains good, emphasizing the importance of continuous heavy metal monitoring to uphold water quality.

Reconnecting the two wetland areas to the Danube River hydrological regime had a positive impact on the zooplankton community, allowing the development of a community similar to that of permanent ecosystems within the Danube Delta Biosphere Reserve, with the Carasuhat area exhibiting this aspect predominantly. These findings provide new perspectives on ecological reconstruction and aquatic ecosystem management and can be valuable for biodiversity conservation and improving aquatic environmental quality.

The nMDS analysis highlighted that chlorophyll "a" and nutrient concentrations in the Zaghen area, water surface and depth in the Carasuhat area, as well as water transparency and heavy metal content in the Uzlina area, were significant parameters in distinguishing the three zones. The RDA analysis demonstrated that PCNM variables, chlorophyll "a", total nitrogen, ammonia, pH, nitrites, transparency, orthophosphates, cadmium, lead, and zinc significantly influenced the composition of zooplankton communities in the studied aquatic systems.

Toxicity test results indicate very low water toxicity in the Zaghen study area. In the future, it is essential to focus on researching the relationship between water and sediment metal levels, bioaccumulation factors (BAFs), and metal levels in different zooplankton species. This would allow us to identify the most sensitive bioindicators, thereby facilitating more effective monitoring and precise assessment of pollution impacts on aquatic ecosystems.

Future Research Directions

The originality of the study lies in its interdisciplinary approach to the three study areas and the evaluation through both physical-chemical and biological parameters. The research contributes significantly to the field of ecology and aquatic ecosystem management, providing a comprehensive perspective on the ecological status and processes occurring in ecological restoration areas. The data concerning the structure and dynamics of zooplankton communities and the ecological status of the Carasuhat and Zaghen ecological restoration areas and Lake Uzlina, as presented in the thesis, can offer valuable information as a basis for developing and implementing appropriate management and conservation measures. In the future, for a more comprehensive ecotoxicological assessment alongside primary consumers such as crustaceans, other species like algae and bacteria (representing producers and consumers) could be added. This would encompass all levels of a food chain within the ecosystem. Ecotoxicological assessment plays a crucial role in determining the risk of contamination to the aquatic environment in the Danube Delta Biosphere Reserve. The results of the conducted studies are highly applicable, both for public authorities and the realm of future research. In this context, we propose long-term monitoring of the two areas to evaluate the progress of ecological restoration, identifying potential issues and intervening where necessary. This approach ensures the proper conservation and protection of these fragile and valuable ecosystems.

REFERENCES

1. Anas, M.U.M., Scott, K.A., Wissel, B. (2013). Suitability of presence vs. absence indicator species to characterize stress gradients: lessons from zooplankton species of boreal lakes. Ecological Indicators, 30, 90–99. https://doi.org/10.1016/j.ecolind.2013.
2. Berzins, B., Pejler, B. (1989). Rotifer occurrence and trophic degree, Hydrobiologia, 182, 171–180.
3. Błędzki, L-A., Rybak, J-I. (2016). Freshwater Crustacean Zooplankton of Europe Cladocera and Copepoda (Calanoida, Cyclopoida): Key to species identification, Springer International Publishing Switzerland, ISBN 978-3-319-29870-2, 923 pp
4. Bondar, C. (1990). Hydrological study. Hydrotechnical works for improving the Razim- Sinoe lake complex exploiting conditions. Study Contract DDNI Tulcea - INMH Bucharest, 229.
5. Brețcan, P., Murărescu, O., Samoilă, E., Olimpia, P., Street, R.C.I., 2008. The modification of the ecological conditions in the Razim-Sinoie lacuster complex as an effect of the anthropic intervention, XXIV th Conference of the Danubian Countries Bled, Slovenia, 12 .
6. Caroni, R., Irvine, K. (2010). The potential of zooplankton communities for ecological assessment of lakes: redundant concept or political oversight? în: Biology and environment: proceedings of the royal Irish Academy. Jstor, 35–53. https://doi.org/10.3318/bioe.2010.110.1.35.
7. Carpenter, S.R., Kitchell J.(1996). The trophic cascade in lakes. Cambridge Univeristy Press, 385 pp.
8. Coleman, J.M., Huh, O.K., Braud, D.(2008). Wetland loss in world deltas. Journal of Coastal Resources, 24, 1–14
9. Damian-Georgescu, A. (1963). Crustacea, Copepoda, Fam.Cyclopidae (forme de apă dulce). Fauna Republicii Populare Romîne. Editura Academiei Republicii Socialiste România, Bucureşti, 205 pp

10. Directiva Cadru Apă 2000/60/CE, a Parlamentului European și a Consiliului din 23 octombrie 2000 de stabilire a unui cadru de politică comunitară în domeniul apei CE
11. Duggan, I.C., Özkundakci, D., David, B.O. (2020) Long-term zooplankton composition data reveal impacts of invasions on community composition in the Waikato lakes, New Zealand. Aquatic Ecology. https://doi.org/10.1007/s10452-020-09803-8
12. Dumitrescu, A. (2003). The Impact of the Social and Economic Policies on the Local People of the Danube Delta and the Necessary Measures, Scientific Annals of the Danube Delta Institute for Research and Development, 9, 75-83.
13. García-Chicote, J., Armengol, X., Rojo, C. (2018). Zooplankton Abundance: A Neglected Key Element in the Evaluation of Reservoir Water Quality. Limnologica, 69, 46–54.
14. Giosan, L., Constantinescu, S., Filip, F., Deng, B.(2013). Maintenance of large deltas through channelization: nature vs. humans in the Danube Delta, Anthropocene,1, 35–45.
15. Gómez-Baggethun, E., Tudor, M., Doroftei, M., Covaliov, S., Năstase, A., Onără, D.F., Cioacă, E. (2019). Changes in ecosystem services from wetland loss and restoration: An ecosystem assessment of the Danube Delta (1960–2010). Ecosysistem Services, 39, 1-17.
16. Gomoiu, M-T. (1996). Facts and remarks on the Danube Delta. Geo-Eco-Marina, 1, 99-113.
17. Gulati, R.D. (1983). Zooplankton and its grazing as indicators of trophic status in Dutch lakes. Environmental Monitoring Assessment, 3, 343–354.
18. Haberman J., Haldna M. (2014). Indices of zooplankton community as valuable tools in assessing the trophic state and water quality of eutrophic lakes: long term study of Lake Vortsjarv. Journal of Limnology 73(2), 263–273. https://doi.org/10.4081/jlimnol.2014.828
19. Hammer, Ø., Harper, D.A.T., Ryan, P.D. (2001). PAST: Paleontological statistics software package for education and data analysis, Palaeontol Electron 4: 1. 31.
20. Kuczynska-Kippen, N., Spoljar, M., Zhang, C., Pronin, M. (2020). Zooplankton functional traits as a tool to assess latitudinal variation in the northern-southern temperate European regions during spring and autumn seasons. Ecological Indicators 117, 106629, https://doi.org/10.1016/j.ecolind.2020.

21. Lampert, W., Sommer, U. (1997). Limnoecology, The ecology of lakes and streams, Oxford University Press, New York.
22. Lampert, W. (1997). Zooplankton research: the contribution of limnology to general ecological paradigms. Aquatic Ecology, 31, 19–27.
23. May, L., O'Hare, M. (2005) Changes in rotifer species composition and abundance along a trophic gradient in Loch Lomond, Scotland, UK. Hydrobiologia 546, 397–404. https://doi.org/10.1007/s10750-005-4282-3
24. Morgan, G.A., Barrett, K.C., Leech, N. L, Gloeckner (G.W. 2019). IBM SPSS for Introductory Statistics Use and interpretation, Ed. Taylor&Francis, 252p.
25. Naselli-Flores, L., Rossetti, G. (2010) Fifty years after the homage to Santa Rosalia: old and new paradigms on biodiversity in aquatic ecosystems, în: Santa Rosalia 50 years on Developments in Hydrobiology, 213, Springer, Netherlands, 246 pp.
26. Negrea, Ş. (1983). Cladocera. Fauna Republicii Socialiste România, Editura Academiei Republicii Socialiste România, Bucuresti, 399 pp.
27. Niculescu, S., Lardeux, C., Hanganu, J. (2017). Alteration and Remediation of Coastal Wetland Ecosystems in the Danube Delta. A Remote-Sensing Approach. Coastal Research Library, Springer International Publishing, 21 (17), 513-554, 10.1007/978-3-319-56179-0ff. ffhal-01598043
28. Oosterberg, W., Staras, M., Bogdan, L., Buijse, A. D., Constantinescu, A., Hanganu, J., Ibelings, B. W., Menting, G. A. M., Năvodaru, I., Torok, L. (2000) Ecological Gradients in the Danube Delta-Present State and Man-Induced Changes. RIZA Report Nr. 2000.015, RIZA the Netherlands, DDNI Romaia and Danube Delta Biopshere Reserve Authority, 166 pp.
29. Ordin nr. 161 din 16 februarie 2006 pentru aprobarea Normativului privind clasificarea calităţii apelor de suprafaţa în vederea stabilirii stării ecologice a corpurilor de apă, publicat în MONITORUL OFICIAL nr. 511 din 13 iunie 2006, https://legislatie.just.ro/Public/DetaliiDocumentAfis/72574
30. Persoone G, Marsalek B, Blinova I, Törökne A, Zarina D, Manusadzianas L, Nalecz-Jawecki G, Tofan L, Stepanova N, Tothova L, Kolar B. (2003). A practical and user-friendly toxicity classification system with microbiotests for natural waters and wastewaters. Environ Toxico, 18(6), 395-402. doi: 10.1002/tox.10141.

31. Romanescu, G. (1999). The Danube Delta: Some Hydromorphodynamic Aspects: Deltaic Changes During the Modern and Contemporary Historical Stages. Suceava University Press, Suceava, Romania, 164 pp.
32. Romanescu, G., Stoleriu, C. (2014). Anthropogenic interventions and hydrological-risk phenomena in the fluvial-maritime delta of the Danube (Romania). Ocean and Coastal Management, 102, 123–130.
33. Rudescu, L. (1960). Trochelminthes (vol II, Fascicula II), Fauna Republicii Populare Romîne, Editura Academiei Republicii Populare România, București, 1192 pp.
34. Scheffer, M. (998). Ecology of shallow lakes. Chapman & Hall, UK, ISBN 0412749203, 346 pp.
35. Schneider, E. (2010). Floodplain restoration of large European rivers, with examples from the Rhine and the Danube. In: Restoration of Lakes, Streams, Floodplains, and Bogs in Europe. Springer, Dordrecht, 185–223.
36. Sladecek, V. (1983). Rotifers as indicators of water quality. Hydrobiologia, 100 (1), 169–201.
37. Spoljar, M., Drazina, T., Lajtner, J., Duic, S.M., Radanovic, I., Wallace, R., Matulic, D., Tomljanovic, T. (2018). Zooplankton assemblage in four temperate shallow waterbodies in association with habitat heterogeneity and alternative states, Limnologica, 71(4), 51–61. https://doi.org/10.1016/j.limno.2018.
38. SR EN ISO 5667-1 (2007). Calitatea apei. Prelevare. Partea 1: Ghid general pentru stabilirea programelor şi a tehnicilor de prelevare
39. Stamou, G., Katsiapi, M., Moustaka-Gouni, M., Michaloudi, E. (2019). Trophic state assessment based on zooplankton communities in Mediterranean lakes. Hydrobiologia, 844, 83–103, https://doi.org/10.1007/s10750-018-3880-9.
40. Staraş, M. (1994). Studiu sectorial privind managementul resurselor pescaresti din Rezervatia Biosferei Delta Dunarii. Banca Europeană pentru reconstructie si dezvoltare si Administratia Rezervației Biosferei Delta Dunării. Seminarii și Workshopuri – Tulcea Sept. 1994
41. Staraş, M., Baboianu, G. (2005). Human induced changes and rehabilitation measures in the Danube Delta, Romania. ICAM Dossier 3, 280–286.
42. Tofan, L., Niță, V., Nenciu, M., Coatu, V., Lazăr, L., Damir, N., Vasile, D., Popoviciu, D.R., Brotea, A.-G., Curtean-Bănăduc, A.M., Avramescu, S., Aonofriesei, F., 2023 - Multiple Assays on Non-Target Organisms to Determine the Risk of Acute

Environmental Toxicity in Tebuconazole-Based Fungicides Widely Used in the Black Sea Coastal Area. Toxics 11, 597. https://doi.org/10.3390/toxics11070597

43. Tudor, I.-M., Ibram, O., Török, L., Covaliov, S., Doroftei, M., Tudor, M., Năstase, A., Năvodaru, I. (2015) Metode de monitorizare a indicatorilor biologici în ecosistemele acvatice ale Deltei Dunării. Cap. 3. pp 95-123. În Tudor I.-M. (ed.), Ghid metodologic de monitorizare a factorilor hidromorfologici, chimici şi biologici pentru apele de suprafaţă din Rezervaţia Biosferei Delta Dunării, ISBN 978-606-93721-8-0, Editura Centrul de Informare Tehnologică Delta Dunării, Tulcea, România, 143 pp.
44. Uhel, R., Spyropoulou, R., Breton, F., Beltrame, C., Arévalo, J., Richard, D., Gómez-Baggethun, E., Martín López, B., Lomas, P., Tomas, P., Ezzine, D., Nichersu, J., Marin, J. (2010). Ecosystem accounting and the cost of biodiversity losses: The case of costal Mediterranean wetlands. European Environmental Agency.