TRANSYLVANIAN REVIEW OF SYSTEMATICAL AND ECOLOGICAL RESEARCH

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Editors

Angela Curtean-Bănăduc, Erika Schneider-Binder & Doru Bănăduc

Sibiu – Romania
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Applied Ecology Research Center,
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International Association for Danube Research	"*Lucian Blaga*" University of Sibiu	Ecotur Sibiu Association	Applied Ecology Research Center of *LB*US	Broward College, Fort Lauderdale

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Scientifical Reviewers

IN MEMORIAM
Angela Curtean-Bănăduc
(1971 – 2023)

Tragically, the period of the preparation of the *Transylvanian Review of Systematical and Ecological Research* 2023 issues was the last period of life for one of its main founders and leaders since 1999, namely ***Angela Curtean-Bănăduc***, who passed away in Clermont-Farrand, France, on 1st November 2023. The others all over the world editors, reviewers, authors and friends of this journal wish to pay here an "in memoriam" loving tribute to someone who was both their very good friend and colleague and the much beloved life partner and wife of ***Doru***.

She was born in Orăștie (Transylvania, Romania) on 27 January 1971, into a caring family of intellectuals whose main educational moral rule, "everything you do, do it right!", deeply influenced her contented golden childhood and teenage years. This happy family and the location of their picturesque, multicultural native city, lying near the Carpathians where the legendary Sarmizegetusa Regia, capital of the ancient Dacians, over the last 2000 years beneficially influenced Romanian dreams and realities – if only to recall here a temple of the Romanian language, "Palia de la Orăștie", the monumental translation of the Old Testament into the Romanian language carried out in the 16th century – created the necessary milieu which inspired ***Angela*** to achieve something important with her life.

The vicinity of one of the largest Danube River tributaries, the scenic Mureș River, gave her the impulse to love the blue like in her gorgeous eyes, rivers, streams, and lakes. The chance to dream, imagine, organize, support and shape directly the lives of people of goodwill involved in the continental waters scientific understanding and protection, and the forces which uphold them, came through the professional opportunities afforded by her studies in chemistry at the "Nicolaus Olahus" Highschool of Orăștie, in ecology at the "Lucian Blaga" University of Sibiu, for her PhD in aquatic ecology at "Ovidius" University of Constanța, and her post-doctoral research in water resources management at "Costin C. Kirițescu" Romanian Academy National Institute of Economic Research in Bucharest, etc.

The water was symbolic for her beautiful blue life on this Blue Planet! Nature was her temple, home and support for many projects in ecology and biology. Macro-invertebrates and fish populate her written output, scientific episodes which are on permanent record in over 200 publications. To give just some examples of what ***Angela*** founded and/or led over the years with fabulous positive and honest energy, efforts and results: *Transylvanian Review of Systematical and Ecological Research* since 1999 including the *Wetlands Diversity* series; *Aquatic Biodiversity International Conference* since 2007; *Acta Oecologica Carpatica* since 2008; environmental *Ecotur Sibiu Association* since the end of the XX century; *Applied Ecology Research Center* in XXI century; *"Lucian Blaga" University of Sibiu Faculty of Sciences* where she worked in teaching and research since 1996, finally for 10 years as a Dean; etc.

Many devoted friends accompanied this genuinely extraordinary beautiful personal and professional life, the hundereds of editors, reviewers and authors all over the world of *Transylvanian Review of Systematical and Ecological Research* being among them.

A unique magic personal and professional match was achieved without a break from 1998 between the beloved ***Angela*** and her husband *Doru*, who not by chance pays his deep loving respect for an unbelievable fabulous shared lifetime experience.

A cruel illness took her too soon from this world, but till the end she encouraging smiled to us with her characteristic kindness and care.

All of us, who had the good fortune to meet and love ***Angela*** in this all too short life, and to benefit from her angelic heart and mind, will keep her in the sunny part of our memories forever!

Family, friends, colleagues and students wish ***Angela*** eternal blue waters on the other side, where sooner or later we shall all meet to again be happy together! God Bless her!

Transylvanian Review of Systematical and Ecological Research
all over the world Editors, Reviewers, Authors and Friends

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Preface

In a global environment in which the climate changes are observed from few decades no more only through scientific studies but also through day by day life experiences of average people which feel and understand allready the presence of the medium and long-term significant change in the “average weather” all over the world, the most comon key words which reflect the general concern are: heating, desertification, rationalisation and surviwing.

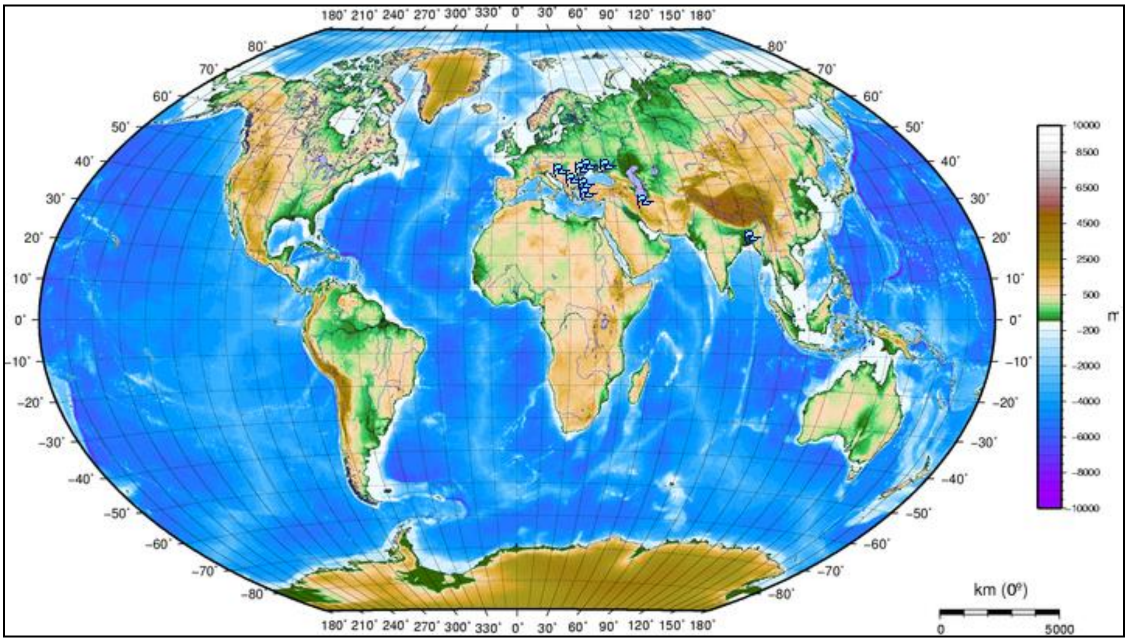
The causes, effects, trends and possibilities of human society to positively intervene to slow down this process or to adapt to it involve a huge variety of aproacess and efforts.

With the fact in mind that these aproaces and efforts shuld be based on genuine scientific understanding, the editors of the *Transylvanian Review of Systematical and Ecological Research* series launch three annual volumes dedicated to the wetlands, volumes resulted mainly as a results of the *Aquatic Biodiversity International Conference*, Sibiu/Romania, 2007-2017.

The therm wetland is used here in the acceptance of the Convention on Wetlands, signed in Ramsar, in 1971, for the conservation and wise use of wetlands and their resources. **Marine/Coastal Wetlands** – Permanent shallow marine waters in most cases less than six metres deep at low tide, includes sea bays and straits; Marine subtidal aquatic beds, includes kelp beds, sea-grass beds, tropical marine meadows; Coral reefs; Rocky marine shores, includes rocky offshore islands, sea cliffs; Sand, shingle or pebble shores, includes sand bars, spits and sandy islets, includes dune systems and humid dune slacks; Estuarine waters, permanent water of estuaries and estuarine systems of deltas; Intertidal mud, sand or salt flats; Intertidal marshes, includes salt marshes, salt meadows, saltings, raised salt marshes, includes tidal brackish and freshwater marshes; Intertidal forested wetlands, includes mangrove swamps, nipah swamps and tidal freshwater swamp forests; Coastal brackish/saline lagoons, brackish to saline lagoons with at least one relatively narrow connection to the sea; Coastal freshwater lagoons, includes freshwater delta lagoons; Karst and other subterranean hydrological systems, marine/coastal. **Inland Wetlands** – Permanent inland deltas; Permanent rivers/streams/creeks, includes waterfalls; Seasonal/intermittent/irregular rivers/streams/creeks; Permanent freshwater lakes (over eight ha), includes large oxbow lakes; Seasonal/intermittent freshwater lakes (over eight ha), includes floodplain lakes; Permanent saline/brackish/alkaline lakes; Seasonal/intermittent saline/brackish/alkaline lakes and flats; Permanent saline/brackish/alkaline marshes/pools; Seasonal/intermittent saline/brackish/alkaline marshes/pools; Permanent freshwater marshes/pools, ponds (below eight ha), marshes and swamps on inorganic soils, with emergent vegetation water-logged for at least most of the growing season; Seasonal/intermittent freshwater marshes/pools on inorganic soils, includes sloughs, potholes, seasonally flooded meadows, sedge marshes; Non-forested peatlands, includes shrub or open bogs, swamps, fens; Alpine wetlands, includes alpine meadows, temporary waters from snowmelt; Tundra wetlands, includes tundra pools, temporary waters from snowmelt; Shrub-dominated wetlands, shrub swamps, shrub-dominated freshwater marshes, shrub carr, alder thicket on inorganic soils; Freshwater, tree-dominated wetlands; includes freshwater swamp forests, seasonally flooded forests, wooded swamps on inorganic soils; Forested peatlands; peatswamp forests; Freshwater springs, oases; Geothermal wetlands; Karst and other subterranean hydrological systems, inland. **Human-made wetlands –** Aquaculture (e. g., fish/shrimp) ponds; Ponds; includes farm ponds, stock ponds, small tanks; (generally below eight ha); Irrigated land, includes irrigation channels and rice fields; Seasonally flooded agricultural land (including intensively managed or grazed wet meadow or pasture); Salt exploitation sites, salt pans, salines, etc.; Water storage areas, reservoirs/barrages/dams/impoundments (generally over eight ha); Excavations; gravel/brick/clay pits; borrow pits, mining pools; Wastewater treatment areas, sewage farms, settling ponds, oxidation basins, etc.; Canals and drainage channels, ditches; Karst and other subterranean hydrological systems, human-made.

The editors of the *Transylvanian Review of Systematical and Ecological Research* started and continue the annual sub-series (*Wetlands Diversity*) as an international scientific debate platform for the wetlands conservation, and not to take in the last moment, some last heavenly "images" of a perishing world …

This volume included variated original researches from diverse wetlands around the world.



The subject areas ( ) for the published studies in this volume.

No doubt that this new data will develop knowledge and understanding of the ecological status of the wetlands and will continue to evolve.

Acknowledgements

The editors would like to express their sincere gratitude to the authors and the scientific reviewers whose work made the appearance of this volume possible.

The Editors

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CORRELATION BETWEEN WATER QUALITY AND THE DIVERSITY INDEX OF THE BOSNA RIVER IN THE ZENICA REGION (BOSNIA AND HERZEGOVINA)

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KEYWORDS: water quality, Spearman corelation coefficient, index of diversity, Bosnia and Herzegovina, Riwer Bosna.

ABSTRACT

Using the Shannon-Weaver index (H'), the link between the physical and chemical components of the biological component in the Bosnia River was examined. The results showed that pH and H' had a strong link (rs = 0.59), while total nitrogen and H' had a poor relationship (rs = 0.44), and COD-Cr had a weak relationship (rs = 0.34). KPK-Cr (I water class) had the lowest parameter concentrations, whereas electrical conductivity (IV-V water class) showed the highest. The values of the biological elements range from a poor 0.267 to a high state of 2.26.

ZUSAMMENFASSUNG: Korrelation zwishen waserquälitetselmenten und diversitäsindex des flusses Bosna in der gegend Zenica (Bosnien und Herzegowina).

Die Beziehung zwischen den physik-chemischen Elementen der biologischen Komponente im Bosnien-Fluss wurde mithilfe des Shannon-Weaver-Index (H') analysiert. Es wurde eine starke Beziehung zwischen pH-Wert und H' (rs = 0,59) und eine schwache Beziehung zwischen Gesamtstickstoff und H' (rs = 0,44) und CSB-Cr mit H' (rs = 0,34) gefunden. Die höchsten Parameterkonzentrationen wurden für die elektrische Leitfähigkeit (IV-V-Wasserklasse) und die niedrigsten für KPK-Cr (I-Wasserklasse) beobachtet. Die Werte der biologischen Elemente reichen von einem schlechten 0,267 bis zu einem hohen Zustand von 2,26.

REZUMAT: Corelația dintre calitatea apei și Indicele de Diversitate al râului Bosna din regiunea Zenica (Bosnia și Herțegovina).

Folosind indicele Shannon-Weaver (H'), a fost examinată legătura dintre componentele fizice și chimice ale componentei biologice din din râul Bosna. Rezultatele au arătat că pH-ul și H' au avut o legătură puternică (rs = 0,59), în timp ce azotul total și H' au avut o relație slabă (rs = 0,44), iar COD-Cr a avut o relație slabă (rs = 0,34). KPK-Cr (clasa I de apă) a avut cele mai scăzute concentrații de parametri, în timp ce conectivitatea electrică (clasa de apă IV-V) a arătat cea mai mare. Valorile elementelor biologice variază de la o stare slabă de 0,267 la o stare ridicată de 2,26.

INTRODUCTION

Many stresses substantially impact freshwater, which is why ongoing technique development, evaluations, and monitoring are crucial (Bănăduc et al., 2022). The largest watercourse in the Zenica area, in Bosnia and Herzegovina, is the Bosna River, which flows from Drivuša through the city. The Bosna River flows through the fields of Sarajevo, Visoko, Kakanj, and Zenica on its upper course. The Bosna River flows through the Zenica region and is around 32 kilometers long. The Kočeva, Babina, and Gračanička rivers are in addition to the Bosna River; the latter only reaches the area under investigation at its confluence. Zenica is categorized as a heavy industrial city that has unreasonably contaminated and deteriorated the environment. The construction of industry and the city in the conditions of a closed basin, with little space for both, creates anthropogenic climate factors that, in their extremity, modify the climate. The northern region of the Zenica basin is home to all industries, with the Arcelor Mittal Zenica company taking up the most space in the industrial zone.

Due to its population density and development, the Bosna River is most exposed to anthropogenic influences, significantly compromising water quality. This condition results from both industrial activities and the influence of the local population. Extensive industrial production processes, such as those in steel mills, must be viewed as sources of complete interaction with the environment. Environmental incidents are becoming more and more noticeable in the Bosna River in the Zenica region. These occurrences show themselves as mass mortality of fish populations, changes in water color noticed by visual inspection, and disagreeable scents. This is the outcome of adding non-native materials to the watercourse, upsetting the natural equilibrium.

Certain freshwater organisms larger than 0.5 mm are known as macroinvertebrate benthos and live on the bottom of aquatic environments. Due to their relatively long lifespan and limited mobility, changes in environmental conditions, alterations in physical water properties (water flow rate, temperature, and light), chemical water properties (nutrient levels, oxygen, and carbon dioxide), as well as seasonal and daily changes in water flow regimes, result in modifications to the qualitative and quantitative structure of the macroinvertebrate community. Under the influence of organic pollution, there is a reduction in species diversity, accompanied by an increase in the number of individuals within the same species. In contrast, clean waters support a greater variety of species but with lower individual abundance (Rosenberg et al., 1993). Consequently, it is possible to evaluate the decline of aquatic ecosystems from one or more causes using the taxonomic makeup of macroinvertebrates.

According to WFD 2000/60/EC, the quality of surface water is determined based on the assessment of its “ecological status”, which encompasses the study of physicochemical elements (thermal conditions, oxygen regime, acidification, nutrients, and specific pollutants), hydro-morphological elements (flow, hydrological regime, river continuity, and morphological conditions) that accompany biological elements (composition and richness of benthic macroinvertebrate fauna). The outcomes form the foundation of future management plans that involve taking all required steps to attain good status

In the rivers Una, Vrbas, Bosna, Drina, and Neretva with Trebišnjica, the research led by Blagojević (1984) showed that the correlations between saprobic parameters and water oxygen content, oxygen saturation, and BOD_5 vary with watercourses biogeographical, regional-hydrographic, and seasonal conditions. Physicochemical parameters, commonly determined in routine monitoring, can be compared with various ecological parameters derived from biomass, diversity, and saprobic index. Furthermore, studies on the relationships between saprobic conditions and chemical factors relevant to the Miljacka River’s oxygen regime have shown no agreement among individual results of measured parameters.

MATERIAL AND METHODS

Twenty samples were collected at five sites along the Bosna River during seasonal aspects, except for total nitrogen, for which 15 samples were taken. The parameters used for assessing water status through intercorrelations are presented in table 1.

Table 1: The applied elements for the analysis of intercorrelations

Hidromorfological elements	River flow Q (m^3/s)
Physico-chemical elements	
Thermal conditions	Water temperature (°C)
Acidification	pH
Oxygen regime	BPK_5 (mg/O_2l) KPK-Cr (mg/O_2l)
–	Electrical conductivity Total suspended solids
Nutrients	Ammonium ion (µg/l) Total nitrogen (µg/l) Total phosphorus (µg/l)
Biological elements	Shannon-Weaver index of diversity (H’)

Table 2 shows the chemical techniques used to test the specified parameters. Air temperature was measured in the field using a glass thermometer, while water temperature was measured using a multifunctional environmental meter – PCE-222 type device.

Water quality assessment is determined by comparing physicochemical parameters with the limit values of substances in current legal regulations. The pH, BOD_5, COD-Cr, and total suspended solids are compared with the maximum permitted concentrations of specific hazardous and harmful substances in surface waters by the Regulation (Official Gazette of the Socialist Republic of Bosnia and Herzegovina, 19/80).

Table 2: Chemical methods for testing water parameters.

Order nr.	Parameter	Measurement unit	Standard-Technique
1.	pH	–	JUS H.Z1.111 – Potenciometric
2.	BPK_5	mg O_2/l	JUS H.Z1.136 – Titrimetric
3.	KPK-Cr	mg O_2/l	JUS H.Z1.165 – Titrimetric
4.	Electrical conductivity	µS/cm	JUS H.Z1.112 – Conductometric
5.	Total suspended solids	mg/l	JUS H.Z1.160 – Gravimetric
6.	Total phosphorus	µg/l	ICP* – Spectrophotometric
7.	NH_4-amonium ion	µg/l	MERCK ** – UV VIS – Spectrophotometric
8.	Total nitrogen	µg/l	MERCK ** – Kjeldahl method

* PERKIN ELMER Analytical Methods for OES-ICP.

** The testing of Water.

Ammonium ion and total phosphorus are compared with the provisions of the Regulation (Official Gazette of the Federation of Bosnia and Herzegovina, 43/07). In contrast, electrical conductivity and total nitrogen are classified according to the Regulation (Official Gazette of the Republic of Srpska, 3/97, 3/98, and 29/00). These comparisons will determine whether there is an environmental burden associated with individual parameters and possible causes of elevated concentrations. The biological elements (Shannon-Weaver index – H') for Type 3 are compared according to the Decision (Official Gazette of the Federation of Bosnia and Herzegovina, 1/14).

The research of the relationship between water quality elements was conducted using statistical tools in the Excel program. The association of variations in the values of two or more variables is called correlation. By applying the correlation coefficient, we aim to determine whether measured physico-chemical parameters can serve as a basis for biological indicators or vice versa.

A complete correlation or functional relationship exists when there is a one-to-one correspondence between every value in variables x and y. When there is a partial correlation, several values of variable y correlate to a given value of variable x. The lower the correlation, the greater the variability of values of variable y that appear with a specific value of variable x.

The relationship between two continuous variables is depicted graphically through a scatter plot of pairs of values of the two continuous variables. Each point on the scatter plot represents a pair of data from one statistical unit. The scatter plot suggests the shape of the relationship between the two variables (Figs. 3-8). A linear relationship between two variables exists if the straight line drawn through the center of the points on the scatter plot best fits the given observations. The correlation coefficient measures the closeness of points to the straight line.

The Spearman rank correlation coefficient, a nonparametric equivalent to the Pearson correlation coefficient, is calculated when one or more of the following conditions is true:

At least one of the variables, x or y, is measured on an ordinal scale;

Neither x nor y follows a normal distribution;

The sample size is small.

We need a measure of association between two variables when this association is not linear.

To estimate the population value of the Spearman coefficient, we use its value calculated on the sample, denoted as rs: we arrange the values of x in ascending order starting from the smallest value and assign successive ranks (numbers 1, 2, 3, ..., n) to them, with identical values receiving the average rank of the values they would take if they were not identical. We assign ranks to the values of variable y in the same manner. rs is the Pearson coefficient between the ranks of x and y.

$$r_s = 1 - \frac{6\sum d_i^{\,2}}{n(n^2-1)}$$

as it is: d_i – difference of ranges, n – number of samples.

Characteristics of the Spearman correlation coefficient: it shares the same features as the Pearson correlation coefficient, denoted as rs, providing a measure of association, not necessarily linear, between variables x and y. rs^2 is not calculated since it does not represent the proportion of total variation in one variable that could be attributed to its linear relationship with others.

Table 3 provides interpretations of linear correlation coefficients, based on which conclusions about the strength and nature of the relationship between the observed variables are drawn. The strength of the association (interpretation) is the same for negative values of the correlation coefficient.

Table 3: Criterion of the strength of the linear relationship between ranked variables expressed through the values of the Spearman correlation coefficient.

Correlation coefficient			Strength of the relationship between variables
1			Total
0.80 ≤	r	< 1	Strong
0.50 ≤	r	< 0.8	Medium strong
0.20 ≤	r	< 0.5	Weak
0.0 ≤	r	< 0.2	Slightly
0			Totally absent

The diversity of macroinvertebrate communities will be represented by the Shannon–Weaver diversity index (H) (Shannon et al., 1949), which utilizes the relative abundance of individual taxa:

$$H' = -\sum P_i \log_2 P_i \text{ as it is } P_i = \frac{ni}{N};$$

as it is: H' – value of the diversity index, Pi – the relative representation of that taxon in the sample and $\log_2 (x) = \log(x)/ \log2$

This index starts at zero if only one species is present in the sample, and as the number of species increases, the index value also increases, reflecting an increase in evenness.

RESULTS AND DISCUSSION

Data on the Bosna River's water level and flow values at the Raspotočje gauge station were provided by the Agency for the Water Area of the Sava River Basin - Sarajevo database, covering the period from January 2014 to April 2015. During the investigated period, fluctuations in the water level of the Bosna River triggered by heavy rainfall and floods were observed. High water levels were recorded, reaching around 520 cm in May 2014 and approximately 320 cm in January 2015, causing widespread flooding (Figs. 1 and 2).

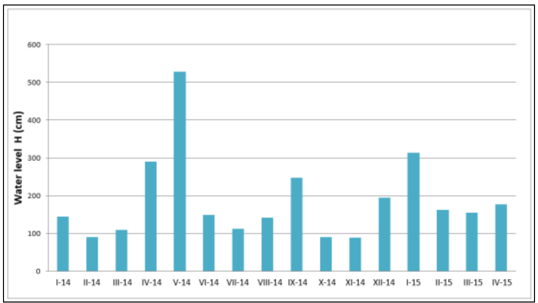

Figure 1: The maximum water levels of the Bosna River.

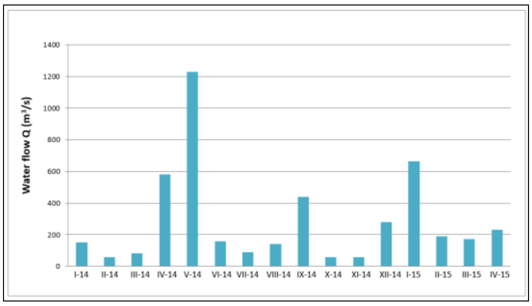

Figure 2: The maximum water flow of the Bosna River.

In the following tables 4, 5, 6, 7, and 8, the values of the studied elements for assessing the water category are presented for five sampling sites collected in annual periods.

Table 4: The values of hydromorphological, physico-chemical, and biological elements of the Drivuša site.

Parameters	08.10. 2014	15.01. 2015	06.05. 2015	23.06. 2015
The flow rates of the Bosna River Q (m^3/s)	27.25	39.10	27.89	15.22
Water temeperature (°C)	12.1	2.6	13.9	12.3
Air temperature (°C)	14.2	3	26.4	22.7
pH	7.6	7.3	7.5	7.4
BPK_5 (mg/O_2l)	1.95	0.35	1.33	1.29
KPK-Cr (mg/O_2l)	4.61	0.78	1.90	1.84
Electrical conductivity (µS/cm)	1,109	1,058.6	970.1	1,258.9
Total suspended solids (mg/l)	8	50	24	34
Amonium ion (µg/l)	2,180	14,010	1,400	1,400
Total nitrogen (µg/l)	–	15.410	2,800	2,800
Total phosphorus (µg/l)	74	76	88.5	107.8
Shannon-Weaver index of diversity (H’)	1.39	1.15	1.86	0.627

Table 5: The values of hydromorphological, physico-chemical, and biological elements of the Raspotočje site.

Parameters	08.10. 2014	15.01. 2015	06.05. 2015	23.06. 2015
Flows of Bosna River Q (m^3/s)	27.42	39.80	28.21	15.63
Water temeperature (°C)	13	1.5	14.7	16.5
Air temperature (°C)	14.25	3.3	30.1	25.3
pH	7.8	7.3	7.5	7.5
BPK_5 (mg/O_2l)	1.73	0.37	0.89	1.29
KPK-Cr (mg/O_2l)	4.10	0.78	1.27	1.84
Electrical conductivity (µS/cm)	1,136	1058.6	931.6	1225.8
Total suspended solids (mg/l)	12	65	18	25
Amonium ion (µg/l)	2,180	16,810	1,400	1,400
Total nitrogen (µg/l)	–	18,210	2,800	2,800
Total phosphorus (µg/l)	74	72	84.0	113.4
Shannon-Weaver index of diversity (H’)	2.11	0.92	1.89	0,267

Table 6: The values of hydromorphological, physico-chemical, and biological elements of the Hotel Metalurg site.

Parameters	08.10. 2014	15.01. 2015	06.05. 2015	23.06. 2015
Flows of Bosna River Q (m^3/s)	28.92	41.30	29.71	17.13
Water temeperature (°C)	12.6	3.9	16.2	17.3
Air temperature (°C)	15.5	3.7	30.4	23.2
pH	7.8	7.2	7.8	7.7
BPK_5 (mg/O_2l)	0.68	0.71	2.67	0.86
KPK-Cr (mg/O_2l)	1.57	1.96	3.81	1.23
Electrical conductivity (µS/cm)	1,136	1,012.6	950.6	1194.3
Total suspended solids (mg/l)	18	28	27	37
Amonium ion (µg/l)	2,800	11,210	1,400	2,800
Total nitrogen (µg/l)	–	12,610	2,800	2,800
Total phosphorus (µg/l)	650	63	79.1	101.2
Shannon-Weaver index of diversity (H’)	1.48	0.92	1.26	0.794

Table 7: The values of hydromorphological, physico-chemical, and biological elements of the Coastal Channel of the Ironworks site.

Parameters	08.10. 2014	15.01. 2015	06.05. 2015	23.06. 2015
Flows of Bosnia river Q (m^3/s)	29.10	41.50	30.10	17.53
Water temeperature (°C)	14.5	3.6	18.2	20.6
Air temperature (°C)	19.2	3.8	32.7	24.3
pH	7.7	7.2	7.7	7.7
BPK_5 (mg/O_2l)	0.95	0.70	3.59	0.86
KPK-Cr (mg/O_2l)	2.35	1.96	5.08	1.23
Electrical conductivity (µS/cm)	1,606	1,083.2	991.1	1,293.9
Total suspended solids (mg/l)	51	34	67	39
Amonium ion (µg/l)	6,550	19,610	2,800	5,600
Total nitrogen (µg/l)	21,010	15,410	7,000	
Total phosphorus (µg/l)	790	77	90.0	146.4
Shannon-Weaver index of diversity (H’)	2.08	0.97	1.52	2.075

Table 8: The values of hydromorphological, physico-chemical, and biological elements of the Banlozi-Jelina site.

Parameters	08.10. 2014	15.01. 2015	06.05. 2015	23.06. 2015
Flows of Bosna River Q (m^3/s)	30.20	42.40	31.21	18.60
Water temeperature (°C)	13.6	2.3	16.6	17.6
Air temperature (°C)	16.5	1.1	30.9	27.2
pH	7.7	8.5	8.0	7.6
BPK_5 (mg/O_2l)	0.71	1.80	1.78	2.24
KPK-Cr (mg/O_2l)	1.57	4.70	2.54	3.20
Electrical conductivity (µS/cm)	1,194	1,035.1	1,013.0	1,330.8
Total suspended solids (mg/l)	36	65	24	33
Amonium ion (µg/l)	5,600	15,410	3,200	4,200
Total nitrogen (µg/l)		16,810	4,200	5,600
Ukupni fosfor (µg/l)	800	69	91.1	121.2
Shannon-Weaver index of diversity (H')	0.92	1.91	2.26	0.809

The studied river Bosna in the Zenica region increases its flow downstream through its entire course, mitigating anthropogenic influences and resulting in the partial flushing of sediment containing different harmful substances. Due to a more prominent food source, this condition leads to a slightly higher diversity of macroinvertebrates at the lower river sites (Tabs. 4-8). In January, the highest flows were recorded at 42.40 m^3/s, leading to significant water levels.

During the study period, the lowest air temperature occurred in January (1.1°C) and the highest in May (32.7°C). Water temperature is directly dependent on air temperature. Lower water temperatures were observed at the upper Bosna River sites. In comparison, higher temperatures were recorded at the lower sites, as expected, due to more prolonged exposure to sunlight, causing downstream water warming (Tabs. 4-8).

The water is weak to moderately basic, ranging from 7.2 to 8.5 (Tabs. 4-8). The pH value fluctuates slightly over the observed period, consistently falling within Class I of water quality. The higher pH values are likely a result of a significant reduction in water quantity, leading to increased concentrations of detergents, which are strongly basic substances (Duran et al., 2000-2001).

BOD_5 values ranged between the values of 0.71 mg/O_2l to 3.59 mg/O_2l, classified as Class II of water quality (Tabs. 4-8). COD-Cr values ranged from 0.78 mg/O_2l to 5.8 mg/O_2l, meeting the requirements of Class I of water quality. There is no evidence of significant organic pollution due to relatively low BOD_5 and COD-Cr values during the observed period. Variations in daily chemical oxygen demand may be attributed to sampling at different times when the quantity of municipal wastewater changes throughout the day (Duran et al., 2000-2001).

In contrast to the oxygen regime, electrical conductivity reached high values from 991.1 µS/cm to 1,606 µS/cm (Tabs. 4-8). It can be classified as Class IV-V of water quality, indicating significant pollution. Increased conductivity values suggest a higher concentration of salts in the water, a result of wastewater discharge accumulating in sediment, adversely affecting aquatic life. Conductivity values are influenced by temperature, pH value, and, to some extent, dissolved carbon dioxide (Trožić-Borovac, 2001).

Total suspended solids (TSS) concentrations ranged between the values of 8 mg/l to 67 mg/l (Tabs. 4-8), falling within the limits for Class II-III of water quality. Determining and analyzing this parameter in water is very important due to the fact that it leads to forming sediment deposits, favoring the creation of anaerobic conditions at the bottom of the aquatic ecosystem.

Ammonium ion concentrations ranged from 1400 µg/l to 19610 µg/l (Tabs. 4-8), exceeding Class IV of water quality limits. Oscillations in ammonium content indicate that sewage and industrial wastewater significantly affect the studied river's water quality. Ammonium is commonly considered an indicator of fresh fecal pollution. Still, increased concentrations can also result from applying fertilizers near the river and wastewater rich in mineral and organic materials. High ammonium values are likely due to the high water levels of the river, leading to flooding of areas around the river and causing increased nutrient concentrations through leaching and accumulation of organic matter from the surrounding vegetation.

Total nitrogen varied during the observed period, ranging from 2,800 µg/l to 21,010 µg/l (Tabs. 4-8), categorized as Class II to IV of water quality. Nitrogen content in water is considered an indicator of water pollution due to insufficiently treated wastewater and excessive fertilizer use.

Total phosphorus levels ranged from 72 µg/l to 800 µg/l (Tabs. 4-8), falling into Class I-II to III-IV of water quality. Phosphorus is an indicator of water pollution, indicating the presence of sewage and artificial fertilizers

The biological diversity index fluctuated from poor at 0.267 at the Raspotočje site (Tab. 5) to high at 2.26 at the Banlozi site (Tab. 8). Differences in diversity and abundance of individuals were observed between seasons. Considering consistent physico-chemical analyses at the sites, variations in diversity index values should be sought in other potential significant influences. Flooding events in 2014 led to changes in hydromorphological elements of water quality, mainly in substrate structure and homogeneity, affecting the composition and structure of macroinvertebrates and other biological communities (Novaković et al., 2015). Specifically, during high water levels in May 2014, a portion of benthic macroinvertebrates was washed away, directly related to increased water levels, as the flow velocity increases with rising water levels (Figs. 1-2), making it difficult for some organisms to resist. After heavy rainfall or floods, drift occurs, the passive transport of macroinvertebrates when the water quantity in the riverbed rises, carrying insects downstream. In this study, the highest number of taxa, individuals, and diversity index values were detected downstream at the Banlozi site of the Bosna River.

Table 9: Comparison of correlation coefficients for the considered relationships between elements.

Orderer nr.	Considered relationships between elements	Spearman’s correlation coefficient and the strength of the relationship between variables
1.	Q – H’	0.24 – weak and positive
2.	T – H’	0.13 – minor and positive
3.	pH – H’	0.59 – medium strong and positive
4.	BPK5 – H’	0.24 – weak and positive
5.	KPK-Cr – H’	0.34 – weak and positive
6.	Electrical conductivity – H’	– 0.24 – weak and negative
7.	Total suspended solids – H’	– 0.11 – minor and negative
8.	Amonium ion – H’	0.13 – minor and positive
9.	Total nitrogen – H’	0.44 – weak and positive
10.	Total phosphorus – H’	– 0.02 – totally absent

The correlation between elements is weak (Tab. 9), except for the established correlation with a moderately strong relationship between pH and H’ (rs = 0.59) (Fig. 3). Within the achieved weak connection, we highlight the total nitrogen – H’ correlation (rs = 0.44) (Fig. 4) and KPK-Cr – H’ correlation (rs = 0.34) (Fig. 5). The same correlation value (rs = 0.24) is achieved for flow (Fig. 6) and BPK_5. Therefore, a statistically significant connection between elements exists for pH and the Shannon-Weaver diversity index, meaning that the most critical influence on H values is the pH. Also, it can be concluded that there is a connection between total nitrogen and KPK-Cr with H’. The low correlation coefficients for temperature (rs = 0.13) indicate that its influence is insignificant for H’. Achieved negative values for electrical conductivity (Fig. 7) and total suspended solids (Tab. 9) imply that H’ increases as the value of these elements decreases. A negative value is observed for total phosphorus and a complete absence of correlation with H’ (Fig. 8).

In the study (Imamović, 2005) on the water quality of the Bosna River, the best correlation was found with the saprobic index for nitrates (r = 0.52), temperature, and pH (r = 0.42). Examining the quality of the Sava River (Ćuk et al., 2014), it was observed that, in addition to physico-chemical indicators, the highest number of taxa is directly connected with the diversity of dominant substrate with a higher proportion of rocks and gravel. The smallest number of taxa inhabits habitats with a proportion of sand and mud without large substrate fractions. Williams and Williams (1998), determined that the composition of the riverbed (size and heterogeneity) and the presence of aquatic vegetation play a dominant role in the composition of macroinvertebrate communities.

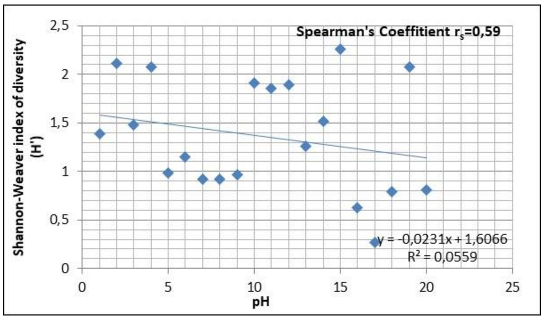

Figure 3: Connection pH – H'.

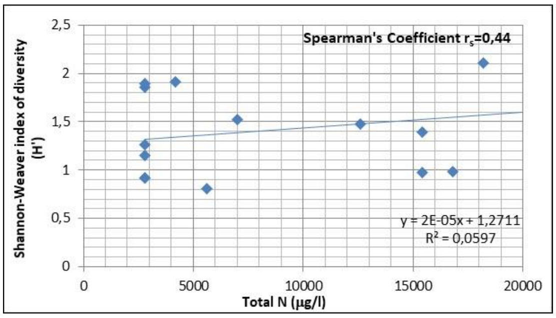

Figure 4: Connection of total N – H'.

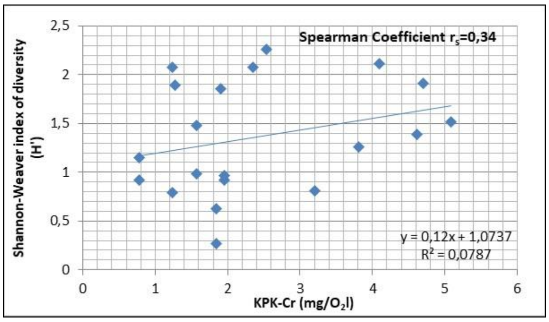

Figure 5: Connection KPK-Cr – H'.

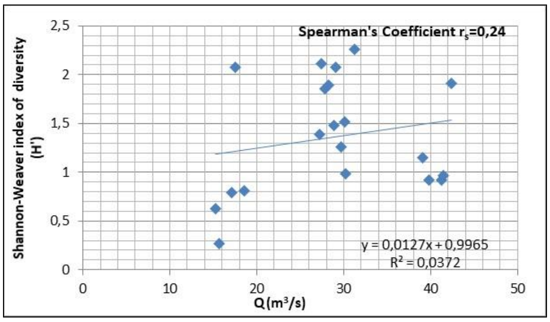

Figure 6: Connection Q – H'.

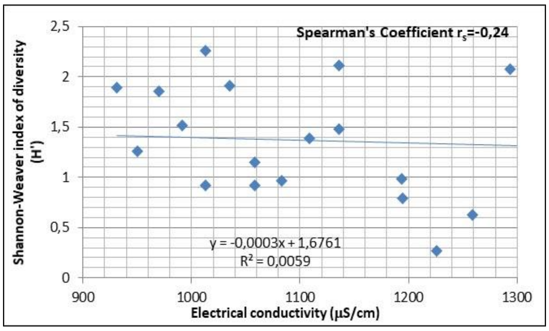

Figure 7: Connection electric conductivity – H'.

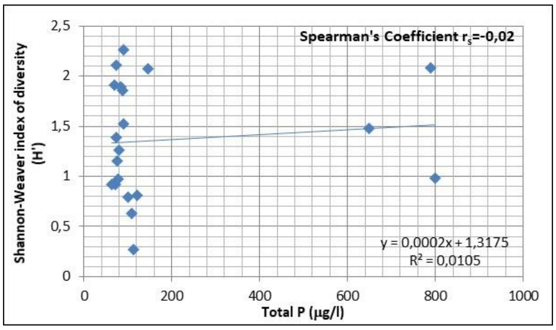

Figure 8: Connection total phosphorus – H'.

As this analysis revealed a significant dispersion of results and negative outcomes, it is necessary to increase the number of sites in further research, analyzing a greater number of samples over a longer period of approximately three years. This is aimed at obtaining more efficient data that would provide a better correlation between ecological parameters.

CONCLUSIONS

Based on the results of the water quality assessment, which included analyses of essential physico-chemical and biological elements of the Bosna River in the Zenica area from October 2014 to June 2015, it can be concluded that multivariate analysis demonstrated the interconnection of water quality elements using the dependent variable with the Shannon-Weaver Diversity Index.

A statistically significant correlation of moderate strength was achieved between pH and the Shannon-Weaver Diversity Index (rs = 0.59). Additionally, there is a connection between total nitrogen and KPK-Cr with the diversity index. In further research, it is necessary to increase the number of sites with a more significant number of samples over a more extended period in different seasonal conditions of watercourses to have more effective data that would provide a better correlation between elements determining water quality. Continuous monitoring of the Bosna River's quality is essential not only for pollution assessment and implementing preventive and practical protection measures but also for more specific requirements to polluters to introduce and implement protective measures preventing or reducing water pollution within permitted concentrations.

REFERENCES

1. Barbour M. T., Gerritsen J., Snyder B. D. and Stribling J. B., 1999 – Rapid bioassessment protocols for use in streams and wadable rivers: periphyton, benthic macroinvertebrates and fish, 2nd edition, EPA 841-B-99-002, Washington D. C., 337.
2. Bănăduc D., Simić V., Cianfaglione K., Barinova S., Afanasyev S., Öktener A., McCall G., Simić S. and Curtean-Bănăduc A., 2022 – Freshwater as a sustainable resource and generator of secondary resources in the 21st century: stressors, threats, risks, management and protection strategies, and conservation approaches, *International Journal of Environmental Research and Public Health*, 19, 16570, 1-30, 10.3390/ijerph192416570.
3. Blagojević S., Dizdarević M. and Pavlović B., 1984 – Odnosi između nekih bioloških i fizičko hemijskih parametara kvaliteta vode u tekućicama Bosne i Hercegovine, Univerziteta u Sarajevu, Godišnjak Biološkog instituta Univezriteta u Sarajevu, 37, 23-30. (in Bosnian)
4. Ćuk R., Tomas D. and Vučković I., 2014 – Kakvoća rijeke Save u 2012, godini, Hrvatske Vode, 88, 97-106. (in Croatian)
5. Duran F. and Salkić A., 2000-2001 – Izvještaji o kontinuiranim mjerenjima zagađenosti zraka i vodotoka u Zenici za 2000-2001 godina, Mjerenja i izvještaj o zagađenosti zraka i vodotoka, Metalurški institut Kemal Kapetanović, Izvještaji broj 005/2000-09/2000, 150. (in Bosnian)
6. EU Water Framework Directive 2000/60/EC of the European Parliament and of the Council establishing a framework for Community action in the field of water policy 2000.
7. Giller P. S. and Malmquist B., 1998 – The Biology of Streams and Rivers, Oxford University Press, Oxford, 256.
8. Imamović A., 2005 – Analiza korelacija ekoloških parametara kvaliteta voda na rijeci Bosni, Javno preduzeće za "Vodno područje slivova rijeka Save", *Voda*, 84, 24-34. (in Bosnian)
9. Novaković B., 2015 – Poplave u Srbiji tokom 2014. godine – Uticaj na zajednice akvatičnih makroinvertebrata, 44 konferencija o aktuelnim problemima korišćenja i zaštite voda, *Voda*, 41-44. (in Bosnian)
10. Odluka o karakterizaciji površinskih i podzemnih voda, referentnim uslovima i parametrima za ocjenu stanja voda i monitoring voda, Službene novine FBiH, br. 1/14, 78. (in Bosnian)
11. Rosenberg D. and Resh V. H., 1993 – Freshwater biomonitoring and benthic macroinvertebrates, Champan Hall, New York, 488.
12. Shanon C. E. and Weaver W., 1949 – The mathematical theori of commucation, The University of Illinois Press, Urbana, I, 11, 19-21, 82-83, 104-107.
13. Trožić-Borovac S., 2001 – Istraživanje makroinvertebrata bentosa rijeke Bosne i pritoka u ocjeni kvaliteta vode, Doktorska disertacija, Prirodno-matematički fakultet Univerzitet u Sarajevu, 260. (in Bosnian)
14. Uredba o klasifikaciji voda i voda obalnog mora Jugoslavije u granicama SR BiH, Službeni list SR BiH, broj 19/80, 374-377. (in Bosnian)
15. Uredba o opasnim i štetnim materijama u vodama, Službeni list Federacije BiH, broj 43/07, 4117-4120. (in Bosnian)
16. Uredba o klasifikaciji voda i kategorizaciji vodotoka, Službeni glasnik Republike Srpske, broj 3/97, 1-16, 3/98 1-2 i 29/00, 1-2, 16 pp). (in Bosnian)
17. Williams D. and Williams N. E., 1998 – Invertebrate communites from freshwater springs what can they contribute to pure applied ecology, U: Studies in Crenobiology. The biology of springs and springbooks, Bachuys Publishers, Leiden, 251-261.

TOXICITY SCREENING OF SURFACE WATERS IN ZAGHEN RESTORATION AREA WITH TOXKIT MICROBIOTESTS

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ABSTRACT

In this study, we report the findings regarding the toxicity of surface waters collected in March 2023 from five sampling stations in the Zaghen restoration area, located within the Danube Delta Biosphere Reserve, Romania. The assessment of acute toxicity was conducted using two microbiotests: the Thamnotoxkit F and the Daphtoxkit F^{TM} magna. Even if the acute toxicity effects are less than 50% and do not allow the calculation of a toxicity score for the water samples, the experiments show a different vulnerability of the two zooplanktonic species to the presence of heavy metals in the water with mortality being higher in *Thamnocephalus platyurus* (20%) than in *Daphnia magna* (15%).

RÉSUMÉ: Évaluer la toxicité de l’eau suprafaciale de la zone de restauration Zaghen en utilisant le test du microbiote Toxkit.

Dans cette étude, nous présentons les résultats concernant la toxicité des eaux de surface prélevées en mars 2023 à partir de cinq stations dans la zone de restauration de Zaghen, située dans la Réserve de Biosphère du Delta du Danube en Roumanie. L’évaluation de la toxicité a été réalisée à l’aide de deux microbiotests: le Thamnotoxkit F et le Daphtoxkit F^{TM} magna. Même si les effets de toxicité aiguë sont inférieurs à 50% et ne permettent pas de calculer un score de toxicité pour les échantillons d’eau, les expériences montrent une vulnérabilité différente des deux espèces zooplanctoniques à la présence de métaux, la mortalité étant plus élevée chez *Thamnocephalus platyurus* (20%) que chez *Daphnia magna* (15%).

REZUMAT: Evaluarea toxicității apelor de suprafață din zona de restaurare Zaghen utilizând microbiotestele Toxkit.

În acest studiu, prezentăm rezultatele referitoare la toxicitatea apelor de suprafață colectate în luna martie 2023 din cinci stații de prelevare din zona de reconstrucție ecologică Zaghen, situată în cadrul Rezervației Biosferei Delta Dunării, România. Evaluarea toxicității acute s-a realizat folosind două microbioteste: Thamnotoxkit F și Daphtoxkit F^{TM} magna. Chiar dacă efectele toxicității acute sunt mai mici de 50% și nu permit calcularea unui scor de toxicitate pentru probele de apă, experimentele dovedesc o vulnerabilitate diferită a celor două specii zooplanctonice față de prezența metalelor grele în apă, mortalitatea fiind mai mare la *Thamnocephalus platyurus* (20%) față de *Daphnia magna* (15%).

INTRODUCTION

The results of this study contribute to the completion of the PhD research topic, titled “Research on zooplankton communities in the ecological restoration areas of Carasuhat (natural flood regime) and Zaghen (controlled flood regime)”, conducted between 2018-023.

The study aimed to assess water toxicity in the Zaghen ecological restoration area using Toxkit microbiotest technology and served as the final part of this PhD thesis.

The main objectives of the thesis were to characterize in space and time the zooplankton communities in aquatic ecosystems subject to ecological reconstruction (Zaghen and Carasuhat) compared to a natural area (Uzlina), in terms of assessing the ecological status of these ecosystems based on the analysis of the main influencing factors (concentration of nutrients and heavy metals).

The data collected will help to develop and implement measures to protect, restore and conserve aquatic resources in accordance with European Union directives and international conventions.

In aquatic environments, heavy metals have received considerable attention because of their toxicity and their tendency to accumulate in biota. While metallic elements are naturally present in the aquatic environment, anthropogenic and industrial inputs increase their presence (Zubcov et al., 2008).

The irreversible nature of this pollution is of particular concern, as once these metals are released into the environment they cannot be recovered. In contrast to organic pollutants, metals are not metabolized; instead, they can be transferred through the food chain and can accumulate in living organisms (Bouthir et al., 2023).

As zooplankton play a crucial role in the processes of metal bioavailability in water, the assessment of water toxicity in zooplankton communities is of great importance.

Metal accumulation by zooplankton occurs by direct adsorption from water or absorption from food and detritus. In addition, zooplankton serve as an important food source, further confirming their role in transferring metals to higher trophic levels (Robin et al., 2012).

Researchers around the world have studied the assessment of anthropogenic disturbances by zooplankton. Due to their position as intermediaries in the trophic chain of aquatic ecosystems, zooplankton play an important role in the transfer of materials between primary producers and higher trophic levels.

Consequently, these organisms can serve as valuable indicators of the overall conditions under which aquatic ecosystems function.

Practical experience has shown that certain pollutants can individually exert their toxic potential even at concentrations below the legal limits (Khangarot and Ray, 1989; Kumar and Han, 2010). Therefore, simply using physicochemical methods to determine the compounds discharged into water is not sufficient to protect aquatic ecosystems.

As a result, biological tests have been developed to directly assess the toxicity and ecological risks of contaminants. These tests integrate the ecological context in a way that conventional chemical analysis cannot, as the latter is based on the measurement of individual, standardized (and pre-selected) chemical substances.

Monitoring the toxicity of wastewater, using both biotests and chemical analysis prior to discharge, could help to assess the efficiency of wastewater treatment systems and protect receiving waters better.

Current approaches to water quality assessment include bio-evaluation and the establishment of safety thresholds for pollutants based on the sensitivity of aquatic biota, as practised in water quality assessment in the EU and the USA.

Algae, daphnids, fish and luminescent bacteria are among the organisms commonly used for aquatic toxicity testing in assessment and monitoring programmes. These tests often include multiple species representing different trophic levels to account for species-specific sensitivities and trophic chain considerations (Tofan et al., 2014; Tofan et al., 2023).

Recent studies, such as that of Park et al. (2023), have utilised integrated assessments of wastewater toxicity using multiple test species representing different trophic levels of aquatic ecosystems.

These tests provide rapid detection tools for wastewater contamination and assist in the determination of toxicity thresholds based on toxicity unit data.

It is important to re-evaluate the current surface water quality standards, particularly for heavy metals such as copper and zinc, as acute toxicity levels for daphnids have shown significant mortality levels similar to those allowed by the quality standards (Tab. 1).

The simultaneous action of multiple metals in aquatic ecosystems or the discharge of industrial effluents into natural receptors can have strong synergistic effects on organisms, as demonstrated by Wang et al. (2020).

This highlights the need for holistic biological testing of wastewater toxicity to ensure sustainable management of aquatic ecosystems.

Considering the presence of metallic elements in the sampled waters from the Zaghen restoration area and the interference of copper and zinc values with the last two quality classes specified in the national standards, it was deemed necessary to conduct microbiotests for biological testing.

Table 1: EC50 - 48 h values for *Daphnia magna* (Khangarot and Ray, 1989), reported in relation to the classification of surface water according to Order 161/2006.

Metals	U.M.	Cls. I	Cls. II	Cls.III	Cls IV	Cls. V	*D. magna* (mg L^{-1})
Cr^{3+}	mg L^{-1}	0.025	0.05	0.1	0.25	>0.25	1.79
Cu^{2+}	mg L^{-1}	0.02	0.03	0.05	0.1	>0.1	0.093
Zn^{2+}	mg L^{-1}	0.1	0.2	0.5	1	>1	0.56
Pb^{2+}	mg L^{-1}	0.005	0.01	0.025	0.05	>0.05	3.61
Cd^{2+}	mg L^{-1}	0.0005	0.001	0.002	0.005	>0.005	1.88
Ni^{2+}	mg L^{-1}	0.01	0.025	0.05	0.1	>0.1	7.29

MATERIAL AND METHODS

The Zaghen study area is located in the eastern part of the city of Tulcea, Romania. It is bounded to the north by the Tulcea Branch of the Danube and to the south by the DJ222C Tulcea-Malcoci road. The project Ecological Restoration in the Zaghen Polder of the Romania-Ukraine Transboundary Biosphere Reserve Danube Delta was implemented from 5 April 2012 to 7 April 2015.

The restoration activities were aimed at bringing water from the Danube River into the enclosure, establishing a suitable hydrological regime typical for flooded areas, restoring natural habitats, conserving biodiversity, and providing ecosystem services in line with the interests of the local community (Dimitriu et al., 2010; Dumitrescu and Cârșmariu, 2014).

The Zaghen study area has a controlled flooding regime, with water supplied by pumping systems from the Danube. It is a relatively isolated wetland surrounded by urban ecosystems. Water samples for heavy metals and ecotoxicological analysis were collected in March 2023 from five different sites in the study area (Fig. 1).

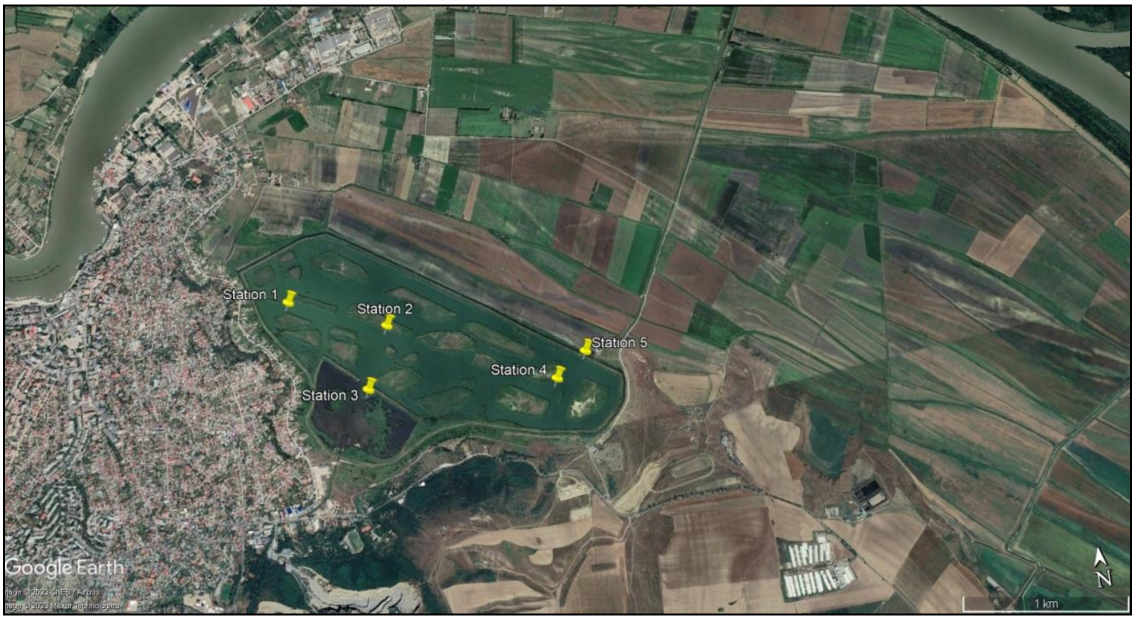

Figure 1: The location of the sampling stations (Google Earth).

Heavy metals analyses (Cd, Zn, Cu, Pb, Ni, and Cr) were performed using a Perkin Elmer Inductively Coupled Plasma ICP-MS ELAN® DRC-e at the Chemistry Laboratory of the Danube Delta National Institute for Research and Development, Tulcea.

The ecotoxicological analysis of the water samples were performed at the Applied Ecology Laboratory of the Faculty of Natural and Agricultural Sciences, “Ovidius” University, Constanța.

Two Toxkit microbiotests, the Daphtoxkit F magna test with the crustacean *Daphnia magna* and the Thamnotoxkit FTM test with *Thamnocephalus platyurus*, were selected for the ecotoxicological studies because of their “culture-free” advantage and user-friendly characteristics.

Both microbiotests were performed according to the standard operating procedures provided in the respective Toxkits (Tofan et al., 2023). The test organisms (*Daphnia magna, Thamnocephalus platyurus*) were provided in the kits in dormant (cryptobiotic) form.

Each Toxkit contained all the disposable materials required to perform the tests. The test organisms were exposed for 24 to 48 hours, depending on the species.

At the end of the test, the number of dead or immobilised organisms was recorded and statistical methods (probit analysis) were used to calculate LC50s and EC50s.

The Daphtoxkit F acute *Daphnia magna* toxicity test is a cost-effective and standardized method for screening the toxicity of chemicals, effluents, surface waters, wastewater, groundwaters, sediment pore waters, and elutriates.

The test is culture-independent, user-friendly and follows ISO standard 6341 and OECD guideline 202. The test procedure involves several steps, including preparation of freshwater standard, hatching of dormant eggs, filling of the test plate with collected water samples, pre-feeding of organisms, transfer of neonates to wells, incubation, evaluation of results, and estimation of EC50 values.

The test provides reliable data for assessing the toxicity of substances to *Daphnia magna* over 24 and 48 hours using a sigmoid function with EC50 calculation application.

The Thamnotoxkit F bioassay is a highly standardised method for performing six acute 24-hour mobility inhibition tests with the freshwater crustacean *Thamnocephalus platyurus*. This cost-effective, culture-independent bioassay conforms to ISO standard 14380.

The test involves several steps, including freshwater standard preparation, cyst hatching, preparation of toxicant dilution series, filling of the test plate, transfer of larvae to wells, incubation, evaluation of results, and estimation of LC50 values.

The test provides reliable data for assessing the toxicity of substances to *Thamnocephalus platyurus* using a sigmoid function with LC50 calculation application.

RESULTS AND DISCUSSION

During the study period from March 2021 to October 2022, a total of 27 species were identified in the Zaghen study area. Most of them were rotifers (20 species), copepods (four species) and cladocerans (three species). A total of 55 species of zooplankton were observed in Carasuhat. Of these, 40 species belong to the taxonomic group of rotifers, 10 species are cladocerans and five species are copepods.

In Uzlina natural lake, the third study area, a total of 46 zooplankton species were identified. Among them, 39 species were rotifers, four species were cladocerans and three species were copepods (Sali, 2023).

Regarding the presence of heavy metals Cd, Zn, Cu, Pb, Ni, and Cr in the study areas, the concentrations were mainly within the limits established for water quality classes I (very good ecological status) and II (good ecological status), with some exceptions for lead, nickel, chromium, and cadmium in a small number of samples.

Among the three study sites, Carasuhat and Uzlina stood out for higher concentrations of heavy metals. This can be attributed to the greater influence of the Danube River as the input of water and sediment from the river is the main carrier of metallic elements, compared to the Zaghen area, which is an isolated ecological system (Sali, 2023).

The values of heavy metal concentrations detected in the Zaghen area in March 2023 are presented in figure 2.We can see that the concentrations of zinc and copper are within quality class I, which matches to a very good ecological condition. As for the concentrations of cadmium, nickel, and chromium, they exceed class I quality and fall into class II quality (good ecological condition). At station 1, which is in the immediate vicinity of residential areas, lead exceeds the limits of quality class II, indicating anthropogenic influence, and falls into quality class III, corresponding to a moderate ecological status.

The results of the bioassays performed are presented in table 2 (EC% and LC%).

Table 2: Determination of acute toxicity effects (EC%) in *Daphnia magna* and *Thamnocephalus platyurus* through testing of water samples containing metallic elements, collected from Zaghen in March 2023; 10% mortality is accepted for Control validation (microbiotests.com).

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*

The results of this research microbiotest toxicity test indicated differential sensitivity between the two analysed species. The combined effect of heavy metals from stations 1 and 2 exhibited higher toxicity to the crustacean species *Thamnocephalus platyurus* compared to species *Daphnia magna*, which showed lower sensitivity to the same combination of heavy metals. This observation is in agreement with existing literature (Persoone et al., 2003).

Significant values of low toxicity leading to mortality have been recorded only for the waters taken from the first two stations:

– *Daphnia magna* (48 hours exposure): 15% mortality;

– *Thamnocephalus platyurus* (24 hours): 20% mortality.

An integrated toxicity assessment (Persoone et al., 2003) could be performed based on toxicity unit (TU) values derived from the results of the applied toxicity tests. The Probit Method, used to calculate the EC50 and its 95% confidence limits, is used to assess the toxicity of water samples of interest. The TU value for a given compound is the concentration at which there is a 50% effect (EC50 of the wastewater expressed as percentage dilution) for a given biological endpoint. The TU value is calculated according to Equation (1) as follows: TU = 100/EC50%. The TU values from each toxicity test are grouped into four toxicity classes: non-toxic (1 TU), slightly toxic (between 1 TU and 10 TU), moderately toxic (between 10 TU and 100 TU) and very toxic (> 100 TU). Each toxicity class is associated with a score: "non-toxic" = 0; "slightly toxic" = 1; "moderately toxic" = 2 and "very toxic" = 3.

Therefore, the toxicity score based on the determination of toxicity units as described above cannot be calculated for our application based on *D. magna* and *T. platyurus* because the mortality results were below 50%.

The Danube Delta Biosphere Reserve ecosystems are exposed to a wide range of human impact categories, resulting in various consequences that interact within the aquatic ecosystem.

Despite observing low mortality rates (below 50%) and water samples being considered as extremely low toxicity for only the first two stations, our findings emphasize the importance of ongoing biological monitoring.

Additionally, to assess contamination risks in the Danube Delta Biosphere Reserve's aquatic ecosystems, we recommend the incorporation of other microbiotests, such as those involving algae and bacteria.

These additional tests can also contribute to the specific evaluation of the efficiency of the wastewater treatment systems discharged into the Danube River throughout all the hydrographic basin, including the city of Tulcea, as well as other direct sources of pollution in the Danube Delta, with the aim of improving the protection of deltaic ecosystems.

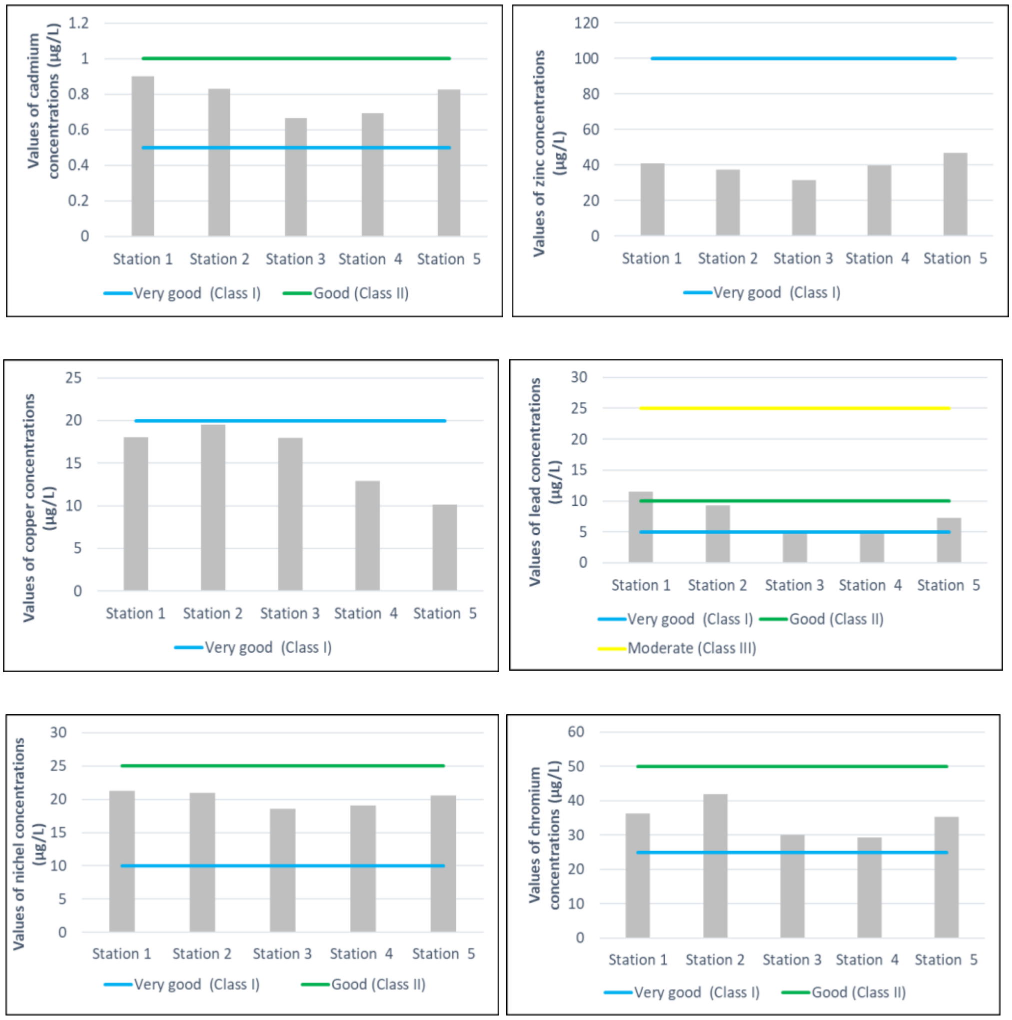

Figure 2: Heavy metals (Cadmium, Zinc, Cooper, Lead, Nickel, and Chromium) concentrations (µg/L) in the surface water samples of the Zaghen study area.

If a correlation is made between the toxicity results (water samples from the first two stations) and the heavy metal content of the samples analysed (Fig. 2), the higher toxicity would be due to Pb, which is in the third quality class for station 1 water, and for Ni, Cr, and Cd the values are in the second quality class for both stations.

CONCLUSIONS

The surface water in the Zaghen area shows extremely low toxicity only for stations 1 and 2, and the species tested show different sensitivity to the presence of heavy metals in the water samples.

Toxkit microbiotests have proven to be practical, reliable, and cost-effective tools for detecting and quantifying the risks associated with polluted waters.

Conducting bioassays with different representatives of the biota is crucial for ecologically meaningful assessments of the potential hazards posed by contaminated environments to the aquatic food chain.

Future studies should focus on exploring the relationship between metal levels in zooplankton, water, and sediment, as well as bioaccumulation factors (BAFs) and metal levels in different zooplankton species, in order to identify the most sensitive bioindicators.

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REFERENCES

1. Bouthir F. Z., Afandi I., Talba S., Labonne M., Masski H., Waeles M. and Lae R., 2023 – First survey of metallic distribution in zooplankton from a south Moroccan area, *Oceanologia*, ISSN 0078-3234, DOI: 10.1016/j.oceano.2023.06.009.
2. Dimitriu M., Scrieciu M. A. and Oprea I. A. 2010 – The ecological reconstruction of the Zaghen Tulcea wetland and its evaluation, *Global Journal of Researches in Engineering*, 10, 5, 14-20.
3. Dumitrescu V. and Cârșmariu A., 2014 – The Lower Danube green corridor. Contributions to the analysis of the restoration opportunities for certain surveyed wetlands in the Danube floodplain, diversified by types of potential, 2nd International Conference Water Resources and Wetlands, 11-13 September, Tulcea, Romania.
4. ISO 6341, 2012 – Water quality-determination of the inhibition of the mobility of Daphnia magna straus (Cladocera, Crustacea) – Acute Toxicity Test. ISO: Geneva, Switzerland, available online: https://www.iso.org/standard/54614.html (accessed on 17 July 2023).
5. ISO 14380, 2011 – Water quality – determination of the acute toxicity to Thamnocephalus platyurus (Crustacea, Anostraca). ISO: Geneva, Switzerland, available online: https://www.iso.org/standard/54613.html (accessed on 17 July 2023).
6. Khangarot B. S. and Ray P. K., 1989 – Investigation of correlation between physicochemical properties of metals and their toxicity to the water flea Daphnia magna Straus, *Ecotoxicology and Environmental Safety*, 18, 2, 109-120.
7. Kumar K. S. and Han T., 2010 – Physiological response of Lemna species to herbicides and its probable use in toxicity testing, *Toxicology and Environmental. Health Sciences*, 2, 39-49.
8. Ordin nr. 161 din 16 februarie 2006 pentru aprobarea Normativului privind clasificarea calităţii apelor de suprafaţă în vederea stabilirii stării ecologice a corpurilor de apă. (in Romanian)
9. Park J., Shin K., Lee H., Choi S., Kim G., Depuydt S., De Saeger J., Heynderickx P. M., Wu D., Asselman J., Janssen C. and Han T., 2023 – Evaluating ecotoxicological assays for comprehensive risk assessment of toxic metals present in industrial wastewaters in the Republic of Korea, *Science of The Total Environment*, 867, 161536, DOI: 10.1016/j.scitotenv.2023.161536.
10. Persoone G., Marsalek B., Blinova I., Törökne A., Zarina D., Manusadzianas L., Nalecz-Jawecki G., Tofan L., Stepanova N., Tothova L. and Kolar B., 2003 – A practical and user-friendly toxicity classification system with microbiotests for natural waters and wastewaters, *Environmental Toxicology*, 18, 6, 395-402, DOI: 10.1002/tox.10141.
11. Robin R. S., Mudulli P. R., Vishmu Vardhan K., Ganguly D., Abhilassh K. R. and Balasubramanian T., 2012 – Heavy metal contamination and risk assessment in the marine environment of Arabian Sea, along the southwest coast of India, *American Journal of Chemistry*, 2, 4, 191-208.
12. Sali I., 2023 – Research on zooplankton communities in the Carasuhat (natural flood regime) and Zaghen (controlled flood regime) ecological reconstruction areas – PhD thesis submitted for public defense, 209.
13. Spreadsheet Instructions for Toxkits Microbiotests LC50 Calculation, available online: http://www.normalesup.org/~vindimian/en_index.html (accessed on 17 July 2023).
14. Test No. 202. 2004 – Daphnia sp. Acute Immobilisation Test, Test No. 202, Daphnia sp. Acute Immobilisation Test|OECD Guidelines for the Testing of Chemicals, Section 2: Effects on Biotic Systems, OECD Library, available online: Oecd-ilibrary.org (accessed on 17 July 2023).
15. 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. and 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.

16. Tofan L., O'Grady E. and Ehlinger T. J., 2014 – Setting up a lab on aquatic ecotoxicology for students, *International Journal of Cross-Cultural Studies and Environmental Communication*, 2, 2, 72-78, DOI: 10.5682/22853324.
17. Wang L., Xing L., Sun J. and Yang L., 2020 – Combined toxicity of copper, cadmium and lead toward Daphnia magna: recommendation for bioassay-based whole effluent toxicity (WET) testing in China, *Journal of Surgical Oncology*, 1-7, DOI: 10.31487/j.JSO.2020.06.
18 Zubcov N., Zubcov E. and Schlenk D., 2008 – The dynamics of metals in fish from Nistru and Prut rivers (Moldova), *Transylvanian Review of Systematical and Ecological Research*, 6, 51-58.

EFFECTS OF LAND USE ON MILLIPEDE COMMUNITIES (SUBPHYLLUM MYRIAPODA, CLASS DIPLOPODA): A REVIEW

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KEYWORDS: millipedes, land use, soil, agriculture, fertilizers, bioindicators, grazing, terrestrial and wetlands land use management.

ABSTRACT

Millipedes are soil macrodetritivores with considerable roles in decomposing organic matter and recycling nutrients. This paper aims to identify the effects of land use on millipedes based on a literature review. Land use changes impact species distribution, diversity, and behavior with consequences for litter decomposition and soil quality. Surrounding landscapes influence the millipedes' community structure. The effects of land use under different ecological contexts and in wetlands and woody riparian zones constitute essential gaps in knowledge.

RÉSUMÉ: Effets de l'usage des terres sur les communautés de diplopodes (Subphyllum Myriapoda, Class Diplopoda): une revue.

Les diplopodes sont des macro-détritivores du sol qui jouent un rôle considérable dans la décomposition de la matière organique et dans le recyclage des nutriments. Cet article vise à identifier des effets de l'usage des terres sur les diplopodes sur la base d'une revue de la littérature. Les changements d'usage des terres influencent la répartition, la diversité et le comportement des espèces, avec des conséquences sur la qualité des sols. Les écosystèmes adjacents influencent la structure des communautés de diplopodes. Les effets d'usage des terres dans différents contextes écologiques et dans les zones humides boisées et les zones riveraines constituent des lacunes critiques dans les connaissances.

REZUMAT: Efectele utilizării terenurilor asupra comunităților de diplopode (Subîncrengătura Myriapoda, Clasa Diplopoda): un review.

Diplopodele sunt macro-detritivore în sol, cu rol considerabil în descompunerea materiei organice și în reciclarea nutrienților. Această lucrare își propune să identifice efectele utilizării terenurilor asupra diplopodelor pe baza analizei literaturii. Schimbările în utilizarea terenurilor influențează distribuția, diversitatea și comportamentul speciilor, având consecințe asupra calității solului. Ecosistemele adiacente influențează structura comunităților de diplopode. Efectele utilizării terenurilor în contexte ecologice diferite și în zone umede și ripariene cu vegetație lemnoasă constituie lacune esențiale în cunoaștere.

INTRODUCTION

Soil macrofauna diversity is extremely important in maintaining soil quality due to its important role in maintaining soil fertility and productivity and its influence on the ecosystem balance (Meitiyani and Dharma, 2018). Soil fauna is often used as a measure of soil quality due to its active role in the decomposition of organic matter, formation of humus, recycling of many nutrient elements, and soil aeration (Dairo and Soyelu, 2017; Meitiyani and Dharma, 2018).

Human activities alter habitat, climate, hydrology, and primary production, causing modifications in the composition of species assemblages (reduced species richness) (Dairo and Soyelu, 2017). Macroinvertebrate communities respond to environmental disturbance induced by land use management (Rossi and Blanchart, 2005). The increasing intensity of agricultural practices and unsuitable soil management result in soil deterioration and biodiversity decline (Sithole et al., 2018). These environmental changes affect the presence and density of the arthropod populations. For example, applying pesticides during agricultural activities strongly influences the diversity and abundance of soil fauna (Meitiyani and Dharma, 2018). Agricultural practices can strongly affect soil's physical and chemical properties (Tóth et al., 2018). Agricultural intensification due to increasing production demands is one of the most significant factors affecting soil biodiversity, with agriculture being Europe's most prevailing form of land management (Tóth et al., 2018).

There are many factors influencing the species richness and abundance of detritivores. Besides pH, microclimate, or habitat structure, local land use impacts the soil macrodetritivore communities, including millipedes (Tóth et al., 2018). Therefore, several studies evaluated the impact of land use on millipedes.

Millipedes are a group of terrestrial invertebrates with great importance, participating in the decomposition and recycling of organic matter. The individuals of this group feed on the plant litter, returning 60% to 90% of organic matter as fecal pellets (Sridhar et al., 2013). They accelerate the decomposition of organic material by soil microbiota and increase soil aeration, improving soil conditions (Stašiov et al., 2014). Millipedes' occurrence depends on factors like the type of ecosystem, humidity, the combination of soil pH and humidity, and the presence of dead wood (Skłodowski and Tracz, 2018). Antunes et al. (2016) pointed out the millipedes' potential role in the production of organic compost because the microflora is provided with critical nutritional components following the physicochemical processes (especially the decreasing of the carbon/nitrogen ratio) that occur due to the fecal pellets millipedes are producing.

Apart from indicating the quality of the natural ecosystems, diplopods are also sensitive to different modifications in semi-natural or artificial habitats, being suitable bioindicators for monitoring the status of the environment and the modifications that appear (Stašiov et al., 2014). The individuals of this group are used as secondary succession bioindicators and in the studies that address the importance of coarse woody debris (Skłodowski and Tracz, 2018).

Habitat type, salt concentration, and soil plasticity are important factors that affect the species composition of diplopod assemblages (Tóth et al., 2018). This group is sensitive to regular disturbances, unfavorable microclimate (especially low humidity), scarce food resources, and monotonous habitat structures. Millipedes actively migrate to open agricultural landscapes, colonizing them (Dauber et al., 2005). In agroecosystems, millipedes usually show low species richness but high density (Tóth et al., 2018). Some millipede species, for example, *Brachyiulus bagnalli*, tolerate drought conditions and human presence, thus preferring disturbed habitats like cereal fields (Tóth et al., 2018). If the food source of millipedes is depleted in agricultural landscapes, they become a pest to the growing crop (Sithole et al., 2018). Millipedes need Ca and Mg for their exoskeleton. Thus, they are vulnerable in habitats with low availability of these minerals. Millipedes prefer to feed on litter with high Ca content (Berg and Hemerik, 2004) and locations close to coarse woody debris, like forests or riparian woody habitats (beneficial from the nutritional point of view) (Jabin et al., 2004; Skłodowski and Tracz, 2018).

In general, more millipede species tend to occur in forest habitats with higher soil fertility levels; millipede community occurrence and structure depend not only on forest management but also on habitat humidity and soil fertility (Skłodowski and Tracz, 2018). The occurrence of millipedes is more strongly affected by the nitrogen content in the litter layer than by the age of the forest, with higher nitrogen content in soil being reflected by a higher species diversity of millipedes (Skłodowski and Tracz, 2018). Some millipede species could be affected by leaf litter quality (Wu et al., 2015).

Soil degradation and deterioration of water quality due to the intensification of agriculture negatively affect the diversity and densities of soil invertebrates and the functioning of food chains. Once the disturbance is reduced, biodiversity may have the ability to recover quickly (Steinwandter et al., 2017).

MATERIAL AND METHODS

The scientific articles that constitute the background of this paperwork were selected from the Web Of Science database. The first set of keywords introduced referred to the Diplopoda taxonomic group: diplopod, “diplopod species”, millipedes, Julid, Polydesmid, Chordeumatid, Glomerid, Polyxenid, Glomeridesmid, Sphaerotheriid, Platydesmid, Siphonocryptid, Siphonophorid, Polyzoniid, Callipodid, Stemmiulid, Spirostreptid, Spirobolid, Siphoniulid, “*Rhinocricus padbergi*”, “*R. padbergi*”. The second set of keywords referred to land use: land-use, landuse, “land use”, managed, pasture, agriculture/agricultural, fertiliser/fertilizer, “set aside fields”, grazing, “alley-cropping”, tillage, “sewage sludge”, “sewage mud”, “grassland management”, “degraded meadow”, “forest compared to plantation”, “disturbance factors”, “gut microbiota”, “pesticide applications”, “field margins”, “riparian”, “forest than plantation”, “sludge application”. The last set of specific keywords had the role of excluding the unnecessary scientific articles that were not related to the subject of the paper: fish, virus, fungi, fossil, chromosomal, and molecular.

The search showed 150 results until 26.08.2020. From the total of 150 scientific articles, 73 were selected based on the following criterion: the keywords introduced in the search must be present in the results section of each article.

Another search was made in September 2023 on Web Of Science with the same keywords shown above in order to update the status of knowledge regarding the studied subject. It resulted that 34 more studies were added on the platform regarding the effects of land use on millipedes from 27.08.2020 to 20.09.2023. For the selection, the same criterion mentioned above was applied. The selection resulted in 13 studies.

From the total of 86 selected studies, 17 scientific papers were not relevant to the subject of this paper and they were not considered.

RESULTS AND DISCUSSION

1 The current state of knowledge regarding the effects of land use on millipedes

1.1 The effects of land use on millipede communities in agricultural context

Due to the numerous environmental modifications caused by anthropogenic activities, an environment-friendly agriculture is recommended instead of conventional agriculture. Applying alternative management forms is sustainable for the soil, maintaining its fertility (Stašiov et al., 2014). Agronomic practices, especially on continuously cropped soil, have negative effects on faunal biodiversity. Environment-friendly farming systems that imply minimum soil tillage, non-chemical pest control strategies, and regular soil tests are viable ways of conserving biodiversity (Dairo and Soyelu, 2017).

Maintaining favorable conditions for macrofauna settlement in cropped land is important for long-term soil health and the sustainability of agricultural production. Monocropping, for example, has adverse effects, such as the build-up of pests and diseases and increasing levels of chemical inputs (Sithole et al., 2018).

Farmers adopt intensive cropping practices such as monoculture, continuous cropping, and conventional tillage to obtain higher production. In the past, cultivation periods were alternated with long fallow periods or rotations with other crops to manage soil fertility. Due to the rising demand for food, the fallow periods and crop rotations were shortened or erased in favor of continuous production, negatively affecting soil (Dairo and Soyelu, 2017).

The effects of land use on millipedes have been addressed since the 1990s. The effects of different types of land use on millipedes, based on literature review, are synthesized in table 1.

Table 1. Effects of different land use on millipede communities.

Types of land use	EFfects on millipede communities	References
slash-and-burn fields vs. slash-and-mulch fields	higher densities in slash-and-burn fields	Trabaniño et al. (1990)
set-aside fields vs. semi-natural grasslands vs. wheat fields	highest total abundance in set-aside fields lowest abundances in grasslands	Tóth et al. (2016)
set-aside fields vs. cereal fields adjacent to set-asides vs. cereal fields vs. semi-natural grasslands	highest abundance in semi-natural grasslands lowest abundance in cereal fields adjacent to set-asides and in set-aside fields	Tóth et al. (2018)
field margins vs. crop	no differences regarding diversity	Smith et al. (2008)
forest margins vs. forest vs. open grassland	higher abundance in forest margins	Skłodowski and Tracz (2018)
Sesbania sp. and pigeon pea mixtures vs. monoculture	higher abundance in the *Sesbania* sp. and pigeon pea mixtures	Sileshi et al. (2008)
maize cultures grown in agroforestry systems vs. miombo woodlands vs. maize	higher abundance in the maize cultures grown in agroforestry systems and miombo woodlands	Sileshi and Mafongoya (2006)
peach palm monoculture vs. agroforestry	higher abundance in peach palm monoculture	Vohland and Schroth (1999)

Table 1 (continued): Effects of different land use on millipede communities.

Types of land use	Efects on millipede communities	References
no-tillage with mulch vs. rotational tillage with mulch vs. conventional tillage	higher numbers of individuals in the areas with no-tillage with mulch and rotational tillage with mulch	Sithole et al. (2018)
no-tillage systems vs. conventional tillage systems	higher activity density (the catch rate per trap) in the no-tillage systems	Manetti et al. (2010, 2013)
no-tillage systems vs. conventional tillage systems	significantly higher abundances in no-tillage systems	Errouissi et al. (2011)
no-tillage systems vs. conventional tillage systems	significantly higher densities in no-tillage systems	Marchão et al. (2009)
no-tillage systems vs. conventional tillage systems	higher numbers of individuals in no-tillage systems	Sharley et al. (2008)
no-tillage systems vs. conventional tillage systems	higher mean densities in no-tillage systems	Brévault et al. (2007)
no-tillage systems vs. rotational tillage systems vs. conventional tillage systems	higher numbers of individuals in no-tillage systems and rotational tillage systems	Zulu et al. (2022)
sustainable agricultural management vs. conventional agricultural management	higher species richness and higher total abundance in the areas with sustainable agricultural management	Stašiov et al. (2014)
organic agricultural management vs. conventional agricultural management	lower abundances in conventional fields surrounded by conventional fields compared to conventional fields surrounded by organic fields	Diekötter et al. (2010)
fallowed fields vs. cultivated fields with frequent soil tillage, application of pesticides and fertilizers	higher numbers of individuals in fallowed fields	Dairo and Soyelu (2017)
managed mixed plantation (no-tillage, minimum weeding) vs. semi-evergreen forest reserve	higher abundance and biomass in plantation	Ashwini and Sridhar (2006)
pastures with alternate grazing-mowing vs. pastures with sheep grazing	higher abundance in pastures with alternate grazing-mowing	Mikula et al. (2014)
local mixture of seeds with herbs vs. commercial mixture of fodder crops vs. spontaneous fallow land	higher abundance in sites with local mixture of seeds with herbs	Mikula et al. (2014)
mulching vs. fertilization vs. no mulching and no fertilization	higher abundance in mulched sites	Mikula et al. (2014)
pure maize crop without fertilization vs. maize crop with mineral fertilization vs. maize crop inter-cropped with a legume cover crop	higher densities in the maize crop inter-cropped with the legume cover crop	Blanchart et al. (2006)

Table 1 (continued): Effects of different land use on millipede communities.

Types of land use	Effects on millipede communities	References
semi-evergreen forests vs. plantations	low carbon to nitrogen ratio and increased content of nitrogen, phosphorus and calcium in the manure of diplopods present in plantations	Sridhar et al. (2013)
cultivated fields vs. grass-dominated fields vs. pine stands vs. hardwood stands	higher abundances in the pine and hardwood forest stands	Callaham et al. (2006)
shrublands vs. grasslands	higher abundances in shrublands	García et al. (2010)
agroforestry ecosystems vs. plantations vs. forests vs. annual crop fields	significantly higher abundances in agroforestry ecosystems, plantations, and forests	Mujeeb Rahman et al. (2012)
managed forest vs. primeval forest	higher species richness in the managed forest	Skłodowski and Tracz (2018)
abandoned pasturelands vs. managed pasturelands	higher numbers of individuals in the abandoned pasturelands	Steinwandter et al. (2017)
forest land vs. homestead garden fields vs. grazing land vs. crop cultivated outfields	highest abundances and diversities in forest land and homestead gardens lowest abundances and diversities in crop cultivated outfields	Bufebo et al. (2021)
secondary vegetation vs. cropping in forest plantation vs. wooded pastures	secondary vegetation presented higher abundances	Duran-Bautista et al. (2023)
permanent crop vs. rotation crop vs. pasture	lowest numbers of individuals in pasture	Forero et al. (2021)
grassland vs. 10-year-old *Eucalyptus* plantation vs. 20-year-old *Eucalyptus* plantation	higher densities in the 10-year-old *Eucalyptus* plantation	Sabatté et al. (2021)
rice paddies vs. natural wetlands	millipedes were present only in the natural wetlands	Bunyangha et al. (2022)

For example, Trabaniño et al. (1990) demonstrated that millipedes were present in higher densities in slash-and-burn fields compared to the slash-and-mulch fields in plains. Dobrovodská et al. (2023) showed that burned plots are unsuitable for millipede communities.

There are a few solutions to provide the appropriate environmental conditions to sustain the biodiversity in agricultural landscapes. The existence of set-aside fields represents such a measure, which preserves and increases the species diversity in agricultural landscapes, and makes a connection between habitats. Set-aside fields are green sections placed in agricultural landscapes and are usually sown with seeds of grass and leguminous species (Tóth et al., 2018). Tóth et al. (2016) found a higher total abundance of millipedes in set-aside fields compared to wheat fields and semi-natural grasslands, while Tóth et al. (2018) demonstrated the positive effects of set-aside fields on most of the soil inhabitant taxa, except millipedes, the individuals of this group being most abundant in the semi-natural grasslands. The advantages of this measure on soil biodiversity are still debatable (Tóth et al., 2018).

The alley-cropping system (polyculture farming) represents another measure to protect and increase biodiversity in agricultural landscapes. It implies a main crop and a secondary crop that are interleaved. The secondary crop is usually a food crop but can also be a tree species, grass, or shrubs. Therefore, complementary habitats are provided for specialist and generalist species (Ashraf et al., 2018). Positive effects of alley-cropping on arthropods in an oil palm plantation (the expansion of palm oil plantations constitutes a threat to biodiversity due to the conversion of forests into plantations, thus the destruction of habitats) were demonstrated, the diversity and abundance of arthropods (including millipedes) being greater in the alley-cropping system than in the monoculture due to the increased habitat heterogeneity, variation in microhabitats and a more optimal microclimatic condition. Also, decomposer arthropod populations would be greater in alley-cropping systems than in monocultures due to a more abundant ground litter, which represents a food source and stabilizes humidity and temperature. This would be beneficial since decomposer invertebrates are crucial components of the soil biota in agricultural landscapes due to their role in increasing soil fertility (Ashraf et al., 2018). According to Sileshi et al. (2008), maize-legume rotations have beneficial effects on millipedes (and soil macrofauna in general) compared to continuously cropped maize, but further research is necessary. This study is consistent with other studies that demonstrated monocultures' negative effects on soil macrofauna.

Contrary to other studies, an older study in central Amazonia (Vohland and Schroth, 1999) showed a higher abundance of millipedes in a peach palm monoculture compared to an agroforestry system because the remains of branches in various stages of decomposition protected the populations of resident diplopods from solar radiation and desiccation.

Dauber et al. (2005) showed that forest edges have an effect on millipedes, the vicinity of forests being essential for them because they serve as refuges and sources of recolonization. In their study, millipede species richness was almost entirely influenced by the composition of the surrounding landscape, being significantly higher in the fallow land compared to the arable land and the grassland. It was concluded that agricultural management does not increase millipede diversity at the landscape level. However, they are caught by "accident" in grassland and arable land traveling from the nearby forests. According to Stašiov et al. (2014), a convenient distribution of arable lands increases the diversity of millipede communities.

The use of techniques such as "conventional tillage" has been shown to cause a significant decrease in spiders, beetles, and diplopods (Brévault et al., 2007; Sharley et al., 2008; Marchão et al., 2009; Manetti et al., 2010; Errouissi et al. 2011; Manetti et al., 2013; Sithole et al., 2018; Fiera et al., 2020; Zulu et al., 2022). Soil management systems, such as conventional tillage, result in the decline of soil organic matter content, deterioration of important soil physical properties, and increase the risk of soil erosion, further affecting soil macrofauna (Sithole et al., 2018). Nowadays, there's a tendency to adopt conservation tillage practices (no-tillage, reduced tillage, and minimum tillage), thus protecting soil from wind and water erosion. The extreme form of reduced tillage (the no-tillage practice) implies that no soil manipulation is performed before planting and before crop residues are left on the soil. Plowing affects soil invertebrates' horizontal and vertical distribution, perturbing their communities and reducing their abundance (Salvio et al., 2011). Manetti et al. (2010) specify that soil degradation increases due to the intensification of agricultural activities carried out with conventional tillage systems, no-till systems being considered less aggressive to soil fauna. Ashwini and Sridhar (2006) demonstrated that an endangered pill millipede species preferred a managed mixed plantation (where organic farming is practiced, which implies no tillage and minimum weeding) to a semi-evergreen forest reserve, showing that organic farming represents a possible solution in limiting the effects of conventional farming on millipede communities. Therefore, management should imply minimum tillage practices such as rotational and strip tillage and biological control instead of chemical application to conserve biodiversity. Regular soil tests are necessary to monitor soil health (Dairo and Soyelu, 2017).

Sometimes, semi-natural grasslands can suffer biodiversity losses due to abandoning traditional management practices, but these situations can be improved by renewed conservation management like grazing or mowing. Van Noordwijk et al. (2017) found an increased richness of millipedes after 17 years of conservation management in grasslands. These results contradict Steinwandter et al. (2018) which stated that the millipedes were most abundant in areas with low sheep grazing intensity. Dobrovodská et al. (2023) showed that regularly mown and grazed plots support millipede diversity. Morón-Ríos et al. (2010) found no detectable effects of seasonal grazing on soil macroinvertebrates. It can be assumed that the intensity of grazing can make the difference, but more studies need to be conducted.

Changes in agricultural land use can result in changes in vegetation. Analyzing the influence of vegetation (which represents plant food sources for detritivores) changes on the gut microbiota of detritivores in the saltmarshes of the German North Sea coast, Dittmer et al. (2012) concluded that the gut microbiota of diplopods is expected to change with the succession of the vegetation in this type of ecosystem.

In the context of replacing native vegetation with pine plantations, Car (2010) concluded that the abundance and species richness of millipedes (in this case, individuals from the Paradoxosomatidae family) did not change between vegetation types in certain conditions, showing that vegetation type is less important for the studied group. It was also concluded that the disturbance of the leaf litter habitat has negative effects on the paradoxosomatid populations. Laossi et al. (2008) showed that plant diversity had no effect on millipedes, but plant biomass strongly affected millipedes in Amazonian pastures.

Consistent with recent studies, in an older study conducted by Emmerling (1995) in the flooded and agricultural sites from the fluvisols of Western Germany, it is mentioned that the increasing gradient of agricultural land use was the main environmental factor that caused the decrease in species diversity, abundances, and fresh biomass of diplopods because of the disturbances caused by agricultural practices in intensively farmed sites. Emmerling (1995) also demonstrated that diplopod diversity was higher in the unflooded sites.

The effects of grazing on litter-feeding soil macrodetritivores are indirect: because grazing affects the plant traits due to biomass removal, trampling, and altered nutrient availability, it can thus affect the litter quality, and palatability. The changes in litter traits caused by grazing impact detritivores' food preferences. However, the changes caused by grazing in litter quality and palatability are stronger in some plant species. Even though soil fertilization itself increases litter palatability (due to the presence of nitrogen, which is found in higher quantities in plant leaves from fertilized areas than in those from unfertilized areas), grazing increases litter palatability in fertilized areas, but not the same happens in the unfertilized areas (Coq et al., 2018). Even though grazing can increase litter palatability for millipedes, Steinwandter et al. (2018) demonstrated that the sheep grazing intensity has a strong effect on the soil macroinvertebrate abundances and community structure, with millipedes being most abundant at the locations with low sheep grazing intensity. However, the abundances of soil macroinvertebrates, in general, were high at the locations with medium and high sheep grazing intensity, proving that the presence of sheep has a general positive effect on soil invertebrates. García et al. (2010) analyzed the effects of grazer species (sheep and goats) and vegetation type (heather, gorse, and grass) on arthropods (including diplopods). The results showed that diplopod populations were not significantly affected by these factors.

Nash et al. (2010) demonstrated that increased pesticide tolerance can appear in some millipede pest species. A study conducted by Salvio et al. (2011) in a soybean plantation under no-tillage addressed the problem of toxic granulated baits with metaldehyde or carbaryl used for slug and pillbug control, which can be harmful to non-target organisms. The study showed that diplopod abundance was not affected by the baits, but further studies are necessary on the effects of these baits on non-target organisms because very little is known about this subject.

Inconsistencies regarding the effects of land use on millipedes appear in literature in some situations. Skłodowski and Tracz (2018) found a higher species richness of millipedes in a managed forest compared to a primeval forest, contrary to the expectations (because species diversity of millipedes is known to be higher in the proximity of dead wood mainly present in the unmanaged forests), managed forests being shadier, more humid, and having a more homogenous structure. There was no difference between millipedes' life traits in the primeval and managed forests (Skłodowski and Tracz, 2018). Chumak et al. (2015) compared species numbers, abundances, and species composition of arthropods (millipedes included) in pristine (virgin) and managed forests. Contrary to the expectations, they did not find significant differences between the two types of forests.

Skłodowski and Tracz (2018) suggest that the managed forests should be protected, and their exploitation should be reduced to very small areas, imitating natural gaps (which should increase the number of millipede species). Removing single trees should be preferred instead of clearcutting (millipedes' richness and density may be higher in the proximity of coarse woody debris). Marichal et al. (2014) demonstrated that soil macroinvertebrates (including diplopods) are affected by landscape dynamics and composition, with the densities of diplopods showing a decrease with deforestation. This study confirmed that due to soil macrofauna's demonstrated correlation between density and soil services, soil macroinvertebrates can represent indicators of soil ecosystem services.

The problem of historical land use was addressed by Kappes et al. (2012), who analyzed a post-mining land and its effects on soil and litter-dwelling fauna in a beech forest, one of the results being that diplopods were more abundant in the small mining pits. They specify that this positive effect may be caused by the fact that pit-like structures have the role of refuges from drought.

1.2 The effects of fertilizers on millipedes

The agricultural, urban, and industrial activities are polluting the soil with residues, which have a negative impact on the environment, thus, the importance of toxicity tests to evaluate the risks to which the environment is exposed is enormous (Coelho et al., 2017). Therefore, studies were conducted to assess the impact of some alternative fertilizers on millipede communities in agricultural landscapes, the organisms belonging to this group being successfully used as bioindicators of soil contamination (Francisco et al., 2015; Christofoletti et al., 2016). This is mainly due to millipede exposure to soil contaminants and their role in transferring them throughout the food chain (Christofoletti et al., 2016; Coelho et al., 2017).

One of the alternative fertilizers studied is the sugarcane vinasse, which represents the main residue from the sugar-alcohol industry. It has an acid pH, high organic matter content and a concentration of salts and metal ions. There are a few studies about the toxic effect of this residue on aquatic organisms and plants, but the information is even poorer when it comes to edaphic fauna. (Christofoletti et al., 2016; Coelho et al., 2017)

Another alternative fertilizer is sewage sludge. It is a residue resulting from wastewater treatment. Studies show that it can be toxic due to the metals, organic compounds and xenobiotics found in it. However, it can be transformed into a biosolid to decrease the number of pathogenic microorganisms. Even as a biosolid, it can contain organic compounds and trace metals; thus, it can still be toxic. (Gaylor et al., 2014; Francisco et al., 2015; Christofoletti et al., 2016; Coelho et al., 2017)

These residues contain high levels of organic matter. Thus, they are widely used as fertilizers as a new disposal alternative but can also contaminate soils (Christofoletti et al., 2016; Coelho et al., 2017). The effect of sewage sludge on soil arthropods is documented even in older research. For example, Andres (1999), showed that millipede densities significantly decreased when an increased concentration of sewage sludge was applied. Millipedes accumulate harmful compounds, especially in the midgut (Christofoletti et al., 2016; Coelho et al., 2017). Thus, much attention has been given lately to assessing the effects of toxic substances in the target organs of millipedes (Francisco et al., 2015).

Coelho et al. (2017) evaluated the sugarcane vinasse and biosolid toxicity using concentrations compatible with the regulation for agricultural use of the studied area (Brazil). The study showed that the biosolid had an effect on some cell layers in the millipedes' midgut, more exactly in the apical and the basal portions of the epithelium (which host the regenerative cells) and also in the hepatic cell layer, but the effect was not significant. On the other hand, the sugarcane vinasse had a much stronger effect on these cell layers than the biosolid. These cell layers showed an increase in the expression of the HSP70 protein, which has an important role in preventing or reversing proteotoxic damage. Thus, the biosolid and the sugarcane vinasse generated a cytoprotective response due to their proteotoxic action.

Christofoletti et al. (2016) evaluated the toxicity of sewage sludge, biosolids and sugarcane vinasse on millipedes' midgut, using concentrations according to the regulations for land application in the studied area (Brazil). As the authors specify, the main tissue responses were significant brush border thickening, induction of epithelial renovation, clustering of hemocytes, accumulation of cytoplasmic granules in hepatic cells, hepatic cells with heteropycnotic nuclei, and cytoplasmic degradation. There were also behavioral changes in the millipedes: the individuals started to avoid burying themselves in soil with biosolid or vinasse.

Francisco et al. (2015) evaluated biosolids' effects on millipedes' perivisceral fat. Histopathological and ultrastructural changes were observed in individuals exposed to the biosolids, as well as pathological changes related to detoxification mechanisms. The exposed individuals died after 90 days of exposure, suggesting the possible toxicity of the biosolids. Loss of cell membranes and cytoplasmic degradation were observed after the millipedes' exposure to the biosolids, as well as a significant increase of the spherocrystals (which are unnecessary substances such as metals stocked in the fat body cells in the form of granules) present in the trophocytes of millipedes. Consistent with other studies, Zulu et al. (2022) showed that millipedes are sensitive to increases in fertilizer application rates.

Sewage sludge used in a variety of concentrations has negative effects on millipedes at the cellular level and can cause death of the animals exposed, as synthesized in tables 2a-c (De Godoy and Fontanetti, 2010; Nogarol and Fontanetti, 2010; Nogarol and Fontanetti, 2011; Perez and Fontanetti, 2011; Bozzatto and Fontanetti, 2012; De Souza and Fontanetti, 2012).

At the cellular level, the most common effects of sewage sludge on millipedes are: frequent points of epithelial renovation at the epithelium level, an increase in the number of cytoplasmatic granules at the hepatic cells level, and a reduction in the number of neutral polysaccharides at the fat body cells level. These three effects can be observed at all concentrations of sewage sludge and all exposure durations. The death of the millipedes before

90 days of exposure can be observed in the cases of 10% and 50% concentrated sewage sludge (Nogarol and Fontanetti, 2010; Nogarol and Fontanetti, 2011; Perez and Fontanetti, 2011; Bozzatto and Fontanetti, 2012; De Souza and Fontanetti, 2012).

Gaylor et al. (2014) conducted a study on the bioaccumulation of Polybrominated Diphenyl Ether (PBDE) in an agricultural soil ecosystem where biosolids are applied. BDE 209 (the major constituent of PBDE) was detected in high levels in millipedes compared to other taxa. BDE-47, BDE-206, 207, and 208 were also found in millipedes.

A study made by Berg and Hemerik (2004) in the Netherlands analyzed the average densities of macroinvertebrates in grasslands at 7, 11, 24, and 29 years after the last fertilization with N, P, and K. It showed that millipedes had higher average densities 24 years after the last fertilization and the lowest average densities at 29 years after the last fertilization, suggesting long-term effects of fertilizers on millipedes. Therefore, caution is needed regarding the use of these residues in agriculture due to their possible negative effects on the environment. Also, waste management techniques need to be improved in order to prevent the negative effects (Francisco et al., 2015; Christofoletti et al., 2016; Coelho et al., 2017).

Table 2a: Tissular effects of sewage sludge on different parts of the midgut of the millipede species *Rhinocricus padbergi* at different sewage sludge concentrations and exposure periods.

Effects / Part of the midgut/concentration and exposure time	Frequent points of epithelial renovation	Increased liberation of secretory vesicles of glycoproteic nature	Presence of calcium released into the lumen	Presence of neutral polysaccharides	Reduction in the number of neutral polysaccharides	Epithelial cells containing calcium	Increase in the number of cytoplasmatic granules
epithelium	+	+	+			+	+
hepatic cells				+			+
fat body cells					+		+
1% 7 days	x	x	x	x	x		
1% 15 days	x				x	x	x
1% 90 days					x		x
10% 7 days	x	x	x	x	x	x	
10% 15 days	x				x	x	x
50% 7 days	x		x	x	x		x
50% 15 days					x	x	

Table 2b: Tissular effects of sewage sludge on different parts of the midgut of the species *Rhinocricus padbergi* at different sewage sludge concentrations and exposure periods.

Effects / Part of the midgut/concentration and exposure time	Increase in the number of hemocytes	Presence of autophagic vacuoles	Increased number of tracheoles	Integrity loss of cellular constituents	Most of the sperochystals contain calcium	Cytoplasmatic vacuolization of the cells	Increase in the quantity of intracellular cranules
epithelium	+	+		+		+	
hepatic cells		+		+			
fat body cells	+		+	+	+		+
1% 15 days	x						
1% 90 days		x	x	x	x		
10% 7 days	x					x	x
10% 15 days	x						
50% 7 days	x						

Table 2c: Tissular effects of sewage sludge on different parts of the midgut of the millipede species *Rhinocricus padbergi* at different sewage sludge concentrations and exposure periods.

Effects Part of the midgut/ concentration and exposure time	Increase in the amount of spherocrystals	Cytoplasmatic degradation	Nuclei with altered morphology	Decrease of proteins	Numerous secretory vesicles of the apocrine type	Several cytoplasmatic granules	Large amount of neutral polysaccharides	Death of the animals before 90 days of exposure
epithelium					+			
hepatic cells						+	+	
fat body cells	+	+	+	+				
10% 7 days	x					x		
10% 15 days	x	x	x	x				
10% 90 days								x
50% 7 days	x	x						
50% 15 days	x	x	x	x	x	x	x	
50% 90 days								x

2. Gaps in knowledge

There are few studies on the impact of land use and soil management systems on millipedes, and we identified several knowledge gaps on this subject. Even though they are very important soil macrodetritivores with major roles in decomposition (De Smedt et al., 2018), millipedes are poorly studied in a landscape context. Knowledge of how land use intensification changes invertebrate communities and trophic interactions in the soil is not enough advanced to guide the development of sustainable agriculture (Lagerlöf et al., 2017). The advantages of set-aside fields on soil biodiversity are still debatable (Tóth et al., 2018). The interactions and effects of soil macrofauna and crop mixtures on soil microbial communities and nutrient fluxes in agricultural ecosystems depend on environmental contexts and are poorly understood. Usually, increasing land use intensity decreases species richness, but patterns are still unclear (De Smedt et al., 2018). More studies are necessary to connect the density of soil invertebrates and beneficial agriculture products. Also, experiments with more crop species are required (Errouissi et al., 2011). Inconsistencies regarding the effects of grazing on millipedes were found in the literature; thus, more studies need to be conducted. Alternative forms of management are thought to have a less negative impact on the environment. However, there is a lack of studies to demonstrate their real advantages for the abundance and diversity of millipedes and soil invertebrates in general (Stašiov et al., 2014).

At the cellular level, even though the reactions of the HSP70 protein can signal environmental alterations and are assessed in a few studies, the studies that assess its expression in diplopods are scarce. More studies are necessary to identify inert and contaminant components contained in alternative fertilizers. Gaylor et al. (2014) mentioned that few studies have examined the accumulation in soil of compounds associated with sewage sludge applied to land, and even fewer studies have addressed the PBDE accumulation in soil biota. Very little is known about the possible effects of molluscicide and crustacicide substances used as baits in plantations on non-targeted organisms (Salvio et al., 2011).

Most studies on soil arthropods and millipedes quantify changes in abundance patterns, while functional responses are rarely addressed (Birkhofer et al., 2016). Greater attention was received by soil arthropod communities in agricultural landscapes, with wetlands, riparian areas, or alpine regions remaining almost unstudied. From all the scientific articles selected, only one paper included wetlands in the study (Bunyangha et al., 2022). Riparian zones and wetlands represent very heterogeneous systems that support highly diverse components of terrestrial life and represent valuable study areas, as direct interactions between terrestrial and aquatic communities can be observed at their level (Ramey and Richardson, 2017). The study of riparian areas and wetlands helps to understand the organization, dynamics, and diversity not only of terrestrial but also of lotic communities (Naiman and Décamps, 1997; Forio et al., 2020; Popescu et al., 2021). Protecting riparian areas and wetlands becomes particularly important in the context of increased anthropogenic pressures. Though such areas occupy a relatively small proportion of the landscape surface, they have a disproportionately higher role in mitigating the effects of pollution and climate change. They are sources of biodiversity, including millipede communities, for other types of landscapes. Millipedes are especially important due to their contribution to soil fertility and nutrient recycling. They have an essential role in the dynamics of terrestrial and wetland ecosystems (Francisco et al., 2015) and in humidifying the soil (Coelho et al., 2017).

Millipede communities are susceptible to environmental changes, and modifications of their community structure occur quickly under anthropogenic pressures. Brévault et al. (2007) suggested that identifying groups of species that can be used as bioindicators of environmental conditions or soil ecological processes would be of great interest. However, millipedes as soil quality monitoring bioindicators still need to be standardized and widely used.

CONCLUSIONS

Our study shows that more millipede species tend to occur in forest and riparian habitats with high humidity and coarse woody debris. Land use changes affect millipedes at the cellular level with consequences on individuals' behavior, species distribution, and community composition due to the sensitivity of this taxonomic group to soil disturbance. Millipedes are more abundant in agricultural lands where aggressive agricultural practices such as tillage are kept to a minimum and sustainable forms of agricultural management are practiced. However, it needs to be clarified if millipedes prefer all the sustainable forms of agricultural management. Set-aside fields represent an example in this regard.

At the cellular level, soil fertilization using alternative fertilizers generates a cytoprotective response in millipedes' midgut and fat body cells. Alternative fertilizers also cause changes in the millipedes' behavior. Thus, the toxic action of alternative fertilizers was demonstrated on millipedes. Therefore, caution is needed towards their use in agriculture.

It was argued that the millipedes' role in litter decomposition can be affected by grazing and soil fertilization since they combined increase litter palatability. The rates of litter decomposition by millipedes in fertilized agricultural landscapes are higher.

Even though wetlands and riparian zones are among the ecosystems most affected by land use modifications, studies on the effects of land use changes on the millipede communities inhabiting these areas are very scarce.

Some clear progress has been made in understanding the effects of land use on millipede communities. However, more studies need to be done to determine the patterns and drivers of community responses under environmental contexts and land use intensities.

REFERENCES

1. Andres P., 1999 – Ecological risks of the use of sewage sludge as fertilizer in soil restoration: effects on the soil microarthropod populations, *Land Degradation & Development*, 10, 1, 67-77.
2. Antunes L. F. D. S., Scoriza R. N., Silva D. G. D. and Correia M. E. F., 2016 – Production and efficiency of organic compost generated by millipede activity, *Ciência Rural*, 46, 815-819.
3. Ashraf M., Zulkifli R., Sanusi R., Tohiran K. A., Terhem R., Moslim R. and Azhar B., 2018 – Alley-cropping system can boost arthropod biodiversity and ecosystem functions in oil palm plantations, *Agriculture, Ecosystems & Environment*, 260, 19-26.
4. Ashwini K. M. and Sridhar K. R., 2006 – Seasonal abundance and activity of pill millipedes (Arthrosphaera magna) in mixed plantation and semi-evergreen forest of southern India, *Acta Oecologica*, 29, 1, 27-32.
5. Berg M. P. and Hemerik L., 2004 – Secondary succession of terrestrial isopod, centipede, and millipede communities in grasslands under restoration, *Biology and Fertility of Soils*, 40, 163-170.
6. Birkhofer K., Dietrich C., John K., Schorpp Q., Zaitsev A. S. and Wolters V., 2016 – Regional conditions and land-use alter the potential contribution of soil arthropods to ecosystem services in grasslands, *Frontiers in Ecology and Evolution*, 3, 150.
7. Blanchart E., Villenave C., Viallatoux A., Barthès B., Girardin C., Azontonde A. and Feller C., 2006 – Long-term effect of a legume cover crop (Mucuna pruriens var. utilis) on the communities of soil macrofauna and nematofauna, under maize cultivation, in southern Benin, *European Journal of Soil Biology*, 42, S136-S144.
8. Bozzatto V. and Fontanetti C. S., 2012 – Sewage sludge toxicity in edaphic organism: analysis of midgut responses in the diplopod Rhinocricus padbergi, *Microscopy Research and Technique*, 75, 7, 869-875.
9. Brévault T., Bikay S., Maldes J. M. and Naudin K., 2007 – Impact of a no-till with mulch soil management strategy on soil macrofauna communities in a cotton cropping system, *Soil and Tillage Research*, 97, 2, 140-149.
10. Bufebo B., Elias E. and Getu E., 2021 – Abundance and diversity of soil invertebrate macro-fauna in different land uses at Shenkolla watershed, South Central Ethiopia, *The Journal of Basic and Applied Zoology*, 82, 1-12.
11. Bunyangha J., Muthumbi A. W., Gichuki N. N., Majaliwa M. J. and Egeru A., 2022 – Soil macroinvertebrate response to paddy rice farming pathways in Mpologoma Catchment, Uganda, *Agronomy*, 12, 2, 312.
12. Callaham Jr. M. A., Richter Jr. D. D., Coleman D. C. and Hofmockel M., 2006 – Long-term land-use effects on soil invertebrate communities in Southern Piedmont soils, USA, *European Journal of Soil Biology*, 42, S150-S156.
13. Car C. A., 2010 – Pine plantations and native millipedes (Diplopoda: Polydesmida: Paradoxosomatidae) in south-eastern New South Wales, Australia, *Australian Journal of Entomology*, 49, 4, 317-323.
14. Christofoletti C. A., Francisco A., Pedro-Escher J., Gastaldi V. D. and Fontanetti C. S., 2016 – Diplopods as soil bioindicators of toxicity after application of residues from sewage treatment plants and ethanol industry, *Microscopy and Microanalysis*, 22, 5, 1098-1110.
15. Chumak V., Obrist M. K., Moretti M. and Duelli P., 2015 – Arthropod diversity in pristine vs. managed beech forests in Transcarpathia (Western Ukraine), *Global Ecology and Conservation*, 3, 72-82.
16. Coelho M. P. M., Moreira-de-Sousa C., de Souza R. B., Ansoar-Rodríguez Y., Silva-Zacarin E. C. M. and Fontanetti C. S., 2017 – Toxicity evaluation of vinasse and biosolid samples in diplopod midgut: heat shock protein in situ localization, *Environmental Science and Pollution Research*, 24, 22007-22017.

17. Coq S., Nahmani J., Resmond R., Segrestin J., David J. F., Schevin P. and Kazakou E., 2018 – Intraspecific variation in litter palatability to macroarthropods in response to grazing and soil fertility, *Functional Ecology*, 32, 11, 2615-2624.
18. Dairo O. S. and Soyelu O. J., 2017 – Consequences of prolonged agronomic practices: faunal composition and abundance in cultivated and fallowed soils, *International Journal of Conservation Science*, 8, 3.
19. Dauber J., Purtauf T., Allspach A., Frisch J., Voigtländer K. and Wolters V, 2005 – Local vs. landscape controls on diversity: a test using surface-dwelling soil macroinvertebrates of differing mobility, *Global Ecology and Biogeography*, 14, 3, 213-221.
20. De Godoy J. A. P. and Fontanetti C. S., 2010 – Diplopods as bioindicators of soils: analysis of midgut of individuals maintained in substract containing sewage sludge, *Water, Air, & Soil Pollution*, 210, 389-398.
21. De Smedt P., Baeten L., Proesmans W., Berg M. P., Brunet J., Cousins S. A. and Verheyen K., 2018 – Linking macrodetritivore distribution to desiccation resistance in small forest fragments embedded in agricultural landscapes in Europe, *Landscape Ecology*, 33, 407-421.
22. De Souza R. B. and Fontanetti C. S., 2012 – Alterations in the fat body cells of Rhinocricus padbergi (Diplopoda) resulting from exposure to substrate containing sewage sludge, *Microscopy and Microanalysis*, 18, 2, 317-323.
23. Diekötter T., Wamser S., Wolters V. and Birkhofer K., 2010 – Landscape and management effects on structure and function of soil arthropod communities in winter wheat, *Agriculture, ecosystems & environment*, 137, 1-2, 108-112.
24. Dittmer J., Lesobre J., Raimond R., Zimmer M. and Bouchon D., 2012 – Influence of changing plant food sources on the gut microbiota of saltmarsh detritivores, *Microbial ecology*, 64, 814-825.
25. Dobrovodská M., Kanka R., Gajdoš P., Krištín A., Kollár J., Stašiov S. and Lieskovský J., 2023 – Factors affecting the biodiversity of historical landscape elements: detailed analyses from three case studies in Slovakia, *Environmental Monitoring and Assessment*, 195, 6, 1-17.
26. Duran-Bautista E. H., Angel-Sanchez Y. K., Bermúdez, M. F. and Suárez J. C., 2023 – Agroforestry systems generate changes in soil macrofauna and soil physical quality relationship in the northwestern Colombian Amazon, *Agroforestry Systems*, 97, 5, 927-938.
27. Emmerling C., 1995 – Long-term effects of inundation dynamics and agricultural land-use on the distribution of soil macrofauna in fluvisols, *Biology and fertility of soils*, 20, 130-136.
28. Errouissi F., Moussa-Machraoui S. B., Ben-Hammouda M. and Nouira S., 2011 – Soil invertebrates in durum wheat (Triticum durum L.) cropping system under Mediterranean semi arid conditions: A comparison between conventional and no-tillage management, *Soil and Tillage Research*, 112, 2, 122-132.
29. Fiera C., Ulrich W., Popescu D., Bunea C. I., Manu M., Nae I. and Zaller J. G., 2020 – Effects of vineyard inter-row management on the diversity and abundance of plants and surface-dwelling invertebrates in Central Romania, *Journal of insect conservation*, 24, 175-185.
30. Forero N., Serrano P. A. and Forero F. E., 2021 – Influence of land use on the diversity and composition of edaphic arthropods in the Granja Tunguavita, *Temas Agrarios*, 26, 1, 80-91.
31. Forio M. A. E., De Troyer N., Lock K., Witing F., Baert L., Saeyer N. D. and Goethals P., 2020 – Small patches of riparian woody vegetation enhance biodiversity of invertebrates, *Water*, 12, 11, 3070.
32. Francisco A., Christofoletti C. A., Neto N. R. and Fontanetti C. S., 2015 – Histopathological changes in the perivisceral fat body of Rhinocricus padbergi (Diplopoda, Spirobolida) triggered by biosolids, *Environmental Science and Pollution Research*, 22, 18590-18600.
33. García R. R., Ocharan F. J., García U., Osoro K. and Celaya R., 2010 – Arthropod fauna on grassland–heathland associations under different grazing managements with domestic ruminants, *Comptes Rendus Biologies*, 333, 3, 226-234.

34. García R. R., Ocharan F. J., Jáuregui B. M., García U., Osoro K. and Celaya R., 2010 – Ground-dwelling arthropod communities present in three types of Cantabrian (NW Spain) heathland grazed by sheep or goats, *European Journal of Entomology*, 107, 2.
35. Gaylor M. O., Mears G. L., Harvey E., La Guardia M. J. and Hale R. C., 2014 – Polybrominated diphenyl ether accumulation in an agricultural soil ecosystem receiving wastewater sludge amendments, *Environmental Science & Technology*, 48, 12, 7034-7043.
36. Jabin M., Mohr D., Kappes H. and Topp W, 2004 – Influence of deadwood on density of soil macro-arthropods in a managed oak–beech forest, *Forest Ecology and Management*, 194, 1-3, 61-69.
37. Kappes H., Clausius A. and Topp W., 2012 – Historical small-scale surface structures as a model for post-mining land reclamation, *Restoration Ecology*, 20, 3, 322-330.
38. Lagerlöf J., Maribie C. and John J. M., 2017 – Trophic interactions among soil arthropods in contrasting land-use systems in Kenya, studied with stable isotopes, *European Journal of Soil Biology*, 79, 31-39.
39. Laossi K. R., Barot S., Carvalho D., Desjardins T., Lavelle P., Martins M. and Grimaldi M., 2008 – Effects of plant diversity on plant biomass production and soil macrofauna in Amazonian pastures, *Pedobiologia*, 51, 5-6, 397-407.
40. Manetti P. L., Faberi A. J., Clemente N. L. and López A. N., 2013 – Macrofauna activity density in contrasting tillage systems in Buenos Aires Province, Argentina, *Agronomy Journal*, 105, 6, 1780-1786.
41. Manetti P. L., López A. N., Clemente N. L. and Faberi A. J., 2010 – Tillage system does not affect soil macrofauna in southeastern Buenos Aires province, Argentina, *Spanish Journal of Agricultural Research*, 8, 2, 377-384.
42. Marchão R. L., Lavelle P., Celini L., Balbino L. C., Vilela L. and Becquer T., 2009 – Soil macrofauna under integrated crop-livestock systems in a Brazilian Cerrado Ferralsol, *Pesquisa Agropecuária Brasileira*, 44, 1011-1020.
43. Marichal R., Grimaldi M., Feijoo A., Oszwald J., Praxedes C., Cobo D. H. R. and Lavelle P., 2014 – Soil macroinvertebrate communities and ecosystem services in deforested landscapes of Amazonia, *Applied Soil Ecology*, 83, 177-185.
44. Meitiyani and Dharma A. P., 2018 – Diversity of soil arthropods in different soil stratification layers, The National Park of Gede Pangrango Mountain, Cisarua Resort, West Java, Indonesia, *IOP Conference Series: Earth and Environmental Science*, 197, 012019.
45. Mikula J., Sarapatka B., Kopecky O. and Tuf I. H, 2014 – Assemblages of millipedes and centipedes according to the type of pasture management: preliminary results from the Czech Republic, *Revue d'Ecologie, Terre et Vie*, 69, 2, 167-172.
46. Morón-Ríos A., Rodríguez M. Á., Pérez-Camacho L. and Rebollo S., 2010 – Effects of seasonal grazing and precipitation regime on the soil macroinvertebrates of a Mediterranean old-field, *European Journal of Soil Biology*, 46, 2, 91-96.
47. Mujeeb Rahman P., Varma R. V. and Sileshi G. W., 2012 – Abundance and diversity of soil invertebrates in annual crops, agroforestry and forest ecosystems in the Nilgiri biosphere reserve of Western Ghats, India, *Agroforestry systems*, 85, 165-177.
48. Naiman R. J. and Decamps H., 1997 – The ecology of interfaces: riparian zones, *Annual Review of Ecology and Systematics*, 28, 1, 621-658.
49. Nash M. A., Hoffmann A. A. and Thomson L. J., 2010 – Identifying signature of chemical applications on indigenous and invasive nontarget arthropod communities in vineyards, *Ecological Applications*, 20, 6, 1693-1703.
50. Nogarol L. R. and Fontanetti C. S., 2010 – Acute and subchronic exposure of diplopods to substrate containing sewage mud: Tissular responses of the midgut, *Micron*, 41, 3, 239-246.
51. Nogarol L. R. and Fontanetti C. S., 2011 – Ultrastructural alterations in the midgut of diplopods after subchronic exposure to substrate containing sewage mud, *Water, Air, & Soil Pollution*, 218, 539-547.

52. Perez D. G. and Fontanetti C. S., 2011 – Assessment of the toxic potential of sewage sludge in the midgut of the diplopod Rhinocricus padbergi, *Water, Air, & Soil Pollution*, 218, 437-444.
53. Popescu C., Oprina-Pavelescu M., Dinu V., Cazacu C., Burdon F. J., Forio M. A. E., Rîşnoveanu G., 2021 – Riparian vegetation structure influences terrestrial invertebrate communities in an agricultural landscape, *Water*, 13, 2, 188.
54. Ramey T. L. and Richardson J. S., 2017 – Terrestrial invertebrates in the riparian zone: mechanisms underlying their unique diversity, *BioScience*, 67, 9, 808-819.
55. Rossi J. P. and Blanchart E., 2005 – Seasonal and land-use induced variations of soil macrofauna composition in the Western Ghats, southern India, *Soil Biology and Biochemistry*, 37, 6, 1093-1104.
56. Sabatté M. L., Massobrio M. J., Cassani M. T. and Momo F. R., 2021 – Macro and mesofauna soil food webs in two temperate grasslands: responses to forestation with Eucalyptus, *Heliyon*, 7, 1.
57. Salvio C., López A. N., Manetti P. L. and Clemente N. L., 2011 – Effects of granulated baits on meso and macrofauna in soybean soil system, *Journal of Environmental Biology*, 32, 6, 793.
58. Sharley D. J., Hoffmann A. A. and Thomson L. J., 2008 – The effects of soil tillage on beneficial invertebrates within the vineyard, *Agricultural and Forest Entomology*, 10, 3, 233-243.
59. Sileshi G. and Mafongoya P. L., 2006 – Long-term effects of improved legume fallows on soil invertebrate macrofauna and maize yield in eastern Zambia, *Agriculture, Ecosystems & Environment*, 115, 1-4, 69-78.
60. Sileshi G. and Mafongoya P. L., 2006 – Variation in macrofaunal communities under contrasting land use systems in eastern Zambia, *Applied Soil Ecology*, 33, 1, 49-60.
61. Sileshi G., Mafongoya P. L., Chintu R. and Akinnifesi F. K., 2008 – Mixed-species legume fallows affect faunal abundance and richness and N cycling compared to single species in maize-fallow rotations, *Soil Biology and Biochemistry*, 40, 12, 3065-3075.
62. Sithole N. J., Magwaza L. S., Mafongoya P. L. and Thibaud G. R., 2018 – Long-term impact of no-till conservation agriculture on abundance and order diversity of soil macrofauna in continuous maize monocropping system, *Acta Agriculturae Scandinavica, Section B-Soil & Plant Science*, 68, 3, 220-229.
63. Skłodowski J. and Tracz H., 2018 – Consequences for millipedes (Myriapod, Dipolopoda) of transforming a primeval forest into amanaged forest – a case study from Białowieża (Poland), *Forest Ecology and Management*, 409, 593-600.
64. Smith J., Potts S. G., Woodcock B. A. and Eggleton P., 2008 – Can arable field margins be managed to enhance their biodiversity, conservation and functional value for soil macrofauna?, *Journal of Applied Ecology*, 45,1, 269-278.
65. Sridhar K. R., Kadamannaya B. S. and Karamchand K. S., 2013 – Nutrient composition of pill millipede manure of the Western Ghats, India, *Journal of Forestry Research*, 24, 539-545.
66. Stašiov S., Hazuchová L., Vician V., Kočík K. and Svitok M., 2014 – Millipede (Diplopoda) communities in agricultural landscape: influence of management form, *Polish Journal of Ecology*, 62, 3, 587-598.
67. Steinwandter M., Rief A., Scheu S., Traugott M. and Seeber J., 2018 – Structural and functional characteristics of high alpine soil macro-invertebrate communities, *European journal of soil biology*, 86, 72-80.
68. Steinwandter M., Schlick-Steiner B. C., Seeber G. U. H., Steiner F. M. and Seeber J., 2017 – Effects of alpine land-use changes: soil macrofauna community revisited, *Ecology and evolution*, 7, 14, 5389-5399.
69. Tóth Z., Hornung E. and Báldi A., 2018 – Effects of set-aside management on certain elements of soil biota and early stage organic matter decomposition in a High Nature Value Area, Hungary, *Nature Conservation*, 29, 1-26.

70. Tóth Z., Hornung E., Báldi A. and Kovács-Hostyánszki A., 2016 – Effects of set-aside management on soil macrodecomposers in Hungary, *Applied soil ecology*, 99, 89-97.
71. Trabaniño C. R., Pitre H. N., Andrews K. L. and Meckenstock D. H., 1990 – Soil-inhabiting phytophagous arthropod pests in intercropped sorghum and maize in southern Honduras, *Turrialba*, 40, 2, 172-183.
72. Van Noordwijk C. G. E., Baeten L., Turin H., Heijerman T., Alders K., Boer P. and Bonte D., 2017 – 17 years of grassland management leads to parallel local and regional biodiversity shifts among a wide range of taxonomic groups, *Biodiversity and Conservation*, 26, 717-734.
73. Vohland K. and Schroth G., 1999 – Distribution patterns of the litter macrofauna in agroforestry and monoculture plantations in central Amazonia as affected by plant species and management, *Applied Soil Ecology*, 13, 1, 57-68.
74. Wu P., Zhang H. and Wang Y., 2015 – The response of soil macroinvertebrates to alpine meadow degradation in the Qinghai–Tibetan Plateau, China, *Applied Soil Ecology*, 90, 60-67.
75. Zulu S. G., Motsa N. M., Sithole N. J., Magwaza L. S. and Ncama K., 2022 – Soil macrofauna abundance and taxonomic richness under long-term no-till conservation agriculture in a semi-arid environment of South Africa, *Agronomy*, 12, 3, 722.

INVESTIGATION OF SOME FISH SPECIES OF SCOMBRIDAE FAMILY IN TERMS OF PARASITES

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ABSTRACT

The purpose of this investigation was to identify the presence of parasite infections and cystic formation in *Scomber japonicus*, *Scomber scombrus*, and *Thunnus thynnus*. *Euryphorus brachypterus* and *Clavellisa scombri* were identified from *Thunnus thynnus* and *Scomber scombrus*, respectively. *Anisakis pegreffii* was identified from *Scomber japonicus* and *Scomber scombrus* by using molecular methods. Additionally, *Anisakis typical* was determined from *Scomber japonicus*.

RÉSUMÉ: Étude de certaines espèces de poissons de la famille des Scombridae en termes de parasites.

Le but de cette enquête était d’identifier la présence d’infections parasitaires et les formations kystiques et les parasites du *Scomber japonicus*, du *Scomber scombrus* et du *Thunnus thynnus*, ont été examinés. *Euryphorus brachypterus* et *Clavellisa scombri* ont été identifiés respectivement sur le thon rouge et le maquereau. *Anisakis pegreffii* ont été signalés sur des maquereaux et des maquereaux par des méthodes moléculaires, *Anisakis typique* des *Scomber japonicus*.

REZUMAT: Investigarea unor specii de pești ale familiei Scombridae din punctul de vedere al paraziților.

Scopul acestei investigații a fost identificarea prezenței infecțiilor parazitare și a formațiunilor chistice și paraziților la *Scomber japonicus*, *Scomber scomber* și *Thunnus thynnus*. *Euryphorus brachypterus* și *Clavellisa scombri* au fost identificate la *Thunnus thynnus* și *Scomber scombrus*. *Anisakis pegreffii* au fost raportate de la *Scomber japonicus* și *Scomber scombrus* prin metode moleculare, *Anisakis typical* la *Scomber japonicus.*

INTRODUCTION

Diseases of different types seen in various fish species are known to be directly related to stress caused by environmental factors (Öktener and Bănăduc, 2023). In natural conditions, fish can migrate to better habitats when exposed to bad conditions, such as a decrease in oxygen level or an increase in ambient temperature. Diseased fish may move to warmer waters where they can increase their body temperature to enhance their inflammatory response.

The aquatic environment has some parameters that directly affect the maintenance of homeostatic balance, growth, and reproduction in fish. Changes in homeostatic balance may pave the way for various diseases or cause different conditions. Among these parameters, the most important ones for fish are temperature, light period, chemical and biological content of water, feed, and stock density (Pozio, 2015). Pathogenic microorganisms and parasites can spread rapidly in aquatic environments by means of contamination of the environment with feces or skin lesions of clinically symptomatic or latently infected fish and can be transmitted to other fish by mouth, gill, or other contact, causing epidemics in a short time, which in turn leads to important economic losses (Robert, 2012; Erer, 2020; Aydın and Pekmezci, 2023).

Türkiye is partly surrounded by the Black Sea, the Aegean Sea and the Mediterranean Sea, and has a rich aquaculture potential with a coastline of 8,333 km in length. Although it changes from year to year, the country's world ranking is in the thirties in terms of total aquaculture production in 2014 and is in the top five among EU countries (FAO, 2016). It is of great importance to gather a wide range of information about fish diseases (parasitic, bacterial, fungal, etc.) to increase the country's share in this ranking and to realize sustainable aquaculture production (Tayar, 2010, 2020).

Parasites, one of the disease factors, can continue their lives in the gill, under the gill cover, gill rakers muscle tissue, the body cavity, internal organs, and the digestive system, especially in the intestines or, in the gallbladder of some species (Garcia et al., 2011; Mladineo et al., 2011; Özak et al., 2012; Şahin and Sağlam, 2016; Fonkwa et al., 2021).

Anisakid nematodes, members of the Anisakidae family, are among the most significant parasitic organisms responsible for some human infections. The nematodes use fish and warm-blooded vertebrates as their hosts, thus have the potential to pass to human body after consumption of the infected organisms. Their final hosts are usually marine mammals. The symptoms in humans include stomach pain, nausea, vomiting, and various abdominal pains. Because the symptoms are similar to those of many other diseases, the diagnosis of the presence of these nematodes is not easy. *Anisakis simplex* has gained public health importance as it has caused an outbreak in humans in the Netherlands due to visceral larval migration. The mature parasites normally live as parasites in sharks and rays, and humans are infected by eating raw herring meat carrying the nematode larvae. Humans infected with this parasite experience severe pain and even death (Wekell, 1994).

Larvae of Anisakid nematode species have been reported so far in different regions of the world (including Africa, Europe, South America, Australia, etc.) and in different fish species Anisakid larvae, particularly those of *Anisakis pegreffii* have been observed in various fish species, especially in Turkish seas (Ütük and Pişkin, 2012; Tepe and Oğuz, 2013; Pekmezci et al., 2014; Yardimci et al., 2014; Şahin and Sağlam, 2016; Becerik et al., 2020; Çelik et al., 2022; Aydın and Pekmezci, 2023).

In the present study, we studied cystic formations obtained from the gills, body cavity, and internal organs of some fish species belonging to the Scombridae family in order to determine the parasitic species in the specimens. It will show us the presence of disease agents that are difficult to prevent by examining the cystic stages of parasitic formations that affect fish health and can later turn into zoonotic structures.

MATERIAL AND METHODS

Study samples. The study was carried out on the parasites detected in chub mackerel (*Scomber japonicus*) and mackerel (*Scomber scombrus)* specimens belonging to the Scombridae family caught from Çanakkale Strait of the North Aegean Sea and in the dead bluefin tuna fish (*Thunnus thynnus*) taken aboard during the hunting season in the Mediterranean Sea. The fish specimens were obtained from the local fish market in Çanakkale, Türkiye during a six-month sampling period.

A total of 267 fish were sampled in the study. All samples were brought to the laboratory for parasitic investigations. Copepod parasites and *Didymozoon* parasites were observed under the gill cover, in the gill lamellae, tongue pad, and gill filaments of mackerel and chub mackerel samples. Only copepodite parasites under the gill covers of the bluefin tuna, and *Didymozoon* parasites in the gill lamellae were also observed. For further analysis, parasites were taken into cyro tubes containing absolute (97.99%) ethanol. The parasites were examined under stereo and light microscopes and photographed. Then, nematode parasites were sent to a diagnostic laboratory (Letgen Biotechnology Laboratory) in tubes containing ethanol for molecular analysis. Some copepod specimens were soaked in lactic acid for 24 hours and then dissected with dissecting needles under a Wild M5 stereomicroscope for morphological investigations. Each extremity of the dissected copepods was placed in glycerin-gelatin between a slide and a coverslip and the coverslip edges were covered with transparent nail polish to obtain permanent preparations.

Morphological analysis. The species determination of the parasitic nematodes was made by considering the anatomical and morphological features of the parasites such as size, general appearance, head and tail structures, oesophagus, and cecum lengths, using the relevant key studies (Setyobudi et al., 2011; Pekmezci et al., 2014; Serracca et al., 2014; Pozio, 2015; Mehlhorn, 2016; Şahin and Sağlam, 2016; Mladineo et al., 2017; Morsy et al., 2017; Pekmezci, 2019; Pekmezci et al., 2019; Pekmezci and Onuk, 2020; Pekmezci, 2021). In particular, the methods of Mladineo et al. (2011; 2017) and Kohn and Nascimento-Justo (2008) were used for the diagnosis of *Didymozoon* parasites.

Identifications and comparisons of the copepods were performed according to Kabata (1979; 2003) and Özak and Yanar (2016). Latin names and synonyms of the parasites and the hosts were checked from online databases (Fishbase, 2022; WoRMS, 2022). Nikon D 90 camera, CMOS (5MP) microscope camera, Zeiss Brand Light Microscope, Canon EOS 1100D connected to Olympus CH30 light microscope were used to obtain photographs of the investigated specimens.

Molecular analysis. COII, ITS region, and 12s rRNA gene regions were amplified using gene-specific primers; the primers 211F: 5’-TTTTCTAGTTATATAGATTGRTTYAT-3’ and 210R: 5’-CACCAACTCTTAAAATTATC-3’ were used for COII; the primers 5’-GTAGGTGAACCTGCGGAAGGATCATT-3’ and NC2: 5’-TTAGTTTCTTTTCCTCCGCT-3’ were used for ITS region; and the primers Anisakis-F: 5’-TGATTTTTAATTTTAGGGTGAAAT-3’ and Anisakis-R: 5’-GGCAATTGATGGATGATTTG-3’ were used for the rrnS gene region.

The NCBI blast program was used for identification of the nematode species (Mattiucci et al., 2011; Piras et al., 2014; Pekmezci, 2021). The reads obtained for the rrnS gene region were contiguous to form a consensus sequence. The Contig Assembly Program (CAP) was used in BioEdit software to perform this process.

In order to determine the phylogenetic analyzes among the nematode species identified by the BLAST analysis of the COII gene region, it was aligned using the CLUSTALW V.1.6 parameter in the GENIOUS 9.0.5 program, and a phylogenetic tree was created in the MEGA 11 program. For this purpose, ITS-RNA, COII, and rrnS-12S gene region sequences of different *Anisakis pegreffii* (Campana-Rouget and Biocca, 1955) and *Anisakis typica* (Diesing, 1860) species were downloaded and recorded in FASTA format using the NCBI nucleotide database for *A. pegreffii* and *A. typica*. *Anisakis pegreffii* (COII: NC0340329.1; ITS: MH211473.1, MF539764.1, MF422217.1, MF803221.1, JQ934869.1, JQ934867.1, JQ934873.1; rrnS 12S: MT312518.1- MT484284.1- MF140359.1) and *A. typica* (AB517571.1) were used as references. In addition, to determine the distance between the species, *Contracaecum rudolphii* (Hartwich, 1964) (COII: EF122202.1; ITS: MH778113.1; rrnS 12S: NC_014870.1) and *Pseudoterranova decipiens* Sensu (Krabbe, 1878) (COII: NC031645.1; ITS: KM273087.1; NC_0316:45.1) were used as the outgroups.

The COII, rrnaS 12S, and ITS gene sequences of the identified nematode species were uploaded to the GenBank database on the NCBI database, and the gene bank accession numbers were obtained for the relevant gene regions.

RESULTS AND DISCUSSION

50 cystic and free nematode parasites, 50 *Didymozoon* cysts, and 80 (53 female and 27 male) parasitic copepods were isolated from the fish samples (Fig. 1). Parasitic copepods isolated from bluefin tuna fish were identified as *Euryphorus brachypterus* (Figs. 2-3), and those from mackerel were *Clavellisa scombri* (Fig. 4). Didymozoons were identified by classical methods, all were identified at genus level as *Didymozoon* sp. was found (Fig. 1c).

The nematodes sampled from chub mackerel (*Scomber japonicus*) were *A. typica* and *A. pegreffii*, and nematodes in mackerel (*Scomber scombrus*) were *A. pegreffii* (Fig. 1b).

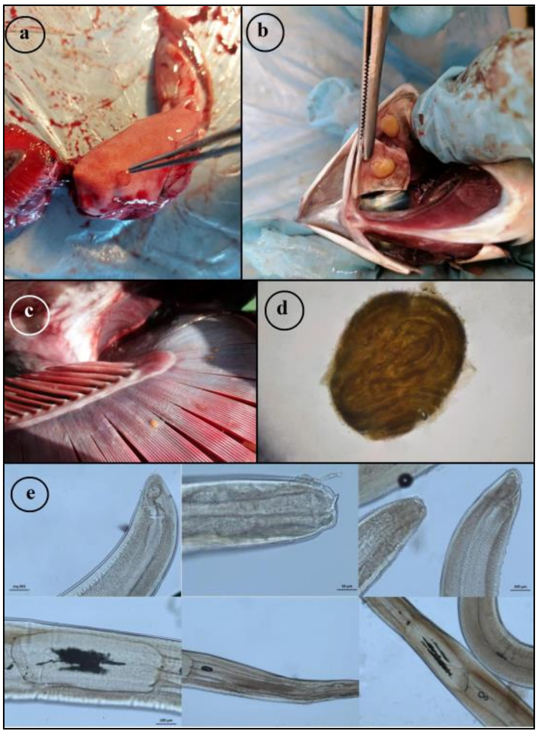

Figure 1: a. Nematode cyst in fish liver, *Didymozoon* parasite cyst on the inner wall of the gill cover, and the gill lamellar, b. Microscopic image of the nematode parasites, c. *Didymozoon*.

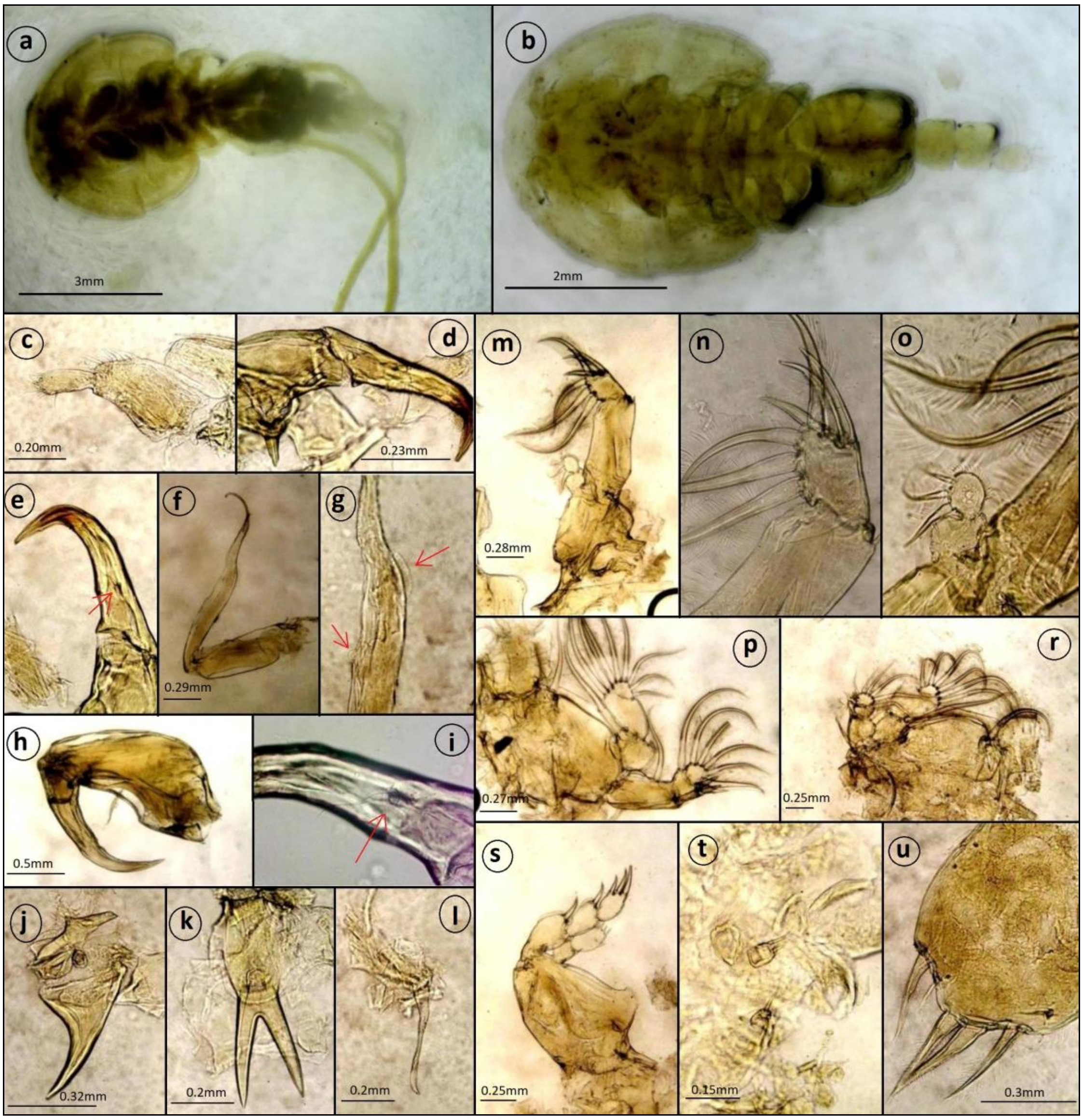

Figure 2: *Euryphorus brachypterus*, a. female, b. male, *Euryphorus brachypterus*, female, c. antennule, d. antenna, e. distal of antenna, f. maxilla, g. seta on maxilla, h. maxilliped, i. distal of maxilliped, j. maxillule, k. sternal furca, l. mandible, m. 1st leg, n. distal of 1st leg, o. distal of 1st leg, p. 2nd leg, r. 3rd leg, s. 4th leg, t. 5th leg, u. caudal ramus.

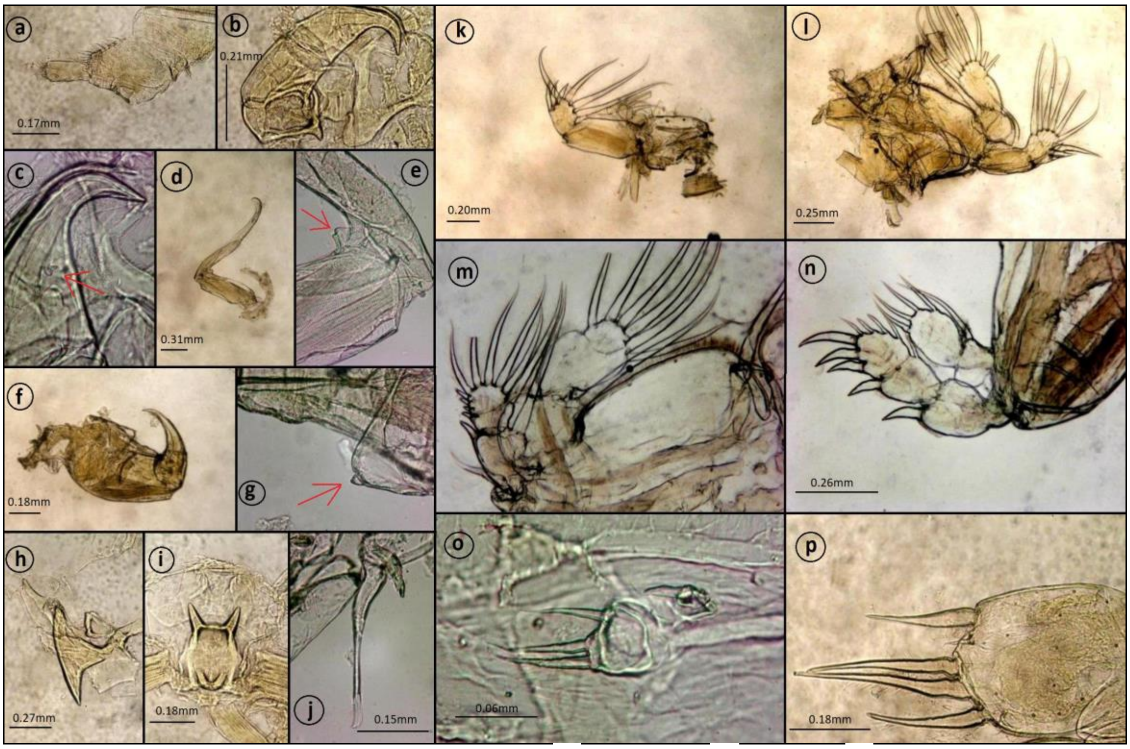

Figure 3: *Euryphorus brachypterus*, male, a. antennule, b. antenna, c. distal of antenna, d. maxilla, e. seta on maxilla, f. maxilliped, g. distal of maxilliped, h. maxillule, i. sternal furca, j. mandible, k. 1st leg, l. 2nd leg, m. 3rd leg, n. 4th leg, o. 5th leg, p. caudal ramus.

Figure 4: *Clavellisa scombri*, a. female, b.male (arrow).

The identified *A. typica* and *A. pegreffii* and the COII, ITS-rRNA and rrnS 12S rRNA sequences of the reference species from the database were aligned separately.

After the alignment, the Neighbor-Joining (NJ) tree was created. Bootstrap value above 50 are shown in the obtained NJ tree (Figs. 5-7). When the nucleotide sequences of the relevant gene region from the NCBI database were compared with the nucleotide sequences we obtained, the species were confirmed to be *A. typica* and *A. pegreffii*.

The GenBank accession numbers of the nematode species are as in the table 1.

Table 1: The GenBank accession numbers of nematode species.

Sampled name	Identified Nematode	Gen regions	Genbank number
Scomber japonicus 1	*A. typica*	COII	OP525256
S. japonicus 2	*A. pegreffii*	COII	OP525266
S. japonicus 3	*A. pegreffii*	COII	OP525267
S. japonicus 4	*A. pegreffii*	COII	OP525268
S. japonicus 5	*A. pegreffii*	ITS	OP176011
Scomber scombrus 1	*A. pegreffii*	rrnS 12S rRNA	OP179783
S. scombrus 2	*A. pegreffii*	COII	OP525261
S. scombrus 3	*A. pegreffii*	rrnS 12S rRNA	OP179784
S. scombrus 4	*A. pegreffii*	COII	OP525262

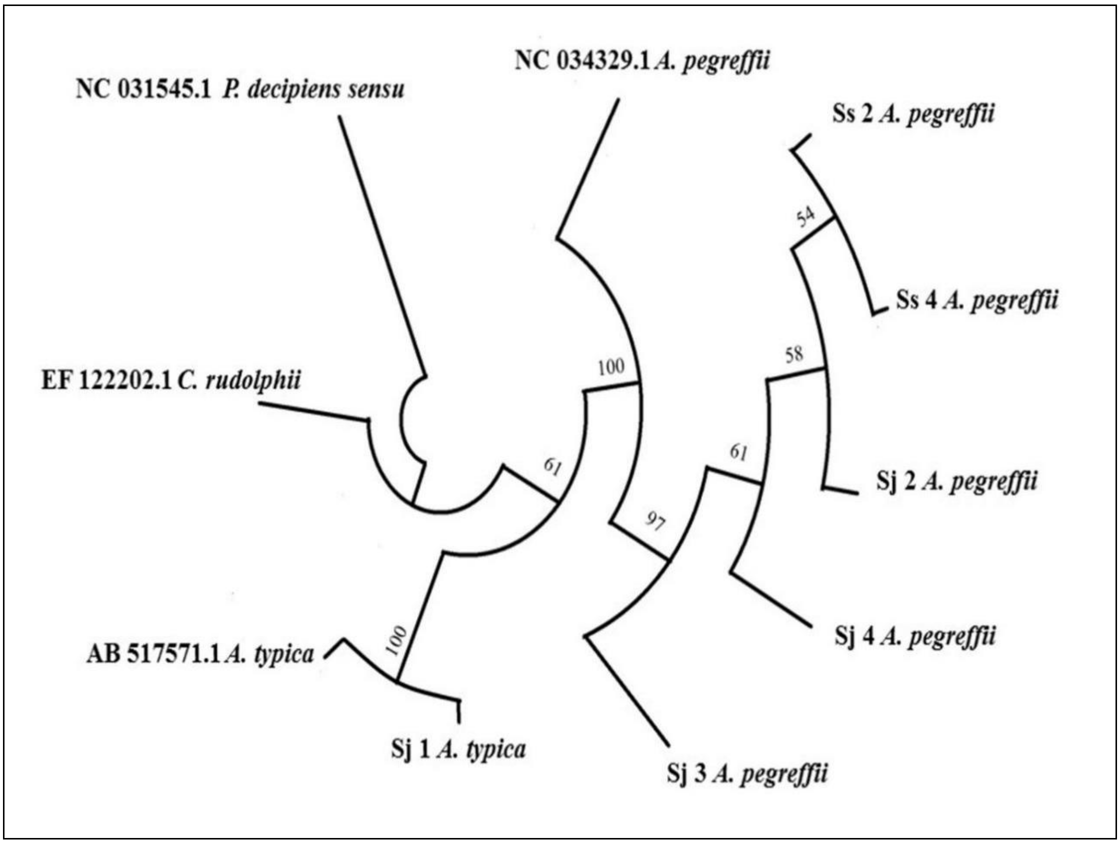

Figure 5: Neighbour-joining tree of COII gene regions of the identified nematode species (NCBI blast program and MEGA 11 program).

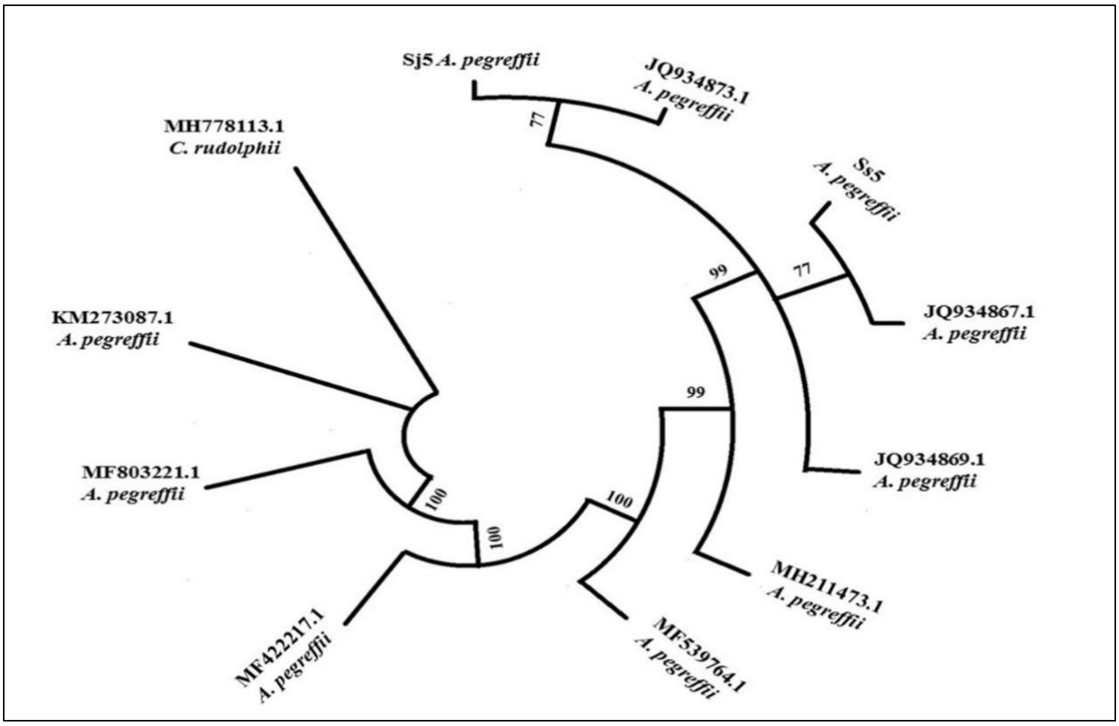

Figure 6: Neighbour-joining tree of ITS-rRNA gene regions of the identified nematode species (NCBI blast program and MEGA 11 program).

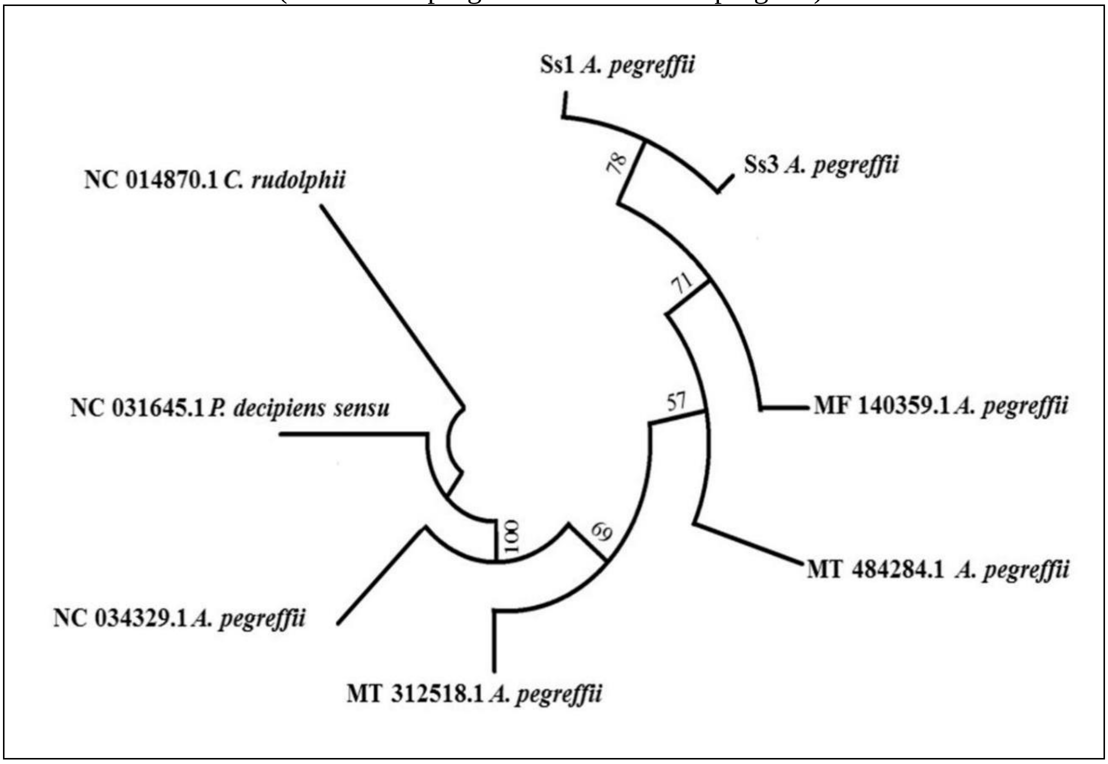

Figure 7: Neighbour-joining tree of the identified nematode species of rrnsS 12S gene regions (NCBI blast program and MEGA 11 program).

DISCUSSION

The study aims to parasitologically examine some fish species belonging to the Scombridae family, which were caught on the coasts of Çanakkale or the surrounding coasts, in terms of fish health. The cystic structures isolated from the gills of the fish were used for the determination of the parasites at the species level.

Cystic structures are one of the stages in which parasites maintain their life cycles by preserving their lives. If a parasite is still alive after the cystic stage, it will continue its life cycle by evolving within the host or moving to another host.

In a research on muscle parasites in marine fish, the molecular characteristics of didymozoids were studied. The differences in the last seven sequences in sequence analysis helped the diagnosis in the study. These differences helped in the molecular differentiation of didymozoids. The molecular separation of each parasite was made easier due to the clear sequence differences. It is the first molecular study to identify didymozoids (Abe et al., 2014).

A new host and a new geographical distribution for three *Didymocyctis* species of the Didymozoidae family were reported in a study on the tuna fish *Thunnus obesus* (Lowe, 1839) helminth parasites (Moreira-Silva et al., 2019). Scombrid fish, which is important in Brazil, may decrease in commercial value due to helminth parasites. Of the determined species, *Didymocystis alalongue* (Yamaguti, 1938) was isolated from the gill raker and operculum, *Didymocystis lamotheargumedoi* (Kohn and Nascimento-Justo, 2008) was isolated from the operculum in three mature forms and *Platocystis vivipara* (Yamaguti, 1970) was isolated from the skin of the dorsal part of the body. *Thunnus obesus* was reported as a new host for *D. alalongue* in the study. The study is also the first record of *Platocystis vivipara* and *D. lamotheargumedoi* in the South Atlantic (Moreira-Silva et al., 2019).

Digenean trematodes belonging to the family Didymozoidae can reduce the commercial value of fish, especially when in muscle tissues (Mota et al., 2019). In this study, the didymozoids isolated from the muscles of chub mackerel were investigated. The study includes 64 chub mackerel, and parasites were detected by the utilization of stereomicroscopy and macroscopy techniques on the muscle tissues. The results showed that the prevalence and abundance of the detected infection were quite low and that didymozoids in muscles differ from those isolated from other organs. Concern has been envisaged that this parasite may cause softening of the muscles, thereby reducing its commercial value and meat quality.

Didymozoid was isolated from the muscle tissue infection of the fish in a study conducted on the Atlantic mackerel *Scomber scombrus*, and parasite-host interaction was studied. Multiple ribbon-like Didymozoid eggs were observed in the connective tissue of *S. scombrus*, mostly embedded between epoxy skeletal muscle fibers. These eggs are at different stages of development, from previtellogenic oocytes to fully embryonated eggs. As a result of molecular studies, it was determined that the eggs belonged to a didymozooid that has not yet been identified. It has been shown that eating mackerel meat will cause displeasure as concurrent severe infection causes muscle damage in the host. However, it has been reported that no situation will endanger public health (Pascual et al., 2006).

Various studies conducted in the coastal waters of Rio de Jeneiro, Brazil investigated the parasite species of different fish species within Didymozoidae family (Nascimento-Justo and Kohn, 2012a, b; Nascimento-Justo et al., 2013; Nascimento-Justo and Konhn, 2014). In these studies on three Scombrid species *T. atlanticus* (Lesson, 1831), *T. albacores* (Bonnaterre, 1788) and *Katsuwonus pelanis* (Linnaeus, 1758) *Didymocystis lamotheargumedoi* n.sp. was isolated, making the study the pioneer of other studies.

Costa et al. (2011) examined and interpreted the helminth parasites of the Atlantic chub mackerel associated with the Mid-North Atlantic, Canary Islands, and other Atlantic regions. In the study conducted on 68 chub mackerel, it was reported that they were infected with 11 different parasitic taxa. The most abundant parasites were the monogenean *Pseudokuhnia minor* (Goto, 1984) in the gills, larval anisakid nematodes in the body cavity, larval tetraphyllidean in the bile duct, and the didymozoid digeneans in the gills. *Didymozoon* cysts were also isolated from the gills of the chub mackerel included in our study. In addition, nematode parasites were also sampled from some chub mackerel. The molecular analysis confirmed that the nematodes were of the species *A. pegreffii* and *A. typica*. Since *Didymozoon* parasites could not be diagnosed on a molecular basis, the naming of parasites was left at the genus level as *Didymozoon* sp.

Şimşek et al. (2021) performed the molecular diagnosis of nematodes they isolated in *Mullus barbatus* (Linnaeus, 1758) in the Mediterranean Sea, using the rrnS gene, and revealed the presence of *Hysterothylacium fabri* (Rudolphi, 1819) larvae for the first time. In the present study, the rrnS gene was used and the nematodes in mackerel were identified as *A. pegreffii*.

Oğuz et al. (2000) isolated the nematode species *A. simplex* in their study on some teleost fish on the coast of Çanakkale. Our study is based on the timeline of parasitological examination of fish samples taken from the local fish market in Çanakkale. In our examination, we determined that there was predominant nematode contamination of *A. pegreffii*. Apart from that, *A. typica* nematode was also isolated.

Çelik et al. (2022) analyzed the nematode parasites isolated from the sea chub mackerel caught from Çanakkale Strait at a molecular level and reported the presence of *A. pegreffii*. In our study, we isolated the species *A. pegreffii* from chub mackerel and mackerel fishes caught from the Çanakkale Strait by using molecular techniques. During our study, we supported our results with both traditional techniques and molecular diagnostic techniques.

CONCLUSIONS

The study aims to parasitologically examine some fish species with high economic value belonging to the Scombridae family, which were caught on the coasts of Çanakkale or the surrounding coasts, in terms of fish health. The cystic structures isolated from the gills of the fish were used for determination of the parasites at species level. Cystic structures are one of the stages in which parasites maintain their life cycles by preserving their lives. If a parasite is still alive after the cystic stage, it will continue its life cycle by evolving within the host or moving to another host.

As a result, the study showed that, considering the previous samplings in the same season and the species, it is necessary to better investigate and consider the environmental and anthropogenic effects, interaction between fish, and internal factors of the host in parasitizing fish. In addition, the change times in water temperature with the effect of global warming showed that parasitic organisms were re-isolated at a time when they did not exist before. The presence of these parasites should play an important role in decrease in the commercial value of fish and once again show the importance of protection and control in our seas.

This study contribute to the identification of possible parasitic disease factors that may be encountered in marine fisheries and to take precautions against health problems that may occur in commercial fisheries.

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REFERENCES

1. Abe N., Okamoto M. and Maehara T., 2014 – Molecular characterization of muscle-parasitizing didymozoids in marine fishes, *Acta Parasitologica*, 59, 2, 354-358, DOI: 10.2478/s11686-014-0234-2.
2. Arda M., Seçer S. and Sarieyyüpoğlu M., 2005 – Fish Diseases, Medisan Yayın Serisi, II. Baskı, Ankara, 142.
3. Aydin C. and Pekmezci G. Z., 2023 – An integrative study of morphological and molecular characterization of Diplectanum aequans (Diplectanidae: Monogenea) infecting European sea bass Dicentrarchus labrax (L.) from Turkish coasts, *Ankara Üniversitesi Veteriner Fakültesi Dergisi*, 70, 3, 245-251. DOI: 10.33988/auvfd.987702.
4. Beceriklisoy G. Ö, Aşti C. and Gönenç B., 2020 – Anisakis spp. infection in Atlantic mackerel (Scomber scombrus, Linnaeus 1758) from the Sea of Marmara, *Journal Of The Turkish Veterinary Medical Society*, 91, 1, 80-85. DOI: 10.33188/vetheder.599455.
5. Costa G., Cavallero S., D'amelio S., Paggi L., Santamaria M. T. G., Perera C. B., Santos M. J. and Khadem M., 2011 – Helminth parasites of the Atlantic chub mackerel, Scomber colias Gmelin, 1789 from Canary Islands, Central North Atlantic, with comments on their relations with other Atlantic regions, *Acta Parasitologica*, 56, 98-104, DOI: 10.2478/s11686-011-0006-1.
6. Çelik E. Ş., Kanat R. E., Dermanci P., Yilmaz D.K., Turgay E., Steinum S.K. and Yilmaz S., 2022 – Effect of Anisakis pegreffii (Nematoda: Anisakidae) on Biochemical and Haematological Characteristics of Chub Mackerel (Scomber japonicus Houttuyn, 1782) Caught in the Dardanelles at Çanakkale, Türkiye, *Çanakkale Onsekiz Mart University Journal of Marine Sciences and Fisheries*, 5, 55-62, DOI: 10.46384/jmsf.1140211.
7. Erer, H., 2020 – Fish Diseases, Atlas Akademi Yayınları, Konya, 196.
8. FAO., 2016 – The state of World Fisheries and Aquaculture, Contributing to food security and nutrition for all, https://www.fao.org/fishery/en/openasfa/cff4968b-b829-41db-83a5-8504a399611e, date of accession 22/05/2022.
9. Froese R. and Pauly D., 2023 – FishBase, World Wide Web electronic publication, www.fishbase.org, version, date of accession 06/2023.
10. Fonkwa G., Kouam K. M., Tchuinkam T., Tomedi E. M. and Tchoumboue J., 2021 – Myxobolus (Myxosporea: Myxobolidae) polyinfection patterns in Oreochromis niloticus in Adamawa-Cameroon, *International Journal of Fisheries and Aquatic Studies*, 9, 1, 123-130, DOI: 10.22271/fish.2021.v9.i1b.2393.
11. Garcia A., Mattiucci S., Damiano S., Santos M. N. and Nascetti G., 2011 – Metazoan parasites of swordfish, *Xiphias gladius* (Pisces: Xiphiidae) from the Atlantic Ocean: implications for host stock identification, *ICES Journal of Marine Science*, 68, 1, 175-182. DOI: 10.1093/icesjms/fsq147.
12. Kabata Z., 1979 – Parasitic Copepoda of British fishes, Ray Society, London. 468.
13. Kabata Z., 2003 – Copepods Parasitic on Fishes, 2nd revised edition, Synopses of the British Fauna, 2nd revised edition, Backhuys, 47, 274.
14. Kohn A. and Nascimento-Justo M. C., 2008 – Didymocyctis lamotheargumedoi n. sp. (Digenea: Didymozoidae) a parasite of three species of scombrid fishes, *Revista Mexicana de Biodiversidad*, 79, 9-14, DOI: 10.22201/ib.20078706e.2008.001.505.
15. Mattiucci S., Paoletti M., Borrini F., Palumbo M., Palmieri R. M., Gomes V., Casati A. and Nascetti G., 2011 – First molecular identification of the zoonotic parasite Anisakis pegreffii (Nematoda: Anisakidae) in a paraffin-embedded granuloma taken from a case of human intestinal Anisakiasis in Italy, *BMC Infectious Diseases*, 11, 82, DOI: 10.1186/1471-2334-11-82.
16. Mehlhorn H., 2016 – Animal parasites: diagnosis, treatment, prevention, Springer, 701.
17. Mladineo I., Šegvić T. and Petrić M., 2011 – Do captive conditions favor shedding of parasites in the reared Atlantic bluefin tuna (Thunnus thynnus)?, *Parasitology International*, 60, 1, 25-33, DOI: 10.1016/j.parint.2010.09.007.

18. Mladineo I., Trumbić Ž., Radonić I., Vrbatović A., Hrabar J. and Bušelić, I., 2017 – Anisakis simplex complex: ecological significance of recombinant genotypes in an allopatric area of the Adriatic Sea inferred by genome derived simple sequence repeats, *International Journal for Parasitology*, 47, 4, 215-223, DOI: 10.1016/j.ijpara.2016.11.003.
19. Moreira-Silva A. M., Nascimento-Justo M. C. and Cohen S. C., 2019 – New host records and new geographic distribution of Didymozoidae (Trematoda), parasites of Thunnus obesus (Scombridae), *Revista Mexicana de Biodiversidad*, 90, DOI: 10.22201/ib.20078706e.2019.90.2789.
20. Morsy K., Badr A. M., Abdel-Ghaffar F., El Deeb S. and Ebead S., 2017 – Pathogenic potential of fresh, frozen, and thermally treated Anisakis spp. type II (L3) (Nematoda: Anisakidae) after oral inoculation into wistar rats: A histopathological study, *Journal of Nematology*, 49, 4, 427-436, DOI: 10.21307/jofnem-2017-092.
21. Mota R., Alves M., Vieira C. P., Vieira J., Tamagnini P. and Saraiva, A., 2019 – Didymozoids in muscle of atlantic chub mackerel (Scomber colias), *Acta Parasitologica*, 64, 308-315, DOI: 10.2478/s11686-019-00049-5.
22. Nascimento-Justo M. C. and Kohn A., 2012 – A new genus and species of the Didymozoidae (Digenea) from the skipjack tuna Katsuwonus pelamis (L.) (Scombridae), *Systematic Parasitology*, 81, 195-201, DOI: 10.1007/s11230-011-9340-9.
23. Nascimento-Justo M. C. and Kohn A., 2012 – Notes on helminth parasites of tuna fishes (Scombridae) in Brazil, *Revista Mexicana de Biodiversidad*, 3, 285-290, DOI: 10.22201/ib.20078706e.2012.1.1146.
24. Nascimento-Justo M. C., Kohn A., De Souza Pereira C., and Flores-Lopes F., 2013 – Histopathology and autoecology of Didymocylindrus simplex (Digenea: Didymozoidae), parasite of Katsuwonus pelamis (Scombridae) in the Southwestern Atlantic Ocean, off South America, *Zoologia*, 30, 3, 312-316, DOI: 10.1590/S1984-46702013000300008.
25. Nascimento-Justo M. C. and Kohn A., 2014 – Monogenoidea and digenea parasites of Thunnus atlanticus (Perciformes, Scombridae) from Rio de Janeiro coast, Brazil, *Neotropical Helminthology*, 8, 2, 339-348, DOI: 10.24039/rnh201482926.
26. Oğuz M., Güre H., Özdemir H., Öztürk M. and Savaş Y., 2000 – Çanakkale ili kıyılarında yakalanan ekonomik öneme sahip bazı teleost balıklarda Anisakis simplex, Rudolphi 1809 araştırılması, *Turkiye Parazitoloji Dergisi*, 24, 431-434. (in Turkish)
27. Öktener A. and Bănăduc D., 2023 – Pollution, fish parasites and fish ecological interdependences in freshwater ecosystems of Turkey, *Water*, ISSN 2073-4441, Section Biodiversity and Functionality of Aquatic Ecosystems, Special Issue Recent Advances in the Aquatic Biodiversity, 15, 1385, doi.org/10.3390/w15071385, 2023.
28. Özak A. A., Demirkale İ. and Cengizler İ., 2012 – Two new records of Myxobolus bütschli, 1882 (Myxozoa, Myxosporea, Myxobolidae) species from Turkey, *Turkish Journal of Zoology*, 36, 2, 191-199, DOI: 10.3906/zoo-1007-30.
29. Özak A. A. and Yanar A., 2016 – Caligid copepods parasitic on fishes of the eastern Mediterranean coast of Turkey, The Turkish part of the Mediterranean Sea, The Turkish part of the Mediterranean Sea marine biodiversity fisheries conservation and governance, Turan C., Salihoğlu B., Özbek E. Ö. and Öztürk B. (eds.), Turkish Marine Research Foundation, İstanbul, 152-166.
30. Pascual S., Abollo E. and Azevedo C., 2006 – Host-parasite interaction of a muscle-infecting didymozoid in the Atlantic mackerel Scomber scombrus L., *ICES Journal of Marine Science*, 63, 1, 169-175, DOI: 10.1016/j.icesjms.2005.08.010.
31. Pekmezci G. Z., Onuk E. E., Bölükbas C. S., Yardimci B., Gürler A. T., Acici M. and Umur S., 2014 – Molecular identification of Anisakis species (Nematoda: Anisakidae) from marine fishes collected in Turkish waters, *Veterinary Parasitology*, 201, 1-2, 82-94, DOI: 10.1016/j.vetpar.2014.01.005.

32. Pekmezci G. Z. and Yardimci B., 2019 – On the occurrence and molecular identification of Contracaecum larvae (Nematoda: Anisakidae) in Mugil cephalus from Turkish waters, *Parasitology Research*, 118, 1393-1402, DOI: 10.1007/s00436-019-06278-x.
33. Pekmezci G. Z., 2019 – Molecular characterization of Hysterothylacium aduncum (Nematoda: Raphidascarididae) larvae infecting Merlangius merlangus euxinus (Linnaeus, 1758) from the Turkish Black Sea coast based on mitochondrial small subunit ribosomal RNA gene analysis, *Journal of Etlik Veterinary Microbiology*, 30, 1, 64-69, DOI: 10.35864/evmd.562427.
34. Pekmezci G. Z. and Onuk E. E., 2020 – Molecular characterization of Anisakis typica (Nematoda: Anisakidae) from the Mediterranean Sea coasts of Turkey: First mitochondrial rrnS sequence data, *Journal of Anatolian Environmental and Animal Sciences*, 5, 2, 241-244, DOI: 10.35229/jaes.738822.
35. Pekmezci G. Z., 2021 – Genetic analysis of the mitochondrial rrnS gene of zoonotic Anisakis pegreffii (Nematoda: Anisakidae) isolated from Micromesistius poutassou (R.) in the Aegean Sea, *Journal of Anatolian Environmental and Animal Sciences*, 6, 2, 266-269, DOI: 10.35229/jaes.913695.
36. Piras M. C., Tedde T., Garippa G., Sanna D., Farjallah S. and Merella P., 2014 – Molecular and epidemiological data on Anisakis spp. (Nematoda: Anisakidae) in commercial fish caught off northern Sardinia (western Mediterranean Sea), *Veterinary Parasitology*, 203, 1-2, 237-40, DOI: 10.1016/j.vetpar.2014.02.003.
37. Pozio E., 2015 – Foodborne nematodes, in Foodborne parasites in the food supply web occurrence and control Woodhead publishing series in Food science, technology and nutrition 165-199.
38. Roberts R. J., 2012 – Fish Pathology, John Wiley & Sons, 439.
39. Serracca L., Battistini R., Rossini I., Carducci A., Verani M., Prearo M., Tomei L., De Montis G. and Ercolini G., 2014 – Food safety considerations in relation to Anisakis pegreffii in anchovies (Engraulis encrasicolus) and sardines (Sardina pilchardus) fished off the Ligurian Coast (Cinque Terre National Park, NW Mediterranean), *International Journal of Food Microbiology*, 190, 3, 79-83, DOI: 10.1016/j.ijfoodmicro.2014.08.025.
40. Setyobudi E., Jeon C. H., Lee C. H., Seong K. B. and Kim J. H., 2011 – Occurrence and identification of Anisakis spp. (Nematoda: Anisakidae) isolated from chum salmon (Oncorhynchus keta) in Korea, *Parasitology Research*, 108, 3, 585-92, DOI: 10.1007/s00436-010-2101-x.
41. Şahin A. G. and Sağlam N., 2016 – Investigation of nematodes in Chub Mackerel (Scomber japonicus Houttuyn, 1782), Horse Mackerel (Trachurus trachurus (Linnaeus, 1758)) and Sardines (Sardinella aurita Valenciennes, 1847) caught in Çanakkale Region, *Aquaculture Studies*, 16, 1, 47-55, DOI:10.17693/yunusae.v16i21959.235769.
42. Şimşek E., Ciloğlu A., Yildirim A. and Pekmezci G. Z., 2018 – Identification and molecular characterization of Hysterothylacium (Nematoda: Raphidascarididae) larvae in bogue (Boops boops L.) from the Aegean Sea, Turkey, *Kafkas Universitesi Veteriner Fakultesi Dergisi*, 24, 4, 525-530, DOI: 10.9775/kvfd.2018.19482.
43. Şimşek E., Sürsal N., Karademir G. K. and Pekmezci G. Z., 2021 – First molecular characterization of Hysterothylacium fabri larvae (Nematoda: Raphidascarididae) in the Mediterranean Sea based on the small subunit ribosomal RNA gene sequence, *Kocatepe Veterinary Journal*, 14, 1, 71-76, DOI: 10.30607/kvj.832003.
44. Tayar M., 2010 – Gıda güvenliği. Marmara Belediyeler Birliği, Ekosan matbaacılık, İstanbul. (in Turkish)
45. Tayar M., 2020 – Gıda güvenliği ve COVID-19, *Veteriner Farmakoloji ve Toksikoloji Derneği Bülteni*, 11, 2, 61. (in Turkish)
46. Tepe Y. and Oğuz M. C., 2013 – Nematode and acanthocephalan parasites of marine fish of the eastern Black Sea coasts of Turkey, *Turkish Journal of Zoology*, 37, 6, 753-760, DOI: 10.3906/zoo-1206-18.

47. Ütük A. E. and Pişkin F. C., 2012 – Molecular detection of Anisakis pegreffii in horse mackerels (Trachurus trachurus) sold for human consumption in Erzurum Province of Turkey, *Kafkas Universitesi Veteriner Fakultesi Dergisi*, 18, 2, 303-307, DOI: 10.9775/kvfd.2011.5466.
48. Wekell M. M., Manger R., Colburn K., Adams A. and Hill W., 1994 – Microbiological quality of seafoods: Viruses, bacteria, and parasites, in Shahidi F. and Botta Jr., (eds.), Seafoods: chemistry, processing technology and quality, Blackie Academic & Professional, London. 196.
49. WoRMS, 2022 – World register of marine species, https://www.marinespecies.org, date of accesion 2022-06-28.
50. Yardimci B., Pekmezci G. Z. and Onur E. E., 2014 – Pathology and molecular identification of Anisakis pegreffii (Nematoda: Anisakidae) infection in the John Dory, Zeus faber (Linnaeus, 1758) caught in Mediterranean Sea, *Ankara Universitesi Veteriner Fakultesi Dergisi*, 61, 233-236, DOI: 10.1501/Vetfak_0000002635.

FISH POPULATION IN THE BELČIŠTA WETLAND – RISK ASSESSMENT, PREVENTION AND MANAGEMENT

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KEYWORDS: Belčišta wetland, fish population, ohrid minnows *(Pelasgus minutus*).

ABSTRACT

Belčišta wetland is the largest wetland habitat in R. of North Macedonia, located 20 km distance from Ohrid Lake and it is a relict remnant of the ancient Desaret Lake, which existed in this area since the Pliocene. This region hosts an endemic freshwater species of the Balcan Penninsula, *Pelasgus minutus*, which is threatened by habitat loss.

There are intentions of artificial introduction of salmonid fishes in Belčišta wetland to develop the recreative fishery and tourism in this area. Introduction and spread of salmonids is a great risk factor to the survival of the ohrid minnows *(Pelasgus minutus*).

RÉSUMÉ: Population de poissons dans la zone humide de Belčišta – évaluation des risques, prévention et gestion.

La zone humide de Belčišta est le plus grand habitat de zone humide de la République de Macédoine du Nord, située à 20 km du lac d'Ohrid et constitue un vestige de l'ancien lac Desaret, qui existait dans cette zone depuis le Pliocène. Cette région abrite une espèce d'eau douce endémique de la péninsule des Balkans, le vairon d'Ohrid (*Pelasgus minutus*), qui est menacée par la perte d'habitat.

Il est prévu d'introduire artificiellement des salmonidés dans la zone humide de Belčišta pour développer la pêche récréative et le tourisme dans cette zone. L'introduction et la propagation des salmonidés constituent un facteur de risque important pour la survie des ménés d'Ohrid (*Pelasgus minutus*).

REZUMAT: Populația de pești din zona umedă Belčišta – evaluarea, prevenirea și managementul riscurilor.

Zona umedă Belčišta este cel mai întins habitat de zone umede din Republica Macedonia de Nord, situat la 20 km distanță de lacul Ohrid și este o rămășiță relictă a lacului antic Desaret, care a existat în această zonă încă din Pliocen. Această regiune găzduiește o specie endemică de apă dulce a Peninsulei Balcanice, *Pelasgus minutus*, care este amenințată prin pierderea habitatului.

Se intenționează introducerea artificială a salmonidelor în zona umedă Belčišta pentru a dezvolta pescuitul recreativ și turismul în această zonă. Introducerea și răspândirea salmonidelor este un mare factor de risc pentru supraviețuirea lui *Pelasgus minutus*.

INTRODUCTION

Belčišta wetland (Blue swamps) (Figs. 1-2) is the largest remaining wetland in R. of North Macedonia. Data on the surface of the wetland varies between 137 ha (Golceva, 2019) and 400 ha (Simovski et al., 2019; Bogner, 2022). It has an altitude of about 760 m and is fed by several springs. The marsh is fed by a few cold springs such as St. Anna, St. John and several others on the eastern edge near the church St. Petka (St. Paraskeva). At the confluence of all these sources the River Matica is formed (Simovski et al., 2019). River Matica is the inflow to River Sateska, the biggest tributary of Lake Ohrid, and the wetland belongs to Lake Ohrid basin. The total amount of water flowing out of the swamps is 5 m^3/sec (Bogner, 2022).

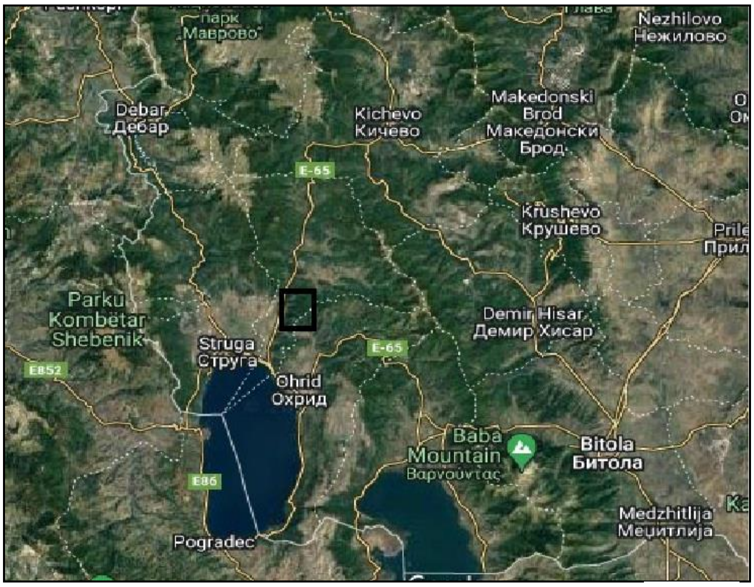

Figure 1: Satellite map – Lake Ohrid watershed (the black square – Belčišta wetland area).

Figure 2: Satellite map – Belčišta wetland.

National strategy for nature protection (2017-2027) recommends that this wetland to be declared as protected and lists it as a major natural heritage in the field of hydrology. The decision should be taken when the new spatial plan of R. of North Macedonia will be done.

MATERIAL AND METHODS

During the period of December 2021 to September 2022, in Belčišta wetland, there has been a total of 176 individuals caught, which were identified as three different species: chub (*Squalius cephalus*), minnow (*Phoxinus lumaireul*) and ohrid minnows (*Pelasgus minutus*) (Fig. 3). Specimens were collected using SAMUS 725 electro fishing device and hand nets, later preserved and stored in 70% ethanol. Identifications follow published morphological species identifications according Kottelat and Freyhof (2007).

Figure 3: Ohrid minnows (*Pelasgus minutus*).

In addition to the small number of species, the fish fauna was also characterized by a small population density.

RESULTS AND DISCUSSION

Present results are a part of detailed study entitled "Ecological status of fish population in Belčišta wetland". During 2022, an investigation was provided on Belčišta wetland and they display as presence of only three fish species with low abundance and low population density. Our main goal on this investigation is to detect pressures and possible dangers of fish populations in the wetland.

Many major human induced activities have had indirect consequences for freshwater wetland, such as overuse of water supply for agriculture activities, the depletion of groundwater, the paving of ephemeral stream channels, deforestation, pollution, etc. (Bănăduc et al., 2022). In course to transform wetland into agriculture fields, in the last decades of 20th century, on Belčišta wetland more meliorative works have been carried out, so that the surface of the wetland has been significantly reduced.

Recently, there are many studies to valuate ecosystem services, sustainable tourism and recreation development in the Belčišta wetland, which proposed many activities based on segmentation and valorisation of factors for the development of several alternative forms of tourism in rural areas. Segmentation is based on the assumption that different types of natural and anthropogenic motifs can attract various types of tourists in the rural area.

The Belčišta wetland is a biodiversity hotspot, hosting endemic freshwater species, *Pelasgus minutus,* which is occurring exclusively here. The genus *Pelasgus* is one of the most ancient genera of the family Leuciscidae. The regions of the oldest colonisation by *Pelasgus* are the drainages of the ancient lakes Ohrid and Prespa and the southernmost part of the Peloponnese (Rodriges et al., 2021).

Currently, 18 distinct molecular lineages are recognized within *Phoxinus* in Europe based on mitochondrial cytochrome c oxidase subunit I (COI) sequences, and at least nine of these molecular lineages can be found in the area of Western Balkans alone, making it one of the most important diversity hot spot for this genus (Vucic et al., 2018; Palandacic et al. 2020).

Apart from these facts, there are intentions of artificial introduction of salmonid fishes in Belčišta wetland to develop the recreative fishery and tourism in this area. Introduction of salmonid fishes is a direct threat to the survival of these small fishes, because they will provoke decreased populations of *Phoxinus lumaireul* and *Pelasgus minutus*.

The fact that until now predominant effort is dedicated to field activities strictly related to management of “charismatic species” for sport fishing, first salmonids. It is furthermore the fishery management focused on and linked to angling activities that seriously risks compromising the survival of these “minor” populations. Local, small and even endemic fish populations represent potential prey of salmonids, illegally released yearly in huge quantities to meet the expectations of anglers (Caputo Barucchi et al., 2022).

For successful protection of Belčišta wetland, it is necessary to reconcile biodiversity conservation and economic development. The biologic importance of the wetland consists of the conservation and increasing biodiversity of the richness of plant and animal species, their diversity and the dimensions of the populations, connected directly to the habitats resulted from the water and its interferences. The wetland supplies food resources that determine colonisation of some shelter habitats, the seasonal establishment and the reproduction of the species that depend of this environment (Costea, 2008).

Some view water as a hostile habitat stemming from our terrestrial way of life, and this is why our rather basic knowledge of these habitats has led to severe difficulties for humankind. This includes wetland vegetation destruction, reclamation, water overuse and pollution (Bănăduc et al., 2022). Wetland fragmentation, contraction, alteration, and loss represent significant stressors impacting aquatic environment abiotic and biotic elements (Bănăduc et al., 2022).

Also, restrictions on water availability are among the most important reasons for conjecturing about the future limit to the human activities and growth of the world population. Climate modification, which is very much tied to human population increase, will induce huge pressure on water resources (Bănăduc et al., 2022).

Protection of Belčišta wetland is the most important national priority in this respect, firstly because it is an important freshwater source in the Lake Ohrid watershed. Belčišta wetland ecosystem belongs to the EMERALD network and has already made an initiative to become a protected area and future NATURA 2000 site (Brajanoska et al., 2018).

CONCLUSIONS

Belčišta wetland is a home of the important and endemic fish species of the Balkan Peninsula, ohrid minnow (*Pelasgus minutes*). Protection of fish populations in Belčišta wetland needs balance between management issues of this ecosystem and its implication on human life, and freshwater conservations.

There are perspectives on Belčišta wetland as a protected area – or nature park in the future.

ACKNOWLEDGEMENTS

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REFERENCES

1. Bănăduc D., Simic V., Cianfaglione K., Barinova S., Afansyev S., Oktener A., McCall G., Simic S. and Curtean-Bănăduc A., 2022 – Freshwater as a sustainable resource and generator of secondary resources in the 21-th century: stressors, threats, risks, management and protection strategies and conservation approaches, *International Journal of Environmental Research and Public Health*, 19, 16570.
2. Bogner D., 2022 – Valorization study of Belcista wetland – Park of nature, Ed. Critical Ecosystem Partnership Fund, 105.
3. Brajanoska R., Matevski V., Avramovski O., Velevski M., Velkovski N., Kostadinovski M., Levkov Z., Melovski L. J., Melovska N., Slavevska Stamenkovik V. and Hristovski S., 2018 – National strategy of biodiversity with Action plan (2018-2023), Ministry of environment and planning of R. North Macedonia, 185.
4. Caputo Barucchi V., Marconi M., Splendiani A., Casari S., Girardi M. and Gandolfi A., 2022 – Mitochondrial DNA suggest uniqueness of a isolated population of the Italian minnow (Phoxinus lumaireul Schinz, 1840) (Teleostei: Cyprinidae) in central Apennines (Italy), *The European Zoological Journal*, 98, 1, 711-718.
5. Costea M., 2008 – Consideration concerning the wetland importance, protection and sustainable management (Romania), *Transylvanian Review of Systematical and Ecological Research*, 6, 155-164.
6. Golceva Z., 2019 – Socio-economic study of Belcista wetland, Ed. Society of ecology and tourism ECOTOURISM 2016 Ohrid, 57.
7. Kottelat M. and Freyhof J. 2007 – Handbook of European Freshwater Fishes, Publications Kottelat, Cornol, Switzerland, 646.
8. National strategy for nature protection (2017-2027), Ministry of environment and planning of R. North Macedonia.
9. Simovski B., Nikolov B. and Mincev I., 2019 – Establishing initial monitoring of the key forest habitat: alluvial forests with common alder (Alnos glutinosa L. Gaertn.) and ash (Fraxinus excelsior L.) in Belcista wetland, Final report, Project: Improving the management and sustainable use of the Belcisko blato (Sini viroj), 25.
10. Palandacic A., Kruckenhauser L., Ahnelt H. and Mikcshi E., 2020 – European minnows trough time: museum collections aid genetic assessment of species introductions in freshwater fishes (Cyprinidae, Phoxinus species complex), *Heredity*, 124, 410-422.
11. Rodriges N. V., Sanda R., Zogaris S. and Vukic J., 2021 – Evolutionary history of the Pelasgus minnow (Teleostei: Leuciscidae) an ancient endemic genus from the Balkan Peninsula, *Molecular phylogenetics and evolution*, 164.
12. Vucic M., Jelic D., Zutinic P., Grandjean F. and Jelic M., 2018 – Distribution of Eurasian minnow (Phoxinus, Cypriniformes) in Western Balkans, *Knowledge and management of aquatic ecosystems*, 419, 11.

DIVERSITY AND CURRENT STATE OF FISH COMMUNITIES OF THE RESERVE "UTRISH" (CAUCASIAN COAST OF THE BLACK SEA, ABRAU PENINSULA)

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KEYWORDS: species richness, diversity, dominance, European black scorpionfish.

ABSTRACT

The article is focused on the current state of biodiversity in the aquatic environment of the Utrish Nature Reserve. Implementation of the multi-level monitoring program for fish and crustacean fauna, conducted in the waters of the Utrish Nature Reserve during the period 2016-2018, using classical methods of biological analysis, as well as visual underwater fish counting, allowed us to assess the overall state of biodiversity in this area as sufficiently stable. The population status of the model indicator species, the European black scorpionfish *Scorpaena porcus*, was also stable during this period, confirming the effectiveness of protective measures in this area. Due to the continued increase in anthropogenic pressure on coastal marine biocenoses, monitoring studies require further continuation.

RÉSUMÉ: Diversité et état actuel des communautés de poissons de la réserve d'Utrish (côte caucasienne de la mer Noire, péninsule d'Abrau).

L'article se concentre sur l'état actuel de la biodiversité dans l'environnement aquatique Utrish de la réserve. La mise en œuvre du programme de suivi à plusieurs niveaux de la faune piscicole et crustacée, réalisé dans les eaux de la réserve d'Utrish au cours de la période 2016-2018, en utilisant des méthodes classiques d'analyse biologique, ainsi que le comptage visuel des poissons sous-marins, a permis d'évaluer l'état général de la biodiversité dans cette zone comme suffisamment stable. L'état de la population de l'espèce indicatrice modèle, la rascasse noire européenne *Scorpaena porcus*, était également stable pendant cette période, confirmant l'efficacité des mesures de protection dans cette zone. En raison de l'augmentation continue de la pression anthropique sur les biocénoses marines côtières, les études de surveillance doivent être poursuivies.

REZUMAT: Diversitatea și starea actuală a comunităților de pești din Rezervația „Utrish" (Coasta Caucaziană a Mării Negre, Peninsula Abrau).

Articolul se concentrează asupra stării actuale a biodiversității în mediul acvatic al Rezervației Utrish. Implementarea programului de monitorizare pe mai multe niveluri a faunei de pești și crustacee, desfășurat în apele Rezervației „Utrish" în perioada 2016-2018, folosind metode convenționale de analiză biologică, precum și numărarea vizuală subacvatică a peștilor, a făcut posibilă evaluarea stării generale a biodiversității în această zonă ca fiind suficient de stabilă. Statutul populației speciei indicator model, peștele scorpion negru European *Scorpaena porcus*, a fost, de asemenea, stabil în această perioadă, confirmând eficacitatea măsurilor de protecție în această zonă. Datorită creșterii continue a presiunii antropice asupra biocenozelor marine de coastă, studiile de monitorizare trebuie continuate.

INTRODUCTION

The problem of studying and preserving biodiversity using monitoring research methods is one of the most pressing global environmental issues due to the constantly growing anthropogenic impact on individual species and the biosphere as a whole (Zakharov and Trofimov, 2019).

The combination of factors such as unique natural conditions, scarce knowledge about the species composition of hydrobionts in the coastal zone, and the abundance of protected reserve waters makes studies of the marine fauna in the coastal zone of the Abrau Peninsula, which marks the beginning of the Caucasus region of the Black Sea, extremely relevant. The Utrish Reserve is located between Cape Bolshoy Utrish and Cape Malyy Utrish and includes a significant part of the coastal zone of the territorial waters of the Black Sea.

Ichthyological monitoring is an important component of environmental monitoring since the most comprehensive information about the overall state of the environment can be obtained by studying biota communities that contain the largest number of sedentary and low – migratory animal species constantly present within the surveyed waters, i.e., benthic communities and ichthyocenosis.

Monitoring of fish community dynamics is the most important component of the study and conservation of aquatic environments, as the analysis of process dynamics allows for identify general patterns of ecosystem changes, coenotic connections, and the biology of individual animal and plant species. Within the framework of ichthyological monitoring programs, the development and implementation of scientific methods for the conservation of ichthyofauna are possible.

Ichthyological studies near the eastern coast of the Black Sea are relatively few and mainly sporadic (Kruglov, 1991; Luzhnyak and Chikhachev, 2000; Plotnikov, 2001; Luzhnyak, 2003; Vasilyeva and Bogorodsky, 2004; Bogorodsky, 2006; Pashkov et al., 2013; Prokofiev, 2016; Karpova et al., 2021). In general, the insufficiency of the study of ichthyofauna can be stated both in the coastal marine zones and in the brackish-water lakes and watercourses of the reserve.

The goal of this study is to assess the state of diversity of the fish community in the Utrish Reserve, which is one of the most important characteristics of community status under the impact of various environmental factors.

The program of comprehensive monitoring ichthyological research in the Utrish Reserve was initiated in 2016 and further continued and developed in 2017–2019.

MATERIAL AND METHODS

Fish populations

The research methods for studying fish populations in the marine waters of the Utrish Nature Reserve included visual underwater observations using photography and videography equipment. During these studies, both surface and free-diving observations were employed. Surface observations involved monitoring the water column and counting fish while in motion on the water's surface, while free-diving allowed for more detailed exploration of the benthic areas. The duration of the counting transect, depending on the benthic landscape, is up to 50 meters.

To increase the accuracy of the counts, underwater compact video and photo cameras were used to record the number of fish in a flock, especially when there were large numbers of individuals. Underwater photography with subsequent image analysis in laboratory conditions was employed to improve the reliability of species identification. The surveys were conducted at six stations in the coastal waters (Fig. 1).

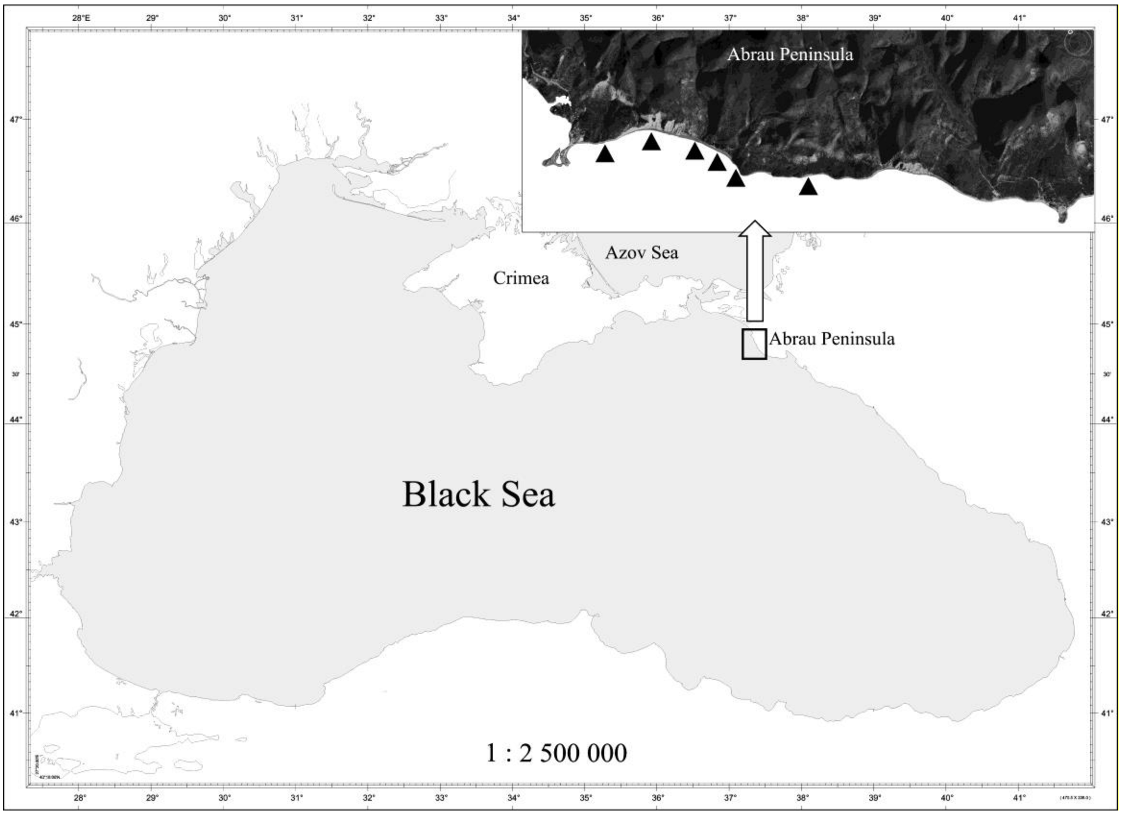

Figure 1: The schematic map of the studied area.

Species richness

In order to analyze indicators of species richness and diversity based on the survey data, the following indices were calculated (Smith and Van Belle, 1984):

1) Margalef index: $I = (S–1)/\ln N$,

where *S* is the total number of recorded species, *N* is the total number of individuals counted.

2) Simpson index: $C = \sum p_i^2$,

where p_i is the evaluation of the significance of each species (abundance or biomass).

3) Shannon-Weaver index: $H = –\sum p_i \times \log_2 p_i$,

where *pi* is the proportion of individuals of the i-th species.

4) Inverse Berger-Parker index: $IBP = N/N_{max}$,

where N*max* is the number of individuals of the dominant species.

Catches analysis

To study the biological status and population characteristics of fish, data from fishing net catches near the boundaries of the reserve's waters within characteristic biotopes for the region were used.

After obtaining ichthyological material, the following types of work were conducted (Pravdin, 1966):

– initial analysis of the catch, including species identification, counting the number of individuals, and determining the total mass of fish for each species;

– biological analysis, including measurements of mass (total, standard length, and fish mass), determination of gender and stage of sexual maturity.

RESULTS AND DISCUSSION

The diversity of fish communities

The species diversity of fish communities consists of two components: species richness, or species density, which is characterized by the total number of species, and evenness, based on the relative abundance or other indicator of species importance and their position in the dominance structure.

One of the main components of biodiversity – species richness or species density – is the total number of species, which is expressed in comparative terms as the ratio of the number of species to the number of individuals (Margalef's species richness index) (Fig. 2).

The highest values of this indicator were observed in spring and summer (in 2016), which may be explained by the active approach of many species to the shore for feeding and spawning. Overall, during the research, fluctuations in this indicator were not significant.

During research, adult individuals of 64 fish species belonging to 46 genera from 36 families and 14 orders were recorded to be more and less regularly present (Tab. 1).

Table 1: The GenBank accession numbers of nematode species.

No.	Order	Species		Number of genus	Number of families
		number	%		
1	Squaliformes	1	1.6	1	1
2	Rajiformes	2	3.1	2	2
3	Acipenseriformes	2	3.1	1	1
4	Clupeiformes	4	6.3	3	2
5	Salmoniformes	1	1.6	1	1
6	Gadiformes	2	3.1	2	2
7	Ophidiiformes	1	1.6	1	1
8	Mugiliformes	4	6.3	2	1
9	Atheriniformes	2	3.1	1	1
10	Beloniformes	1	1.6	1	1
11	Gasterosteiformes	2	3.1	1	1
12	Scorpaeniformes	2	3.1	2	2
13	Perciformes	36	56.3	24	16
14	Pleuronectiformes	4	6.3	4	4
Total		64	100	46	36

The family Gobiidae, with ten species, is characterized by great species richness; the families Labridae and Blenniidae have five species each. Four species were recorded for the family Mugilidae, and three species for the families Sparidae and Clupeidae. Three other families are represented by two species, and the remaining families by one species each. Thus, representatives of ten families out of 36 constitute about 60% of species richness.

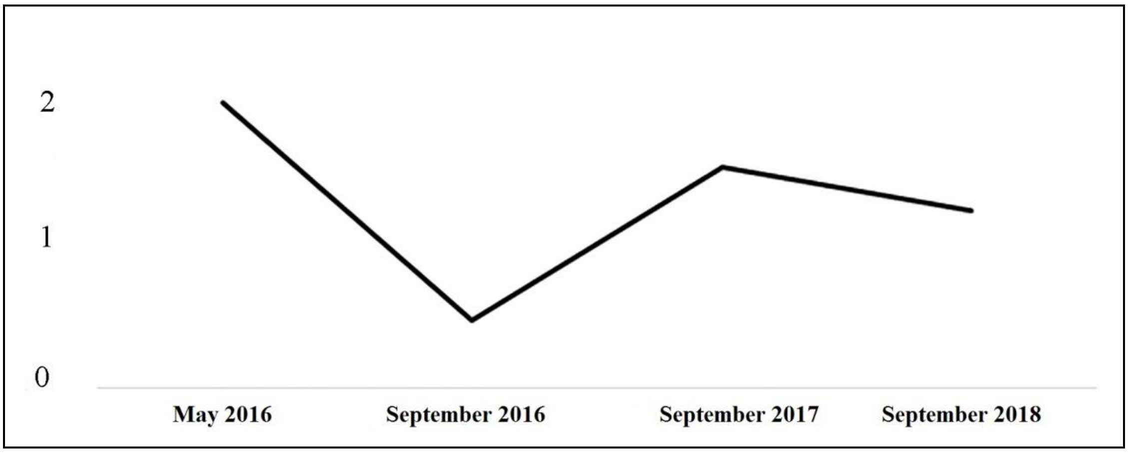

Figure 2: Temporal dynamics of the fish species richness index in the water area of the Utrish Nature Reserve.

To assess differences in the species structure of fish communities, a dominance-diversity curve analysis was used (Fig. 3).

It is known that the more steeply a curve falls, the lower the overall diversity (in terms of evenness) and the stronger the dominance of one or several species. The distribution over different time periods is represented by an intermediate S-shaped curve that corresponds to the log-normal type of estimate of the significance of individual species, which indicates the complex nature of differentiation and overlapping of niches and the presence of developed fish communities. According to the obtained data, the lowest homogeneous community structure was observed in September 2016.

The main indicators of species diversity – Simpson’s Dominance Index (C), Pielou’s Evenness Index (E), Shannon-Wiever Species Diversity Index (H), Berger-Parker Index (IBP) – were calculated based on long-term data series of census data (Fig. 4).

Dominance indicators (Simpson’s and Berger-Parker indices) in fish communities during the observation period reached their highest values in 2016, followed by a decrease and a slight increase in the evenness of communities, which was reflected in the dynamics of the Pielou’s Index. Overall, the fluctuations can be considered insignificant and caused by local hydrological and biocenotic conditions.

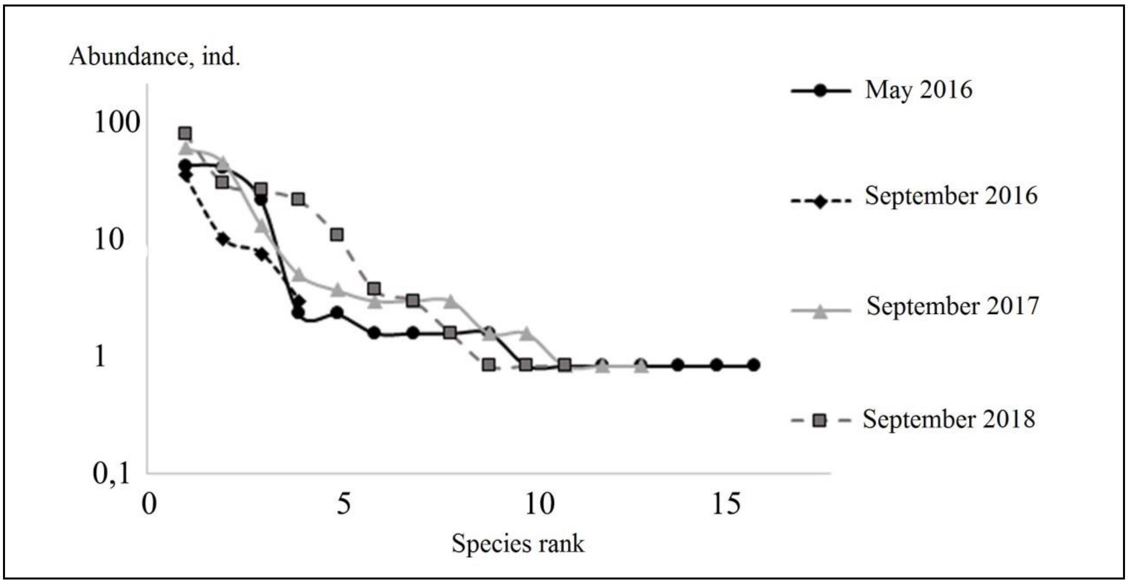

Figure 3: Dominance-diversity curve in fish communities
in the area of the Utrish Nature Reserve in different periods.

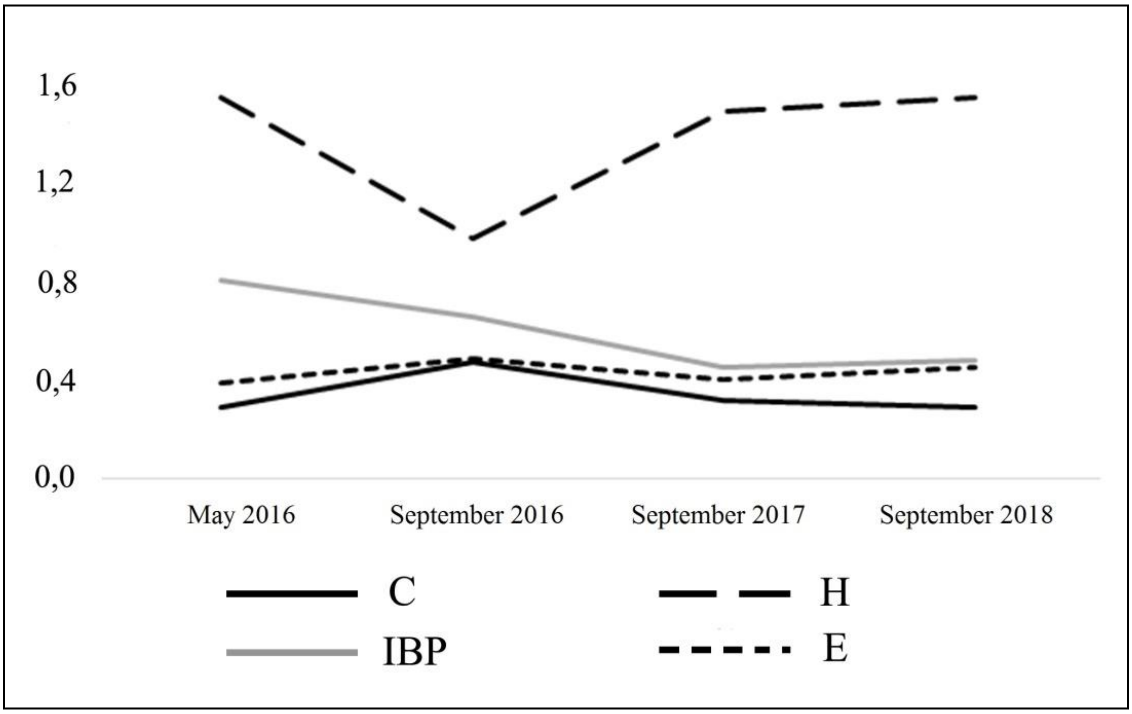

Figure 4: Dynamics of species diversity indices.

The black scorpionfish population condition

Studying the biological characteristics of indicator species is an important source of information about the overall condition of aquatic organisms as a result of the complex impact of natural and anthropogenic factors. In the reserve's marine environment, the black scorpionfish *Scorpaena porcus* Linnaeus, 1758, can be classified as such species due to its abundance and benthic sedentary lifestyle. The black scorpionfish is one of the most widespread predators, inhabiting all types of coastal biotopes, and is a model species for assessing the state of biota in various marine habitats. In May and September 2016, among the fish of this species, individuals of the size class 100-110 mm predominated; three other size classes – 110-120 mm, 120-130 mm, and 190-200 mm noticeably prevailed over the rest. In September 2017, an abundance of larger size groups (140-150 mm, 160-170 mm, and 170-180 mm) was observed, with the 120-130 mm size class being dominant. At the end of the warm period in 2018, the scorpionfish exhibited an abundance of size groups of 110-120 mm (in both females and males) and 120-130 mm (in females). A significant proportion of individuals in larger size classes was also observed, primarily 170-180 mm, as well as 140-150 mm, 150-160 mm, and 200-210 mm, with the 140-150 mm size class being numerous in both males and females, while the others were predominantly females.

Overall, the characteristics of the size-frequency structure of the scorpionfish population in different years are quite similar (Fig. 5), indicating the stability of the species and the overall favorable state of the environment.

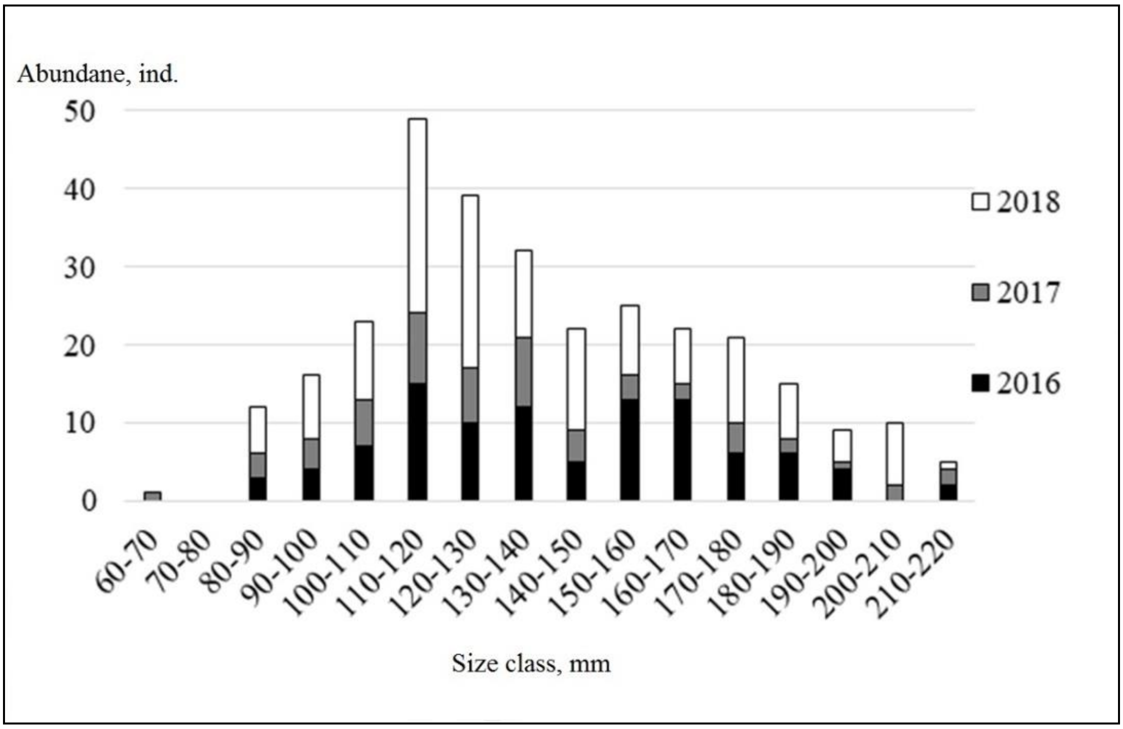

Figure 5: Size-frequency composition of the scorpionfish population in 2016-2018.

CONCLUSIONS

The complex ichthyological studies have confirmed the effectiveness of measures for the protection of marine, brackish, and freshwater biocenoses on the Utrish Reserve as a center of conservation of the gene pool of natural fauna of the North Caucasus region. It should be emphasized once again that Utrish is the only reserve with a marine area on the Black Sea coast of the Krasnodar Territory, which is clearly insufficient to ensure the ecological safety of this region. This area contains several well-preserved marine biocenoses, of which fish communities are an integral component. During the warm season, there is a spawning and growth of juvenile summer-producing fish, which account for about 95% of the number of species found in the reserve's water area, while the remaining species reproduce during the cold period. Due to the uniqueness of the coastal biotopes of the reserve, adjacent to the Abrau Peninsula, it is advisable to establish a kilometer-wide buffer zone along the perimeter of the water boundaries of the reserve's water area due to the specific nature of the habitat, namely its mobility.

The passage of vessels and crafts negatively affects both the reproducers during the spawning period as well as the fish during the early stages of ontogeny. The main negative factors of navigation include the stressful factor of noise pollution, mechanical damage to passive hydrobionts during the operation of propellers and water intake for cooling ship power units, chemical pollution when fuels and lubricants enter the water, etc.

Localized biotopes with specific fish communities, whose life cycles are directly linked to these biocenoses, have been found in the reserve's water area and therefore require special protection. However, further research is required to clarify the specific structural characteristics of these communities and their mapping.

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REFERENCES

1. Bogorodsky S. V., 2006 – Detection of Parablennius incognitus (Blennidae) off the eastern coast of the Black Sea, northern Abkhazia, *Journal of Ichthyology*, 46, 1, 22-28. (in Russian)
2. Vasilyeva E. D. and Bogorodsky S. V., 2004 – Two new species of gobies (Gobiidae) in the ichthyofauna of the Black Sea, *Journal of Ichthyology*, 44, 5, 599-606. (in Russian)
3. Karpova E. P., Boltachev A. R., Ablyazov E. R., Gubanov V. V. and Bykhalova O. N., 2021 – Current state of the ichthyofauna of the Utrish Nature Reserve and adjacent sea waters, Terrestrial and marine ecosystems of the Abrau Peninsula: history, condition, protection, State Nature Reserve "Utrish", Anapa, 5, 241-248. (in Russian)
4. Kruglov M. V., 1991 – Bottom coastal ichthyocenes of the Black Sea: composition and current state, *Proceedings oft he All-Russian school on technical means and methods of research of the World Ocean*, Moscow, 2, 165. (in Russian)
5. Luzhnyak V. A., 2003 – Ichthyofauna of rivers and estuaries of the Black Sea coast of Russia, *Journal of Ichthyology*, 43, 4, 457-463. (in Russian)
6. Luzhnyak V. A. and Chikhachev A. S., 2000 – Species composition of the ichthyofauna of reservoirs on the Black Sea coast of Russia, Main problems of fisheries and protection of fishery reservoirs of the Azov-Black Sea basin, Rostov-on-Don, AzNIIRKh, 73-84. (in Russian)
7. Pashkov A. N., Reshetnikov S. I. and Makhrov A. A, 2013 – On the issue of occurrence in the waters of the Krasnodar region of the four-striped Chromogobius quadrivittatus (Steindachner, 1863) (Pisces, Gobiidae), *Proceedings of the VII International Conference "Modern fishery and environmental problems of the Azov-Black Sea region"*, Kerch, 83-87. (in Russian)
8. Plotnikov G. K., 2001 – Ichthyofauna of various aquatic ecosystems of the North-West Caucasus, Krasnodar, KubSU, 166. (in Russian)
9. Pravdin I. F., 1966 – Guide to the study of fish, Moscow, Food industry, 376. (in Russian)
10. Prokofiev A. M., 2016 – Gobius cruentatus (Gobiidae) in the Russian waters of the Black Sea coast of the Caucasus, *Journal of Ichthyology*, 56, 1, 117-120. (in Russian)
11. Smith E. P. and G. Van Belle, 1984 – Nonparametric estimation of species richness, *Biometrics*, 40, 119-129.
12. Zakharov V. M. and Trofimov I. E. 2019 – Biodiversity assessment: study of developmental stability, Moskow, Partnership of scientific publications KMK, 160. (in Russian)

ACUTE AND CHRONIC EFFECTS OF PESTICIDES ON NON-TARGET AQUATIC ORGANISMS

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KEYWORDS: pesticides, toxicity, aquatic organisms, non-target organisms.

ABSTRACT

Pesticides are widely used to protect crops from pests, ensure food security, and increase agricultural productivity. However, the application of these chemicals can lead to unintended adverse effects on non-target aquatic organisms. This scientific manuscript provides a comprehensive review of the acute and chronic effects of pesticides on aquatic non-target organisms, focusing on their behavior, physiology, reproductive success, and populations dynamics. Understanding these effects is critical to developing effective strategies to minimize the ecological risks associated with pesticide use.

ZUSAMMENFASSUNG: Akute und chronische Auswirkungen von Pestiziden auf Nicht-Ziel Wasserorganismen.

Pestizide werden häufig eingesetzt, um Nutzpflanzen vor Schädlingen zu schützen, die Ernährungssicherheit zu gewährleisten und die landwirtschaftliche Produktivität zu verbessern. Die Anwendung dieser Chemikalien kann jedoch unbeabsichtigt zu schädlichen Auswirkungen auf Nicht-Ziel Wasseroganismen führen. Daher ist das Ziel des vorgelegten Manuskripts, einen umfassenden Überblick über die akuten und chronischen Auswirkungen von Pestiziden auf Nicht- Ziel-Wasserorganismen vorzulegen und sich dabei auf deren Verhalten, ihre Physiologie, den Fortpflanzungserfolg sowie die Populationsdynamik zu konzentrieren. Das Verstehen dieser Auswirkungen ist von entscheidender Bedeutung für die Entwicklung wirksamer Minderungsstrategien zur Verringerung der mit dem Pestizideinsatz verbundenen ökologischen Risiken.

REZUMAT: Efecte acute și cronice ale pesticidelor asupra organismelor acvatice nețintă.

Pesticidele sunt utilizate pe scară largă pentru a proteja culturile de dăunători, asigurând securitatea alimentară și îmbunătățind productivitatea agricolă. Cu toate acestea, aplicarea acestor substanțe chimice poate duce, din neatenție, la efecte adverse asupra organismelor acvatice nețintă. Acest manuscris științific își propune să ofere o revizuire cuprinzătoare a impactului acut și cronic al pesticidelor asupra organismelor acvatice nețintă, concentrându-se pe comportamentul, fiziologia, succesul reproductiv și dinamica populațiilor acestora. Înțelegerea acestor efecte este crucială pentru dezvoltarea strategiilor eficiente de atenuare pentru a minimiza riscurile ecologice asociate cu utilizarea pesticidelor.

INTRODUCTION

The pollution with chemicals, is an important cause of the ecosystems' deterioration and ecosphere's biogeochemical cycles disturbance with long term major negative impacts (Curtean-Bănăduc et al., 2017). Among them, pesticides play a main role in agriculture, but their impact on non-target organisms has caused concern worldwide (Khoshnood et al., 2015). Aquatic organisms, including fish, amphibians, invertebrates, and plants, are often the main victims of pesticide contamination through runoff and drift (Zaller and Brühl, 2019).

Pesticides play a main role in agriculture, but their impact on non-target organisms has caused high concern. Aquatic organisms, including fish, amphibians, invertebrates, and plants, are the main victims of pesticide contamination through runoff and drift. (Kaur et al., 2029)

There are several categories of pesticides that are identified by their chemical structure. Some of them are persistent and remain in the environment for a long time after their use, others are not persistent and there are no or only traces of them after use. Many pesticides have been found to have the ability to bioaccumulate, i.e. they can pass through the food chain in ecosystems and sometimes be found in areas far away from their place of use. On the other hand, it has been shown that many pesticides can enter ground and surface waters outside their application site through runoff, drift and leaching from agricultural land. Many studies have shown that various pesticides can also affect non-target organisms. Since runoff eventually reaches rivers, lakes, wetlands, and the marine environment, terrestrial use of pesticides could have negative effects on aquatic non-target organisms. However these negative effects may vary depending on the presence and/or amount of other environmental factors such as salinity, temperature, suspended solids, sediments, etc., and also depend on the species, age, developmental stage and some other biological factors. (USEPA, 2002)

This manuscript aims to summarize the current state of knowledge on the acute and chronic effects of pesticides on aquatic non-target organisms.

Acute effects

When an organism is exposed to an inappropriate condition or pollutant, it can lead immediately or in a short time to changes in behavior, physiology, biochemistry, histology, and many other areas called acute effects. Sometimes such changes are severe and irreversible and can lead to the death of the exposed organisms, and sometimes the changes are mild and reversible when the contaminant is removed from the environment. It is well established that many pesticides are lipophilic and can therefore affect the nervous, immune, cardiovascular, and reproductive systems of organisms, as well as causing mutagenicity and carcinogenicity with adverse health effects. (Khoshnood, 2017)

Histopatology

Acute exposure to pesticides can lead to cell and tissue damage in non-target aquatic organisms such as fish and amphibians. It has been shown that even acute exposure to sublethal concentrations of the herbicide atrazine can cause such tissue damage in the gills, kidneys, intestines, and liver of Caspian Kutum, *Rutilus frisii kutum* larvae and fingerlings (Khoshnood et al., 2014). For example, acute exposure to sublethal concentrations of atrazine caused several gill tissue damages in Caspian Kutum, *Rutilus frisii kutum* fingerlings, including detachment of lamellar epithelium, thickening of lamellae, edema in lamellae, and filament, hypertrophy of pavement cells of lamellae; necrosis, lamellar fusion, hyperplasia of the filament epithelium, club-shaped lamellae, detachment of the epithelium of the gill rackers, hyperplasia of the lamellar epithelium and shrinkage of the lamellae (Fig. 1). Atrazine can also cause histopathological changes in the kidney (Khoshnood, 2015) (Fig. 2), brain and liver of fish (Ahmad, et al., 2021).

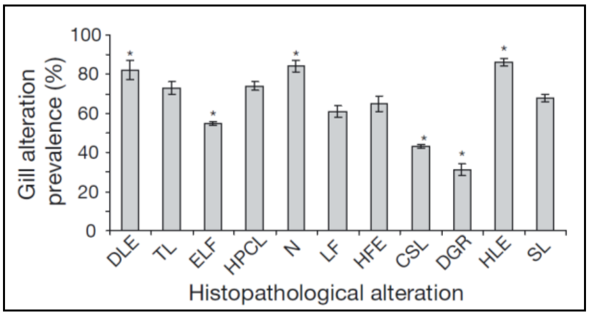

Figure 1: Prevalence of histopathological changes in the gills of *R. frisii kutum* fingerlings after acute exposure to sublethal concentrations of the herbicide atrazine: DLE: detachment of lamellar epithelium, TL: thickening of lamellae, ELF: edema in lamellae and filaments, HPCL: hypertrophy of pavement cells of lamellae; N: Necrosis, LF: lamellar fusion; HFE: hyperplasia of the filament epithelium, CSL: club-shaped lamellae, DGR: detachment of the epithelium of the gill rackers, HLE: hyperplasia of the lamellar epithelium, and SL: shrinkage of the lamellae (Khoshnood et al., 2015).

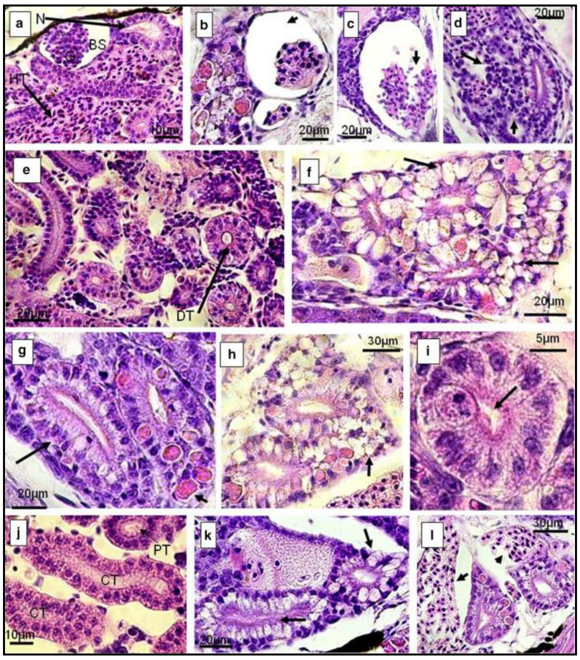

Figure 2: Normal histological structure (a, e, j) and histopathological changes (b-d, f-i, k-l) in the kidney of *R. frisii kutum* larvae after 96 h of atrazine exposure. Head kidney consists of haematopoietic tissue and kidney tubules (a). After the Bowman's capsule, the tubular system of the nephron consists of short neck tubule (a), the proximal tubule (j), which has cuboidal epithelial cells with an apical brush border, the distal tubule (e), whose epithelial cells have no brush border, and the collective tubule (j). Bowman's capsule is the first part of the nephron that surrounds the capillary network, the glumerulus (a). the tissue changes after exposure to atrazine were as follows: reduction in size of the glomerulus and enlargement of Bowman's space (b); necrosis of the glomerulus (c); necrosis and destruction of the haematopitic tissue of the head kidney (d); swelling, hypertrophy, vacuolization and aggregation of hyaline droplets in the epithelial cells of the renal tubules (f–h, k); hyperplasia of the renal tubular epithelial cells (g); necrosis of the renal tubular epithelial cells (h); reduction in luminal space and complete obstruction of the lumen in the renal tubules (i, k); hyperemia (l). BS Bowman's space, G Glumerulus, HT haematopoietic tissue, N neck, DT distal tubule, PT proximal tubule, CT collective tubule, (→): Tissue changes (Khoshnood, 2015).

Another study on the acute effects of sublethal concentrations of alpha-cypermethrin (an insecticide and indoor residual spray) on frogs showed histopathological changes in the kidney, spleen, heart, and tongue (Riaz et al., 2022). Based on a study on the effects of subchronic exposure to environmentally relevant concentrations of the herbicide atrazine, it was clarified that atrazine causes histological damage in the liver and testes of the neotropical fish *Astyanax altiparanae* (Destro et al., 2021).

Behaviour

Exposure to pesticides can significantly affect the behavior of non-target aquatic organisms. Studies have documented changes in feeding behavior, locomotion, and avoidance behavior following pesticide exposure (Sanchez-Bayo and Goka, 2006). For example, changes in swimming behavior, gasping for air at the surface, loss of balance and rapid movements of the operculum and fins have been observed in fish after exposure to many pesticides. In addition, there are reports of reduced egg production and hatching of fish, as well as abandonment of nest and brood (Sanchez-Bayo and Goka, 2006).

Physiology

Pesticides can disrupt the physiological mechanisms of aquatic organisms. Changes in respiration rate, metabolic activity, and ion regulation have been observed, leading to growth disturbances, reduced immune response and increased susceptibility to disease (Liess and Von der Ohe, 2005). One of the well-described effects of pesticides, particularly in amphibians, is the impairment of the endocrine system. For example, the herbicide atrazine has been shown to impair the endocrine system of suburban frogs, even at low ecologically relevant doses, resulting in hermaphroditic and demasculinized frogs (Hayes et al., 2002). In addition, previous studies have shown that some herbicides such as atrazine and glyphosate, can interefer with the development and reproduction of freshwater organisms, including fish. These studies have shown that atrazine can disrupt reproduction in addition to its other effects such as mortality, growth disturbances, biochemical complications, behavioral change, and even genetic changes. In addition, studies have shown that the herbicide atrazine can affect osmoregulation and hydromineral balance in freshwater crabs (Waring and Moore, 2004). Destro et al. (2021) showed that exposure to low concentrations of atrazine induced oxidative stress in the gills, liver and muscles of yellow-tailed tetra fish. In addition, atrazine caused minor endocrine disruption, mainly a change in the triiodothyronine/thyroxine (T3/T4) ratio in *Astyanax altiparanae* (Destro et al., 2021).

Reproductive success

Pesticides pose a threat to the reproductive success of non-target aquatic organisms. Reduced fecundity, altered sex ratio, abnormal development, and reduced hatching success have been observed, potentially affecting population dynamics (Gilliom, 2007). As previously mentioned, the herbicide atrazine could significantly alter the reproductive system of exposed frogs and fish by impairing testosterone production, resulting in impaired reproduction (Hayes et al., 2002).

Ecological implications

Acute effects of pesticides can lead to immediate mortality or sublethal effects that contribute to long-term ecological consequences. Population declines, disrupted food webs and shifts in species composition can be the result of pesticide exposure (Stehle and Schulz, 2015).

CHRONIC EFFECTS

Bioacumulation

Pesticides with long half-lives tend to bioaccumulate in non-target aquatic organisms, leading to increased concentrations over time and causing biomagnification through the food chain. This bioaccumulation can lead to chronic toxicity as certain pesticides persist in the food chain (Beketov et al., 2013). In addition to the negative effects of such criteria, the bioaccumulation of pesticides in aquatic organisms through the consumption of marine and fish food could have a direct impact on the health of humans and aquatic/terrestrial predator species).

Developmental abnormalities

Chronic exposure to pesticides can lead to developmental disorders in aquatic organisms, affecting growth rates, morphology and overall fitness (Brogan et al., 2019). Known developmental abnormalities caused by pesticides include atrazine-induced changes in the gonads of freshwater frogs. It has been shown that such changes can occure following exposure to low and sub-lethal concentrations of a pesticide (Hayes et al., 2002).

Reproductive impairment

Prolonged exposure to pesticides can lead to reproductive impairment, including reduced fertility, impaired gonadal development, and altered breeding behavior (Gibbons et al., 2015).

Sublethal effects

Chronic exposure to pesticides can lead to sublethal effects such as impaired immune function, increased susceptibility to disease and impaired general health (Relyea, 2005). After exposure to sublethal doses, the organism reacts differently depending on age, sex, developmental stage, species, etc. Reversible and/or irreversible cellular and tissue changes may occure following exposure of an organism to sublethal doses. These changes in fish include gill, kidney and digestive tissue damage, changes in hepatic enzyme activity, and hemorrhagic lesions along with the behavioral changes already mentioned (Khoshnood et al., 2015).

MITIGATION STRATEGIES

Various strategies can be used to mitigate the harmful effects of pesticides on non-target aquatic organisms (Munn and Gilliom, 2001):

– Integrated Pest Management (IPM).

Promoting IPM practices can reduce reliance on pesticides by incorporating alternative pest control methods, thus minimizing the overall impact on the environment.

– Buffer zones.

Establishing buffer zones between agricultural fields and water bodies can reduce the runoff of pesticides into aquatic ecosystems, protecting non-target organisms.

– Pesticide alternatives.

Promoting the use of less toxic and more environmentally friendly pesticide alternatives can minimize risks to non-target aquatic organisms.

– Monitoring and regulation.

Regular monitoring of pesticides use and their impact on aquatic ecosystems enables informed decision-makers and the establishment of regulations to protect non-target organisms.

CONCLUSIONS

Understanding the acute and chronic effects of pesticides on non-target aquatic organisms is critical for sustainable pest management and protecting the integrity of aquatic ecosystems. By adopting appropriate mitigation strategies, it is possible to find a balance between agricultural productivity and the protection of non-target organisms, ultimately promoting a healthier environment for all.

REFERENCES

1. Ahmad W., Sultan A., Jan S., Ali A., Rahman I., Usman H., Sher A., Khan N. S., Khan Z. and Khan A., 2021 – Environmental risk of atrazine (herbicide) to brain, gills and liver tissues of fish, Ctenopharyngodon idella (Valenciennes, 1844), *Journal of Geoscience and Environment Protection*, 9, 1-16, DOI: 10.4236/gep.2021.92001.
2. Barzman M., Bàrberi P., Birch A. N. E., Boonekamp P. M., Dachbrodt-Saaydeh S., Graf B., Hommel B., Jensen J. E., Kiss J., Kudsk P., Lamichhane J. R., Messéan A., Moonen A. C., Ratnadass A., Ricci P., Sarah J. L. and Sattin M., 2015 – Eight principles of integrated pest management, *Agronomy for Sustainable Development*, 35, 4, 1199-1215, DOI: 10.1007/s13593-015-0327-9.
3. Beketov M. A., Kefford B. J., Schäfer R. B. and Liess M., 2013 – Pesticides reduce regional biodiversity of stream invertebrates, *Proceedings of the National Academy of Sciences*, USA, 110, 27, 11039-11043, DOI: 10.1073/pnas.1305618110.
4. Brogan A. P., Hogue S. L., Vekaria R. M., Reynolds I. and Coukell A., 2019 – Understanding payer perspectives on value in the use of pharmaceuticals in the United States, *Journal of Managed Care & Specialty Pharmacy*, 25, 12, 1319-1327, DOI: 10.18553/jmcp.2019.25.12.1319.
5. Curtean-Bănăduc A., Irimia-Hurdugan O. and Bănăduc D., 2017 – Management of the persistent organic pollutants – strategies, programs, regulations and actions, chapter 5, in Transfer of knowledge in the field of persistent organic pollutant – Good Practices – Curtean-Bănăduc A. (ed.), ISBN 978-606-12-1441-6, 77-89.
6. Destro A. L. F., Silva S. B., Gregório K. P., de Oliveira J. M., Lozi,A. A., Zuanon J. A. S., Salaro A. L., da Matta S. L., Gonçalves R. V. and Freitas M. B., 2021 – Effects of subchronic exposure to environmentally relevant concentrations of the herbicide atrazine in the Neotropical fish Astyanax altiparanae, *Ecotoxicology and Environmental Safety*, 208, 111601, DOI: 10.1016/j.ecoenv.2020.111601.
7. Gibbons D., Morrissey C. and Mineau P., 2015 – A review of the direct and indirect effects of neonicotinoids and fipronil on vertebrate wildlife, *Environmental Science and Pollutant Research*, 22, 103-118, DOI: 10.1007/s11356-014-3180-5.
8. Gilliom R. J., 2007 – Pesticides in U.S. streams and groundwater, *Environmental Science and Technology*, 41, 10, 3408-14, DOI: 10.1021/es072531u.
9. Hayes T. B., Collins A., Lee M., Mendoza M., Noriega N., Stuart A. A., Vonk A., 2002 – Hermaphroditic, demasculinized frogs after exposure to the herbicide atrazine at low ecologically relevant doses, *The Proceedings of the National Academy of Sciences*, 99, 8, 5476-5480, DOI: 10.1073/pnas.082121499.
10. Kaur R., Mavi G. K., Raghav S. and Khan I., 2019 – Pesticides classification and its impact on environment, *International Journal of Current Microbiology and Applied Sciences*, 8, 3, 1889-1897.
11. Khoshnood Z., Jamili S., Khodabandeh S., Mashinchian Moradi A., Motallebi Moghanjoghi A., 2014 – Histopathological effects and toxicity of atrazine herbicide in Caspian Kutum, Rutilus frisii kutum fry, *Iranian Journal of Fisheries Sciences*, 13, 3, 702-718, DOI: jifro.ir/article-1-1728-en.html.
12. Khoshnood Z., Jamili S. and Khodabandeh S., 2015 – Histopathological effects of atrazine on gills of Caspian kutum Rutilus frisii kutum fingerlings, *Diseases of Aquatic Organisms*, 113, 3, 227-34, DOI: 10.3354/dao02850.
13. Khoshnood Z., 2015 – Histopathological alterations in the kidney of Caspian kutum, Rutilus frisii kutum, larvae and fingerlings exposed to sublethal concentration of atrazine, *Bulletin of Environmental Contamination and Toxicology*, 94, 2, 158-63, DOI: 10.1007/s00128-014-1431-2.

14. Khoshnood Zahra., 2017 – Effects of Environmental Pollution on Fish: A Short Review, *Transylvanian Review of Systematical and Ecological Research*, 19, DOI: 10.1515/trser-2017-0005.
15. Liess M. and Von Der Ohe P. C., 2005 – Analyzing effects of pesticides on invertebrate communities in streams, *Environmental Toxicology and Chemistry*, 24, 4, 954-65, DOI: 10.1897/03-652.1
16. Munn M. D. and Gilliom R. J. 2001 – Pesticide toxicity index for freshwater aquatic organisms, U.S. Geological Survey, *Water-Resources Investigations Report*, 01-4077.
17. Relyea R.A., 2005 – The lethal impact of Roundup on aquatic and terrestrial amphibians, *Ecological Applications*, 15, 4, 1118-1124, DOI: 10.1890/04-1291.
18. Riaz A., Majeed M., Riaz M. A., Iqbal A. and Usman Ali Ashfaq U. A., 2022 – Histopathological alteration in organs of adult male frog after exposure to alpha-cypermethrin, *Pure and Applied Biology*, 11, 1, 175-180.
19. Sanchez-Bayo F. and Goka K., 2006 – Ecological effects of the insecticide imidacloprid and a pollutant from antidandruff shampoo in experimental rice fields, *Environmental Toxicology and Chemistry*, 25, 6, 1677-87, DOI: 10.1897/05-404r.1.
20. Silva V., Mol H. G. J., Zomer P., Tienstra M., Ritsema C. J. and Geissen V., 2019 – Pesticide residues in European agricultural soils – A hidden reality unfolded, *Science of the Total Environment*, 653, 1532-1545, DOI: 10.1016/j.scitotenv.2018.10.441.
21. Solomon K. R., Carr J. A., Du Preez L. H., Giesy J. P., Kendall R. J., Smith E. E. and Van Der Kraak G. J., 2008 – Effects of atrazine on fish, amphibians, and aquatic reptiles: a critical review, *Critical Reviews in Toxicology*, 38, 9, 721-72, DOI: 10.1080/10408440802116496.
22. Stehle S. and Schulz R., 2015 – Agricultural insecticides threaten surface waters at the global scale, *Proceedings of the National Academy of Sciences*, 112, 5750-5755, DOI: 10.1073/pnas.1500232112.
23. USEPA (US Environmental Protection Agency), 2002 – Reregistration eligibility science chapter for atrazine environmental fate and effects chapter, Washington, D. C.
24. Waring C. P. and Moore A., 2004 – The effect of atrazine on Atlantic Salmon (Salmo salar) smolts in freshwater and after sea water transfer, *Aquatic Toxicology*, 66, 93-104, DOI: 10.1016/j.aquatox.2003.09.001.
25. Zaller J. G. and Brühl C. A., 2019 – Editorial: Non-target effects of pesticides on organisms inhabiting agroecosystems, *Frontiers in Environmental Science*, 7, 1-3.

ASSESSMENT OF THE EFFICIENCY OF 5TH (20 KM) HILSA SHAD (*TENUALOSA ILISHA*) SANCTUARY OF THE PADMA RIVER (BANGLADESH) FOR SUSTAINABLE POLICY FORMULATION AND CONSERVATION MANAGEMENT

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KEYWORDS: Hilsa, sanctuary, *Tenualosa ilisha*, Padma River, Bangladesh.

ABSTRACT

The studied area is the single largest fishery in Bangladesh. The present work was conducted at two points of the Padma River (Sureshwar and Tarabunia) from July 2019 to June 2021 to monitor the efficiency of the 5th Hilsa sanctuary. Proportion of spent rate, length frequency, larvae and Jatka abundance, CPUE of larvae, the length-weight relationship of Jatka, plankton composition, and physico-chemical parameters of water were assessed and found adequate in the Hilsa sanctuary.

RÉSUMÉ: Évaluation de l’efficacité du 5ème sanctuaire (20 km) d’alose hilsa (*Tenualosa ilisha*) de la rivière Padma, Bangladesh pour la formulation de politiques durables et la gestion de la conservation.

La zone êtudiée est la plus grande pêcherie du Bangladesh. Les présents travaux ont été menés en deux points de la rivière Padma (Sureshwar et Tarabunia) de juillet 2019 à juin 2021 pour contrôler l’efficacité du 5ème sanctuaire de Hilsa. Le pourcentage de taux d’épuisement, la fréquence de longueur, l’abondance des larves et du Jatka, la CPUE des larves, la relation longueur-poids du Jatka, la composition du plancton et les paramètres physico-chimiques de l’eau ont été évalués et jugés satisfaisants dans le sanctuaire de Hilsa.

REZUMAT: Evaluarea eficacității celui de-l cincilea (20 km) sanctuar pentru *Tenualosa ilisha* de pe râul Padma (Bangladesh) pentru formularea politicilor durabile și managementului conservativ.

Zona studiată este cea mai mare zonă de pescuit din Bangladesh. Studiul de față a fost efectuat în două puncte ale râului Padma (Sureshwar și Tarabunia) din iulie 2019 până în iunie 2021 pentru a monitoriza eficiența celui de-al 5-lea sanctuar pentru Hilsa. Procentul de consum, frecvența lungimii, abundența larvelor și Jatka, CPUE a larvelor, relația lungime-greutate a Jatka, compoziția planctonului și parametrii fizico-chimici ai apei au fost evaluate și considerate satisfăcătoare în sanctuarul Hilsa.

INTRODUCTION

Human activities have had a significant negative impact on aquatic ecosystems, jeopardizing their fragile balance and biodiversity (Rasiga et al., 1999; Onciu and Radu, 2006; Paltenea et al., 2008; Gumpinger and Scheder, 2008; Soolutayo, 2012; Kulkarni Balasaheb et al., 2017; Adom, 2018; Tsybulskiy et al, 2023). Pollution from a variety of sources, including industrial discharge, agricultural runoff, and urban trash, introduces hazardous compounds into bodies of water, including heavy metals, pesticides, and plastics (Zubcov et al., 2008; Astratinei and Varduca, 2008; Iepure and Selescu, 2009; Bashir, 2020; Öktener and Bănăduc, 2023). All these contaminants disturb aquatic food chains, injure aquatic animals, and reduce water quality, resulting in eutrophication, hypoxia, and algal blooms (Clarkson, 1995; Smith et al., 1999; González et al., 2021). Overfishing, which is driven by commercial demands and unsustainable practices, depletes fish populations while disrupting the structure and function of marine ecosystems (Pauly et al., 2002; Bardey, 2020). Habitat loss due to dredging, coastal development, deforestation, etc., exacerbates the reduction of aquatic biodiversity (Halpern et al., 2008). The cumulative consequences of these human-induced stresses endanger the health and resilience of aquatic ecosystems, compromising the supply of critical ecological services on which human societies rely (MEA, 2005; Bănăduc et al., 2022; Boeraș et al., 2024). It is vital to develop holistic management approaches and enact strong rules to prevent these harmful impacts and support the sustainable stewardship of aquatic resources for present and future generations.

Rivers and stream ecosystems are critical for biodiversity and delivering essential ecosystem services (Constanza et al., 2011; Alan Yeakley et al., 2016; Vári et al., 2022). Conservation activities focused at safeguarding these freshwater habitats are critical for preserving their ecological integrity and ensuring sustainable water resource management. According to Waters (1996), good river ecosystems not only support various aquatic species, but also help to purify water, regulate floods, and move sediment. Furthermore, the preservation of riparian vegetation along riverbanks is critical for stream bank stabilization, erosion prevention, and riparian terrestrial wildlife habitat (Naiman et al., 1993; Curtean-Bănăduc et al., 2014). Effective conservation techniques, such as habitat restoration, pollution management, and sustainable water use practices, are critical for maintaining the health and resilience of river and stream ecosystems (Vörösmarty et al., 2010). By following these steps, we can protect rivers and streams' biodiversity and ecological functioning for short and long term.

The Padma River is a natural, free-flowing river. This river is one of the major rivers of Bangladesh., located in the north-western part of this country. It is 120 km long and about four to six km wide. The shape of the river has changed significantly over the last 30-35 years. A satellite image of a river change at a NASA site (NASA Earth Observatory) shows that the river changed from a relatively thin straight line to a braided shape. Recently, it has become straight up again. It is formed at the confluence of the Ganga and Jamuna rivers in India. It later merged with the Meghna River in Bangladesh and ended in the Bay of Bengal. Thousands of people besides farmers and fishermen depend on this river. The common perception of general people is that the character of the Padma River is aggressive in terms of its impact on the riparian communities. Due to the wild nature of the river, countless farms, property, and homes have been destroyed and countless lives lost in recent decades, a high number of people have been displaced in other safer areas. This Padma River is famous too for its Hilsa Shad (or Rupali Hilsa), with a highly economic and ecologic importance (Gragnani and Michele 2021).

Following the formulation of The Hilsa Fisheries Management Action Plan (HFMAP) by Bangladesh Fisheries Research Institute (BFRI), four sanctuaries and four breeding grounds were established in 2002-2003. Subsequently, two new nursery grounds (5th and 6th) were implemented by the Department of Fisheries under the supervision of BFRI.

The survey was conducted from BFRI, Riverine Station, Chandpur to assess the previously identified Fifth Hilsa Nursery Ground. That is 20 km wide from the confluence of Meghna, Padma, and Dakatia River to the Sureshwar area. The physico-chemical parameters of water, plankton observations, Jatka CPUE, length-frequency percentage, Jatka length-weight relationship have been studied; juvenile Hilsa is locally called Jatka.

After analysing the collected data from the study area (for two consecutive years), the previously identified nursery grounds were re-evaluated.

This study will serve as crucial documentation to formulate policy for the sustainable conservation of Hilsa fishery resources in the Padma River through sustainable management adoption.

MATERIAL AND METHODS

Study sites and duration

Two sites were selected for conducting the study: a) Tarabunia and b) Sureswar. The study was conducted from July 2019 to June 2021 (Figs. 1-3).

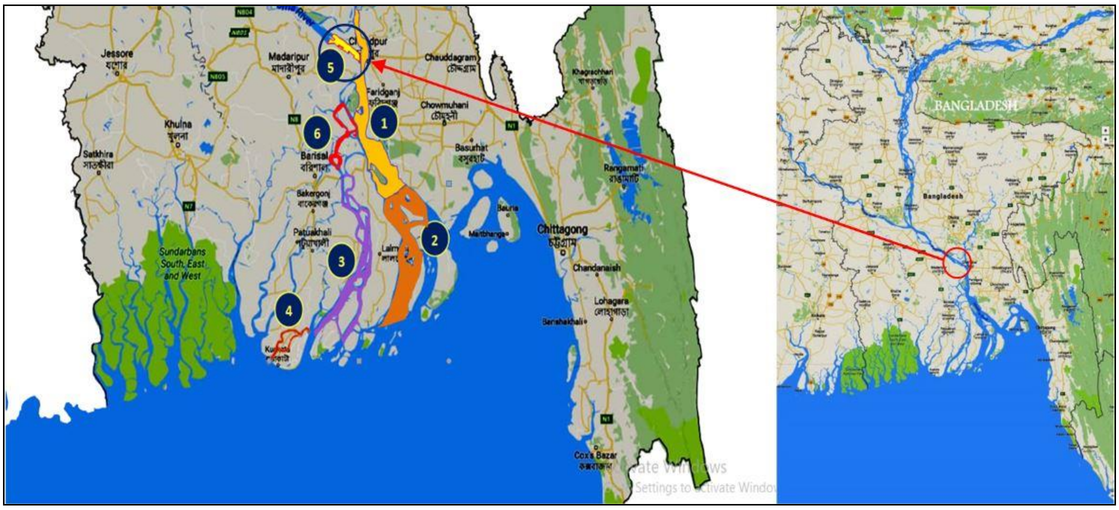

Figure 1: Nursey Grounds
(5th Hilsa Sanctuary, 20 km)
of Padma River.

Figure 2: The studied area detail.

Figure 3: Field work in the study area.

A) Physico-chemical parameters of water

Chemical parameters such as DO, CO_2, pH, Total Alkalinity, Total Hardness, TDS, and Conductivity, as well as physical factors such as air temperature, water temperature and transparency, were monitored directly in each sampling station. HACH (Model FF2, USA) water test kit was used to determine both physical and chemical characteristics.

B) Plankton

During the study, plankton samples were collected from the sampling station via 50-micron plankton net following the standard drop count method. Considering the qualitative and quantitative aspects, one ml concentrated plankton samples were placed in Sedgwick-Rafter counting cells and observed under an electronic microscope (Amscope binocular biological microscope). Ward and Whipple (1959) and Prescott (1962) were used to identifying plankton.

C) Jatka observation

i) CPUE of Jatka: CPUE of Jatka Hilsa were collected by BFRI Experimental Net from the sanctuary and estimated by calculating the amount of Jatka (kg per 100 m net per hour). All the Jatka Hilsa were treated in accordance with the the BFRI rules ethics committee.

ii) Length frequency and percentage: Length frequency data were collected from sampling station. The total length of Hilsa larvae and Jatka was measured by measuring scale and total body weight was taken with an electric digital balance for each fish after the specimens were dried on blotting paper.

iii) Length-weight relationship of Jatka: The relationship between the total length (TL) and total body weight (BW) of fish was estimated by using the following parabolic equation: $BW = aTL^b$; where TW = Body weight of fish in (g); TL = Total length of fish in (cm); a = Proportionality constant (intercept); b = An exponent indicating isometric growth when equal to 3, regression coefficient (slope). The association degree between TL and BW was calculated by the determination coefficient (r^2). Value of theexponent ‘b’ provides information on fish growth. When $b = 3$, the increase in weight is isometric, otherwise it is allometric (positive allometric if $b > 3$, negative allometric if $b < 3$).

Data analysis

Data was analysed using Microsoft Excel 2010, with representations in pie charts and line diagrams. Additionally, calculations for the length-weight relationship were conducted.

RESULTS AND DISCUSSION

A) Physico-chemical parameters of water. Much work has already been done in Bangladesh to check the river-based physico-chemical parameters. However, relatively little work has been done on the big rivers like Padma, Meghna, and Jamuna. Even less noticeable in the case of the river Padma.

The air and water temperatures varied at both Padma sampling sites, being lower from December to January. Sureswar exhibited higher levels of dissolved oxygen (DO), pH, and transparency compared to the Tarabunia site. Free carbon dioxide showed minor fluctuations in both locations throughout the year. Total alkalinity and total hardness were nearly identical in both sites (Fig. 4).

B) Plankton identification and composition. The present study revealed that among the 13 phytoplanktonic groups studied, Bacillariophyceae was found to be highest (21%) followed by Cyanophyceae (18%), Chlorophyceae (16%), and Zygnematophyceae (11%), Dinophyceae (7%), Euglenophyceae and Coscinodiscophyceae (5%), Trebouxiophyceae and Mediohyceae (4%), Xanthophyceae, Ulvophyceae and Chrysophyceae (2%). Among the four zooplanktonic groups studied, Rotifera shared the highest percentage (37%) followed by Copepoda (36%) and Cladocera (27%) (Tabs. 1 and 2) (Figs. 5-7).

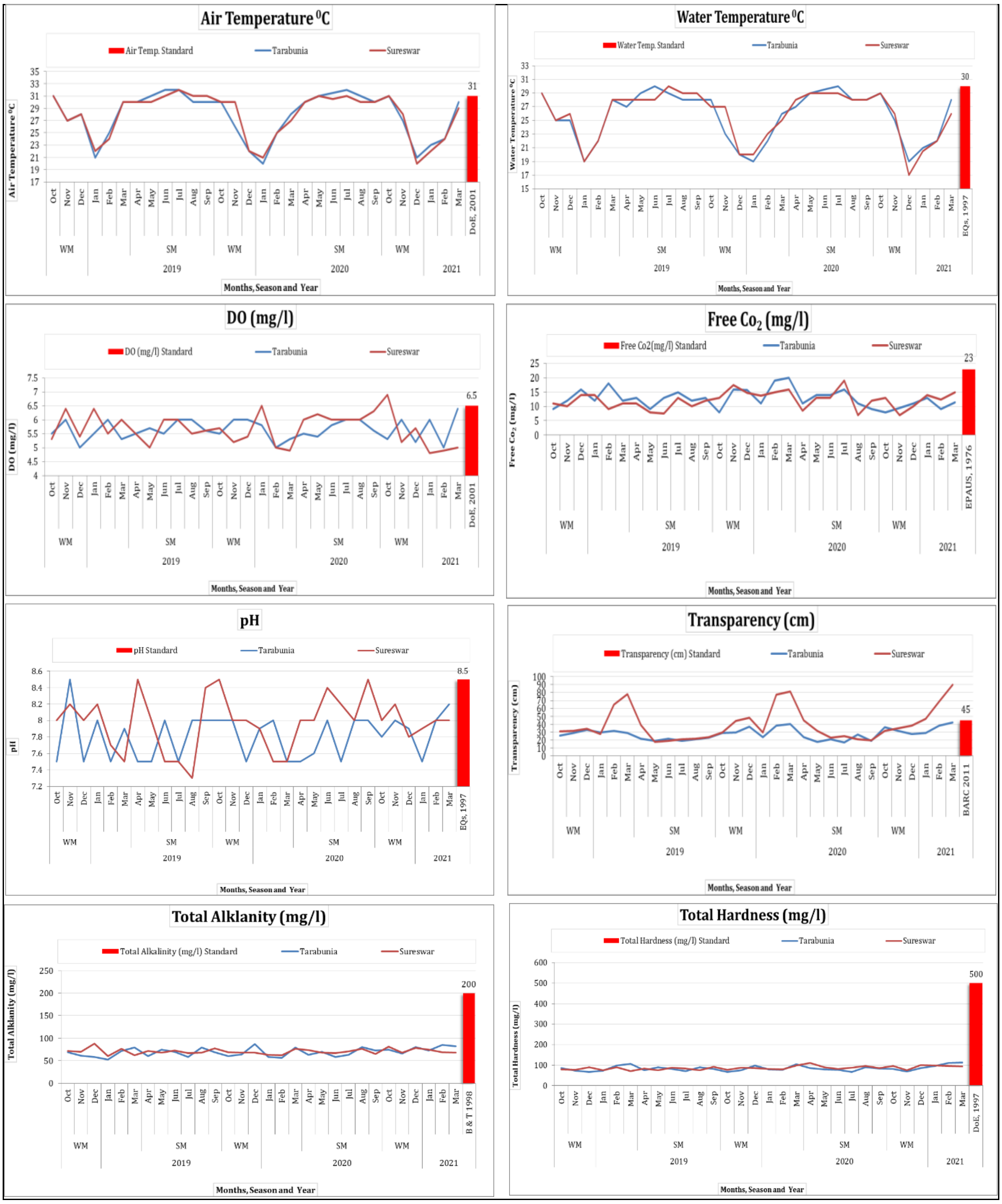

Figure 4: Average water quality parameters of Padma River (2018 to 2021). Eight Parameters (Air Temperature, Water Temperature, Dissolved Oxygen, Carbon Dioxide, pH, Transparency, Total Alkalinity, and Total Hardness) are showing the changes (location and season-wise). Bangladesh standard is highlighted with red bar and data labels. WM = Winter Monsoon, SM = Summer Monsoon (Banglapedia, 2022; National Geographic Society; Eurekalert Organization, 2002).

Table 1: Phytoplankton of Padma River.

Serial number	Phytoplankton group	Species name
1.	Bacillariophyceae	*Amphora* sp., *Asterionella* sp., *Bacillaria* sp., *Cyclotella* sp., *Diatoma* sp., *Fragillaria* sp., *Gomphonema* sp., *Navicula* sp., *Nitzschia* sp., *Striatella* sp., *Synedra* sp., *Tabellaria* sp.
2.	Cyanophyceae	*Anabaena* sp., *Aphanocapsa* sp., *Arthrospira* sp., *Gomphosphaeria* sp., *Merismopedia* sp., *Microcystis* sp., *Nostoc* sp., *Oscillatoria* sp., *Polycystis* sp.
3.	Chlorophyceae	*Ankistrodesmus* sp., *Coelastrum* sp., *Coelastrum* sp., *Microspora* sp., *Pediastrum* sp., *Scenedesmus* sp., *Selenestrum* sp., *Tetraedron* sp., *Volvox* sp.
4.	Chrysophyceae	*Uroglena* sp.
5.	Conjugatophyceae	*Spirogyra* sp., *Staurastrum* sp.
6.	Coscinodiscophyceae	*Coscinodiscus* sp., *Melosira* sp., *Triceratium* sp.
7.	Dinophyceae	*Ceratium* sp., *Dinophysis* sp., *Peridinium* sp., *Prorocentrum* sp.
8.	Euglenophyceae	*Phacus sp.*, *Euglena* sp., *Trachelomonas* sp.
9.	Mediophyceae	*Skeletonema* sp., *Thalassiosira* sp.
10.	Trebouxiophyceae	*Actinastrum* sp., *Oocystis* sp.
11.	Ulvophyceae	*Ulothrix* sp.
12.	Xanthophyceae	*Tribonema* sp.
13.	Zygnematophyceae	*Closterium* sp., *Cosmarium* sp., *Muogeotia* sp., *Spirogyra* sp., *Staurastrum* sp., *Zygnema* sp.

Table 2: Zooplankton of Padma River.

Serial number	Zooplankton group	Species name
1.	Rotifera	*Asplanchna* sp., *Brachionus* sp., *Filinia* sp., *Keratella* sp.
2.	Cladocera	*Bosmina* sp., *Daphnia* sp., *Moina* sp.
3.	Copepoda	*Cyclops* sp., *Diaptomus* sp., *Naupleus* sp.,*Mesocyclops* sp.

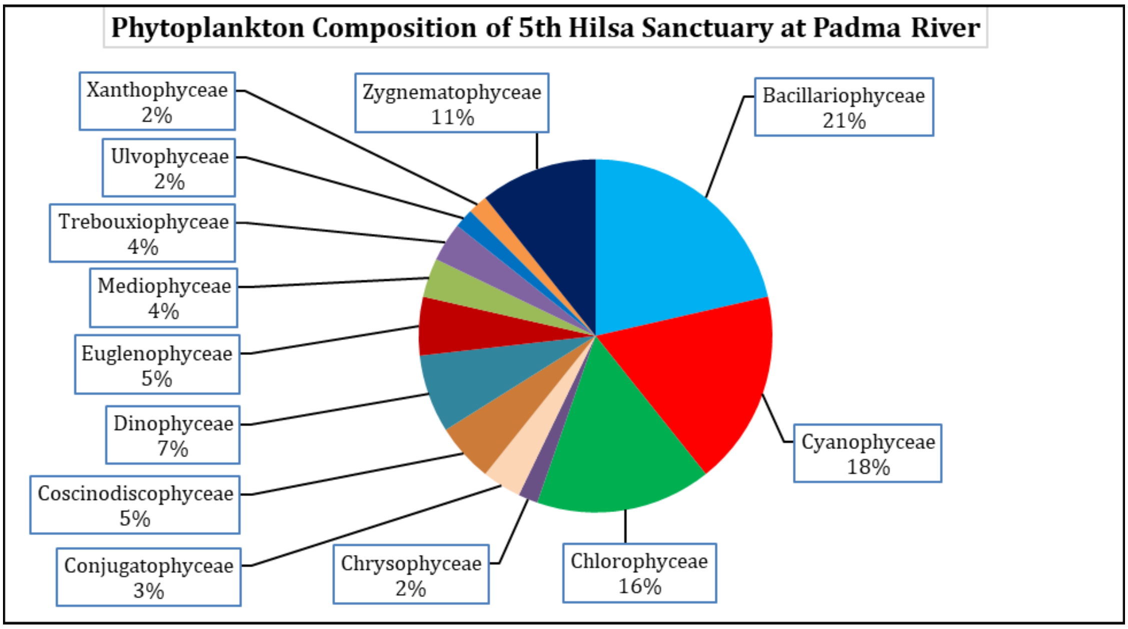

Figure 5.

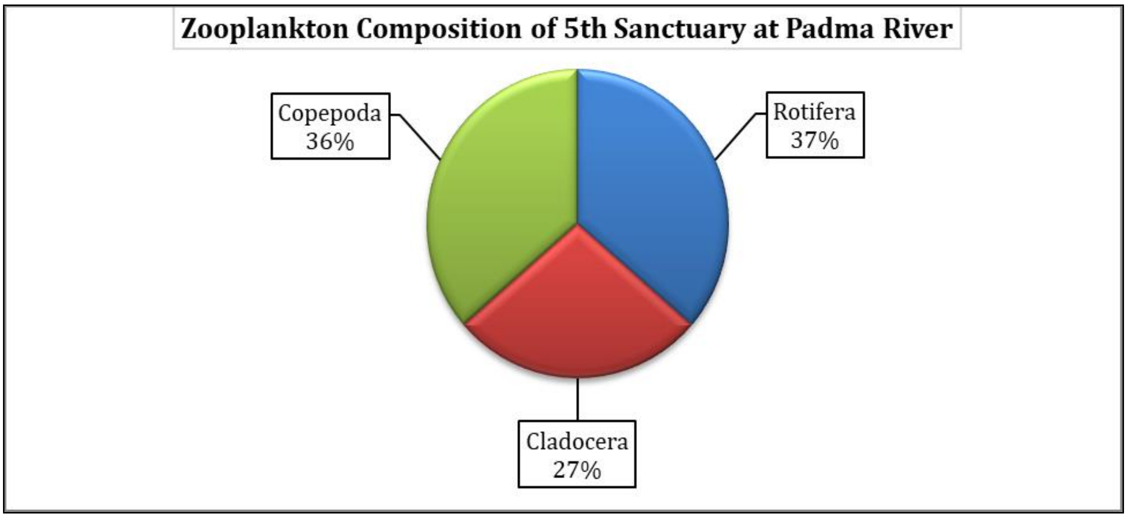

Figure 6.

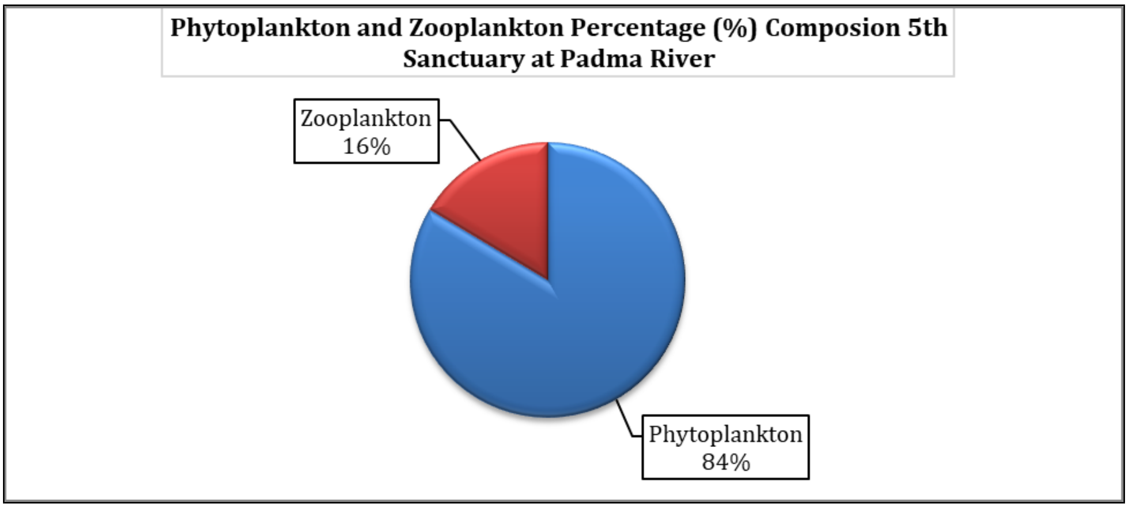

Figure 7.

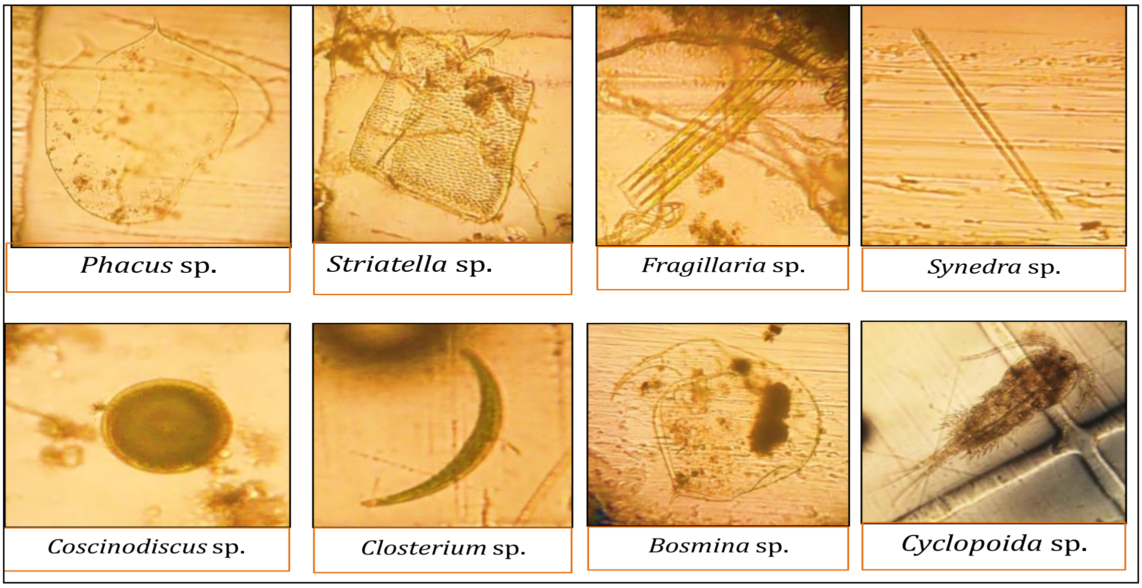

Figure 8: Pictorial view of identified Zooplankton and Phytoplankton species from 5th (20 km) Hilsa Sanctuary of Lower Padma River.

C) Jatka Observation

i) CPUE and change percentage of Jatka (2019-2020-2021)

Jatka's CPUE (kg/hour/100 m net) at the 5th Sanctuary is set to be 4.2 kg in 2019, 4.5 kg in 2020, and 4.8 kg in 2021. Jatka's CPUE has gradually increased. Compared to 2019, the CPUE of Jatka in Padma River has increased by 6.14 percent in 2020 and 11.9 percent in 2021 (Fig. 9).

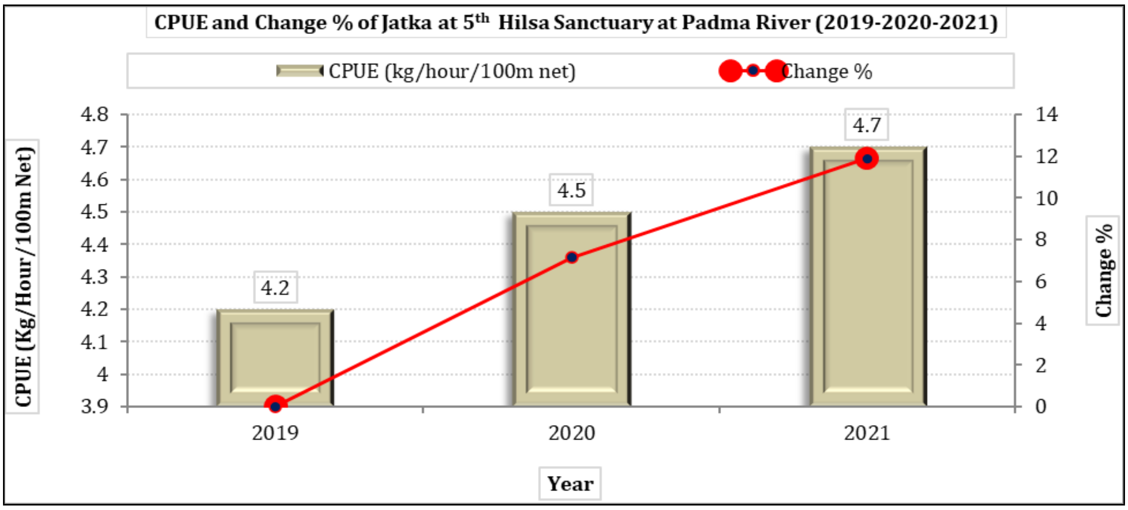

Figure 9.

ii) Length frequency percentage (%) (2019-2021)

Jatka ranged from 11 to 22 cm length sizes were found 5th Hilsa Sanctuary in 2019. In the Tarabunia Region in 2019, and 2020 numbers 214, 212 Jatka Hilsa's length frequencies have been made, respectively. Jatka, 13 cm in length size was found to be the most common size (37%) followed by 14 cm (33%), 15 cm (12.6%), and 12 cm (9%) in 2019. Length frequency percentage gradually decreased from 16 to 22 cm (1.4-0.4%). The lowest percentage of Jatka (0.4%) was recorded as 17 cm length sizes.

Jatka ranged from 11 to 18 cm length were found 5th Hilsa Sanctuary in 2020. Jatka, 12 cm in length was found to be the most common size (33%) followed by 14 cm (28%), 13 cm (24%) and 11 cm (8%) in 2020. Length frequency percentage gradually decreased from 15 to 18 cm (3-1%). The lowest percentage of Jatka (1%) was recorded as 17-18 cm length sizes.

In the case of Tarabunia, it was found that in the year 2019, the presence of 12, 13, 14, and 15 cm Jatka was higher and as a percentage, it was 9, 37, 33, and 12.5 cm respectively. In 2020, 12 (33%), 13 (24%), and 14 cm (28%). In these two years 2019-20, it is seen that the presence and abundance of 12 to 15 cm Jatka were more in Tarabunia (Fig. 10).

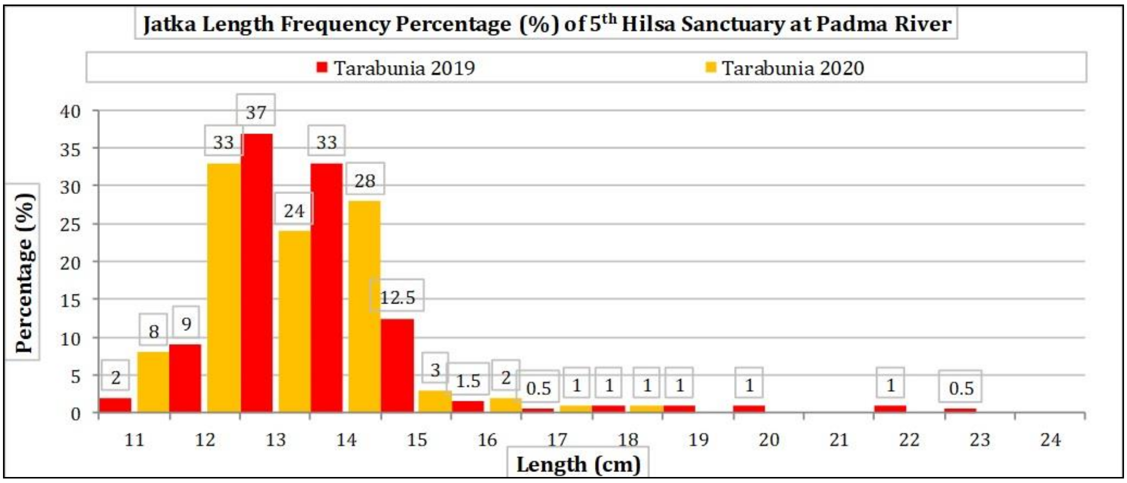

Figure 10.

iii) Length-weight relationship of Jatka

In this study, the length-weight relationship of Jatka in the Hilsa Sanctuary has been established. The maximum length of Jatka was recorded as 23 cm approximately. The value of ‘b’ greater than 3.0 indicates positive allometric growth. In the current study, the value of ‘b’ was calculated as 2.88, which indicates the bad condition of Jatka in the Padma River in 2019 (Fig. 11). The value of ‘b’ was calculated as 3.07, which indicates the good condition of Jatka in the Padma River in 2020 (Fig. 12). In the current study, the value of ‘b’ was calculated as 2.83, which indicates the bad condition of Jatka in the Padma River in 2021 (Fig. 13).

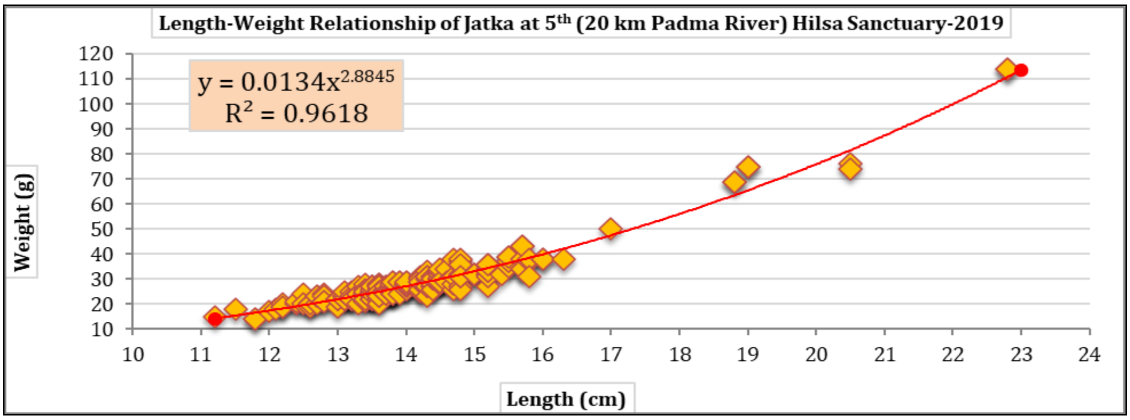

Figure 11.

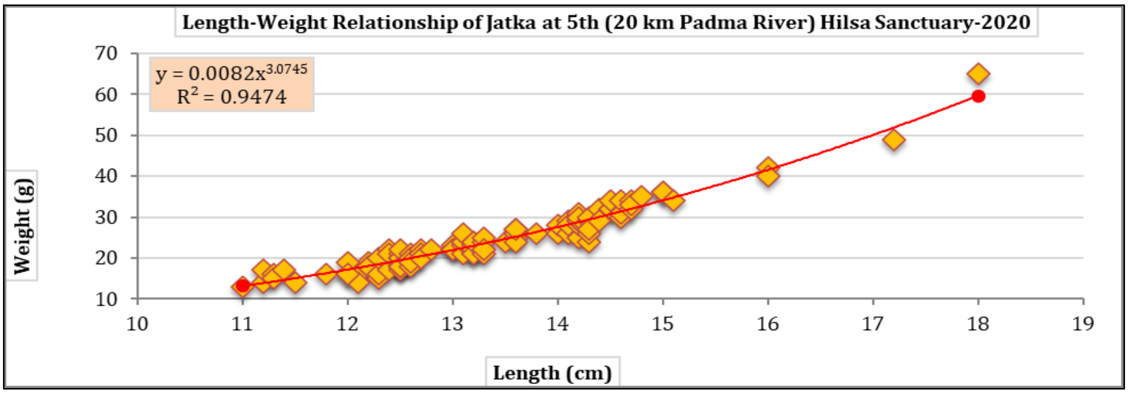

Figure 12.

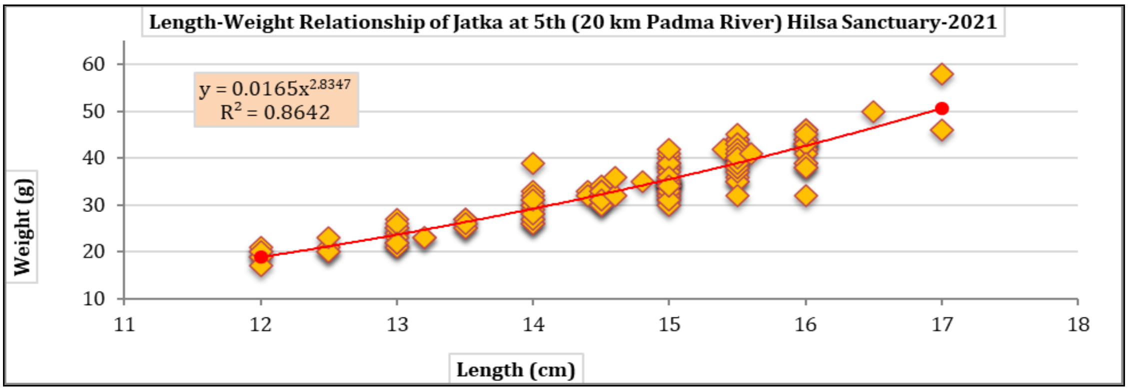

Figure 13.

The physico-chemical properties of the water studied in the Padma River were more or less the same by the standards of Bangladesh. According to DoE (2001) and EQS (1997), air and water temperatures at all sites were within the standard range. DoE standards in all site areas were within the allowed limits (DoE, 2001). CO_2 values were recorded lower than the standard (EQS, 1997). Transparency, hardness, and alkalinity were also within reasonable limits, including the pH value (Rahman, 1992; DoE, 1997; EQS, 1997). The recorded values of various physical and chemical parameters of the Padma River were within the standard values of the rivers in Bangladesh. This is conducive to the normal physiological life of river aquatic life. According to Rahman (2018), in 2015 (September to December), the air and water temperatures in the Sureshwar area of Meghna River were 22.1-32°C and 21.5-31.5°C, the pH and dissolved oxygen ranges were 7.3-7.7 and 5.2-5.8 mg/l, CO_2 was 17.4-19.6 mg/l and total hardness and alkalinity ranges were 58-75 mg/l and 72.5-89.3 mg/l respectively.

At the same time, air and water temperatures in the Tarabunia area of the Meghna River were 22-32.5°C and 21.7-31.2°C, the pH and dissolved oxygen ranges were 7.4-7.8 and 5.1-5.9 mg/l, CO_2 was 17.3-18.4 mg/l and total hardness and alkalinity ranges were 60-80 mg/land 74-84 mg/l respectively. The values of all the parameters mentioned in the above reference match the results obtained in this study. In the case of temperature, one-and-a-half limits were exceeded, but all other standards were within the approved standard of Bangladesh River water as directed by (EQS, 1991; EQS, 1997; ESR ,1997; DoE, 1997; DoE, 2001).

According to (JICA, 1992), during the month of February-May, Chlorophyceae (in the case of phytoplankton) and Brachionidae (in the case of zooplankton) were dominant in the Rajshahi Region of the Padma River. According to (Ahmed and Alfasane, 2004), Munshiganj in the upper reaches of the Padma River was dominant by phytoplankton's group: Chloropyceae (10 species and 38.46%), and Bacillariophyceae (9 species and 34.61%) in 2002. According to (Rahman and Huda, 2012), in the case of phytoplankton in Manikganj of Padma river in 2010-2011, Bacillariophyceae (10 species, average value 3,3421 ind/100 l) was dominant. In the case of zooplankton, Rotifera (10 species, average value 348.6 ind/100 l) was dominant. Flura et al. (2016) has reported that the Mawa, Godagari, and Pakshi areas of the Padma River were dominated by Chlorophyceae (25 and 50%) (Phytoplankton group) and Rotifera (8 and 45%) (Zooplankton group) in 2014. NATP 2 (2018) reported that Munshiganj in the upper reaches of the river Padma was dominated by Chlorophyceae (25) (Phytoplankton group) and Rotifera (8) zooplankton (Zooplankton groups) in 2016. The period from 2002 to 2011 shows that in the upper reaches of the river Padma, in Munshiganj, in Manikganj, Bacillariophyceae is the dominant group for phytoplankton. In the case of zooplankton, Rotifera is singularly dominant. Again, it is seen that in the period from 2014 to 2017, Chlorophyceae is the dominant group in the case of phytoplankton in the upper reaches of the river Padma, in Munshiganj, and Mawa, Godagari, Pakshi region. In the case of zooplankton, the Rotifera alone are dominant. Bacillariophyceae (21%) also dominate in the present study, and in the case of zooplankton, Rotifera (36%) was dominant. The proportion of plankton was phytoplankton 84 and zooplankton 16%.

According to (Rahman et al., 2017), the CPUE of Jatka in the Meghna River from 2002 to 2016 was 0.94 to 3.25 kg respectively. Although it declined slightly in 2006 and 2007, Jatka has steadily increased. The current study shows that in 2019-2021, the CPUE is 4.2-4.7 kg. That means that the environment for Jatka growth in the Padma River is in the best condition.

Not much works has been done about the length frequency percentage rate of Jatka. According to Rahman et al. (2017), 15-20 cm size Jatka was found in the Meghna River from 0.39 to 2.11%, 21-25 cm size Jatka has been found in Meghna River from 1 to 17.27%. In the present study, in 2019, 11 cm size Jatka (2%), 12 cm (9%), 13 cm (36%), 14 cm (33%), 15 cm (12.7%), 18 cm (1.4%) and 22 cm (1%) were found in Padma River, and in 2020, 11 cm size Jatka (8%), 12 cm (33%), 13 cm (24%), 14 cm (28%), 15 cm (3%), 16 cm (2%) and 18 cm (1%) were found in Padma River.

In the case of Jatka, not much has been done about the length-weight relationship. Haldar and Rahman (1998) reported that the value of Jatka (2-18 cm) 'b' in the Chandpur, Bhola, Kuakata-Barisal region was 3.41. Amin et al. (2000) reported that the 'b' value of 365 Jatka Hilsa in the Meghna River (3.50-15.50 cm in length) was 3.09. In the present study, the 'b' value of 205 Jatka Hilsa in 2020 was 3.06 (positive allometric). According to Narejo et al. (2008), the 'b' values of male Hilsa are 3.03 and 3.61 in the length-weight relationship of 10.1-50 cm fish of 260 Jatka Hilsa in Indus River of Pakistan; the value of 'b' of female Hilsa is 3.06 and 3.63 cm in length. The river environment, fish food, breeding, the life cycle can be more or less the quality of the variety. In the present study, all the values of 'b' were allometric positive.

CONCLUSIONS

The survey was conducted from BFRI, Riverine Station, Chandpur to re-evaluate and to monitor the efficiency of the previously identified nursery grounds. Research has been conducted on physico-chemical parameters of water, plankton observation, Jatka CPUE, length-frequency percentage, Jatka length-weight relationship. The results of all the above-mentioned topics were satisfactory; the functioning of the nursery ground remained stable.

It may be mentioned here that after the formulation of the Hilsa Fisheries Management Action Plan by BFRI, four sanctuary and four breeding grounds were set up in the light of necessary recommendations (HFMAP, 2002). Later, two new nursery grounds (5th and 6th) were implemented by the Department of Fisheries under the supervision of BFRI. Therefore, the results of this research project will undoubtedly serve as important evidence and reference for future research, policy, strategy, and implementation for Hilsa fish conservation.

Recommendations: i) Effective research needs to be continued to keep the Jatka and Brood Hilsa conservation management in all Hilsa-friendly rivers of Bangladesh; ii) Proper measures should be taken to protect the fishery resources from river salinity; iii) Timely action should be taken in the context of climate change; iv) The new nursery grounds of Hilsa needs to be identified through further investigation; v) Above all, necessary and timely steps should be taken to maintain the productivity of the nursery ground.

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REFERENCES

1. Adom D., 2018 – The human impact and the aquatic biodiversity of lake Bosomtwe: rennaisance of the cultural traditions of Abono (Ghana)? *Transylvanian Review of Systematical and Ecological Research, 20*, 1, 87-110.
2. Ashfaque A. and Almujaddade A., 2004 – Ecological studies of the river Padma at Mawa Ghat Munshiganj II, Primary productivity, phytoplankton standing crops and diversity, *Pakistan Journal of Biological Sciences*, 7, 1870-1875, DOI: 10.3923/pjbs.2004.1870.1875.
3. Alan Yeakley J., Ervin D., Chang H., Granek E. F., Dujon V., Shandas V. and Brown D., 2016 – Ecosystem services of streams and rivers, *River science: research and management for the 21st century*, 335-352.
4. Amin S. N., Rahman M. A., Haldar G. C., Nahar S., Dewan S. and Mazid M. A., 2000 – Population dynamics of jatka (juvenile hilsa) in the Meghna River, Bangladesh, *Asian Fisheries Science*, 13, 4, 383-390.
5. APHA (American Public Health Association), 1995 – Standard methods for the examination of water and wastewater, 14th Edit, American Public Health Association, 1015 Eighteenth Street, N. W. Washington, D. C., 2036.
6. Millenium Ecosystem Assessment, 2005 – *Ecosystems and human well-being*, 5, 563, Island Press, Washington D. C.
7. Astratinei V. and Varduca I., 2008 – Effect of metal pollution on aquatic microorganisms: a case study in mining areas (Romania), *Transylvanian Review of Systematical and Ecological Research*, 6, 109.
8. Bănăduc D., Simić V., Cianfaglione K., Barinova S., Afanasyev S., Öktener A., McCall G., Simić S. and Curtean-Bănăduc A., 2022 – Freshwater as a sustainable resource and generator of secondary resources in the 21st century: Stressors, threats, risks, management and protection strategies, and conservation approaches, *International Journal of Environmental Research and Public Health*, 19, 24, 16570.
9. Banglapedia, 2022 – Monsoon (Bangla and English), The National Encyclopaedia of Bangladesh, https://bn.banglapedia.org/index.php/.
10. Bardey D. J., 2019 – Overfishing: pressure on our oceans, *Research in Agriculture Livestock and Fisheries*, 6, 3, 397-404.
11. Bashir I., Lone F. A., Bhat R. A., Mir S. A., Dar Z. A., Dar S. A., 2020 – Concerns and threats of contamination on aquatic ecosystems, Bioremediation and biotechnology: sustainable approaches to pollution degradation, 1-26.
12. Boeraș I., Burcea A., Bănăduc D., Florea D. I., Curtean-Bănăduc A., 2024 – Lotic ecosystem sediment microbial communities’ resilience to the impact of wastewater effluents in a polluted European hotspot – Mureș Basin (Transylvania, Romania), *Water*, 16, 3, 402, https://doi.org/10.3390/w16030402.
13. Clarkson T. W., 1995 – Environmental contaminants in the food chain, *The American Journal of Clinical Nutrition*, 61, 3, 682S-686S, DOI: 10.1093/ajcn/61.3.682s.
14. Costanza R., Kubiszewski I., Ervin D., Bluffstone R., Boyd J., Brown D., Chang H., Dujon V., Granek E., Polasky S. and Shandas V., 2011 – Valuing ecological systems and services, *F1000 biology reports*, 3, DOI: 10.3410/B3-14.
15. Curtean-Bănăduc A., Schneider-Binder E. and Bănăduc D., 2014 – The importance of the riverine ligneous vegetation for the Danube Basin lotic ecosystems, in Cianfaglione K. (ed.), L’importanza degli Alberi e del Bosco, Cultura, scienza e coscienza del territorio, Temi Ed., Trento, Italia, ISBN: 978-88-973772-63-9, I-II, 187-210.
16. DoE (Department of Environment), 2001 – The general overview of pollution status of river of Bangladesh, Government of the People’s Republic of Bangladesh, Ministry of Environment and Forest, Department of Environment, Dhaka, Bangladesh.

17. DoE (Department of Environment), 1997 – Environment conservation rules, E. C. R. – schedule 3, standards for water, Department of Environment of Bangladesh, https://www.elaw.org/system/files/Bangladesh, Environmental Conservation Rules.
18. ECR., 1997 – The Environment Conservation Rules, Government of the People's Republic of Bangladesh, Ministry of Environment and Forest, Government of Bangladesh. https://www.elaw. org/ system/files/Bangladesh, Environmental Conservation Rules.
19. EQS (Environmental Quality Standard), 1997 – Government of the People's Republic of Bangladesh, Ministry of Environment and Forest, Department of Environment, Gazette, registered. DA-1, Dhaka, Bangladesh.
20. EQS., 1991 – Environmental quality standards for Bangladesh, Department of Environment, Government of the People's Republic of Bangladesh, 43.
21. Eurekalert Organization., 2002 – Ocean temperatures affect intensity of the South Asian monsoon and rainfall, News Release, 6-Nov-2002.NASA/Goddard Space Flight Center, https://www.eurekalert.org/news-releases/519226.
22. Flura Alam M. A., Hossain M. R. A., Rubel A. K. M. S. A., Tanu M. B. and Khan M. H., 2016 – Assessment of physicochemical conditions and plankton populations of the river Padma, Bangladesh, *Asian Australasian Journal of Bioscience and Biotechnology*, 1, 1, 86-94.
23. González R., Durante C., Arcagni M., Juncos R., Seco Pon J., Crespo E. and Narvarte M., 2021 – Effects of pollution in aquatic food chains, *Anthropogenic Pollution of Aquatic Ecosystems*, 61-89, https://doi.org/10.1007/978-3-030-75602-4_4.
24. Gragnani M. and Evangelista M., 2021 – Introduction to Geographical Indications (GIs) and the benefits of the Lisbon System, Lisbon Webinar Series, https://www.wipo.int/edocs/mdocs/mdocs/en/wipo_webinar_lisbon_2021_1/wipo_webinar_lisbon_2021_1_presentation.pdf.
25. Gumpinger C. and Scheder C., 2008 – Decline of biodiversity as a result of various human impacts related to river regulation-exemplified by several small river catchments (Austria), *Transylvanian Review of Systematical and Ecological Research*, 6, 141.
26. Haldar G. C. and Rahman M. A., 1998 – Ecology of Hilsa, Tenualosa ilisha (Hamilton), in Proceedings of BFRI/ACIAR/CSIRO Workshop on Hilsa Fisheries Research in Bangladesh held on 3-4 March 1998 at Bangladesh Agricultural Research Council, Dhaka, Bangladesh, Mazid M. A. and Blaber S. J. M. (eds), Bangladesh Fisheries Research Institute, BFRI proceedings series no. 6, 11-19.
27. Halpern B. S., Walbridge S., Selkoe K. A., Kappel C. V., Micheli F., d'Agrosa C., Bruno J. F., Casey K. S., Ebert C., Fox H. E. and Fujita R., 2008 – A global map of human impact on marine ecosystems, *Science*, 319, 5865, 948-952.
28. Hilsa Fisheries Management Action Plan for Bangladesh (HFMAP), 2002 – Fourth Fisheries Project, World Bank, Report no. 38.10 September.
29. Iepure S. and Selescu L., 2009 – Relationship between heavy metals and hyporheic invertebrate community structure in the middle basin of the Arieș River (Transylvania, Romania), *Transylvanian Review of Systematical and Ecological Research*, 7, 125-148.
30. JICA, 1992 – Ecology, 14, EPA-2, Draft Final Report, Government of the People's Republic of Bangladesh, Flood Action Plan, https://openjicareport.jica.go.jp/pdf/11044849_04.pdf.
31. Balasaheb K., Atul B., Ashok J. and Rahul K., 2017 – Present status of intertidal biodiversity in and around Mumbai (West Coast of India), *Transylvanian Review of Systematical and Ecological Research*, 19, 1, 61-70.
32. Naiman R. J., Decamps H. and Pollock M., 1993 – The role of riparian corridors in maintaining regional biodiversity, *Ecological applications*, 3, 2, 209-212.
33. Narejo N. T., Lashari P. K. and Jafri S. I. H., 2008 – Morphometric and meristic differences between two types of Palla, Tenualosa ilisha (Hamilton) from river Indus, Pakistan, *Pakistan Journal of Zoology*, 40, 1.

34. NASA earth Observatory, World of Change: Padma River, https://earthobservatory.nasa.gov/world-of-change/PadmaRiver.
35. National Geographic Society, Monsoon, Resource Library, Encyclopedic Entry, https://www.nationalgeographic.org/encyclopedia/monsoon/print/.
36. NATP 2. 2018 – Study of the climate change impact on fisheries resources and fisher`s especially women and children in selected climate hotspot zone of Bangladesh, PIU, National Agricultural Technology Program, Phase II Project (NATP-2), Bangladesh Agricultural Research Council (BARC), New Airport Road, Farmgate, Dhaka, 1215, Bangladesh.
37. Öktener A. and Bănăduc D., 2023 – Ecological interdependence of pollution, fish parasites, and fish in freshwater ecosystems of Turkey, *Water*, 15, 7, 1385, DOI: 10.3390/w15071385.
38. Onciu T. M. and Radu A., 2006 – Retezat Mountains (Romania) glacial lakes zooplankton biodiversity, *Transylvanian Review of Systematical and Ecological Research*, 3, 49.
39. Paltenea E., Viforeanu A., Bulgaru C. and Jecu E., 2008 – Swamps biodiversity of the White Nile (Sudan), *Transylvanian Review of Systematical and Ecological Research*, 6, 81.
40. Pauly D., Christensen V., Guénette S., Pitcher T. J., Sumaila U. R., Walters C. J., Watson R. and Zeller D., 2002 – Towards sustainability in world fisheries, *Nature*, 418, 6898, 689-695.
41. Prescott G. W., 1962 – Algae of the Western Great Lakes area, Revised, Brown W. M. C. (ed.) 135 South Locust Street, Dubuque, Lowa, 977.
42. Rahman M. A. and Huda M. E., 2012 – Study of the seasonal variations in physicochemical and biological aspects of the Padma River at Paturia Ghat, Manikganj, *Jahangirnagar University Environmental Bulletin*, 1, 55-66.
43. Rahman M. S., 1992 – Water Quality Management in Aquaculture, Bangladesh, Brac Prokashona, 84.
44. Rahman M. A., Pramanik M. M. H., Hasan M. M. and Mahmud Y., 2018 – Physicochemical properties and plankton abundance in sanctuary and non sanctuary areas in the river Meghna, Bangladesh, *Asian-Australasian Journal of Food Safety and Security*, 2, 2, 79-85.
45. Rahman M. A., Pramanik M. M. H., Flura, Ahmed T., Hasan M. M., Khan M. H. and Mahmud Y., 2017 – Impact assessment of twenty-two days fishing ban in the major spawning grounds of Tenualosa ilisha (Hamilton, 1822) on its spawning success in Bangladesh, *Journal of Aquaculture Research and Development*, 8, 6, 1-12.
46. Rasiga A., Momeu L., Péterfi Ş. and Kozma A., 1999 – Biodiversity of algae of the upper and middle course of the Olt River, *Transylvanian Review of Systematical and Ecological Research*, 31-48.
47. Smith V. H., Tilman G. D. and Nekola J. C., 1999 – Eutrophication: impacts of excess nutrient inputs on freshwater, marine, and terrestrial ecosystems, *Environmental pollution*, 100, 1-3, 179-196.
48. Soolutayo V., 2012 – Effects of human activities on the biodiversity of a tropical man made lake (Esinminrin Dam), *Transylvanian Review of Systematical and Ecological Research*, 14, 175-186.
49. Tsybulskiy O., Trylis V., Hupalo O. and Savytskiy O., 2023 – Biological diversity of Sula River (Ukraine) under different hydrological conditions, *Transylvanian Review of Systematical and Ecological Research*, 25, 2, 65-76.
50. Vári Á., Podschun S. A., Erős T., Hein T., Pataki B., Iojă I. ., Adamescu C. M., Gerhardt A., Gruber T., Dedić A. and Ćirić M., 2022 – Freshwater systems and ecosystem services: challenges and chances for cross-fertilization of disciplines, Ambio, 51, 1, 135-151, DOI: 10.1007/s13280-021-01556-4.
51. Vörösmarty C. J., McIntyre P. B., Gessner M. O., Dudgeon D., Prusevich A., Green P., Glidden S., Bunn S. E., Sullivan C. A., Liermann C. R. and, Davies P. M., 2010 – Global threats to human water security and river biodiversity, *Nature*, 467, 7315, 555-561.
52. Ward H. B. and Whipple G. C., 1959 – Freshwater Biology, John Willey and Sons. Inc. New York, London, 2, 12-48.

53. Zubcov N., Zubcov E. and Schlenk D., 2008 – The dynamics of metals in fish from Nistru and Prut rivers (Moldova), *Transylvanian Review of Systematical and Ecological Research*, 6, 51.