TRANSYLVANIAN REVIEW OF SYSTEMATICAL AND ECOLOGICAL RESEARCH

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Doru Bănăduc, Robert J. Wolf & Teodora Trichkova

Sibiu – Romania
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IN MEMORIAM

Bjørn Grothaug Andersen
(1924 – 2012)

Bjørn Grothaug Andersen was a Norwegian university teacher of Quaternary geology and glaciology who made opening contributions to glacial geology and the in depth understanding of climate change.

Skiing in the winter and fishing in the summer with his father and brothers in his childhood and teenage periods, adored the mountains in Stavanger region, several times he crossed Trollgaren in Ryfylke, this stunning moraine that is fitting to its name. He questioned how it was shaped, and was said by the farmers of the area that it was truly Trolls that had set up the winding fence of gigantic stones, His interest for this natural phenomenon in addition to many others in turn led to his curiosity and attention for nature in general and Ice Age in special.

In 1951 he married Astrid E. Kruse Andersen which supports his activities all along their common life. Subsequently to a research fellowship at Yale University in 1954-1956, *Andersen* was a teaching quaternary geology at the University of Oslo from 1956 to 1970, then at the University of Bergen for nine years until his retirement. He supervised the geological institutes both in Oslo and Bergen. He was in charge for the geological education of over 30 year groups of students, and he unrelenting participated to the academic world activities until autumn 2011, when he was hit by a cancer and he was in poor health.

All along his activity, his students and colleagues benefited from his expertise and international contacts. He conducted field trips to the Antarctic, South America, New Zeeland, Greenland, etc. *Andersen* kept in contact with some of his students until his death.

Andersen's first scientific journey to the South Pole came after a period of small Norwegian interest in the Antarctic after the great achievements of Roald Amundsen in 1911-1912. *Andersen* was the next Norwegian to visit the Pole after the Amundsen successful mission. An America expedition which reached the Pole a week before *Andersen* revered his realisations by naming a mountain escarpment (Andersen Escarpment) after him.

He accompanied along his activity a party of scientists doing research in Norway, Chille, New Zeeland, Greenland, etc.

Among such trips, in 2005 *Andersen* with a group of scientists went on a research voiage to Greenland to measure the ice coverage in connection to climatic change.

All his research led to a succession of works, among them papers in the respected international scientific journals *Nature* and *Science*. In addition, to two significant books on glacial geology, one about the Ice Age in Norway (Ice age Norway: landscapes formed by ice age glaciers) and an international textbook on the World Ice Age (The Ice Age World: an introduction to Quaternary history and research with emphasis on North America and Northern Europe during the last 2.5 million years) were published. Among numerous valuable scientific papers, Amundsen's first paper is one of the most basic papers in Norwegian glacial geology, even thought it was published before C-14 dating was available.

Bjørn Grothaug Andersen was a dedicated naturalist all his adult life, letting an important scientific legacy in his field of activity.

The Editors

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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.

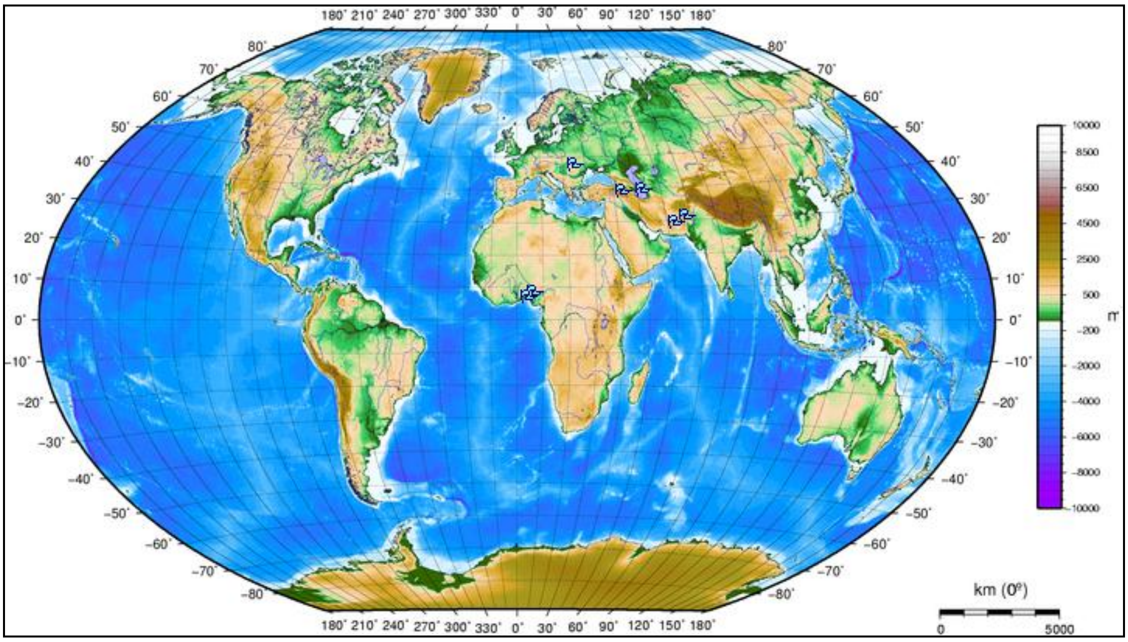
The causes, effects, trends and possibilities of human society to positively intervene to slow down this process or to adapt to it involve a huge variety of aproacess and efforts.

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.

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The Editors

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CORRELATION OF ELEMENT RECORDS IN ARAGONITIC BIVALVE SHELLS OF *CERATODERMA* AS ENVIRONMENTAL PROXIES

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KEYWORDS: mineral, toxicity, aquatic organism, sea, correlation.

ABSTRACT

The correlation of 60 elements recorded in aragonitic bivalve shells of *Ceratoderma* was tested to identify the trends of their availability in coastal areas of the southwest Caspian Sea in order to more efficiently assess them as a proxy of pollution. An inductively coupled plasma mass spectrometer (ICP-MS) (Agilent 8900-Agilent Technologies, Palo Alto, CA, USA) was used for their quantification. A multi-variate cluster test was used to check the similarity of the elements in the clade membership. The results of this study show highly significant positive and negative correlations among the elements in aragonitic bivalve shells of *Ceratoderma* as a bio-indicator part of an aquatic organism. This would be helpful for saving time and cost especially when utilized as environmental proxies.

RÉSUMÉ: Corrélation des enregistrements d'éléments dans les coquilles de bivalves aragonitiques de *Ceratoderma* en tant que proxys environnementaux.

La corrélation d'enregistrements de 60 éléments dans les coquilles de bivalves aragonitiques de *Ceratoderma* a été testée pour trouver la relation et les tendances de leur disponibilité dans les zones côtières du sud-ouest de la mer Caspienne afin de trouver leur relation pour les évaluer plus rapidement comme indicateur de pollution. Un spectromètre de masse à plasma à couplage inductif (ICP-MS) (Agilent 8900-Agilent Technologies, Palo Alto, Californie, États-Unis) a été utilisé pour leur quantification. Un test de cluster multivarié a été utilisé pour vérifier la similarité des éléments appartenant au clade. Les résultats de cette étude montrent des corrélations positives et négatives significatives élevées entre les éléments des coquilles de bivalves aragonitiques de *Ceratoderma* en tant que bio-indicateur d'un organisme aquatique, ce qui serait utile pour économiser du temps et des coûts, en particulier lorsqu'il est utilisé comme proxy environnemental.

REZUMAT: Corelarea înregistrărilor elementelor în scoicile aragonitice ale bivalvelor de *Ceratoderma* ca proxi-uri de mediu.

Corelația înregistrărilor celor 60 de elemente în cochiliile aragonitice ale bivalvelor de *Ceratoderma* a fost testată pentru a găsi relația și tendințele disponibilității lor în zonele de coastă din sud-vestul Mării Caspice, pentru identificarea relației lor pentru a le evalua mai repede ca proxi ale poluării. Pentru cuantificarea lor a fost utilizat un spectrometru de masă cu plasmă cuplat inductiv (ICP-MS) (Agilent 8900-Agilent Technologies, Palo Alto, CA, SUA). Un test cluster multivariat a fost folosit pentru a verifica similitudinea elementelor din componența cladei. Rezultatele acestui studiu arată corelații pozitive și negative semnificative între elementele din cochiliile de bivalve aragonitice de *Ceratoderma* ca parte bio-indicator a unui organism acvatic, ceea ce ar fi util pentru economisirea timpului și a costurilor, mai ales atunci când sunt utilizate ca proxi-uri de mediu.

INTRODUCTION

Bivalves as well as other marine invertebrates can acquire trace elements from the surrounding water and substrate (Ghosn et al., 2020). These species can serve as bioindicators to estimate trace element availability in various locations by accumulating the contaminants in a sedentary manner that does not result in death Khan et al., 2022). All these elements might come from environment through water, substrate, or food (Ghosn et al., 2020). To be utilized as a bioindicator, all species must have the same elemental content as the surrounding marine environment, specifically the water, across all locations and circumstances in the research area (Lomartire et al., 2022). The exploration of element correlation in shells of bivalves has gained increasing attention in recent years (Nour, 2020). Researchers have focused on understanding how different elements interact and how they are incorporated into the shell structure of bivalves, with the goal of elucidating both the environmental conditions and biological processes that influence shell formation (Clark et al., 2020).

Previous studies have shown a wide range of elements existing in different bivalve shells, including calcium, magnesium, strontium, and various trace metals (Piwoni-Piórewicz et al., 2021). The correlation between all these elements can provide insights into the history of a bivalve’s environment, such as temperature variations, salinity levels, and pollution exposure (Zhao et al., 2020). Although factors such as genetics and physiological processes influence certain characteristics of bivalve shells, external environmental factors also affect the composition and structure of the shell (Clark et al., 2020). Furthermore, significant correlations between specific elements and shell characteristics were identifies and observed, shedding light on the biomineralization process in bivalves (Wang et al., 2022; Dai et al., 2020).

This study attempts to look at the correlation and dispersion of certain elements along the coastal area of the southwest Caspian Sea using the aragonitic bivalve shells of *Ceratoderma*.

MATERIAL AND METHODS

Sampling of *Ceratoderma* bivalve shells was done seasonally throughout four seasons at four stations located on the southwest coast of the Caspian Sea (Astara, Anzali, Kiashahr, and Chabaksar) (Fig. 1). The spatial coordinates of each station were precisely determined by GPS in the first sampling and sampling was done from each station with three repetitions.

The collected samples were stored in a plastic bag and taken to the laboratory. After species identification based on the Invertebrate Atlas of the Caspian Sea (Birshtein et al., 1971) the shells were dried, coded, and measured. A digital scale with an accuracy of four decimal places was utilized to weigh the samples and a digital caliper with an accuracy of two decimal places was used to measure their length. Samples with similar age classes were grouped together after washing and were kept in closed Falcon tubes which were washed previously with double distilled water.

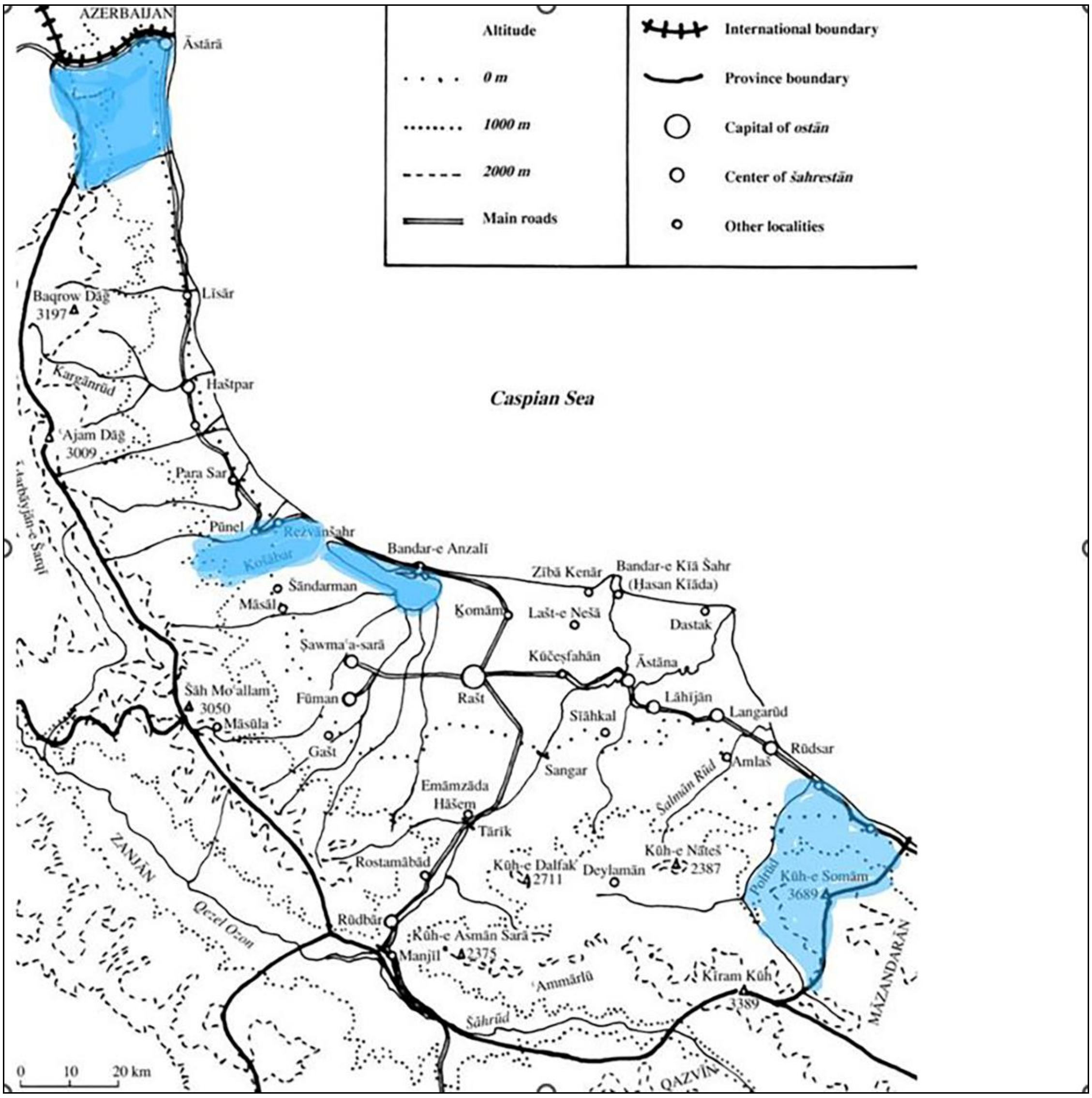

Figure 1: Sampling areas highlighted as blue colour.

The samples obtained from the fin were digested with a mixture of nitric acid (65% HNO_3) and perchloric acid (60% $HClO_4$) (2:1 v/v) in a water bath at 70°C in order to degrade organic matter. Distilled water was added to the solution to make a volume of 50 mL. The following trace elements were determined in these samples: Ag, Al, As, B, Ba, Be, Bi, Ca, Cd, Ce, Co, Cr, Cs, Cu, Dy, Er, Eu, Fe, Ga, Gd, Hf, Hg, Ho, In, Ir, K, La, Li, Lu, Mg, Mn, Mo, Na, Nb, Nd, Ni, Os, P, Pb, Pd, Pr, Pt, Re, Rh, Ru, S, Sb, Sc, Se, Sm, Sn, Rb, Sr, Ta, Tb, Te, Th, Ti, Tl, Tm, U, V, W, Y, Yb, Zn, and Zr. They were quantified using an inductively coupled spectrometer (ICP-MS), supplied by Agilent as one of their 7500 series machine. The operational conditions for the ICP-MS were optimized in accordance with the manufacturer's instructions. The recovery of the analytical methods was verified utilizing the certified reference materials DORM-5 (National Research Council Canada), REE-1 (Natural Resources

Canada), and single-element standards (Merck) in the case of elements not included in the previous two. According to Yang et al., (2016), the limits of detection (LOD) and quantification (LOQ) of each element were calculated through the analyte concentration that corresponded to three and ten times the standard deviation of ten independent blank measurements respectively. The recovery values for the analytes examined ranged between 96% and 103%. The linearity test showed R^2 values above 0.995 for the elements analysed. In the case of rare earth elements, extremely low concentrations were determined. The blanks were similarly processed, and the concentrations were determined with standard solutions prepared in the same matrix.

A boxplot was used to detect outlier data. The data were checked for normality and homogeneity of variance, and data transformation was done if necessary. A correlation test was also used to calculate the correlation and relationship between data. A cluster analysis was performed using the Euclidean distance as the clustering method to determine the possible grouping of trace elements. All statistical analyses were done with SPSS software (Version 26. SPSS Inc., Chicago, IL) and a significance level of 5% was considered. Excel (Version 2016) was used to draw the graph.

Since all animal handling was done under the Iranian and European guidelines on animal welfare, and the waste matter was used for distinctive purposes, no ethical committee approval was necessary.

RESULTS AND DISCUSSION

Utilizing aragonitic bivalve shells of *Ceratoderma* can validate a powerful proxy response to environmental variations. For the objectives of this investigation, we used a constant seasonal growth model. However, variations in growth rates among individuals in different sites and/or seasons may generate an offset in element chemical records. This should be considered while interpreting data (Tab. 1).

Table 1: The length, weight and age range of *Ceratoderma* in the station in different seasons.

Station	Season	Length range (mm)	Weight range (gr)	Age range (year)
Astara	Spring	8.7-24	0.6-2.0	1-4
	Summer	7.5-28.5	0.05-2.6	1-4
	Autumn	0.8-2	0.03-2	1-4
	Winter	0.67-2.6	0.06-2.6	1-4
Anzali	Spring	0.59-2.3	2.7-0.02	1-3
	Summer	7-24	0.05-3.3	1-3
	Autumn	0.59-2.3	0.02-2.7	1-3
	Winter	1.2-2.5	0.2-2.1	1-3
Kiashahr	Spring	16-25	0.2-2.1	1-3
	Summer	12-25	0.2-2	1-3
	Autumn	1-2.2	0.1-1.1	1-3
	Winter	0.83-2.2	0.04-1.7	1-3
Chaboksar	Spring	9-28	0.11-2.1	1-4
	Summer	7.9-25.6	0.05-3	1-4
	Autumn	1-2.3	0.2-2.2	1-4
	Winter	0.47-2.5	0.02-1.4	1-4

Concentrations of the elements found in the shells of *Ceratoderma* are listed highest to lowest based on the mean concentration and the upper and lower quartiles, expressed in µg/kg except Ca with expressed mg/kgin (Tab. 2).

Table 2: The concentrations of the elements determined in the shells of *Ceratoderma*, based on the mean concentration minimum, maximum, and standard deviation.

Element	N	Minimum	Maximum	Mean	Standard deviation
Al (ppb)	44	15.00	8855357.14	3263809.21	3462769.30
Na (ppb)	44	321028.04	4559202.30	1916205.16	1427612.22
Sr (ppb)	44	3071.20	2895438.87	892340.37	951565.85
K (ppb)	44	32646.05	1651788.99	581615.02	591305.65
Mg (ppb)	44	112410.07	988478.45	423808.17	282886.81
S (ppb)	44	78.53	909375.00	318254.63	327867.42
Ca (ppm)	44	228404.85	492417.76	298984.77	73251.40
P (ppb)	44	3916.41	665397.32	244276.31	245956.09
Ti (ppb)	44	0.95	707943.93	164167.09	192783.64
Fe (ppb)	44	23200.00	330172.41	120591.02	69436.37
B (ppb)	44	14048.53	513934.43	94119.96	104085.31
Zn (ppb)	44	1277.14	266174.31	92122.97	95377.01
Sb (ppb)	44	8.78	215691.96	68104.58	74855.32
Ba (ppb)	44	7812.50	97692.31	52521.20	28095.39
Mn (ppb)	44	5468.75	227643.93	39318.36	44655.63
Cr (ppb)	44	4.77	363934.43	27406.14	60945.03
Co (ppb)	44	1.28	351639.34	20443.81	58624.47
Zr (ppb)	44	1.17	22015.18	7603.93	8235.17
As (ppb)	44	6.12	25982.14	7533.08	8460.30
Cu (ppb)	44	4.96	132592.62	6812.09	20645.84
V (ppb)	44	716.96	17142.86	6397.66	5977.95
Li (ppb)	44	854.70	14520.14	3178.43	2530.24
Pb (ppb)	44	96.56	56250.00	3041.75	8329.75
Rb (ppb)	44	4.82	94723.53	2239.43	14267.52
Ni (ppb)	44	5.92	6697.25	1989.73	2310.24
Mo (ppb)	44	28.96	2413.76	825.06	861.77
Hg (ppb)	44	0.66	3750.00	769.42	985.36
Ga (ppb)	44	78.53	3985.62	718.90	1003.06
Y (ppb)	44	7.35	2107.76	620.73	661.93
Ag (ppb)	44	30.41	2208.95	420.85	421.29
Cs (ppb)	44	4.26	3128.46	130.45	464.93
La (ppb)	44	44.72	1361.79	113.54	194.45
U (ppb)	44	14.23	205.07	96.62	41.30
Nd (ppb)	44	6.15	1116.54	87.43	165.04

Table 2 (contunued): The concentrations of the elements determined in the shells of *Ceratoderma*, based on the mean concentration minimum, maximum, and standard deviation.

Element	N	Minimum	Maximum	Mean	Standard deviation
Sc (ppb)	44	10.54	1052.21	87.20	155.35
Se (ppb)	44	12.64	377.95	85.96	71.23
Pd (ppb)	44	9.95	143.68	83.29	39.00
Lu (ppb)	44	20.00	140.19	77.76	32.28
Te (ppb)	44	3.26	140.19	75.09	37.90
Be (ppb)	44	6.00	140.19	74.51	39.22
Th (ppb)	44	1.30	272.64	70.19	55.00
Ta (ppb)	44	1.16	140.19	70.19	42.83
Ce (ppb)	44	7.15	140.19	67.26	43.34
Sn (ppb)	44	6.76	300.24	67.19	58.96
Cd (ppb)	44	10.00	140.19	62.09	45.71
W (ppb)	44	6.00	140.19	60.61	46.58
Pr (ppb)	44	1.14	299.00	60.46	64.02
Sm (ppb)	44	0.91	215.24	60.11	56.41
Tb (ppb)	44	0.63	140.19	59.35	49.06
Yb (ppb)	44	0.71	140.19	59.04	51.04
Tl (ppb)	44	0.95	140.19	58.50	48.97
Gd (ppb)	44	0.50	226.60	57.65	59.49
Bi (ppb)	44	4.00	140.19	57.48	49.96
Dy (ppb)	44	1.37	157.52	56.30	55.50
Tm (ppb)	44	7.03	140.19	56.00	50.79
Os (ppb)	44	7.20	140.19	55.99	50.88
Ru (ppb)	44	3.17	140.19	55.47	51.45
Nb (ppb)	44	6.00	140.19	55.15	51.64
Er (ppb)	44	0.50	140.19	53.99	54.52
Hf (ppb)	44	0.61	140.19	53.54	53.17
Eu (ppb)	44	0.51	140.19	53.37	54.09
Ir (ppb)	44	1.26	140.19	53.25	53.38
Re (ppb)	44	1.51	140.19	53.09	53.54
Ho (ppb)	44	0.53	140.19	52.54	54.26
In (ppb)	44	0.50	140.19	52.09	54.44
Rh (ppb)	44	0.54	140.19	52.06	54.47
Pt (ppb)	44	0.72	140.19	51.95	54.58

The majority of water entering the Caspian Sea flows through the rivers Sefidrood and Anzali Lagon in the southern zone (Ataei et al., 2019). Given their geographical location, they flow through the majority of the south Caspian Sea's urban, agricultural, and industrial sectors, potentially polluting it (Nematollahi et al., 2021). Domestic and industrial activities in developing countries release enormous amounts of harmful pollutants, including trace elements, into waterways (Akhtar et al., 2021). Moreover, trace metals with higher concentrations in these zones, such as Al, Cd, As, and Hg, are frequently found in crude oil (Saleh et al., 2021) which seems to indirectly come with the currents. Furthermore, the discharge of agricultural wastewater is tainted with pesticides or fertilizers, in which trace metals are abundant (Fu et al., 2021). Considering these points, the presence of elements such as As, Cd, and Hg could be attributed to the river's closeness to more developed sectors.

The figure 2 grouped all elements except Al in one major group which was followed by two sub-groups; first sub-group included Na and Sr and the second included all major group elements except Na and Sr. On the clustering analysis, two groups were established. Except for Al which stands separately, the similarity between the groups may be due to natural factors, a common origin, and physicochemical properties of the elements (Bakhshalizadeh et al., 2023).

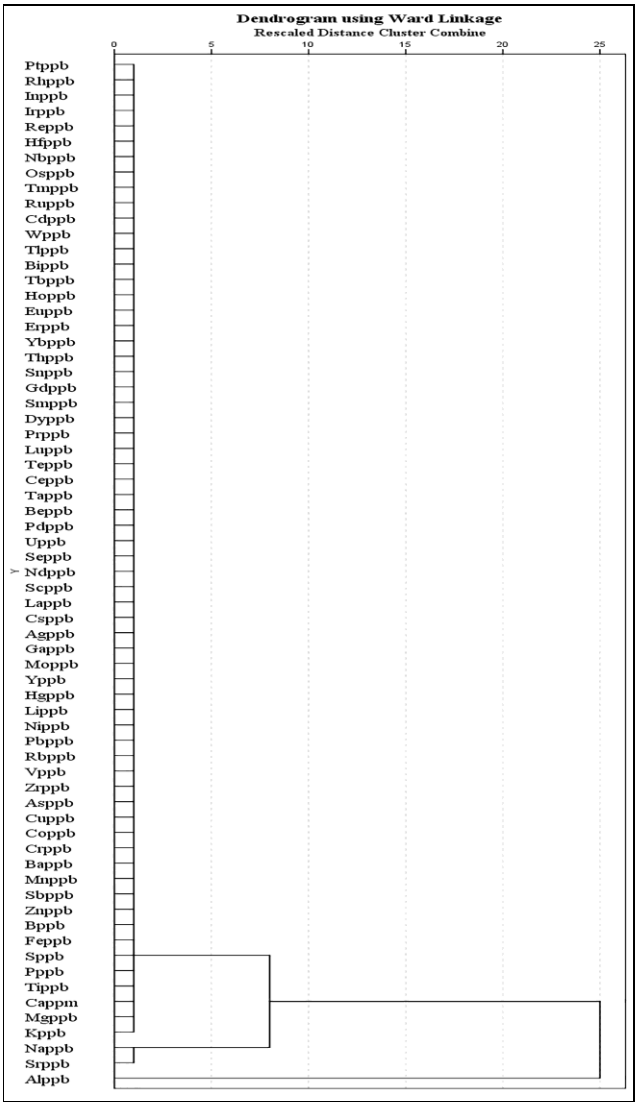

Figure 2: Trace element grouping in shells of *Ceratoderma* based on the Ward linkage.

Finding the correlation among elements in bio-indicator aquatic organisms save time and costs when used as environmental proxies (Lomartire et al., 2021). Here, the correlation was not seen in elements of aragonitic bivalve shells such as B, Ba, Pd, U, Ag, Fe, Co, Cs, Ga, La, Se, although strong positive correlations ($r \geq 0.9$ $n = 44$ $p < 0.05$) were seen among: Ca with Lu and Cd; Gd with Th; Ho with Be, Lu, Ca, Cd and Gd; Li with Ca; Pr with Gd and Th; Sm with Th, Gd, Ho and Pr; Sn with Th, Gd, Sm and Pr; Tb with Lu, Ca, Cd, Ho and Sm; Te with Cd, Ho and Tb; Tl with Be, Lu, Ca, Cd, Gd, Ho, Sm, Tb and Te; Bi with Be, Lu, Ca, Cd, Gd, Ho, Sm, Tb, Tl and Te; Ce with Lu, Ca, Cd, Ho, Tb, Tl and Bi; Cr with Co; Cu with Co and Cr; Dy with Be, Th, Cd, Gd, Ho, Pr, Sm, Sn, Tb, Tl, Bi; Er with Be, Lu, Ca, Cd, Gd, Ho, Pr, Sm, Tb, Tl, Bi, Ce and Dy; Eu with Be, Lu, Ca, Cd, Gd, Ho, Sm, Tb, Tl, Bi, Ce, Er and Dy; Hf with Be, Lu, Ca, Cd, Ho, Tb, Te, Tl, Bi, Ce, Er, Eu and Dy; Hg with Ca, Cd, Ho, Tl, Bi, Eu and Hf; In with Be, Lu, Ca, Cd, Ho, Tb, Te, Tl, Bi, Ce, Er, Eu, Hf, Hg and Dy; Ir with Be, Lu, Ca, Cd, Ho, Tb, Te, Tl, Bi, Ce, Er, Eu, Hf, Hg and Dy; K with Lu, Ca, Cd, Ho, Tb, Te, Tl, Bi, Ce, Er, Eu, Hf, Hg, Ir, In and Dy; Mg with Lu, Ca, Cd, Ho, Tb, Te, Tl, Bi, Ce, Er, Eu, Hf, In, Ir, K and Dy; Mn with Li; Mo with Lu, Cd, Ho, Tb, Te, Tl, Bi, Ce, Er, Eu, Hf, In, Ir, K, Mg and Dy; Na with Cd, Ho, Tb, Tl, Bi, Er, Eu, In, Ir, K, Mg and Mo; Nb with Be, Lu, Ca, Cd, Ho, Tb, Te, Tl, Bi, Ce, Er, Eu, Hf, Hg, In, Ir, K, Mg, Mo and Dy; Nd with Cs and La; Ni with Lu, Cd, Ho, Tb, Tl, Bi, Eu, In, Ir, K, Mo and Nb; Os with Be, Lu, Ca, Cd, Ho, Tb, Te, Tl, Bi, Ce, Er, Eu, Hf, Hg, In, Ir, K, Mg, Mo, Nb, Ni and Dy; P with Lu, Cd, Ho, Tb, Tl, Bi, Ce, Er, Eu, Hf, In, Ir, K, Mg, Mo, Nb, Os and Dy; Pb with Cs, La and Nd; Pt with Be, Lu, Ca, Cd, Ho, Tb, Te, Tl, Bi, Ce, Er, Eu, Hf, In, Ir, K, Mg, Mo, Nb, Ni, P, Os and Dy; Re with Be, Lu, Ca, Cd, Ho, Tb, Te, Tl, Bi, Ce, Er, Eu, Hf, In, Ir, K, Mg, Mo, Nb, Ni, P, Pt, Os and Dy; Rh with Be, Lu, Ca, Cd, Ho, Tb, Te, Tl, Bi, Ce, Er, Eu, Hf, In, Ir, K, Mg, Mo, Nb, Ni, P, Pt, Os, Re and Dy; Ru with Be, Lu, Ca, Cd, Ho, Tb, Te, Tl, Bi, Ce, Er, Eu, Hf, In, Ir, K, Mg, Mo, Nb, Ni, P, Pt, Os, Re, Rh and Dy; S with Na; Sb with Lu, Ca, Cd, Ho, Tb, Tl, Bi, Ce, Er, Eu, Hf, Hg, In, Ir, K, Mg, Mo, Nb, Ni, P, Pt, Os, Re, Rh, Ru and Dy; Sc with Cs, La, Nd and Pb; Rb with Cs, La, Nd, Pb and Sc; Sr with S and Na; Ta with Cd, Ho, Tb, Bi, Eu, Hf, In, Ir, K, Mg, Mo, Nb, Os, Pt, Re, Rh, Ru, Sb and Sr; Ti with Lu, Ca, Cd, Ho, Li, Tb, Tl, Bi, Ce, Dy, Er, Eu, Hf, In, Ir, K, Mg, Mn, Mo, Nb, Ni, P,Pb, Pt, Os, Re, Rh, Ru and Sb; Tm with Be, Lu, Ca, Cd, Ho, Tb, Te, Tl, Bi, Ce, Dy, Er, Eu, Hf, Hg, In, Ir, K, Mg, Mo, Nb, Ni, P, Pt, Os, Re, Rh, Ru and Sb; V with Be, Lu, Ca, Cd, Ho, Tb, Te, Tl, Bi, Ce, Dy, Er, Eu, Hf, Hg, In, Ir, K, Mg, Mo, Nb, Ni, P, Pt, Os, Re, Rh, Ru and Sb; W with Be, Lu, Ca, Cd, Ho, Tb, Te, Tl, Bi, Ce, Dy, Er, Eu, Hf, Hg, In, Ir, K, Mg, Mo, Nb, Ni, P, Pt, Os, Re, Rh, Ru and Sb; Y with Be, Lu, Ca, Cd, Gd, Ho, Sm, Tb, Te, Tl, Bi, Ce, Dy, Er, Eu, Hf, In, Ir, K, Mg, Mo, Nb, Ni, P, Pt, Ta, Ti, Tm, V, W, Os, Re, Rh, Ru and Sb; Yb with Ca, Cd, Gd, Pr, Sm, Tb, Tl, Bi, Ce, Dy, Er, Eu, Hf, In, Ir, K, Mg, Mo, Nb, Ni, P, Pt, Ta, Ti, Tm, V, Y, W, Os, Re, Rh, Ru and Sb; Zn with Be, Lu, Ca, Cd, Gd, Ho, Pr, Sm, Tb, Tl, Bi, Ce, Dy, Er, Eu, Hf, Hg, In, Ir, K, Mg, Mo, Nb, Ni, P, Pt, Ta, Ti, Tm, V, Y, Yb, W, Os, Re, Rh, Ru and Sb; Zr with Be, Lu, Ca, Cd, Ho, Sm, Tb, Te, Tl, Bi, Ce, Dy, Er, Eu, Hf, Hg, In, Ir, K, Mg, Mo, Nb, Ni, P, Pt, Ta, Ti, Tm, V, Y, Yb, W, Zn, Os, Re, Rh, Ru and Sb; Al with Be, Lu, Ca, Cd, Gd, Ho, Sm, Tb, Te, Tl, Bi, Ce, Dy, Er, Eu, Hf, Hg, In, Ir, K, Mg, Mo, Nb, Ni, P, Pt, Ta, Ti, Tm, V, Y, Yb, W, Zn, Zr, Os, Re, Rh, Ru and Sb; As with Lu, Ca, Cd, Ho, Sm, Tb, Tl, Bi, Ce, Er, Eu, Hf, Hg, In, Ir, K, Mg, Nb, Ni, P, Pt, Ta, Ti, Tm, V, Y, Yb, W, Zn, Zr, Os, Re, Rh, Ru and Sb; B with Co, Cr and Ni. Strong negative correlations ($r \leq -0.9$ $n = 44$ $p < 0.05$) were found among: Na with Cd, Ho, Tb, Tl, Bi, Er, Eu, Hf, In, Ir, K, Mg and Mo; P with Na; Pt, Rh, Ru, Re with Na; S with Cd, Ho, Tb, Tl, Bi, Er, Eu, Hf, In, Ir, K, Mg, Mo, Nb, Os, P, Pt, Re, Rh and Ru; Sb with Na and S; Sr with Cd, Tb, Tl, Hf, In, Ir, K, Mo, Nb, Os, Pt, Re, Rh, Ru and Sb; Ti with S and Na; Tm with S and Na; V with S and Na; W with S and Na; Yb with S and Na; Zn with S and Na; Zr with Sr, S, and Na; Al with Sr, S, and Na; As with S and Na.

Further research in this field could focus on exploring the relationships between different elements in bivalve shells and how these interactions may influence shell structure and function. Investigating the mechanisms by which bivalves incorporate and regulate elemental uptake in their shells could also provide valuable insights into potential adaptation strategies in response to changing environmental conditions.

CONCLUSIONS

In conclusion, the literature on elementcorrelation in the shell of bivalves contributes to our understanding of the complex interactions between organisms and their environment. This research not only has significant implications for environmental monitoring and biomonitoring efforts but also opens up new avenues for exploring the physiological and ecological significance of elemental composition in bivalve shells. Further research in this field is warranted to uncover the underlying mechanisms and potential applications for conservation and management efforts.

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BIOCHEMICAL PROFILING OF SOME COMMERCIAL MARINE FIN FISH SPECIES IN LAGOS (NIGERIA)

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ABSTRACT

The proximate mineral compositions of *Scomber scombrus*, *Pseudotholitus typus*, and *Chrysichthys nigrodigitatus* in Lagos were analyzed using standard methods. Moisture content was 58.12%, 75.28%, and 74.68% respectively. Crude protein content was not significantly different among the species. *Scomber scombrus* had the highest crude fat and was the only species with detectable crude fiber (2.04%). Ash contents were similar across species. Carbohydrate content was lowest in *Chrysichthys nigrodigitatus* (3.48%) and highest in *Scomber scombrus* (7.42%). *Pseudotholitus typus* had the highest total macro elements. Mineral patterns varied, with all minerals except sodium being significantly higher in *Scomber scombrus* and *Pseudotholitus typus* compared to *Chrysichthys nigrodigitatus*. This indicates that nutritional quality changes are associated with variations in mineral content.

RÉSUMÉ: Profilage biochimique de quelques espèces commerciales de poissons marins à nageoires à Lagos.

Les compositions proximales et minérales de *Scomber scombrus*, *Pseudotholitus typus* et *Chrysichthys nigrodigitatus* à Lagos ont été analysées en utilisant des méthodes standards. La teneur en eau était respectivement de 58,12%, 75,28% et 74,68%. La teneur en protéines brutes n'était pas significativement différente entre les espèces. *Scomber scombrus* avait la plus forte teneur en matières grasses brutes et était la seule espèce avec des fibres brutes détectables (2,04%). Les teneurs en cendres étaient similaires d'une espèce à l'autre. La teneur en hydrates de carbone était la plus faible chez *Chrysichthys nigrodigitatus* (3,48%) et la plus élevée chez *Scomber scombrus* (7,42%). *Pseudotholitus typus* avait les macro-éléments totaux les plus élevés. Les profils minéraux varient, tous les minéraux sauf le sodium étant significativement plus élevés chez *Scomber scombrus* et *Pseudotholitus typus* que chez *Chrysichthys nigrodigitatus*. Cela indique que les changements de qualité nutritionnelle sont associés à des variations de la teneur en minéraux.

REZUMAT: Profilul biochimic al unor specii de pești marini comerciali din Lagos.

Compozițiile proximale și minerale ale speciilor *Scomber scombrus*, *Pseudotholitus typus* și *Chrysichthys nigrodigitatus* din Lagos au fost analizate utilizând metode standard. Conținutul de umiditate a fost de 58,12%, 75,28% și respectiv 74,68%. Conținutul de proteine brute nu a fost semnificativ diferit între specii. *Scomber scombrus* a avut cea mai mare cantitate de lipide brute și a fost singura specie cu fibre brute detectabile (2,04%). Conținutul de cenușă a fost similar între specii. Conținutul de carbohidrați a fost cel mai scăzut la *Chrysichthys nigrodigitatus* (3,48%) și cel mai ridicat la *Scomber scombrus* (7,42%). *Pseudotholitus typus* a avut cele mai multe macroelemente totale. Structura tuturor mineralelor a variat, cu excepția sodiului, fiind semnificativ mai mare la *Scomber scombrus* și *Pseudotholitus typus* comparativ cu *Chrysichthys nigrodigitatus*. Acest lucru arată că modificările calității nutriționale sunt asociate cu variații ale conținutului de minerale.

INTRODUCTION

The malnutrition situation is intense in developing countries with low levels of protein and mineral intake. As a result there is an urgent need to find a way of raising the protein and mineral intake of the average citizen from 5.5 g/head/day to 35 g/head/day (Moratiel et al., 2020). The World Health Organization reported that about two billion people across the globe are suffering from mineral and vitamin deficiencies and the majority of these cases are in third world countries (WHO, 2014). Chronic malnutrition (protein energy malnutrition, micronutrient deficiencies, and over nutrition) was found to impact 38% of Nigeria's population (Rufai et al., 2022). Meanwhile, more than 41% of the total animal protein intake is obtained from fishery products because it is relatively cheaper than other animal meat. and the total fish consumption rate has risen to 2.66 million metric tons annually (Moruf et al., 2019). Fish is one of the most affordable sources of protein, micro-nutrients, and other essential nutrients crucial to human health. According to Ogundiran et al. (2014), marine fish are generally cheaper and much more abundant than fresh water fish in Nigeria.

The nutritional characteristics of fish and fishery products are of vital interest to consumers. The nutritional value of fish comprises of the contents of moisture, dry matter, protein, lipids, vitamins, and minerals plus the caloric value, while minerals are essential nutrient components of many enzymes and metabolism and it contributes to the growth of fish (Opeyemi, 2020). Fish of various species provide different levels of nutrients to their consumers and nutritive values of a fish varies with season (Opeyemi, 2020).

In human nutrition, chemical elements that are required for the normal maintenance of human body are refered to as essential elements (Jiang et al., 2015). These elements (K, Ca, Mg, Na, Fe, Zn, Cu, and Mn) participate in several biochemical reactions. Calcium, magnesium and phosphorus are crucial in formation of bones and teeth, sodium and potassium work together in transmission of nerve impulses and keeping electrolyte balance, zinc is mostly found as a catalyst in enzyme reactions, and iron forms part of the hemoglobin molecule which transports oxygen around the body (Cobas et al., 2022). When these elements are not adequately provided to the human body through dietary intake, the individual suffers from mineral deficiency diseases such as anemia, osteoporosis, goiter, stunted growth, and genetic disorders (Shrestha et al., 2021).

Several scientific studies on the chemical components of fish have been conducted on for different freshwater and marine fish species around the world. In Nigeria, Opeyemi (2020) conducted a study on comparative analysis of the proximate and elemental composition of five fish species, *Clarias gariepinus, Lates niloticus, Sarotherodon galilaeus, Heterotis niloticus,* and *Oreochromis niloticus,* which were collected from Ero Reservoir Ikun Ekiti. Ayeloja et al. (2023) focused their study on *Auchenoglanis occidentalis, Labeo parvus,* and *Chrysichthys nigrodigitatus*. Biochemical assays of the moisture, ash, crude protein, fibers, lipid, and different elements (Fe, Na, K, Ca, Mg, Zn, Mn, and Cu) of the fillets were carried out. All the fish species examined turned out to be rich sources of protein, moisture, lipid, ash, and minerals and met important requirements for human nutritional needs. They belong to the high-protein, (18-23%), high moisture, and low oil category. Information on the nutritional value of local key species before common postharvest processing and preservation methods in the country is limited or often missing from nutrient composition databases (Fakoya et al., 2019). Hence, the present research aimed to provide a preliminary assessment of the proximate composition, some minerals and their relationship in raw marine fin fish species in Lagos, Nigeria.

The Lagos area is a well known Nigerian zone for fishing and fish trade (Fola-Matthews et al., 2024) and for environment related problems (Uwadiae and Chidolue, 2024).

MATERIAL AND METHODS

Sample collection

The fish samples used for this study include *Scomber scombrus, Pseudotholitus typus,* and *Chrysichthys nigrodigitatus*. They were obtained from Makoko Jetty of the Lagos Lagoon. The lagoon lies between latitudes 6º26' and 6º39'N and longitudes 3º29' and 3º50'E. Lagos Lagoon is a part of a continuous system of lagoons and creeks' lying along the coast of Nigeria and it is an open tidal estuary situated within the low-lying coastal zone of Nigeria (Moruf and Lawal-Are, 2017). The samples were transported in a 20 L bucket to the laboratory and processed within four hours of the collection. The fresh samples were identified using Identification Guides (Schneider, 1990).

Chemical analysis

The determination of the percentage proximate composition was analyzed chemically according to the method of analysis described by the Association of Official Analytical Chemist (AOAC, 2006) while the percentage mineral elemental concentration was determined using (AAS) Atomic Absorption Spectrophotometer and calculated in ppm (µg/g dry weight).

Statistical data analysis

Data were analyzed by descriptive analysis and Duncam multiple range test (DRMT). Statistical software package (SPSS version 17, Chicago, USA) was employed in the analysis. Differences were considered significant at an alpha level of 0.05. All means were given with ± standard error.

RESULTS AND DISCUSSION

Proximate composition

The proximate composition of some selected commercial marine fin fish species in Lagos is shown in table 1. It includes moisture, protein, fat, fibre, ash, and carbohydrate content. The result of the proximate compositions obtained in the present investigation clearly demonstrate that, the proportion of protein content was dominating over carbohydrates and lipid contents in all the studied marine fin fish species. The percentage moisture content in *S. scombrus*, *P. typus*, and *C. nigrodigitatus* were 58.12 ± 0.37%, 75.28 ± 1.22% and 74.68 ± 0.35% respectively. These values were higher than what was reported by Lawal-Are et al. (2018) for the whole body (69.55 ± 2.93%) of *Squilla aculeata calmani* from the same habitat. The crude protein contents of *S. scombrus* (15.01 ± 0.60%), *P. typus* (13.87 ± 1.38%) and *C. nigrodigitatus* (4.51 ± 0.72%) are similar to that reported for raw (16.02 ± 2.24%) but higher than that in the grilled specimen (14.98 ± 1.12%) *Cardisoma armatum* (Moruf et al., 2021a). The protein content of the studied fish species showed no significance difference ($p > 0.05$) when compared among the three species. The higher lipid content (16.57 ± 1.05%) of *S. scombrus* reveals involvement in energy production at the cellular level. Crude fiber was only detected in *S. scombru*s with the value of 2.04 ± 0.02% similar to 2.47% reported for flesh of *Panulirus regius* from Lagos area Atlantic Ocean (Moruf et al., 2021b).

The ash content in the present study was not significantly different among the marine fin fish species and ranges from 0.85 ± 0.06% (*S. scombrus*), 0.90 ± 0.03% (*C. nigrodigitatus*) and 1.20 ± 0.61% (*P. typus*). The lowest carbohydrate content was recorded in *C. nigrodigitatus* (3.48 ± 0.77%) and significantly different from what was recorded for *S. scombrus* (7.42 ± 0.56%) and *P. typus* (7.29 ± 1.28%), which were all lower than 37.55-42.8% obtained in whole organism of *Squilla aculeata calmani* (Lawal-Are et al., 2018).

In this study, the proximate compositions of the investigated marine fin fish species show a significant difference especially in crude fat and crude fiber. Generally, the biochemical composition of any organism is known to reflect its nutritional quality and is being influenced by several biotic and abiotic factors including species, season, size of the animal, food, temperature, and stage in the life cycle (Banu et al., 2016).

Table 1: Proximate composition of some commercial marine fin fish in Nigeria; Mean ± Standard, values with different superscripts across row are significantly different at ($p < 0.05$).

Parameters (%)	*Scomber scombrus*	*Pseudotholitus typus*	*Chrysichthys nigrodigitatus*
Moisture	58.12 ± 0.37^a	75.28 ± 1.22^b	74.68 ± 0.35^b
Protein	15.01 ± 0.6^a	13.87 ± 1.38^a	14.51 ± 0.72^a
Crude fat	16.57 ± 1.05^a	2.35 ± 0.55^b	6.43 ± 0.04^c
Crude fibre	2.04 ± 0.02^a	0.00 ± 0.00^b	0 ± 0.00^b
Total ash	0.85 ± 0.06^a	1.20 ± 0.61^a	0.90 ± 0.03^a
Carbohydrate	7.42 ± 0.56^a	7.29 ± 1.28^a	3.48 ± 0.77^b

Mineral concentrations

The result of the mineral content is contained in table 2. The highest total macro element was recorded in *P. typus* (790.23 mg 100 g-1) while the lowest in *C. nigrodigitatus* (366.26 mg 100 g-1). Potassium was the highest concentrated mineral in *S. scombrus* and *P. typus* with values of 371.72 ± 1.06 mg 100g-1 and 328.61 ± 0.61 mg 100g-1 while phosphorus was the highest concentrated mineral in *C. nigrodigitatus* with the value of 101.75 ± 7.64 mg 100g-1. The pattern of mineral contents in *S. scombrus* and *P. typus* was potassium > phosphorus > sodium > magnesium > calcium; while for *C. nigrodigitatus*, it was phosphorus > sodium > potassium > magnesium > calcium. All the investigated minerals (except sodium) were significantly higher ($p < 0.05$) in *S. scombrus* and *P. typus* compared to that in *C. nigrodigitatus*. This result is similar and conforms to the report of Ogundiran et al. (2014), which indicates that the marine fish species in southwestern Nigeria can be a good source of macro minerals for human health.

Table 2: Proximate composition of some commercial marine fin fish in Nigeria; Mean ± Standard, values with different superscripts across row are significantly different at ($p < 0.05$).

Parameters (mg $100g^{-1}$)	*Scomber scombrus*	*Pseudotholitus typus*	*Chrysichthys nigrodigitatus*
Calcium	12.43 ± 3.18^a	12.96 ± 3.30^a	8.86 ± 25.85^b
Magnesium	78.31 ± 1.6^a	86.31 ± 1.52^a	67.34 ± 6.03^b
Potassium	371.72 ± 1.06^a	328.61 ± 0.61^a	90.23 ± 7.33^b
Phosphorus	215.11 ± 3.55^a	267.21 ± 2.24^a	101.75 ± 7.64^b
Sodium	89.17 ± 27.97^a	95.14 ± 15.51^a	98.08 ± 4.74^a
Total	766.74	790.23	366.26

Relationship between proximate and mineral compositions

Tables 3, 4 and 5 shows the correlation matrixes between the proximate and mineral compositions of *S. scombrus* and *P. typus* compared to that of *C. nigrodigitatus* respectively. In each case, there were approximately perfect positive relationships between proximate and mineral components. In *S. scombrus*, a perfect relationship was exhibited by crude fiber with Ca (0.99), Mg (1.00), K (0.95), P (1.00), and Na (0.97) at 0.05 significance level. The same was applicable to ash content. In *P. typus*, fat and carbohydrate contents showed positive relationships with all the minerals except potassium (–0.11). In *C. nigrodigitatus*, protein contents showed positive relationships with calcium (0.58), phosphorus (0.54), and sodium (0.72). This finding is comparable to the report on the macro and micro-nutrients of the flesh of *Callinectes amnicola* from Southwest Nigeria (Moruf et al., 2019).

Table 3: Correlation coefficient among the proximate and mineral compositions in *Scomber scombrus*.

	Moi.	Prot.	Fat	Fibre	Ash	NFE	Ca	Mg	K	P	Na
Moisture	1	–	–	–	–	–	–	–	–	–	–
Protein	0.94	1	–	–	–	–	–	–	–	–	–
Crude Fat	–0.92	–0.72	1	–	–	–	–	–	–	–	–
Crude Fibre	0.12	–0.24	–0.50	1	–	–	–	–	–	–	–
Total Ash	0.74	0.46	–0.95	0.75	1	–	–	–	–	–	–
NFE	–0.02	–0.37	–0.37	0.99	0.65	1	–	–	–	–	–
Ca	–0.04	–0.39	–0.36	0.99	0.64	1.00	1	–	–	–	–
Mg	0.20	–0.16	–0.57	1.00	0.80	0.98	0.97	1	–	–	–
K	0.41	0.07	–0.74	0.95	0.91	0.90	0.90	0.97	1	–	–
P	0.07	–0.28	–0.46	1.00	0.72	1.00	0.99	0.99	0.94	1	–
Na	0.36	0.02	–0.70	0.97	0.89	0.92	0.92	0.98	1.00	0.96	1

Table 4: Correlation coefficient among the proximate and mineral compositions in *Pseudotholitus typus*.

	Moi.	Prot.	Fat	Ash	NFE	Ca	Mg	K	P	Na
Moisture	1	–	–	–	–	–	–	–	–	–
Protein	–0.55	1	–	–	–	–	–	–	–	–
Crude Fat	–0.93	0.82	1	–	–	–	–	–	–	–
Total Ash	0.95	–0.78	–1.00	1	–	–	–	–	–	–
NFE	–0.42	–0.53	0.05	–0.11	1	–	–	–	–	–
Ca	0.85	–0.90	–0.99	0.97	0.12	1	–	–	–	–
Mg	0.82	–0.93	–0.97	0.96	0.17	1.00	1	–	–	–
K	0.95	–0.78	–1.00	1.00	–0.11	0.97	0.96	1	–	–
P	0.89	–0.87	–0.99	0.99	0.05	1.00	0.99	0.99	1	–
Na	0.85	–0.91	–0.98	0.97	0.13	1.00	1.00	0.97	1.00	1

Table 5: Correlation coefficient among the proximate and mineral compositions in *Chrysichthys nigrodigitatus*.

	Moi.	Prot.	Fat	Ash	NFE	Ca	Mg	K	P	Na
Moisture	1	–	–	–	–	–	–	–	–	–
Protein	–0.21	1	–	–	–	–	–	–	–	–
Crude Fat	0.99	–0.35	1	–	–	–	–	–	–	–
Total Ash	0.56	0.69	0.43	1	–	–	–	–	–	–
NFE	–0.34	–0.85	–0.20	–0.97	1	–	–	–	–	–
Ca	–0.92	0.58	–0.97	–0.19	–0.06	1	–	–	–	–
Mg	–0.20	–0.92	–0.05	–0.92	0.99	–0.20	1	–	–	–
K	0.31	–0.99	0.44	–0.62	0.79	–0.66	0.87	1	–	–
P	–0.94	0.54	–0.98	–0.24	–0.01	1.00	–0.15	–0.62	1	–
Na	–0.83	0.72	–0.90	0.00	–0.25	0.98	–0.38	–0.79	0.97	1

CONCLUSIONS

The proximate parameters of the studied marine fin fish species are similar to mineral element concentrations in *Scomber scombrus* and *Pseudotholitus typus*, with calcium, magnesium, potassium, phosphorus and sodium, contributing significantly to the nutritional qualities of the fishes.

The positive relationship in the nutritional quality indicates that changes in proximate composition are associated with changes in the mineral contents of the marine fish species.

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MORPHOLOGICAL VARIATION AND LENGTH-WEIGTH RELATIONSHIP OF *LABEO ANGRA* (HAMILTON, 1822) SAMPLED FROM THREE DIFFERENT RIVERS OF DJNAIPUR (BANGLADESH)

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KEYWORDS: condition factor, *Labeo angra*, length-weight, morphometric, meristic.

ABSTRACT

We sampled 150 *Labeo angra* fish from the Punarbhaba, Atrai and Dhepa rivers of Dinajpur, Bangladesh, with total lengths (TL) ranging between 10.6 and 16.7 cm, and determined their length-weight relationships and morphological variation. 17 characters out of 18 morphometric characters showed significant variation ($p < 0.05$), while three out of eight meristic characters differed among the populations. In the case of morphometric proportions, three out of seven morphometric proportions showed significant variation. The growth was positive allometric ($b > 3$) for the Punarbhaba River, isometric ($b = 3$) for the Atrai River, and negative allometric ($b < 3$) for the Dhepa River. The coefficient of determination (r^2) for the Punarbhaba, Atrai, and Dhepa rivers were 0.942, 0.920, and 0.942, respectively, indicating a high degree of correlation between length and weight.

RÉSUMÉ: Variation morphologique et relation longueur-poids du *Labeo angra* (Hamilton, 1822) échantillonné dans trois rivières différentes de Djnaipur (Bangladesh).

Nous avons échantillonné dans les rivières Punarbhaba, Atrai et Dhepa de Dinajpur, Bangladesh, 150 poissons *Labeo angra* mesurant entre 10,6 et 16,7 cm de longueur totale (TL) et déterminé leurs relations longueur-poids et leurs variations morphologiques. 17 caractères morphométriques sur 18 présentent une variation significative ($p < 0,05$) tandis que trois caractères méristiques sur huit diffèrent au sein de la population. Dans le cas des proportions morphométriques, trois proportions morphométriques sur sept présentent une variation significative. La croissance était allométrique positive ($b > 3$) pour Punarbhaba, isométrique ($b = 3$) pour Atrai et allométrique négative ($b < 3$) pour la rivière Dhepa. Les coefficients de détermination (r^2) des rivières Punarbhaba, Atrai et Dhepa étaient respectivement de 0,942, 0,920 et 0,942, ce qui montre un degré élevé de corrélation entre la longueur et le poids.

REZUMAT: Variația morfologică și relația lungime-greutate la *Labeo angra* (Hamilton, 1822) prelevați din trei râuri diferite din Djnaipur (Bangladesh).

Am colectat în râurile Punarbhaba, Atrai și Dhepa din Dinajpur, Bangladesh, 150 de pești *Labeo angra* cu lungimea totală (TL) cuprinsă între 10,6 și 16,7 cm și am determinat relația lungime-greutate și variația morfologică a acestora. 17 caractere morfometrice din 18 au prezentat variații semnificative ($p < 0,05$), în timp ce trei caractere meristice din opt diferă între populații. În cazul proporțiilor morfometrice, trei proporții morfometrice din șapte au prezentat o variație semnificativă. Creșterea a fost alometrică pozitivă ($b > 3$) pentru Punarbhaba, izometrică ($b = 3$) pentru Atrai și alometrică negativă ($b < 3$) pentru râul Dhepa. Coeficientul de determinare (r^2) pentru râurile Punarbhaba, Atrai și Dhepa a fost de 0,942, 0,920 și 0,942, indicând un grad ridicat de corelație între lungime și greutate.

INTRODUCTION

Labeo angra is a species of fish in the family Cyprinidae, commonly known as the Angra and locally reffered to as Kharsa, Angrot, and Kharish in Bangladesh (Talwar and Jhingran, 1991; Rahman, 1989). This species is found in Northern India, Bangladesh, Nepal, Myanmar, and Pakistan, and it has also been reported from Afghanistan (Froese and Pauly, 2010). In Bangladesh, the species is abundant in the streams of Sylhet, Mymensingh, Dinajpur, and Rangpur districts, as well as in rivers and streams of other districts. It is an herbivorous, column-feeding freshwater fish that is also benthopelagic and potamodromous in nature. (Devi and Boguskaya, 2009)

The body of this fish is elongated and cylindrical. Live mature fish are brownish along the back, side, and abdomen, with a yellowish color and a black or bluish stripe along the flanks, extending from the eye to the caudal fin base. This species has been known to reach usually a maximum length of about 18.5 cm, and occasionally more than 22 cm, and it breeds normally during monsoon months from July to August in India. This species is commercially important as it serves as both an important food and sport fish. Although *Labeo angra* is classified as least concern by the IUCN (2015), the abundance of this species is gradually decreasing in nature due to over-exploitation, habitat degradation, aquatic pollution, siltation, climate change, and other ecological reasons (Ahamed et al., 2023). Furthermore, the use of small mesh sizes, fishing methods, fishing during the breeding season, intensive fishing, and illegal fishing methods such as poisoning, grenadine fishing, and dynamiting contribute to the decline of fish stocks. Therefore, it is time to take urgent steps to conserve the species of our riverine fish biomass and restore to their habitats (Ahamed et al., 2017).

To expand conservation approaches, knowledge of the biology, morphology, and population structure of any species is essential (Ahamed et al., 2020) for understanding short-term and ecologically influenced variations. As the availability of the *Labeo angra* species decreases, day by day attention is needed to conserve the fish. Since there is no prior information regarding the morphological variations and length-weight relationships of *Labeo angra* among the Atrai, Punarbhaba, and Dhepa rivers, the aim of this study is to investigate the variations in morphometric and meristics characters, as well as the growth patterns of *Labeo angra* among the three river stocks. Therefore, this study was conducted with the following objectives: to determine the morphometric and meristic variation of *Labeo angra* collected from the Punarbhaba, Atrai, and Dhepa rivers in the Dinajpur district of Bangladesh, and to assess the length-weight relationship of *Labeo angra* collected from these rivers.

MATERIAL AND METHODS

Experimental period and working station

The total experimental work was conducted over a twelve month period. Samples were collected from three stations in Bangladesh from September 2019 to August 2020. Morphometric and meristic studies, image capture, measurement of length and weight, and all other related tasks were performed in the Fish Biodiversity Laboratory at the Bangladesh Fisheries Research Institute (BFRI), Freshwater Substation, Saidpur, Nilphamari.

Sources and collection of fish

The fish samples were collected from three different wild areas in the Dinajpur District, namely the Atrai, Punarbhaba, and Dhepa rivers. In this study, a total of 150 experimental specimens were collected (50 specimens from each location) (Fig. 1, Tab. 1). Samples were collected with the assistance of local fishermen using standard fishing gear, such as cast nets and hand nets.

Table 1: The sample collection site and number of specimens collected from different sources.

Population number	Sample collection site	Number of specimens
1.	Atrai River, Mohanpur, Dinajpur.	50
2.	Punarbhaba river, Kanchan, Dinajpur.	50
3.	Dhepa River, Ashramghat, Dinajpur.	50

Figure 1: Map showing the geographical region of the three rivers of Dinajpur District.

Samples preservation

The collected fish samples were taken to the laboratory at the Bangladesh Fisheries Research Institute. The fish samples were then labeled with the date and kept in a refrigerator for further study.

Morphological studies

Fifty specimens from each location were used for morphological studies following the standard procedures described by Appa Rao (1966) and Dwivedi and Menezes (1974), with slight modifications. The morphometric and meristic characters were recorded by using a measuring scale, electronic balance, needle, and magnifying glass (Hossen et al., 2017).

Measurement of morphometric characteristics

The fishes were brought to the laboratory in a frozen condition. The frozen fish samples were kept in a metallic tray a thaw and then immersed in water for a short period. At that point, the water from the body surface of each fish was blotted dry with tissue paper. Samples were then kept at room temperature to dry, in order to avoid bias during the weighing process. The morphometric characters were estimated using a centimeter scale and an electronic balance (Fig. 2). Finally, all the data were entered into a Microsoft Excel spreadsheet. The estimated morphometric parameters and their descriptions are provided in table 2.

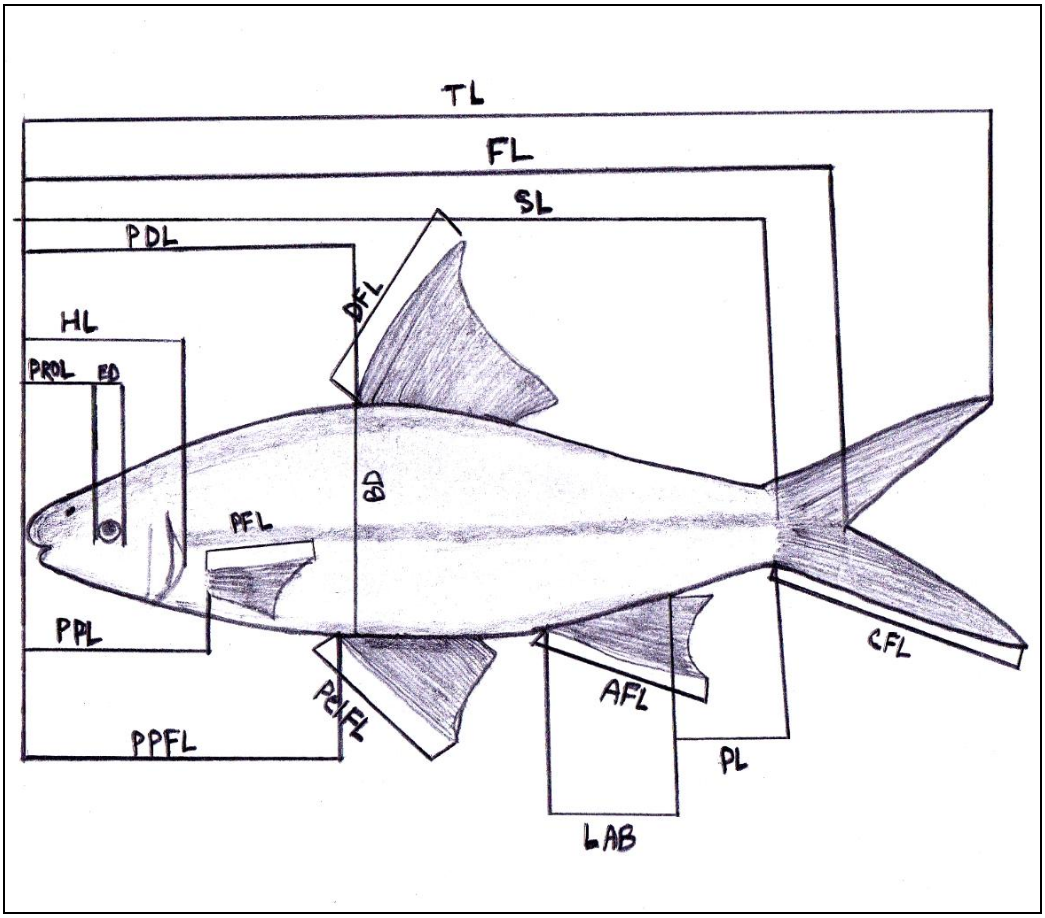

Figure 2: Schematic representation of the abbreviation of morphometric measurements of *Labeo angra*.

Table 2: Some morphometric parameters with abbreviations and description.

Parameter	Parameter description
Total length (TL)	Distance from the tip of the snout to the top of the caudal fin ray.
Body weight (BW)	Total weight of the body.
Fork length (FL)	Distance from the tip of the snout to the end of the middle caudal fin rays.
Standard length (SL)	Distance from the tip of the snout to the end of the vertebral column.
Body depth (BD)	The most swollen bit of the body.
Head length (HL)	Distance from the tip of the snout to the posterior margin of the opercula.
Eye length (ED)	Diameter of the eye.
Pectoral fin length (PFL)	Length of the longest fin ray of the pectoral fin.
Pelvic fin length (PelFL)	Length of the longest fin ray of the pelvic fin.
Dorsal fin length (DFL)	Length of the longest fin ray of the dorsal fin.
Anal fin length (AFL)	Length of the longest fin ray of the anal fin.
Caudal fin length (CFL)	Length of the longest fin ray of the caudal fin.
Pre-orbital length (PrOL)	Distance from the tip of the snout to the anterior margin of the eye.
Pre-pectoral length (PPL)	Distance from the tip of the snout to the insertion of the pectoral fin.
Pre-pelvic length (PPFL)	Distance from the tip of the snout to the insertion of the pelvic fin.
Pre-dorsal length (PDL)	Distance from the tip of the snout to the anterior end of the first dorsal fin base.
Peduncle length (PL)	Vertical distance from the anterior part to the posterior part of the anal fin.
Length of anal base (LAB)	Distance from the end of the anal fin base to the beginning of the caudal fin base.

Measurement of meristic characteristics

Eight meristic characters were estimated for the experiment. A magnifying glass and needle were used to count the fin rays and scales; in the case of fin rays, only the principal rays (both hard and soft rays) were counted as separate rays. The estimated meristic characters and their descriptions are given in table 3 and figure 3.

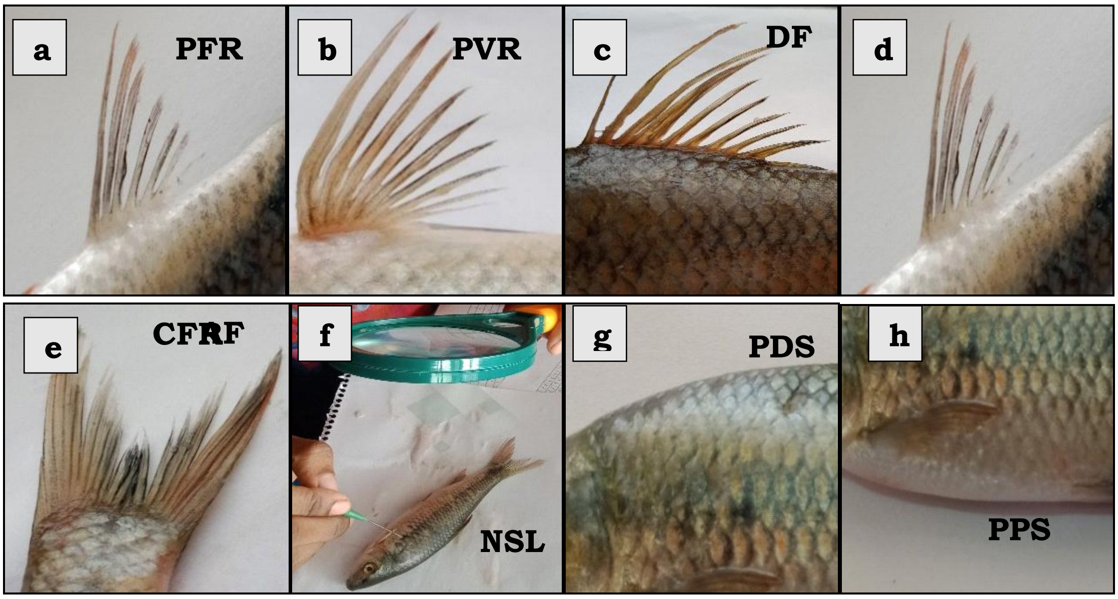

Figure: 3 Schematic representation of the meristic measurements of *Labeo angra*.

Table 3: Meristic parameters with the abbreviation

Characters	Abbreviation
Number of pectoral fin rays	PFR
Number of pelvic fin rays	PVR
Number of dorsal fin rays	DFR
Number of anal fin rays	AFR
Number of caudal fin rays	CFR
Number of scales on lateral line	NSLL
Pre-dorsal scale	PDS
Pre-pelvic scale	PPS

Measurement of length and weight

The fish samples were placed in a metallic tray for measuring total length and body weight. The total length was measured in the laboratory using a centimeter scale, and the weight of each individual fish was recored in grams using an electronic balance. The data for total length and body weight were entered into a Microsoft Excel spreadsheet.

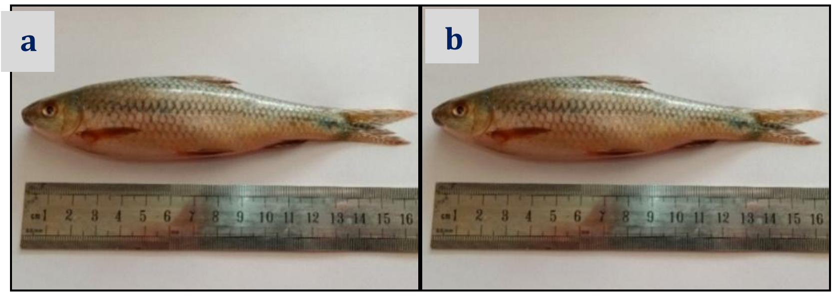

Figure 4: (a) Measurement of length and (b) weight of *Labeo angra* in laboratory by using a measuring scale and electronic balance.

Length-weight relationship

The relationship between total length (TL) and body weight (BW) was calculated using the expression:

$$BW = a\ TL^b$$

where BW is body weight in grams, TL is total length in centimeters, and a and b are constants. Parameters a and b of the length-weight relationship were estimated using liner regression analysis based on natural logarithms:

$$\ln(BW) = \ln(a) + b\ (\ln(\ TL))$$

where ln(BW) is the dependent variable and ln(TL) is the independent variable; ln(a) and b are the intercept and slope of the log-log linear relationship, respectively. The slope was estimated using the formula for slope:

$$b = [n\sum XY - \sum X\sum Y] / [n\sum X^2 - (\sum X)^{2]}$$

where, X = ln (TL),

Y = ln (BW), and n is the number of observations in the sample. The intercept, ln(a) was estimated as

$$\ln(a) = -b$$

The value of a in the equation of the length-weight relationship was estimated as:

$$a = \exp\ (\ln a)$$

The coefficient of determination (r^2) is the square of the correlation coefficient (r). The r^2 value lies between 0 and 1, relating the proportion of the variation of the dependent variable which can be explained by the variation in the independent variable. To express the degree of linear association between two variables, the correlation coefficient, r was estimated by:

$r = [\ n\sum XY - \sum X\sum Y] / \sqrt{}\ ([n\sum X^2 - (\sum X)^2]\ [n\sum Y^2 - (\sum Y)^2])$ (Le Cren, 1951).

Data analysis

All types of statistical analyses for each species were performed using Excel 2010, PAST (Paleontological Statistics, version 3.0), and SPPS (Statistical Package for social science, version 22.0) software. Differences in morphometric characters and meristic counts of fish were analyzed using analysis of variance (ANOVA) to detect the significance of differences among the various regions.

RESULTS AND DISCUSSION

Morphometric variations

Eighteen morphometric characters were recorded from the samples collected from three wild populations of *Labeo angra* are shown in tables 4 and 5.

Among the eighteen morphometric characters, seventeen characters (TL, BW, FL, SL, BD, PFL, PelFL, DFL, AFL, CFL, PL, HL, ED, PrOL, PPL, PPFL, and PDL) were found to be significant, while one morphometric character (LAB) was non-significant ($p < 0.05$) among the three rivers. Morphometric characters revealed that TL, BW, FL, SL, BD, PFL, PelFL, DFL, AFL, CFL, HL, PPFL, and PDL were significantly higher ($p < 0.05$) in *Labeo angra* collected from the Punarbhaba and Atrai rivers compared to those fishes collected from the Dhepa River, with no significant variation between the Punarbhaba and Atrai river. Conversely, the morphometric characteristics such as CFL, PrOL, and PPL in *Labeo angra* from Atrai River were significantly different ($p < 0.05$) compared to Punarbhaba and Dhepa rivers. The highest PL and lowest ED were found in the Labeo angra sampled from the Punarbhaba River compared to those sampled from both the Atrai and Dhepa Rivers.

Table 4: Mean values of eighteen morphometric characters as recorded from *Labeo angra* of three wild populations (n = 50 for each population); values in each row with different superscripts (a, b and c) differs significantly ($p < 0.05$).

Morphometric characters	Mean values (cm) of population (Mean ± SE)				F-values	P-values
	Punarbhaba River	Atrai River	Dhepa River	Average		
Total length (TL)	$14.19±0.15^{b}$	$14.23±0.15^{b}$	$13.12±0.20^{a}$	13.85±0.17	13.42	0
Body weight (BW)	$27.31±0.99^{b}$	$30.05±1.02^{b}$	$21.84±0.20^{a}$	26.4±0.74	17.43	0
Fork length (FL)	$12.30±0.13^{b}$	$12.49±0.14^{b}$	$11.37±0.17^{a}$	12.05±0.15	15.96	0
Standard length (SL)	$11.50±0.13^{b}$	$11.60±0.14^{b}$	$10.61±0.17^{a}$	11.24±0.15	14.37	0
Body depth (BD)	$1.80±0.04^{b}$	$1.82±0.03^{b}$	$1.68±0.03^{a}$	1.77±0.03	4.98	0.008
Pectoral fin length (PFL)	$2.07±0.03^{b}$	$2.09±0.02^{b}$	$1.9±0.03^{a}$	2.02±0.03	14.65	0
Pelvic fin length (PelFL)	$2.01±0.03^{b}$	$2.08±0.02^{b}$	$1.88±0.03^{a}$	1.99±0.03	14.45	0
Dorsal fin length (DFL)	$2.58±0.03^{b}$	$2.62±0.03^{b}$	$2.4±0.04^{a}$	2.533±0.03	14.65	0
Anal fin length (AFL)	$1.85±0.03^{b}$	$1.82±0.02^{b}$	$1.71±0.03^{a}$	1.793±0.03	8.63	0
Caudal fin length (CFL)	$3.18±0.04^{b}$	$3.41±0.05^{c}$	$2.99±0.06^{a}$	3.193±0.05	16.08	0
Peduncle length (PL)	$1.94±0.04^{b}$	$1.83±0.02^{a}$	$1.74±0.03^{a}$	1.837±0.03	10.52	0
Head length (HL)	$2.60±0.04^{b}$	$2.65±0.03^{b}$	$2.47±0.03^{a}$	2.573±0.03	6.78	0.002
Eye diameter (ED)	$0.67±0.01^{a}$	$0.70±0.01^{b}$	$0.71±0.01^{b}$	0.693±0.01	6.93	0.001
Pre orbital length (PrOL)	$1.13±0.01^{a}$	$1.18±0.02^{b}$	$1.09±0.01^{a}$	1.133±0.01	8.95	0
Pre pectoral length (PPL)	$2.58±0.03^{a}$	$2.66±0.04^{b}$	$2.47±0.03^{a}$	2.57±0.03	8.14	0
Pre pelvic length (PPFL)	$5.56±0.07^{b}$	$5.61±0.06^{b}$	$5.20±0.08^{a}$	5.457±0.07	10.66	0
Pre dorsal length (PDL)	$5.11±0.06^{b}$	$5.04±0.06^{b}$	$4.71±0.06^{a}$	4.953±0.06	11.61	0
Length of anal base (LAB)	1.05±0.01	1.25±0.20	0.95±0.02	1.083±0.08	1.71	0.184

Different morphometric proportions of *Labeo angra* in wild populations.

The proportions of seven morphometric characters (TL: FL, TL: SL, TL: HL, TL: BD, SL: HL, SL: BD, and HL: ED) from three wild populations were analyzed to observe the morphometric variation, as shown in table 5. Except for five morphometric proportion characters (TL: SL, TL: HL, TL: BD, SL: HL, and SL: BD), only two proportions (TL: FL and HL: ED) exhibited significant variation among the *Labeo angra* from the three rivers.

The proportion of total length to fork length (TL: FL) was significantly higher ($p < 0.05$) in *Labeo angra* from Punarbhaba and Dhepa rivers compared to those collected from Atrai River. The proportion of SL: HL showed significant variation ($p < 0.05$) in *Labeo angra* sampled from the Punarbhaba River compared to those fishes sampled from the Dhepa River, which was not significantly different from that of the Atrai River. Additionally, the proportion of HL: ED was significantly different and higher ($p < 0.05$) in *Labeo angra* collected from the Punarbhaba and Atrai compared to those collected from the Dhepa.

Table 5: Mean ± SE in parenthesis of different morphometric proportions of *Labeo angra* of three wild populations; [a, b, c] Values in each row with different superscripts differs significantly ($p < 0.05$).

Morpho-metric characters	Mean values (cm) of the population (Mean±SE)				F-values	P-values
	(Tukey HSD)					
	Punarbhaba River	Atrai River	Dhepa Rriver	Average		
TL: FL	$1.15±0.003^{b}$	$1.14±0.003^{a}$	$1.15±0.003^{b}$	1.15±0.003	8.4	0.000*
TL: SL	1.23±0.002	1.23±0.003	1.24±0.003	1.23±0.003	2.78	0.066
TL: HL	5.51±0.099	5.38±0.032	5.30±0.036	5.40±0.056	2.76	0.067
TL: BD	7.80±0.108	7.89±0.094	7.84±0.077	7.84±0.093	0.77	0.465
SL: HL	$4.46±0.077^{b}$	$4.39±0.026^{ab}$	$4.29±0.032^{a}$	4.38±0.045	3.04	0.051
HL: ED	$3.93±0.076^{b}$	$3.79±0.041^{b}$	$3.49±0.064^{a}$	3.74±0.060	12.96	0.000*
SL: BD	6.48±0.084	6.43±0.078	6.34±0.062	6.42±0.075	0.9	0.409

Meristic variations

Eight meristic characters (PFR, PeFR, AFR, CFR, DFR, NSLL, PDS, and PPS) calculated from samples collected from the three rivers of wild populations of *Labeo angra* are shown in table 6. Among the eight meristic characters, three (DFR, PDS, and PPS) were found to be significantly different, while five (PFR, PeFR, AFR, CFR, and NSLL) were non-significant ($p<0.05$) among the three stocks. The meristic character DFR was significantly higher ($p<0.05$) in *Labeo angra* collected from the Atrai River compared to those collected from the Punarbhaba and Dhepa rivers. The meristic character PDS significantly differed ($p<0.05$) in *Labeo angra* sampled from the Punarbhaba River compared to those collected from the Atrai and Dhepa rivers. On the other hand, the meristic character PPS in *Labeo angra* showed significant variation in the Atrai River compared to those collected from the Punarbhaba and Dhepa rivers.

Table 6: Mean ± SE of eight meristic characters as recorded from *Labeo angra* of three wild populations (n = 50 for each population); Values in each row with different superscripts (a, b, and c) differs significantly ($p < 0.05$).

Meristic characters	Mean values (no.) of populations (Tukey HSD)				F-values	P-values
	Punarbhaba River	Atrai River	Dhepa Rriver	Average		
No. of pectoral fin rays (PFR)	16±0.0	16±0.0	16±0.0	16±0.0	–	–
No. of Pelvic fin rays (PeFR)	9±0.0	9±0.0	9±0.0	9±0.0	–	–
No. of Dorsal fin rays (DFR)	12.64±0.07^{a}	13±0.0^{b}	12.64±0.07^{a}	12.76±0.05	13.78	0.000*
No. of Anal fin rays (AFR)	7±0.0	7±0.0	7±0.0	7±0.0	–	–
No. of Caudal fin rays (CFR)	19±0.0	19±0.0	19±0.0	19±0.0	–	–
No. of Scales on lateral line (NSLL)	41.82±0.05	41.84±0.05	41.8±0.06	41.82±0.05	0.12	0.89
Pre-dorsal scale (PDS)	13.6±0.07^{b}	13.06±0.09^{a}	13.16±0.09^{a}	13.27±0.08	11.77	0.000*
Pre-pelvic scale (PPS)	11.8±0.08^{b}	11.46±0.09^{a}	11.94±0.08^{b}	11.73±0.08	9.48	0.000*

Total length and body weight of *Labeo angra* of three different regions

The investigation analyzed a total of 150 individuals of *Labeo angra* from three different regions of Bangladesh. The fish individuals were different in size; in one stock, all fish were similar in size, while the total populations of individuals of different sizes. The total length and body weight of the Punarbhaba River stock ranged from 12 to 16.7 cm and from 14.81 to 47.05 g, respectively. In the Atrai River stock, the total length and body weights ranged from 12.2 to 16.2 cm and 18.97 to 48.84 g, respectively. Finally, the total length and body weight of fish from the Dhepa River was 10.6 to 16.3 cm and 11.89 to 39.17 g, respectively (Tab. 7).

Table 7: The record of size range and number of individuals of *Labeo angra* collected from three different regions of Dinajpur District, Bangladesh.

Sl. No.	Location	Number of fishes	Size range of fishes		Mean±SE	
			Length (cm)	Weight (g)	Length (cm)	Weight (g)
1.	Punarbhaba River, Kanchan	50	12 to 16.7	14.81 to 47.05	14.19±0.15	27.31±0.99
2.	Atrai River, Mohanpur	50	12.2 to 16.2	18.97 to 48.84	14.23±0.15	30.05±1.02
3.	Dhepa River, Ashramghat	50	10.6 to 16.3	11.89 to 39.17	13.12±0.20	21.84±0.99
4.	All populations	150	10.6 to 16.7	11.89 to 48.84	13.85±0.11	26.40±0.64

Length-weight relationship of *Labeo angra*

The "b" values varied among stocks, ranging from 2.674 to 3.207. The lowest value was found in the Dhepa River (2.674), and the highest value was observed in the Punarbhaba River (3.207) (Figs. 4-7). The coefficient of determination (r^2) ranged from 0.920 to 0.942, with the lowest value found in the Atrai River and the highest values in Punarbhaba and Dhepa rivers (Tab. 8). The highest value of the coefficient of determination (r^2 = 0.942) in the Punarbhaba and Dhepa rivers indicated a strong correlation between body weight and total length (Tab. 8). Conversely, the coefficient of determination (r^2 = 0.920) found in Atrai River also suggested a strong relationship between body weight and total length, similar to that observed in the other two rivers.

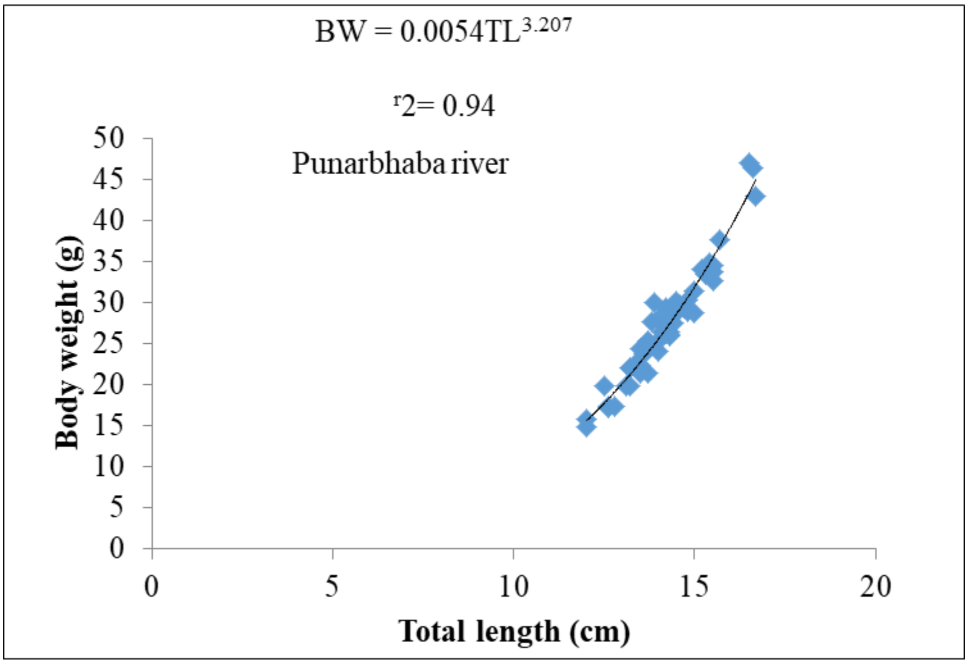

Figure 4: Linear relationship between the total length (TL) and body weight (BW) of *Labeo angra* in Punarbhaba River.
In this plot, total populations were 50 and the value of "b" = 3.207 and r^2 = 0.942.

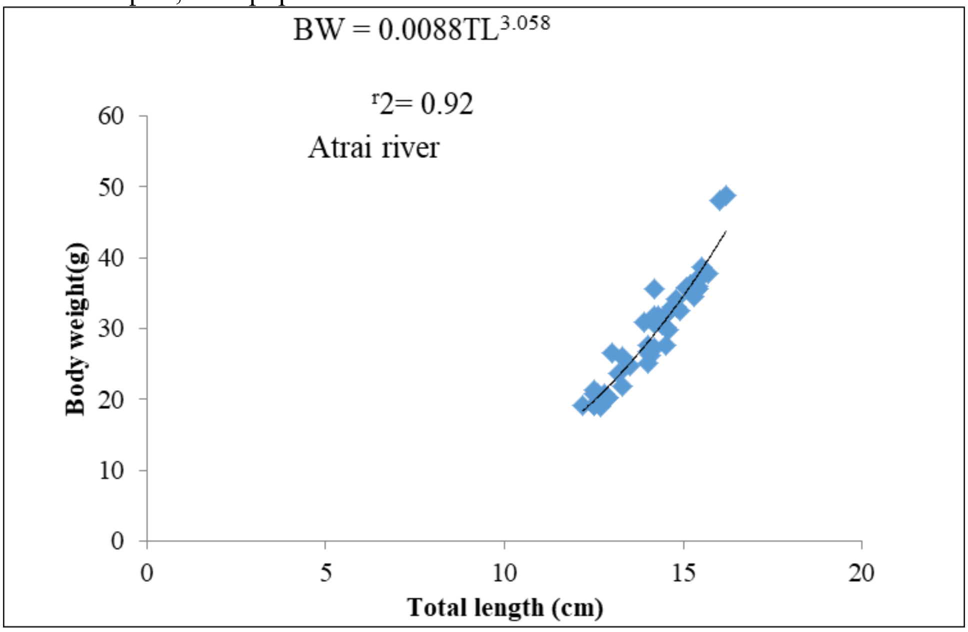

Figure 5: Linear relationship between the total length (TL) and body weight (BW) of *Labeo angra* in Atrai River.
In this plot, total populations were 50 and the value of "b" = 3.058 and r^2 = 0.920.

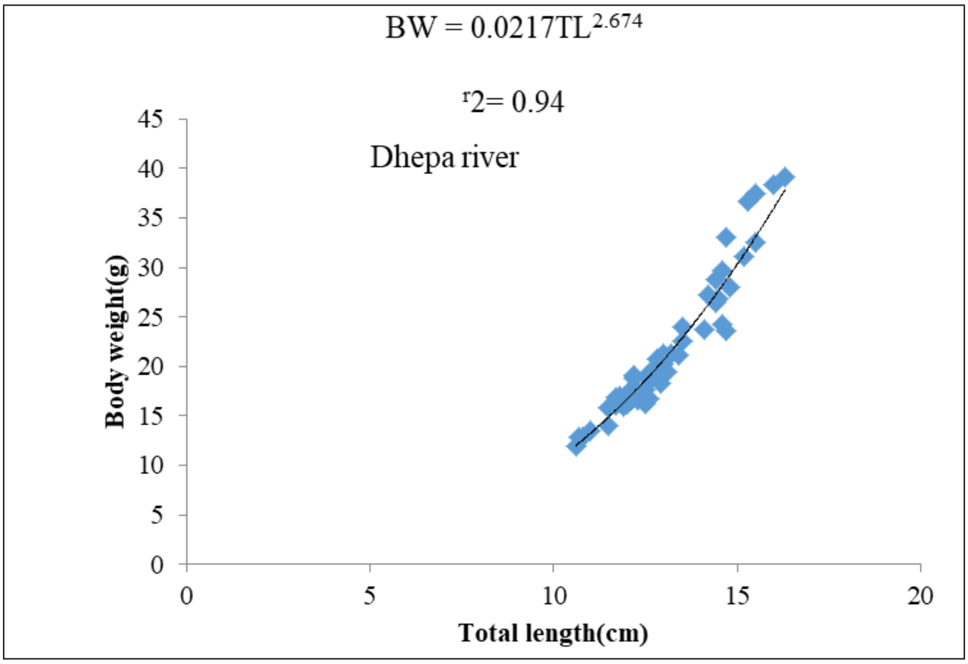

Figure 6: Linear relationship between the total length (TL) and body weight (BW) of *Labeo angra* in Dhepa River.

In this plot, total populations were 50 and the value of "b" = 2.674 and r^2 = 0.942.

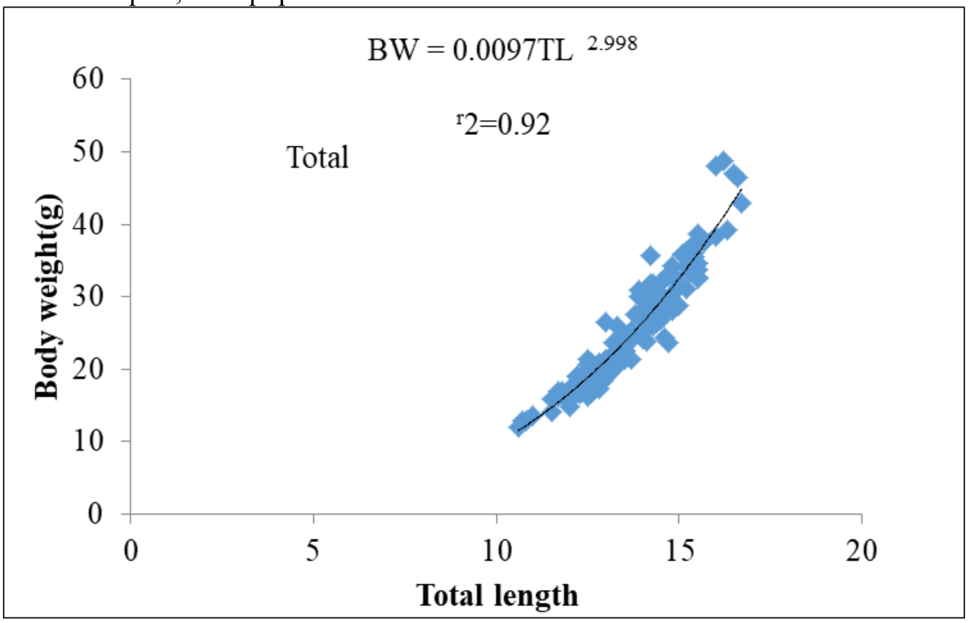

Figure 7: Generalized relationship between the body weight (BW) and total length (TL) of *Labeo angra* from three different regions of Dinajpur, Bangladesh.

In this plot, total populations were 150 and the value of "b" = 2.998 and r^2 = 0.92.

Table 8: Linear equation and power curve equations between total length (TL) and body weight (BD) with coefficient of determination (r^2) of different geographical regions of Bangladesh.

Location	Linear equation	Power curve	Slope, b	Coefficient of determination (r^2)
Punarbhaba River, Kanchan	lnBW=3.207 lnTL-5.2233	BW=0.0054TL3.207	3.207	0.942
Atrai River, Mohanpur	lnBW=3.0578 lnTL-4.7371	BW=0.0088 TL3.058	3.058	0.92
Dhepa River, Ashramghat	lnBW=2.674 lnTL-3.8303	BW=0.0217TL2.674	2.674	0.942
All populations	lnBW=2.998 lnTL-4.6348	BW=0.0097TL2.998	2.998	0.926

Growth pattern inference for *Labeo angra* of three different regions

The growth pattern of each region or stock was presented by the confidence interval of the "b" value in the length-weight relationship equation. When the confidence interval indicates b = 3, the growth of the stock is isometric; when b > 3, the growth is positive allometric; and when b < 3, the growth is negative allometric. The "b" value in the fish stock of Punarbhaba River was 3.207, indicating that the growth of this stock is positive allometric. In the Atrai River, the "b" value was 3.058, so the growth pattern of this stock is isometric. Finally, the "b" value of Dhepa River stock was 2.674, indicating that the growth pattern is negative allometric (Tab. 9).

Table 9: Parameters of the length-weight relationships of *Labeo angra* and their growth pattern in the three studied regions.

Sampling location	Number of fishes	Slope, b	CI of b at 95%	Growth pattern inference
Punarbhaba River, Kanchan	50	3.207	2.9760-3.4387	Positive allometric
Atrai River, Mohanpur	50	3.058	2.7955-3.3203	Isometric
Dhepa River, Ashramghat	50	2.674	2.4822-2.8663	Negative allometric
All populations	150	2.998	2.8597-3.1353	Isometric

Among eighteen morphometric characters, seventeen characters (TL, BW, FL, SL, BD, PFL, PelFL, DFL, AFL, CFL, PL, HL, ED, PrOL, PPL, PPFL, and PDL) were found to be significantly different, while one morphometric character (LAB) was found to be non-significant among the three rivers. There was less variation in morphometric characters of *Labeo angra* between the Punarbhaba and Atrai rivers compared to those collected from the Dhepa River. The Dhepa River population exhibited much more variation compared to the other two rivers stocks, which may be due to differences in location, habitat conditions, food availability, and environmental factors. The similarities between the stocks from the Punarbhaba and Atrai rivers could attributed to their common origin and similar environmental impacts (alkalinity, current pattern, temperatures, and turbidity), as well as food availability, as reported by Mir et al. (2013) and Naeem et al. (2012).

Significant morphometric variations were observed in *Barilius bendelisis* collected from the rivers Gaula and Kosi of the Kumaon region of the Central Indian Himalayas. In contrast to the present study, no significant differences were observed in the head length (HL), length of pectoral fin (PFL), length of pelvic fin (PVFL), or eye diameter (ED); however a significant difference was observed in the length of anal fin base (LAFB) of *Barilius bendelisis* obtained from the rivers Gaula and Kosi (Saxena et al., 2015). In the case of *Labeo ariza* among the stocks of Atrai, Jamuna, and Kangsha rivers reported by (Ahammad et al., 2018); head length, pre-orbital length and maximum body depth of *Cirrhinus reba* among the stocks of Brahmaputra River, Padma River, Karatoya River, and Jamuna River (Ethin et al., 2019).

Morphometric proportions

In the present study, except for TL: SL, TL: HL, TL: BD, and SL: BD, only TL: FL, SL: HL, and HL: ED showed significant variation in *Labeo angra* collected from the three rivers. The observed proportions of morphological characteristics of *Labeo angra* showed only three significant differences. Most of the proportions do not show significant differences likely due to the similar size of fish. In agreement with the present study, no significant variation was recorded in the ratio of TL: SL of *Ompok pabda* among the population of Tentulia, Baleshwer, Payra, and Halda rivers. On the other hand, significant variations were observed in TL: HL and SL:HL of *Ompok pabda* (Chaklader et al., 2016). The varying results of morphological proportions of fish is likely a result of originating from different locations (Naeem et al. 2012).

Meristic characters

In this study, three meristic characters (DFR, PDS, and PPS) were found significant, and five meristic characters (PFR, PeFR, AFR, CFR, and NSLL) were non-significant among the stocks of Atrai, Punarbhaba, and Dhepa rivers.

Similar to the present study, no significant differences were observed in the number of rays in the anal fin, pectoral fin, ventral fin, caudal fin, and the number of scales in the lateral lines of *Chromis chromis* collected from the middle Adriatic (Dulcic, 2005). In *Cirhinus cirrhosus*, there was no significant difference found in the number of scales on lateral line (NSoLL), fin rays in caudal fin (FRCF), fin rays in anal fin (FRAF), or fin rays in pelvic fin (FRPelF) from the hatchery stock of Jessore, baor stock of Gopalganj and river stock of Faridpur (Gain et al., 2017). Among the *Macrognathus pancalus* from four populations from Dhakuria beel, Jessore, Bohnni boar, Gopalgonj, the Arial kha river, Madaripur and the Nabaganga river, Jhenidah, there were no significant differences reported in the anal fin rays (Sarower-E-Mahfuj et al., 2019). Additionally, there were no significant differences reported in pectoral fin rays, caudal fin rays, and scales in lateral lines of *Cirrhinus reba* among the stocks of Brahmaputra, Karatoya, Padma, and Jamuna Rivers. In the present study, it was also observed that the pectoral fin rays (16), pelvic fin rays (9), anal fin rays (7), and caudal fin rays (19) remained constant among the three populations of *Labeo angra*, which was also reported by Ethin et al. (2019). The meristic characteristics of fishes can be influenced by many factors, such as environmental parameters, particularly temperature (Sfakianakis et al., 2011) and genetic factors. Therefore, the observed morphological variations in the present study may be attributed to environmental or genetic differences, or both, among the populations.

Length-weight relationship of *Labeo angra*

In the current study, the total length and weight of fish samples from Punarbhaba River ranged from 12 to 16.7 cm and 14.81 to 47.05 g, from the Atrai River, ranged from 12.2 to 16.2 cm and 18.97 to 48.84 g, and from the Dhepa River, ranged from 10.6 to 16.3 cm and 11.89 to 39.17 g respectively. Ara (2016) reported the total length of *Barilius bendelisis* (7 to 15.6 cm), *Barilius barna* (4.70 to 9.70 cm), *Barilius tileo* (6.18 to 10.8 cm). In this study, the generalized relationships of total length and body weight of *Labeo angra* were: Punarbhaba River, $BW = 0.0054TL^{3.2074}$, Atrai River, $BW = 0.0088\ TL^{3.0579}$, and Dhepa River, $BW = 0.0217TL^{2.6743}$. Ara (2016) conducted an experiment in the Atrai River and observed the relationship between the total length and body weight of *Barilius bendelisis* ($BW = 0.020TL^{2.778}$), *Barilius barna* ($BW = 0.017TL^{2.768}$), and *Barilius tileo* ($BW = 0.026TL^{2.5884}$). In another study, Soomro et al. (2007) reported the length-weight relationship for male ($BW = 0.0039TL^{3.159}$), female ($BW = 0.0072TL^{2.958}$), and combined fish ($BW = 0.0054TL^{3.05}$) of a catfish, *Eutropiichthyes vacha* from Indus River, Sindh, Pakistan.

The value “b” is 3.207 in the Punarbhaba River, indicating that the growth of fish is positive allometric. In the Atrai River “b” value was 3.058, indicating isometric growth, while the “b” value for the fish of Dhepa River was 2.674, indicating negative allometric growth. Mir et al. (2013) found positive allometric growth ($b > 3$) for males, females, and pooled sexes of *Labeo rohita* collected from six drainages of the Ganga Basin, India.

In the present study, high values of the co-efficient of determination (r^2) were observed in all regression analyses. The r^2 values found were 0.942 for the Punarbhaba River, 0.920 for the Atrai River, 0.942 for the Dhepa River, and 0.926 for all populations combined. The r^2 values obtained from the fish samples of the studied regions were close to 1, indicating a strong correlation.

Subba et al. (2009) observed a high correlation ($r^2 = 0.924$) between the length and weight of *Gudusiago danahiae* collected from Biratnagar, Nepal. Sheikh and Ahmed (2018) reported a coefficient of determination (r^2) of 0.972 for males and 0.926 for females of *Schizothorax plagiostomus* collected from the River Jhelum in Kashmir Valley,

Factors such as feeding, sex, maturity, specimen number, area, seasonal effects, degree of stomach fullness, habitat, health, and general fish condition, as well as differences in the observed length ranges of the specimen may affect the length-weight relationships of fish (Hasan et al., 2020). In this study, the growth performances variation from the river to river is likely due to differences in locations, variations of the ecological conditions of the habitats, availability of food, and physiology of the animals.

CONCLUSIONS

The present study findings revealed that morphological characters did not show significant variation between the Punarbhaba and Atrai rivers, and the growth performance of *Labeo angra* in these two rivers was better than that of the Dhepa River. This similarity may be due to the common origin of the rivers, availability of food, and similar environmental impacts. Conversely, morphological variation and negative allometric growth observed in Dhepa River may be due to location differences, ecological conditions of the habitats, availability of food, or physiology of the fish. This study is the first attempt to provide important information on the morphological variations and growth performances of *Labeo angra* from three different rivers of Dinajpur, which will be valuable for the fishery biologists and various government and non-government organizations in sustainable production, management, and conservation of *Labeo angra*.

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MINIȘ RIVER (NERA/DANUBE BASIN) ICHTHYOFAUNA DYNAMIC OVER ONE CENTURY

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KEYWORDS: Carpathians, lotic system, human impact, fish communities dynamic.

ABSTRACT

Compared to older data sources, 13 new fish species for the Miniș River were found: *Phoxinus phoxinus*, *Hucho hucho*, *Barbus balcanicus*, *Rutilus rutilus*, *Carasius gibelio*, *Leucaspius delineatus*, *Ameiurus nebulosus*, *Pseudorasbora parva*, *Cottus gobio*, *Rhodeus amarus*, *Alburnus alburnus*, *Chondrostoma nasus*, *Cobitis elongata*. Eight species known here from the past were still found: *Salmo trutta*, *Squalius cephalus*, *Alburnoides bipunctatus*, *Gobio obtusirostris*, *Barbatula barbatula*, *Eudontomyzon danfordi*, *Sabanejewia balcanica*, *Romanogobio banaticus*. The unnatural structure of the fish communities and the identified changes of the fish zonation along Miniș River reveal a complex set of impacts from human activities on this lotic ecosystem.

RÉSUMÉ: Rivière Miniș (Bassin de la Nera/Danube) dynamique des connaissances sur l'ichtyofaune sur un siècle.

13 nouvelles espèces de poissons ont éte trouvées dans rivière Miniș, en comparison avec des sources de données plus anciennes: *Phoxinus phoxinus*, *Hucho hucho*, *Barbus balcanicus*, *Rutilus rutilus*, *Carasius gibelio*, *Leucaspius delineatus*, *Ameiurus nebulosus*, *Pseudorasbora parva*, *Cottus gobio*, *Rhodeus amarus*, *Alburnus alburnus*, *Chondrostoma nasus*, *Cobitis elongata*. Huit espèces connues ici du passé ont encore été trouvées: *Salmo trutta*, *Squalius cephalus*, *Alburnoides bipunctatus*, *Gobio obtusirostris*, *Barbatula barbatula*, *Eudontomyzon danfordi*, *Sabanejewia balcanica*, *Romanogobio banaticus*. La structure non naturelle des communautés de poissons et certaines des modifications identifiées dans la zonation des poissons le long de la rivière Miniș révélent un ensemble complexe d'activités humaines ayant un impact sur cet écosystème lotique.

REZUMAT: Râul Miniș (bazinul Nera/Dunăre) dinamica cunoașterii ihtiofaunei de-a lungul unui secol.

Au fost găsite 13 specii noi de pești pentru râul Miniș, în comparație cu datele unor surse mai vechi: *Phoxinus phoxinus*, *Hucho hucho*, *Barbus balcanicus*, *Rutilus rutilus*, *Carasius gibelio*, *Leucaspius delineatus*, *Ameiurus nebulosus*, *Pseudorasbora parva*, *Cottus gobio*, *Rhodeus amarus*, *Alburnus alburnus*, *Chondrostoma nasus*, *Cobitis elongata*. Opt specii cunoscute aici din trecut au fost încă regăsite: *Salmo trutta*, *Squalius cephalus*, *Alburnoides bipunctatus*, *Gobio obtusirostris*, *Barbatula barbatula*, *Eudontomyzon danfordi*, *Sabanejewia balcanica*, *Romanogobio banaticus*. Structura nenaturală a comunităților de pești și unele dintre modificările identificate ale zonelor ihtiologice de-a lungul râului Miniș dezvăluie impactul unui set complex de activități umane asupra acestui ecosistem lotic.

INTRODUCTION

The 27 km long Miniș River (its drainage basin covers an area of 244 km²) springs in the Romanian south-west Carpathians in the Rolului Peak (963 m) area, crossing the Aninei Mountains in its middle course through Miniş Gorges, and meets the Nera River on the territory of Bozovici Commune; the Nera Basin to which the Miniş basin belongs, being a very complex and relatively isolated geographical area (*; **; ***; Posea, 2006; Ujvari I, 1972; Diaconu, 1971).

The particular environmental characteristics of this geographical region push the evolutionary processes in founding and preserving valuable fish species communities in this zone (Bănăduc et al., 2023).

The ability, devotion, and perseverance of ground-breaking and revolutionary academic, historical famous scientists like Grigore Antipa and Petru Mihai Bănărescu, to indicate only two of the most iconic Romanian ichthyologists, as well as the succeeding generations of experts in fish biology and ecology, headed to the launch and developing in Romania a remarkable ichthyology school with outstanding scientific results. The nearly three centuries of extensive fish-related field work and analyses have led to the accumulation of some useful information in this respect, and novel research can take the comparative advantages in principle from the previous results obtained in different sampling campaigns.

The geographically remote and isolated hydrographic basins of the Carpathian Mountains are more or less known but in general are characterised by relatively numerous conservative and economic valuable fish species communities (Curtean-Bănăduc et al., 2019; Koščo et al., 2014; Bănăduc et al., 2013) in the more and more numerous cases when anthropogenic impacts are not a major driving force there (Bănăduc et al., 2023, 2021, 2020; Bănăduc 2010; Popa et al., 2019; Curtean-Bănăduc et al., 2014).

This paper intends to refresh and fill a gap between the past and present, namely between the relatively far away in time ichthyological data (Antipa, 1909; Bănărescu, 1964), and the present, in this geographicaly remote and relatively isolated watershed, regarding fish fauna composition and distribution dynamic in the Miniș River.

MATERIAL AND METHODS

This ichthyofauna research was conducted between 2022-2024, mainly in the project "Îmbunătăţirea stării de conservare a speciilor şi habitatelor de interes conservativ din Parcul Naţional Cheile Nerei-Beuşniţa şi ariile naturale de interes comunitar şi naţional suprapuse prin revizuirea planului de management integrat" POIM Cheile Nerei-Beușnița/"Improving the conservation status of species and habitats of conservation interest in the Cheile Nerei-Beușnița National Park and the overlapping natural areas of European Community and national interest by revising the integrated management plan" POIM Cheile Nerei-Beușnița.

The presence/absence of diverse fish species in the Miniș River was reported based on electrofishing sampling (with a Hans Grassl IG 600 TL device), in time (45 minutes)/effort unit. The sampling was conducted at 18 sampling sections of 100 m in length every two to three kilometers starting from the river springs area to its outflow in the Nera River. After fast visual identification of the sampled fish, all of them were released unharmed in their habitat of origin.

RESULTS

The researched fish species were found in the below (Tab. 1) 17 lotic sampling stations and in one lentic station.

Table 1: (A) Fish species reported by Antipa in the study area at the end of the 19th century (Antipa, 1909). (B) Fish species reported by Bănărescu in the study area in the first part of the 20th century (1964). (C) Fish species idetifified in the period 2022-2024. (–) No historical data or no fish sampled.

Sampling stations	Location, coordinates and altitude	Sampling stations habitat characteristics	Fish species
M1	Miniș springs wetland area N45º01.190’ E21º48.222’ 655 m altitude	Muddy substrate, dominated by fallen vegetation from trees (leaves, branches, etc.). Abundant riparian vegetation, consisting of trees and shrubs (alders, beeches, blackberryes). The lotic sector crosses a forest. No anthropogenic changes were observed. The water was very shallow in the sampling periods to can allow the fish presence.	A – B – C –
M2	2 km downstream the springs area N45º01.320’ E21º49.105’635 606 m	Substrate dominated by boulders, gravel and sand, with abundant, luxuriant riparian vegetation consisting of trees (beech, alder) and shrubby vegetation. There are small waterfalls that contribute to the oxygenation of the water. Stable banks, without signs of erosion. No anthropogenic impact was observed.	A – B *Salmo trutta* C *Salmo trutta* A – B – C *Phoxinus phoxinus*
M3	4 km downstream the springs area N45º01.815’ E21º50.523’ 562 m	Substrate dominated by gravel and sand, as well as boulders. There were observed vegetation depositions. The riparian vegetation is well represented and in good condition, containing trees and shrubs, stable banks, without signs of erosion. Anthropogenic impact is present as forestry roads crossing the minor bed of the Miniș River.	A – B *Salmo trutta* C *Salmo trutta* A – B – C *Phoxinus phoxinus*

Table 1 (continued): (A) fish species signalled by Antipa in the study area (A) at the end of the 19th century (Antipa, 1909), (B) fish species signalled in the area by Bănărescu in the study area in the first part of the 20th century (1964), (C) Miniş River sampling stations location, present habitat characteristics and fish species presence in the period 2022-2024; (–) no old data or no fish sampled.

Sampling stations	Location, coordinates and altitude	Sampling stations habitat characteristics	Fish species
M4	8 km downstream the springs area N 45º02.056' E 21º51.265' 530 m	Substrate consisting of boulders and gravel, and covered by organic matter. Riparian habitat in favourable condition. Abundant riparian vegetation, consisting predominantly of tall trees and shrubs. No anthropogenic impact was observed. Stable banks, without signs of erosion.	A – B *Salmo trutta* C *Salmo trutta* A – B – C *Phoxinus phoxinus*
M5	10 km downstream the springs area N 45º01.795' E 21º51.681' 513 m	Substrate consisting of gravel, sand and boulders. Substrate covered by organic matter. Riparian habitat in favourable condition. Abundant riparian vegetation consisting predominantly of tall trees, shrubs and herbaceous vegetation. No anthropogenic impact was observed. Stable banks, without signs of erosion.	A – B *Salmo trutta* C *Salmo trutta* A – B – C *Phoxinus phoxinus* A – B – C – *Hucho hucho* A – B – C – *Barbus balcanicus*
M6	Upstream Cârşa Waterfall N 45º01.612' E 21º53.152' 462 m	Substrate consisting of gravel and boulders, isolated muddy substrate. Substrate covered by organic matter. Riparian habitat in favourable condition. Abundant riparian vegetation consisting predominantly of tall trees, shrubs and herbaceous vegetation. No anthropogenic impact was observed.	A – B *Salmo trutta* C *Salmo trutta* A – B – C *Phoxinus phoxinus* A – B – C – *Hucho hucho* A – B – C – *Barbus balcanicus* A – B – *Squalius cephalus* C – *Squalius cephalus* A – B – C – *Rutilus rutilus*

Table 1 (continued): (A) fish species signalled by Antipa in the study area (A) at the end of the 19th century (Antipa, 1909), (B) fish species signalled in the area by Bănărescu in the study area in the first part of the 20th century (1964), (C) Miniș River sampling stations location, present habitat characteristics and fish species presence in the period 2022-2024; (–) no old data or no fish sampled.

M7	Upstream Gura Golumbului Lake N 45º01.125' E 21º54.264' 434 m	Substrate consisting of gravel, boulders and sand. Substrate covered by organic matter. Riparian habitat in favourable condition. Abundant riparian vegetation, consisting predominantly of trees, shrubs, and herbaceous vegetation. Anthropogenic impact present: old bridge providing access to the property. Stable banks, without signs of erosion.	A – B *Salmo trutta* C *Salmo trutta* A – B – C *Phoxinus phoxinus* A – B – C – *Hucho hucho* A – B – C – *Barbus balcanicus* A – B – *Squalius cephalus* C – *Squalius cephalus* A – B – C – *Rutilus rutilus*
M8	Gura Golumbului Lake N 45º00.781' E 21º54.900' 430 m	Substrate consisting of mud and sand with aquatic vegetation. Riparian vegetation specific to the lentic ecosystem consisting predominantly of reeds/ grassy vegetation, surrounded by trees. Stable banks, without signs of erosion. The Miniș lotic ecosystem was transformed into a lentic one by blocking the river course with a concrete dam, about 20 m high. Present fish species are characteristic of the lentic ecosystem, including the new-comer invasive species. The dam is equipped with relatively functional fish ladders.	A – B – C *Phoxinus phoxinus* A – B – *Squalius cephalus* C – *Squalius cephalus* A – B – C – *Rutilus rutilus* A – B – C – *Carasius gibelio* A – B – C – *Leucaspius delineatus* A – B – C – *Ameiurus nebulosus* A – B – C – *Pseudorasbora parva*

Table 1 (continued): (A) fish species signalled by Antipa in the study area (A) at the end of the 19th century (Antipa, 1909), (B) fish species signalled in the area by Bănărescu in the study area in the first part of the 20th century (1964), (C) Miniș River sampling stations location, present habitat characteristics and fish species presence in the period 2022-2024; (–) no old data or no fish sampled.

M9	Downspstream Gura Golumbului Lake N 45º00.709’ E 21º54.962’ 423 m	Substrate consisting of boulders, gravel, sand, and isolated mud. Abundant organic load present on the substrate. Riparian habitat in favourable condition. Abundant riparian vegetation, consisting predominantly of tall trees (*Salix alba*), reeds and grassy vegetation. Stable banks, without signs of erosion. The human impact is represented by the modification of the minor and major river beds, as well as the configuration of the river, through the presence of hydrotechnical construction.	A – B – C *Phoxinus phoxinus* A – B – *Squalius cephalus* C – *Squalius cephalus* A – B – C – *Rutilus rutilus* A – B – *Alburnoides bipunctatus* C – *Alburnoides bipunctatus* A – B – *Gobio obtusirostris* C – *Gobio obtusirostris* A – B – C – *Barbus balcanicus*
M10	Miniș-Poneasca rivers confluence N 45º00.607’ E 21º57.266’ 354 m	Substrate consisting of boulders and gravel. Riparian habitat in favorable condition. Abundant riparian vegetation consisting predominantly of tall trees, shrubs and herbaceous vegetation. Stable banks, without signs of erosion. The confluence with Poneasca stream. No anthropogenic impact was observed in the area.	A – B – C *Phoxinus phoxinus* A – B – *Squalius cephalus* C – *Squalius cephalus* A – B – C – *Rutilus rutilus* A – B – *Alburnoides bipunctatus* C – *Alburnoides bipunctatus* A – B – *Barbatula barbatula* C – *Barbatula barbatula* A – B – C – *Barbus balcanicus*

Table 1 (continued): (A) fish species signalled by Antipa in the study area (A) at the end of the 19th century (Antipa, 1909), (B) fish species signalled in the area by Bănărescu in the study area in the first part of the 20th century (1964), (C) Miniș River sampling stations location, present habitat characteristics and fish species presence in the period 2022-2024; (–) no old data or no fish sampled.

M11	Upstream trout farm Miniș N 45º00.221’ E 21º58.131’ 322 m	Substrate consisting of boulders and gravel. Riparian habitat in favorable condition. Abundant riparian vegetation consisting predominantly of tall trees, shrubs and herbaceous vegetation. Stable banks, without signs or erosion. The confluence with Poneasca River is here. The presence of the Miniș trout farm can represent a threat through the possible escape of invasive species or species that are not characteristic for this river sector.	A – B – C – *Cottus gobio* A – B – *Eudontomyzon danfordi* C – *Eudontomyzon danfordi* A – B – C *Phoxinus phoxinus* A – B – *Squalius cephalus* C – *Squalius cephalus* A – B – C – *Rutilus rutilus* A – B – *Alburnoides bipunctatus* C – *Alburnoides bipunctatus* A – B – *Barbatula barbatula* C – *Barbatula barbatula* A – B – C – *Barbus balcanicus*
M12	Downstream trout farm Miniș N 44º59.453’ E 21º58.733’ 310 m	Substrate consisting of gravel and sand. Riparian habitat in favourable condition. Abundant riparian vegetation, consisting predominantly of tall trees and grassy vegetation. Stable banks, without signs of erosion. No anthropogenic impact was observed in the area.	A – B – C – *Cottus gobio* A – B – C *Phoxinus phoxinus* A – B – *Squalius cephalus* C – *Squalius cephalus* A – B – *Alburnoides bipunctatus* C – *Alburnoides bipunctatus* A – B – *Sabanejewia balcanica* C – *Sabanejewia balcanica* A – B – C – *Barbus balcanicus*

Table 1 (continued): (A) fish species signalled by Antipa in the study area (A) at the end of the 19th century (Antipa, 1909), (B) fish species signalled in the area by Bănărescu in the study area in the first part of the 20th century (1964), (C) Miniş River sampling stations location, present habitat characteristics and fish species presence in the period 2022-2024; (–) no old data or no fish sampled.

M13	Upstream Busu Pension N 44º58.602' E 21º59.121' 297 m	Substrate consists of gravel and sand, isolated boulders and mud. Water has high organic load. On certain sections, reduced water speed, relatively laminar water flow. Riparian habitat in favourable condition. Abundant riparian vegetation, consisting predominantly of tall trees and grassy vegetation. Stable banks, without signs of erosion. No anthropogenic impact was observed.	A – B – C – *Cottus gobio* A – B – *Barbatula barbatula* C – *Barbatula barbatula* A – B – *Alburnoides bipunctatus* C – *Alburnoides bipunctatus* A – B – *Sabanejewia balcanica* C – *Sabanejewia balcanica* A – B – C – *Barbus balcanicus*
M14	Downstreamm Busu Pension N 44º57.426' E 21º59.410' 270 m	Substrate consisting of boulders and sand. Water depth from 40 cm to 1.4 m in pits. Width between 10-14 m. High turbidity of the water. Riparian habitat in favourable condition. Abundant riparian vegetation, consisting predominantly of tall trees and shrubs. High degree of water shading. Stable banks, without signs of erosion. The anthropogenic impact consists of a forestry road crossing through the minor river bed.	A – B – C – *Cottus gobio* A – B – *Barbatula barbatula* C – *Barbatula barbatula* A – B – *Alburnoides bipunctatus* C – *Alburnoides bipunctatus* A – B – *Squalius cephalus* C – *Squalius cephalus* A – B – *Sabanejewia balcanica* C – *Sabanejewia balcanica* A – B – C – *Barbus balcanicus* A – B – C – *Rhodeus amarus*

Table 1 (continued): (A) fish species signalled by Antipa in the study area (A) at the end of the 19th century (Antipa, 1909), (B) fish species signalled in the area by Bănărescu in the study area in the first part of the 20th century (1964), (C) Miniș River sampling stations location, present habitat characteristics and fish species presence in the period 2022-2024; (–) no old data or no fish sampled.

M15	2 kn upstream Bozovici locality N 44º56.900' E 21º59.895' 268 m	Substrate consisting predominantly of sand and gravel, isolated boulders. Average depth 40-60 cm to 1.3 m in pits. Width between 10-12 m. High turbidity of the water. Riparian habitat in favorable condition. Abundant riparian vegetation, consisting predominantly of tall trees, shrubs, herbaceous vegetation. High degree of water shading. Stable banks, without signs of erosion. No anthropogenic impact was identified.	A –, B – C – *Cottus gobio* A –, B – C – *Phoxinus phoxinus* A – B – *Barbatula barbatula* C – *Barbatula barbatula* A – B – *Alburnoides bipunctatus* C – *Alburnoides bipunctatus* A – B – *Squalius cephalus* C – *Squalius cephalus* A – B – *Sabanejewia balcanica* C – *Sabanejewia balcanica* A –, B – C – *Barbus balcanicus* A – B – *Romanogobio banaticus* C – *Romanogobio banaticus*
M16	Bozovici locality N 44º55.944' E 22º00.176' 260 m	Substrate consisting predominantly of cobblestones and sand. Average depth 30 cm-1.2 m. Width between 10-12 m. High turbidity of the water. Riparian habitat in favorable condition. Abundant riparian vegetation, consisting predominantly of tall trees, shrubs, herbaceous vegetation. High degree of water shading. Stable banks, without signs of erosion. The human impact is represented by household waste present on the river banks.	A –, B – C – *Cottus gobio* A – B – *Eudontomyzon danfordi* C – *Eudontomyzon danfordi* A – B – *Barbatula barbatula* C – *Barbatula barbatula* A – B – *Alburnoides bipunctatus* C – *Alburnoides bipunctatus* A –, B – C – *Alburnus alburnus* A – B – *Squalius cephalus* C – *Squalius cephalus* A – B – *Sabanejewia balcanica* C – *Sabanejewia balcanica* A – B – *Romanogobio banaticus* C – *Romanogobio banaticus* A –, B – C – *Rhodeus amarus*

Table 1 (continued): (A) fish species signalled by Antipa in the study area (A) at the end of the 19th century (Antipa, 1909), (B) fish species signalled in the area by Bănărescu in the study area in the first part of the 20th century (1964), (C) Miniş River sampling stations location, present habitat characteristics and fish species presence in the period 2022-2024; (–) no old data or no fish sampled.

M17	2 km upstream Miniş-Nera rivers confluence N 44º55.087' E 21º59.971' 250 m	Substrate consisting predominantly of cobblestones and sand. Average depth 30 cm-1.4 m. Width between 10-12 m. High turbidity of the water. Organic load deposited on the substrate, bioderma present. Riparian habitat in favorable condition. Abundant riparian vegetation, consisting predominantly of tall trees (*Alnus glutinosa*, *Salix alba*) shrubs, grassy vegetation. High degree of water shading. Stable banks, without signs of erosion. Anthropogenic impact: the presence of an access road that facilitates the passage of motorized vehicles through the minor river bed.	A – B – *Barbatula barbatula* C – *Barbatula barbatula* A – B – *Alburnoides bipunctatus* C – *Alburnoides bipunctatus* A –, B – C – *Alburnus alburnus* A –, B – C – *Barbus balcanicus* A – B – *Gobio obtusirostris* C – *Gobio obtusirostris* A – B – *Squalius cephalus* C – *Squalius cephalus* A – B – *Sabanejewia balcanica* C – *Sabanejewia balcanica* A – B – *Romanogobio banaticus* C – *Romanogobio banaticus* A –, B – C – *Rhodeus amarus* A – B – C – *Chondrostoma nasus* A – B – C – *Cobitis elongata*

Table 1 (continued): (A) fish species signalled by Antipa in the study area (A) at the end of the 19th century (Antipa, 1909), (B) fish species signalled in the area by Bănărescu in the study area in the first part of the 20th century (1964), (C) Miniș River sampling stations location, present habitat characteristics and fish species presence in the period 2022-2024; (–) no old data or no fish sampled.

M18	Miniș-Nera rivers confluence N 44º54.766' E 22º00.090' 245 m	Substrate consisting mainly of sand, gravel, and silt. Average depth 20 cm-1 m. Width between 10-15 m. Water high turbidity. Riparian habitat in favorable condition. Abundant riparian vegetation, consisting mainly of grassy vegetation with tall trees (*Alnus glutinosa*, *Salix alba*) and shrubs. Stable banks, without signs of erosion. Anthropogenic impact: the presence of an access road through which motorized vehicles pass through the minor riverbed. Active riverbed mineral exploitation present in the vicinity of this sampling station.	A – B – *Barbatula barbatula* C – *Barbatula barbatula* A – B – *Alburnoides bipunctatus* C – *Alburnoides bipunctatus* A –, B – C – *Alburnus alburnus* A – B – C – *Barbus balcanicus* A – B – *Gobio obtusirostris* C – *Gobio obtusirostris* A – B – *Squalius cephalus* C – *Squalius cephalus* A – B – *Sabanejewia balcanica* C – *Sabanejewia balcanica* A – B – *Romanogobio banaticus* C – *Romanogobio banaticus* A –, B – C – *Rhodeus amarus* A –, B – C – *Chondrostoma nasus* A – B – C – *Cobitis elongata*

DISCUSSION

Natural, semi-natural and human induced environmental conditions and ichthyofauna differ in space and in time in the Carpathians hydrographical net (Afanasyev et al., 2023; Bănăduc et al., 2021, 2017, 2013, 2012, 2010, 2017, Didenko et al., 2014; Popa et al., 2019, 2015, 2013; Curtean-Bănăduc and Bănăduc, 2008; Simalcsik and Bates, 1973) the ecosystems characteristics differences controlled the founding of fish zonation grounded on the key indicator fish species. The Carpathians' lotic systems springing in mountain areas, like the river studied here, have five such specific fish zones: brown trout zone, grayling and mediterranean barbell zone, nase zone, barbel zone, and carp zone (Bănărescu, 1964). Some deviations from this natural zones were identified in this study, and are commented below.

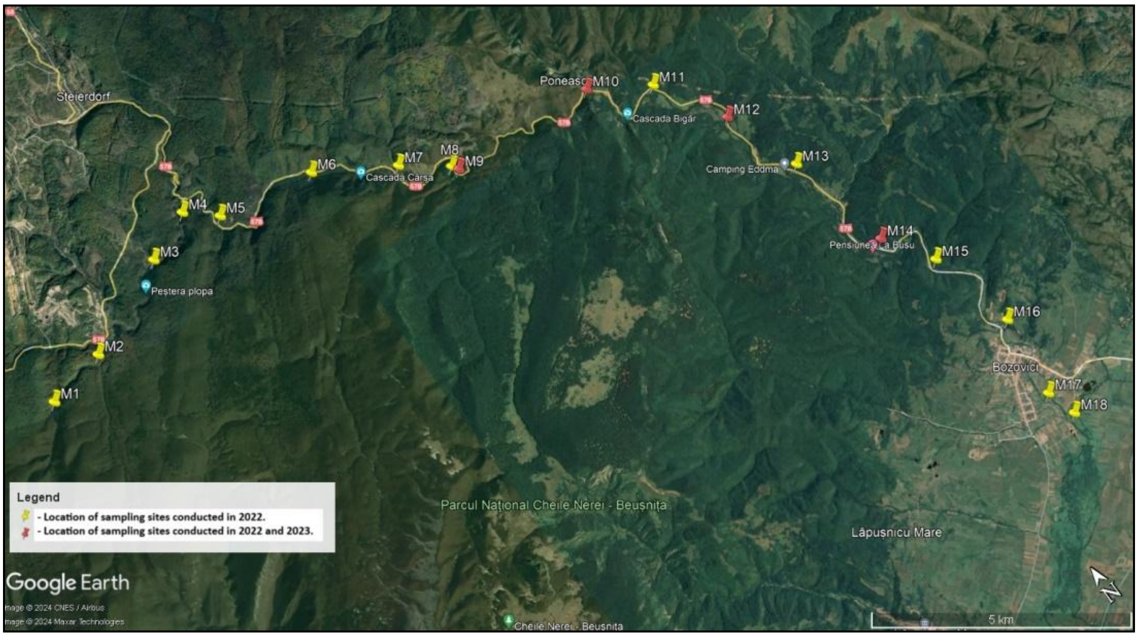

Figure 1: Sampling stations along the Miniș River.

The Miniș River springs area (**M1**) is an altitude wetland area with soft substrate and fluctuating low water levels, a good enough reason to explain the absence of fish here.

The downstream springs sector (**M2-4**) cover a mountainous habitat and the most characteristic fish species *Salmo trutta* appeared along the last century. The absence of *Cottus gobio* here can be explained by continuous introductions of trout over long periods, which in the long-term might eliminate this much less aggressive fish. *Cottus gobio* appear downstream where the variation of the habitat allows it to hide and survive. The appearance of *Phoxinus phoxinus* downstream in the last decades is interesting and can be a sign of climate change.

Downstream (**M5-7**), the significant increase of the water flow and the diversification of the habitat characteristics constantly enriched the fish diversity. *Squalius cephalus* was known here in the last century, but lately species like *Barbus balcanicus*, and *Rutilus rutilus* appeared. *Rutilus rutilus* presence can be explained from the downstream lake influence. The upstream *Salmo trutta* and *Phoxinus phoxinus* species remain in these sectors too. A totally unexpected, and unknown until now, was the appearance of *Hucho hucho*, the flagship species for the Danube Basin that has been decreasing constantly as abundance and range over the last century (Curtean-Bănăduc et al., 2019; Bănăduc et al., 2013).

In the **M8** area is the major human induced impact on the Miniș River, namely the anthropogenic Gura Golumbului Dam lake. The building of a dam here converts a free-flowing lotic sector of the Miniș River into a lentic habitat, supporting impoundment-dwelling fish that free-flowing river segments might not naturally sustain. This lake is now an efficient sediment trap; sediments wrongly managed here can adversely impact the area covered by the lake and the downstream lotic segments' fish habitats. This impoundment transformed the landscape of the Miniș Watershed, fragmenting it and modifying its water and sediment flow. This important factor impacts considerably the river ecosystems, moderately affecting the migratory fish species and the connectivity among diverse fish populations under normal water and sediment management conditions. However, these impacts become far more pronounced under "accidental" mismanagement scenarios (Bănăduc et al., 2024). Next to the over a century presence here of *Squalius cephalus* and *Gobio obtusirostris*, new species including *Rutilus rutilus, Carasius gibelio, Leucaspius delineates, Ameiurus nebulosus*, and *Pseudorasbora parva* appeared lately mainly due to the presence of the dam lake and its water mismanagement (Bănăduc et al., 2024). *Salmo trutta* disappears from here to downstream of the river. This is the first time that *Phoxinus phoxinus* was found here.

The natural changings in the lotic habitats due to increasing of the water flow and the habitat diversity, and the influence of the lake presence and its poor water and sediment management induce significant changes in fish diversity. In **M9-10,** the well known species in the last century *Squalius cephalus* remain present till the end of the Miniș River. The newly-recorded species in the last decades, *Phoxinus phoxinus,* occupies the most lower river sector. The new-comer, *Rutilus rutilus,* due to the lake proximity remain here. *Gobio obtusirostris,* well known here from the past and now more encouraged also due to the proximity of the lake, is present here too. *Barbus balcanicus* is still present now too, even if it was not known in the past. The known presence in the last century till now of *Alburnoides bipunctatus* was noted.

In **M11-16,** *Cottus gobio* unknown until the present, appears only recently in the middle part of this Carpathian stream and not like usually in upper sectors; previously unrecorded in this area, *Cottus gobio* has only recently been identified in the middle reaches of the studied river. This may be a relatively new entrance from the Miniș tributaries or the much downstream Nera River. Interesting is a new species appearance in this sector, unknown in the last century until now, namely *Eudontomyzon danfordi*, which will appear only once more in a much downstream station near the confluence with Nera River. *Phoxinus phoxinus* continues its presence only in the uppermost two stations of this sector, disappearing downstream, to reappear accidentally, in a much lower single station. In this sector the well-known species in the last century, *Squalius cephalus*, known here in the last century until now, remain present until the confluence of the Miniș River with the Nera River. The new-comer, *Rutilus rutilus,* is present due to the lake proximity; M11 being the last lower station where it was found now in the studied river. *Alburnoides bipunctatus* continues its known presence over a century all along this sector and to the end of the river. *Barbatula barbatula* continues its presence all along this sector and to the end of the river. *Barbus balcanicus* continues its presence all along this sector and to the end of the river. From M12, *Sabanejewia balcanica,* known here over a century, appears for the first time in the river and continues to be present in all this sector and to the end of this river. For the first time, *Rhodeus amarus* appears and continues to be found till the end of this sector and of the river. A new appearance in M15 is *Romanogobio banaticus,* well known in the last century here, continues to be present till the end of this sector and of the river. *Alburnus alburnus* not known until now in this river, appears here.

In **M17-18**, the lowest Miniș River sector, the well known for over a century fish species, like *Barbatula barbatula, Alburnoides bipunctatus, Gobio obtusirostris, Squalius cephalus, Sabanejewia balcanica* and *Romanogobio banaticus* continued to be present. These not known until now species are present here as in some upstream sectors: *Alburnus alburnus*, *Barbus balcanicus*, and *Rhodeus amarus.* For the first time, these unknown until now species appeared in this river sector: *Chondrostoma nasus* and *Cobitis elongata.* The improving of fish species richness is due to the changing of habitats and near proximity of Nera River confluence.

In summary, a series of 13 new fish species for this river watershed were identified in this study, in comparison with older reliable data sources: *Phoxinus phoxinus, Hucho hucho, Barbus balcanicus, Rutilus rutilus, Carasius gibelio, Leucaspius delineatus, Ameiurus nebulosus, Pseudorasbora parva, Cottus gobio, Rhodeus amarus, Alburnus alburnus, Chondrostoma nasus*, and *Cobitis elongata*. Only eight species that were known here from past records were still found: *Salmo trutta*, *Squalius cephalus*, *Alburnoides bipunctatus*, *Gobio obtusirostris, Barbatula barbatula, Eudontomyzon danfordi, Sabanejewia balcanica*, and *Romanogobio banaticus*.

Antipa (1909) makes a general coverage of ichtyofauna of Romania since the beginning of the 19th century without covering the Miniș River basin; this being covered in detail since the middle of the 20th century by Bănărescu (1964).

This research almost doubled the number of fish species recorded as present in the Miniș River.

Special attention should be given to some species of high conservation interest such as: *Hucho hucho, Chondrostoma nasus* and *Romanogobio banaticus,* which has a diminishing distribution (Bănăduc et al., 2023).

In the upper presented context, the alien invasive species *Carasius gibelio, Ameiurus nebulosus*, and *Pseudorasbora parva* (Drăgan et al., 2024; Anastasiu et al., 2017) should be continuously monitored.

The skewed structure of the fish communities and some of the identified modifications of the fish zonation along the Miniș River reveal that a complex set of human activities impact this lotic ecosystem. Based on these new data, an adjusted management plan of the Miniș Watershed should be built and integrated at the level of the Nera River watershed management plan.

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GENDER, AGE, AND SEASONAL VARIATION IN SCALE CHARACTERISTICS OF *ALBURNUS SELLAL* HECKEL, 1843 FROM THE TIGRIS RIVER (TURKEY) A GEOMETRIC MORPHOMETRIC STUDY

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KEYWORDS: Cyprinidae, landmark, morphometric, geometry, scale, shape, Turkey.

ABSTRACT

In this study, 37 female and 40 male *Alburnus sellal* individuals from the Tigris River were analyzed. Using geometric morphometrics, significant differences in scale size between sexes were found, but none in shape. Seasonal and age groups showed significant differences in both size and shape, with females and autumn individuals having larger scales. Scale size increased with age. PCA showed variation across age, season, and sex, while CVA and DFA revealed shape differences between age and seasonal groups, but not between sexes.

RESUMEN: Variación sexual, de edad y estacional en las características de escamas de *Alburnus sellal* Heckel, 1843 del río Tigris (Turquía): un estudio morfométrico geométrico.

En este estudio, se analizaron 37 individuos hembras y 40 machos de *Alburnus sellal* del río Tigris. Utilizando morfometría geométrica, se encontraron diferencias significativas en el tamaño de las escamas entre los sexos, pero no en la forma. Los grupos estacionales y de edad mostraron diferencias significativas tanto en tamaño como en forma, con las hembras y los individuos del otoño teniendo escamas más grandes. El tamaño de las escamas aumentó con la edad. El análisis PCA mostró variaciones según la edad, la estación y el género, mientras que el CVA y el DFA revelaron diferencias en la forma entre los grupos de edad y de estación, pero no entre los sexos.

REZUMAT: Sexul, vârsta și variația sezonieră a caracteristicilor dimensionale la *Alburnus sellal* Heckel, 1843 din râul Tigru (Turcia) un studiu morfometric geometric.

În acest studiu, au fost analizați 37 de indivizi femele și 40 de masculi de *Alburnus sellal* din râul Tigru. Folosind morfometrie geometrică, s-au găsit diferențe semnificative între mărimea solzilor între sexe, dar nu și în forma lor. Grupurile sezoniere și de vârstă au prezentat diferențe semnificative atât în mărime, cât și în formă, femelele și indivizii de toamnă având solzi mai mari. Mărimea solzilor a crescut odată cu vârsta. Analiza PCA a arătat variații în funcție de vârstă, sezon și sex, în timp ce analizele CVA și DFA au evidențiat diferențe de formă între grupurile de vârstă și de sezon, dar nu și între sexe.

INTRODUCTION

Image techniques have revealed numerous new features of fish scales. Fish scales differ depending on the sex, age, diet, habitat, and genetic makeup of the fish. The silhouette of the scales is important not only in species discrimination in systematic studies but also in revealing intra-specific variations and determining differentiation between populations (Roberts, 1993, Braeger et al.2017; Ibáñez and Jawad, 2018; Ibáñez et al. 2023). Consequently, fish scales are an important tool in identifying and monitoring fish populations (Trueman and Moore, 2007).

Geometric morphometric methods for analyzing fish scales have been shown to be a reliable and practical tool for distinguishing between challenging genera, species, geographic variants, and local populations. In addition, these methods are also effective for assessing the effects of habitat on scale morphology, as well as for indicating age and seasonal variation. These analyses provide important biometric data related to scale shape (Bilici, 2020; Dörtbudak et al., 2022; Rohlf and Marcus, 1993; Vignon, 2012; Zelditch et al., 2004).This method is also more economical, easier, and harmless compared to other methods, allowing for sampled fish to be released again and for the inspection and monitoring of many samples from populations (Bilici et al., 2016; Çicek et al., 2016; Ibáñez et al., 2007, 2009; Poulet et al., 2005; Staszny et al., 2013).

The Sellal bleak, *Alburnus sellal* Heckel, 1843, also known as "Gümüşbalığı" in Turkey, belongs to the Leuciscidae family and is native to the Euphrates, Tigris, Zoreh, Persis, and Hormuz River basins (Turkey, Syria, Iraq, and Iran) (Coad, 2010; Çiçek et al., 2023a, b; Jouladeh-Roudbar et al., 2020; Saad et al., 2023; Shahraki et al., 2022, 2023). This species inhabits lakes, reservoirs, and all kinds of streams and rivers from the cold Anatolian highlands down to the subtropical Shatt al Arab and Iranian Gulf rivers (Çiçek et al. 2023b; Saad et al. 2023).

This fis species, frequently reported as *Albumus mossulensis* in several studies (Coad, 1996; Kaya et al. 2016; Kuru, 1978), was finnally determined to be a synonym for *Alburnus sellal* mainly due to significant overlaps in lateral line, gill rake, anal fin ray, and also vertebra number intervals (Bogutskaya, 1997). Subsequent morphological and molecular studies further supported Bogutskaya's interpretation, leading to its acceptance as a synonym of the *Alburnus sellal* species (Bektas et al., 2020; Mangit and Yerli, 2018; Mohammadian-Kalat et al., 2017).

Alburnus sellal has been the subject of extensive and multidisciplinary research, with studies delving into various aspects (Banaee et al., 2023; Banaee et al., 2014; Bostanci et al., 2015; Dane and Şişman, 2020; Esmaeili et al., 2018; Mangit and Yerli, 2018; Mousavi-Sabet et al., 2013). Additionally, numerous investigations into its biology and ecology have been carried out in Iraq (Jawad, 2004; Mohamed et al., 2016), Iran (Esmaeili and Ebrahimi, 2006; Ergene, 1993; Mousavi-Sabet et al., 2013; Parsa et al., 2011), and Turkey (Basusta and Cicek, 2006; Ozdemir et al., 1993; Parmaksız et al. 2018; Türkmen and Akyurt, 2000; Uçkun and Gökçe, 2015; Yıldırım et al., 2003; Yıldırım et al., 2007).

This research aims to evaluate the effectiveness of using a landmark-based, geometric morphometric approach to describe fish scale morphology in *Alburnus sellal* and to distinguish differences between seasons, age groups, and male and female individuals.

MATERIAL AND METHODS

In this research, we collected 77 specimens (37 female and 40 male) of *Alburnus sellal* from the Tigris River (Fig. 1).

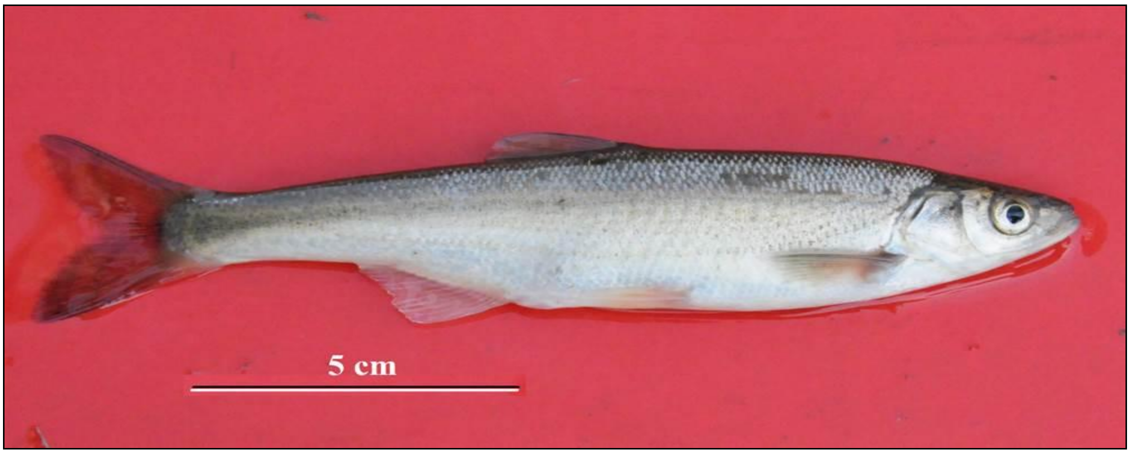

Figure 1: The overall body appearance of *Alburnus sellal* (Tigris bream), Tigris River (photo E. Ünlü).

Samples localities are shown in figure 2, and the seasonal, sexual, and age distributions of the samples, as well as some water parameters of the locality where they were collected, are given in table 1.

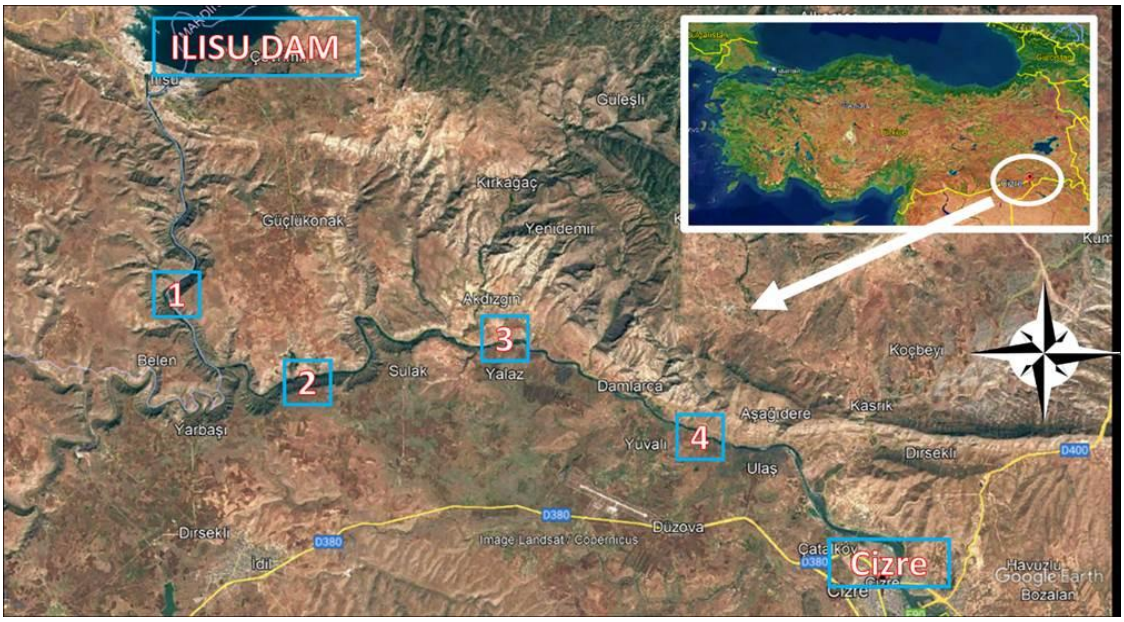

Figure 2: Map of the study area were the smples were obtained.
Sample localities (1-Tigris River (Güçlükonak), 2-Tigris River (Güçlükonak), 3-Tigris River (Akdizgin), 4-Tigris River (Damlarca).

Table 1: Samples distribution and water parameters of the study.

Season	Female	Male	Total
Autumn (November)	2	–	2
Winter	1	–	1
Spring (April)	22	25	47
Summer (July)	13	14	27

	Age				
	II	III	IV	V	VI
Sample number	12	45	17	2	1

Date	Water temperature (°C)	pH	Dissolved oxygen (O_2)	$\%O_2$	Electrical conductivity (EC) µS/cm
26.04.2021	17.7	8.3	9.11	101	306
01.07.2021	24.6	7.86	7.67	97.2	474
04.11.2021	13.5	8.2	8.62	96.8	365

The sex of each fish was determined by observing their gonads. Scales from the front and upper sections of the lateral lines of the dorsal fins were taken to determine their age and morphology. The fish scales tissue was cleaned with 5% NaOH for two hours, then washed with distilled water, and immersed in 96% ethanol for several minutes to remove any remaining water. Following this the scales were placed between two slides and photographed by an stereo microscope (Olympus SZX7, Tokyo, Japan) and a digital camera (OLYMPUS Camedia C-5060 5.1 MP w/4x Optical Zoom, Tokyo, Japan) under 20× and 40× magnifications. Images were analyzed by geometric morphometric procedure (Zelditch et al., 2004; Rohlf and Marcus, 1993; Bookstein, 1991). Subsequently, six landmarks (Fig. 3) were digitized using tpsDig ver. 2.32 (Rohlf, 2015) software, and Procrustes analysis was conducted. Following the separation of shape and size (centroid size = CS)of the samples, Procrustes ANOVA, PCA, CVA/MANOVA, and DFA analyses were performed using Morpho J1.06d (Klingenberg, 2011). R Core Team (2019) and Jamovi Ver. 2.4 (2023) programs.

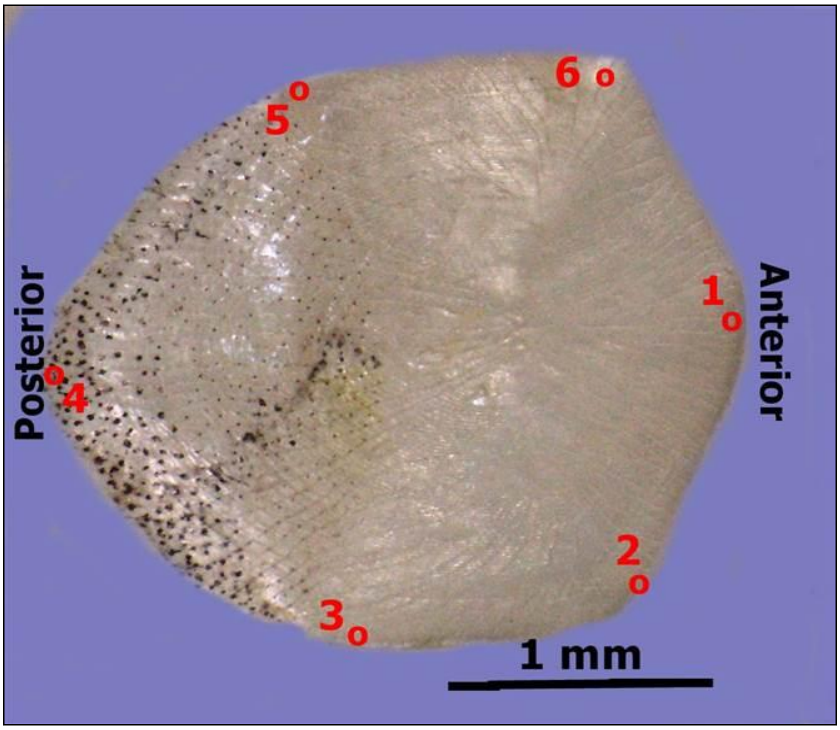

Figure 3: Landmark definitions used in the fish scales.

RESULTS

When the results of Procrustes ANOVA are examined, no significant difference in size (LogCS) (p = 0.1960) and shape (p = 0.9350) between sexes is found. Groups based on season and age are significant in size (p < 0001), but not for shape (Tab. 2).

Table 2: Procrustes ANOVA results of scales for amos groups (df: Degree of freedom, F: Goodal's F, CS: Centroid Size, P: p value).

Procrustes ANOVA						
		df	F	P (param.)	Pillai tr.	P (param.)
Sex	CS	1	1.70	0.1960		
	Shape	8	0.37	0.9350	0.06	0.8330
Season	CS	3	9.78	<.0001		
	Shape	24	1.26	0.1822	0.31	0.5086
Age	CS	4	13.89	<.0001		
	Shape	32	0.43	0.9978	0.16	0.9996

Scale size is larger in females than in males, and the summer and winter groups' scales are larger than other groups. Scale size increases with age groups (Fig. 4).

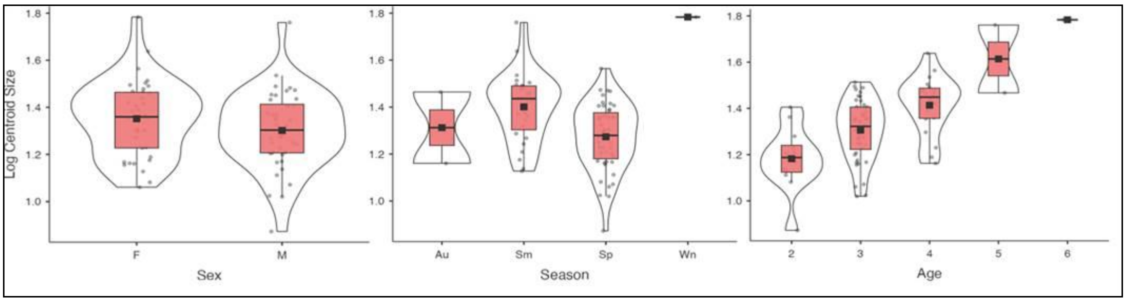

Figure 4: Box-violin chart of scale's size of amos groups.

In PCA analysis, when examined by age, first two PC explain 49.4%, by season, first two PC explain 49.7% and by sex, first two PC explain 50.2% of the total variation (Tab. 3).

Table 3: PCA resulsts of sclaes for amos groups.

	PC	Eigenvalue	% variance
Sex	1	0.0019	30.8
	2	0.0012	19.4
	3	0.0010	16.5
Season	1	0.0017	29.5
	2	0.0012	20.2
	3	0.0010	16.5
Age	1	0.0019	30.4
	2	0.0012	19.0
	3	0.0010	16.7

When PCA plots are examined, there is no clear separation between the groups along the PC1 and PC2 axes (Fig. 5).

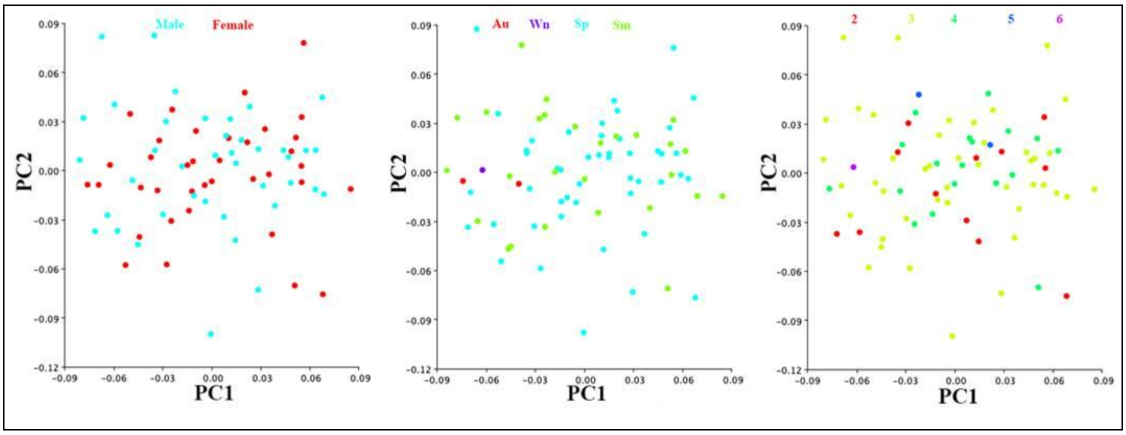

Figure 5: Scatter plot of principal component analysis (PCA)
showing the distribution of scale shapes by sex, age, and season.

When looking at the CVA results, there is not clear separation and significant difference between any groups (Tab. 4, Fig. 6).

Table 4: CVA results of scales for amos groups (Mah. Dist.: Mahalanobis distance, Proc. Dist.: Procrustes distance, p val: Permutation p-value).

Age								
	2		3		4		5	
	Mah.Dist/ p val.	Proc.Dist/ p val.	Mah.Dist/ p val.	Proc.Dist/ p val.	Mah.Dist/ p val.	Proc.Dist/ p val.	Mah.Dist/ p val.	Proc.Dist/ p val.
3	0.6658/ 0.8921	0.0150/ 0.9398	–	–	–	–	–	–
4	0.6631/ 0.8821	0.0168/ 0.9119	0.3960/ 0.9815	0.0100/ 0.9823	–	–	–	–
5	1.4983/ 0.9156	0.0477/ 0.7395	1.1786/ 0.9602	0.0363/ 0.9007	1.9158/ 0.9501	0.0356/ 0.8516	–	–
6	1.8235/ 0.8713	0.0702/ 0.6626	1.8328/ 0.9497	0.0662/ 0.6868	1.1345/ 0.8262	0.0716/0.3921	2.2266/ 1.0000	0.0752/ 0.6629
Season								
	Au		Sm		Sp			
Sm	2.5778/0.1333	0.0792/ 0.0923	–	–	–	–	–	–
Sp	2.4285/ 0.2009	0.0781/0.0763	0.7899/ 0.2211	0.0196 / 0.3625	–	–	–	–
Wn	2.5473/ 0.6631	0.0614/0.6631	1.6775/ 0.9826	0.0631/ 0.7042	2.1015/ 0.8228	0.0734/ 0.4500	–	–
Sex								
	Female		–		–		–	
Male	0.4914/ 0.8114	0.0109/ 0.9034	–	–	–	–	–	–

CVA graphs show that the groups are very similar in terms of scales and there is a lot of overlap (Fig. 6).

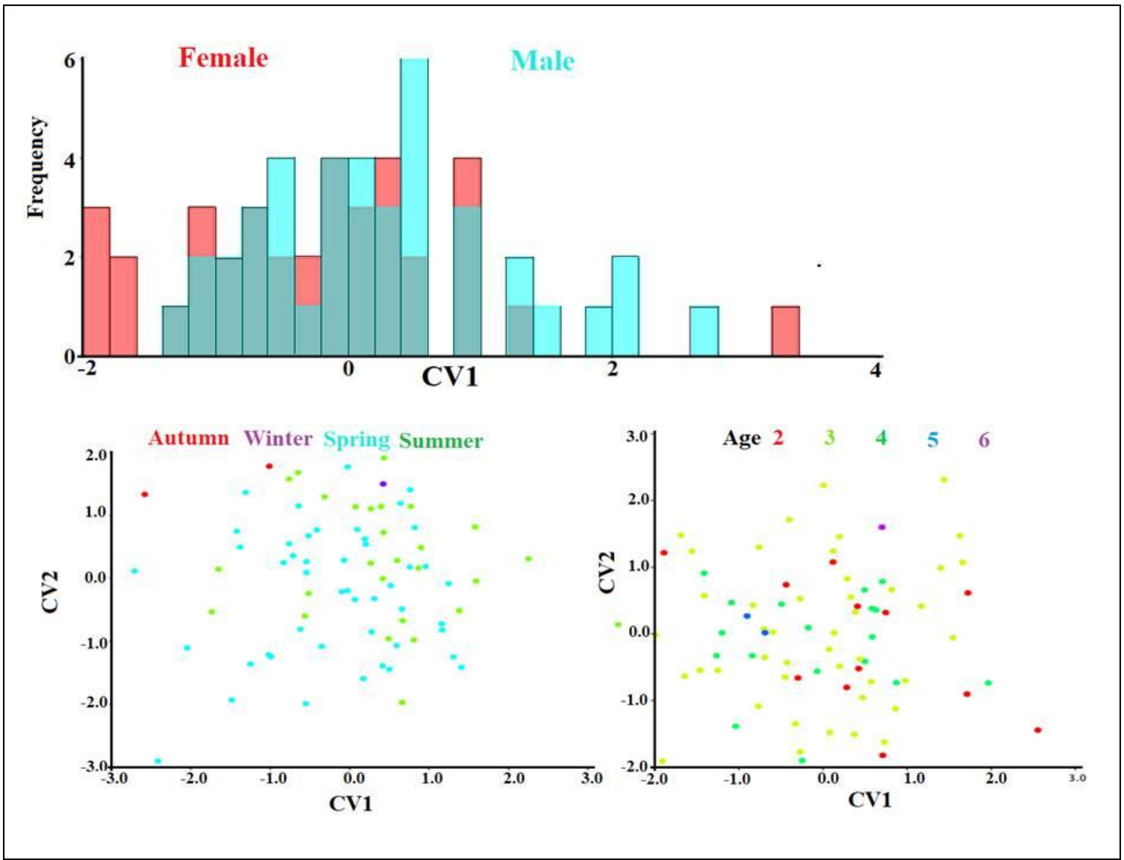

Figure 6: CVA plots of scales for amos groups.

Upon reviewing the DFA results, there are not significant differences between any studied groups (Tab. 5, Fig. 7). Looking at the warp line graphs (Fig. 7) produced by the DF analysis, it shows that there are no significant differences in scale shape between the groups.

Table 5: DFA results of scales for amos groups (Mah. D.: Mahalanobis distance, Proc. D.: Procrustes distance, T^2: T-square, Par. p: Parametric p value, Perm. p: Permutation p-value).

Age					
		2	3	4	5
	T^2	4.0500	–	–	–
3	Param. p	0.8904	–	–	–
	Perm. p (Proc./T^2)	0.9450/0.8780	–	–	–
4	T^2	2.7109	2.2702	–	–
	Param. p	0.9740	0.9785	–	–
	Perm. p (Proc./T^2)	0.9010/0.9690	0.9820/0.9800	–	–
5	T^2	16.6296	2.2724	4.5819	–
	Param. p	0.5931	0.9807	0.9265	–
	Perm. p (Proc./T^2)	0.7130/0.5820	0.9100/0.9840	0.8550/0.9530	–
6	T^2	79.0948	2.5351	8.6745	0.0582
	Param. p	0.1159	0.9732	0.7320	0.8494
	Perm. p (Proc./T^2)	0.6590/0.2080	0.6740/0.9210	0.3960/0.7110	0.6790/0.3330
Season					
		Au	Sm	Sp	
	T^2	16.9733	–	–	–
	Param. p	0.1958	–	–	–
	Perm. p (Proc./$T^{2)}$	0.0870/0.1880	–	–	–
	T^2	14.0309	10.7168	–	–
	Param. p	0.1906	0.3077	–	–
	Perm. p (Proc./$T^{2)}$	0.0790/0.2240	0.3840/0.3240	–	–
	Sex	–	–	–	–
	Female	–	–	–	–
	T^2	0.0373	3.0994	4.5932	–
	Param. p	0.8785	0.9635	0.8581	–
	Perm. p (Proc./$T^{2)}$	0.6420/0.3220	0.6770/0.9960	0.4540/0.8150	–
Sex					
		Female	–	–	–
	T^2	4.6405	–	–	–
	Param. p	0.8330	–	–	–
	Perm. p (Proc./$T^{2)}$	0.9020/0.8490	–	–	–

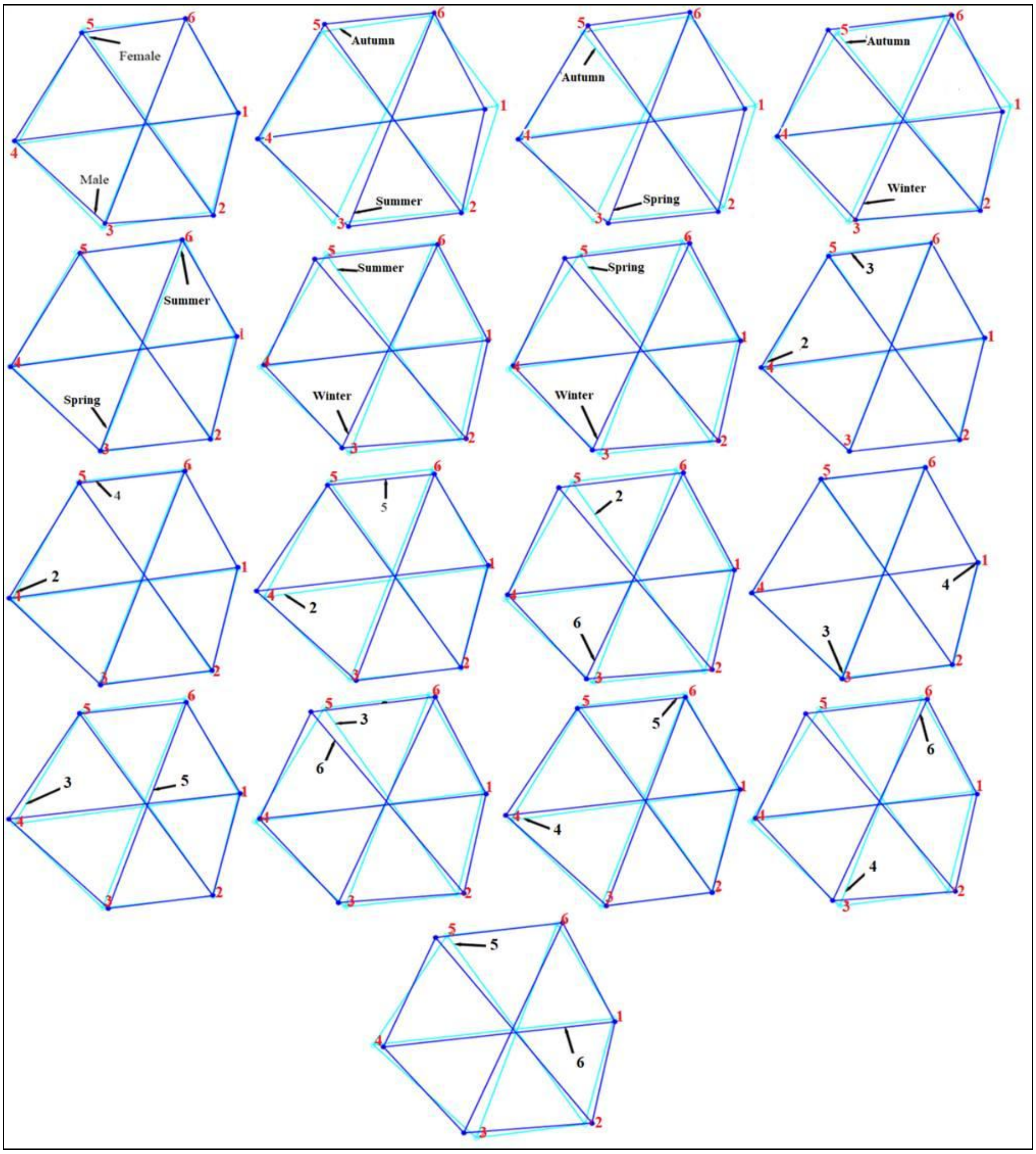

Figure 7: Warp line of scale shape difference between groups of amos.

DISCUSSION

Fish scales contain small growth rings that allow us to determine the age of the fish. These growth rings are typically arranged around a center and are composed of $CaCO_3$ compounds (Carbonara and Follesa, 2019; Chen et al., 2022). Variations in these rings occur because fish scales generally grow excessively when feeding is abundant, typically during spring and summer, and slow down or stop altogether when feeding is inadequate, especially during winter (Gümüş et al., 2002). As the structure of annual growth rings in fish scales is influenced by environmental conditions, this type of differentiation can be significant based on the physicochemical parameters of the environment and feeding (Staszny et al., 2012). In this sense, changes in the shape of fish scales can allow for differentiation in populations (Ibáñez et al., 2007; Ibáñez et al., 2009). Additionally, inter/intraspecific morphological variability may indicate genetic differences among samples or can respond to environmental conditions within the framework of phenotypic plasticity (Carro et al., 2018; Staszny et al., 2013).

Geometric morphometrics is very important in fish scales studies because it allows for the aqurate quantitative analysis of shape and size variation in a way that traditional morphometrics cannot achieve (Carro et al., 2018; Moreira et al., 2020). This method provides a detailed and comprehensive understanding of the shape and size changes in fish scales, which can be used to address questions related to taxonomy, evolution, and ecology. Additionally, geometric morphometrics allows for the visualization and analysis of complex patterns of shape variation, making it a valuable tool for researchers studying fish scales (Çiçek et al., 2017; Ibáñez and Jawad, 2018;Ibáñez et al., 2023) applied geometric morphometric methods successfully on *Capoeta trutta* and *Capoeta umbla* species. In the present study, it was achieved on *Acanthobrama marmid* species at the same success. In the size analysis performed according to sex, it was seen that female samples were larger than males. These results show that fish species can be successfully distinguished by morphometric geometric analysis.

This type of analysis has been used successfully in previous studies. For example, studies on fish scale and otolith morphometry and geometry (Bilici, 2020; Çiçek et al., 2017; Dörtbudak and Özcan, 2018; Ibáñez et al., 2019; Richards and Esteves, 1997; Staszny et al., 2012; Teimori, 2016; Wichard et al., 2005) have yielded important results in this field. In addition, studies examining the relationship between fish size and otolith morphometry (Staszny et al., 2012, 2013) were also effective in determining the species.

When the ANOVA results of the study were analysed, no significant difference was found between sex in terms of size and shape. Seasonal and age-based groups were significant in terms of size ($p < 0001$), but not in terms of shape.

Scale size is larger in females than in males, and the summer and winter groups have larger scales than all other groups. Scale size increases with age groups.

In PCA analysis, the first two PCs explained 49.4% of the total variation when analysed by age, the first two PCs explained 49.7% when analysed by season and the first two PCs explained 50.2% when analysed by sex.

When CVA results are analysed, there is no clear distinction and no significant difference between any of the groups. CVA graphs show that the groups are very similar in terms of scales and there is a lot of overlap.

When the CFA results are analysed, it is seen that there are no significant differences between any groups. Looking at the warp line graphs produced by the DF analysis, it is seen that there are no major differences between the groups in terms of scale shape.

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TRACE METAL CONTAMINATION IN TWO FISH SPECIES FROM EPE LAGOON (SOUTH-WEST NIGERIA): HEALTH RISK ASSESSMENT

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KEYWORDS: metals, bioaccumulation, food safety, marine, risk, assessment.

ABSTRACT

This study investigates trace metal concentrations in water, sediment, and fish from the Epe Lagoon, Nigeria. Sediments showed the highest levels, with iron being the most abundant, followed by zinc, manganese, copper, nickel, chromium, and lead. Significant differences in zinc accumulation factors were observed between *Clarias gariepinus* and *Tilapia mariae*. Bio-sediment accumulation factors (BSAF) for all metals were below one, with copper and iron showing the highest bioaccumulation in both fish species. The estimated daily intake of metals was below reference doses, with the highest target hazard quotient (THQ) for iron and the lowest for chromium, showing no significant differences between species.

RÉSUMÉ: Trace de contaminants métalliques dans deux espèces de poissons de la lagune Epe (sud-ouest Nigeria): évaluation des risques pour la santé.

Cette étude examine les traces de métaux dans l'eau, les sédiments et les poissons de la lagune d'Epe, au Nigeria. Les sédiments présentaient les niveaux les plus élevés, le fer étant le plus abondant, suivi du zinc, du manganèse, du cuivre, du nickel, du chrome et du plomb. Des différences significatives dans les facteurs d'accumulation du zinc ont été observées entre *Clarias gariepinus* et *Tilapia mariae*. Les facteurs d'accumulation des biosédiments (BSAF) pour tous les métaux étaient inférieurs à un, le cuivre et le fer présentant la bioaccumulation la plus élevée chez les deux espèces de poissons. L'absorption quotidienne estimée de métaux était inférieure aux doses de référence, avec le quotient de risque cible (THQ) le plus élevé pour le fer et le plus faible pour le chrome, ne montrant aucune différence significative entre les espèces.

REZUMAT: Contaminarea cu urme de metale la două specii de pești din laguna Epe (sud-vestul Nigeriei): evaluarea riscurilor pentru sănătate.

Acest studiu investighează urmele de concentrații de metale în apă, sediment și pești din laguna Epe, Nigeria. Sedimentele au prezentat cele mai înalte concentrații, fierul fiind cel mai abundent, urmat de zinc, mangan, cupru, nichel, crom și plumb. S-au observat diferențe semnificative în factorii de acumulare de zinc între *Clarias gariepinus* și *Tilapia mariae*. Factorii de acumulare de bio-sediment (BSAF) pentru toate metalele au fost sub unu, cuprul și fierul prezentând cea mai mare bioacumulare la ambele specii de pești. Aportul zilnic estimat de metale a fost sub dozele de referință, cu cel mai mare coeficient de pericol țintă (THQ) pentru fier și cel mai mic pentru crom, nefiind diferențe semnificative între specii.

INTRODUCTION

Addressing trace metal contamination in lagoon ecosystems is critical for safeguarding their ecological integrity and ensuring the safety of resources for local communities. Trace metal pollution is a critical environmental issue that poses significant risks to aquatic ecosystems and human health. Among the various contaminants, lead and chromium are of particular concern due to their high toxicity, persistence, and ability to bioaccumulate in the environment (Ali and Khan, 2018). Lead and chromium are particularly hazardous due to their deleterious effects on aquatic organisms and human health. According to Okereafor et al. (2020), lead exposure in aquatic environments can impair physiological and neurological functions in fish, leading to reduced growth, reproduction, and survival rates. Similarly, chromium is toxic and can cause severe neurological damage in fish and higher trophic-level organisms, including humans who consume contaminated fish (Pandey, 2017).

Aquatic habitats are significant drivers of global biodiversity and rank among the most vulnerable ecosystems (Sen and Dhote, 2024) due to overexploitation, water contamination, flow alterations by hydraulic structural activities, habitat destruction or degradation and invasion of non-native species (Kolawole and Iyiola, 2023; Mukherjee et al., 2023). Globally, freshwater bodies are enriched with more than 10,000 fish species (Chaigneau et al., 2023), accounting for over 40% of the world's fish communities and 25% of the diverse invertebrate population on a global scale (Chaigneau et al., 2023). Fresh and brackish water fish species hold significant commercial importance around the world (del Monte-Luna et al., 2016) and in Nigeria, playing a vital role in the country's economy, food security, and local livelihoods. These fish species thrive in the diverse aquatic ecosystems found throughout Nigeria, including rivers, lakes, and coastal lagoons (Adaka et al., 2015; Olopade, 2013; Olopade et al., 2015).

While previous studies have highlighted the widespread contamination of Nigeria's aquatic ecosystems with trace metals (Edo et al., 2024; Bawa-Allah, 2023; Amadi et al., 2022), comprehensive data on the levels and bioaccumulation patterns of these metals in the Epe Lagoon remain limited. Understanding the extent of contamination and the bioaccumulation dynamics is essential for assessing the ecological health of the lagoon and the potential risks to human populations relying on its resources. This study aims to address this knowledge gap by analyzing trace metal concentrations in water, sediment, and two fish species (*Clarias gariepinus* and *Tilapia mariae*) from the Epe Lagoon, a vital ecosystem supporting biodiversity and local communities in Lagos, Nigeria. By examining bioaccumulation patterns, this research seeks to provide a clearer understanding of the environmental impacts of trace metals and propose actions for mitigating pollution, protecting ecosystem health, and safeguarding the well-being of local communities.

MATERIAL AND METHODS

Study area

Epe Lagoon lies between latitudes 6°23'27"N-6°41'45"N and longitudes 2°42'20"E-3°42'21"E with a surface area of 243 km^2 and an average depth of about 1.80 m. The Epe Lagoon is sandwiched between two other lagoons, the Lekki Lagoon (freshwater) in the east and the Lagos Lagoon (brackish water) in the west. It supports a major fishery in Lagos State, Nigeria, and it is also used as a transportation route for people, goods, and timber from Epe to other places in South-Western Nigeria (Olopade et al., 2015).

Based on increasing anthropogenic effects from heaps of domestic and solid waste dumps, six sampling stations close to settlements were chosen for this research (Fig. 1). Each site is approximately 1.5 km apart.

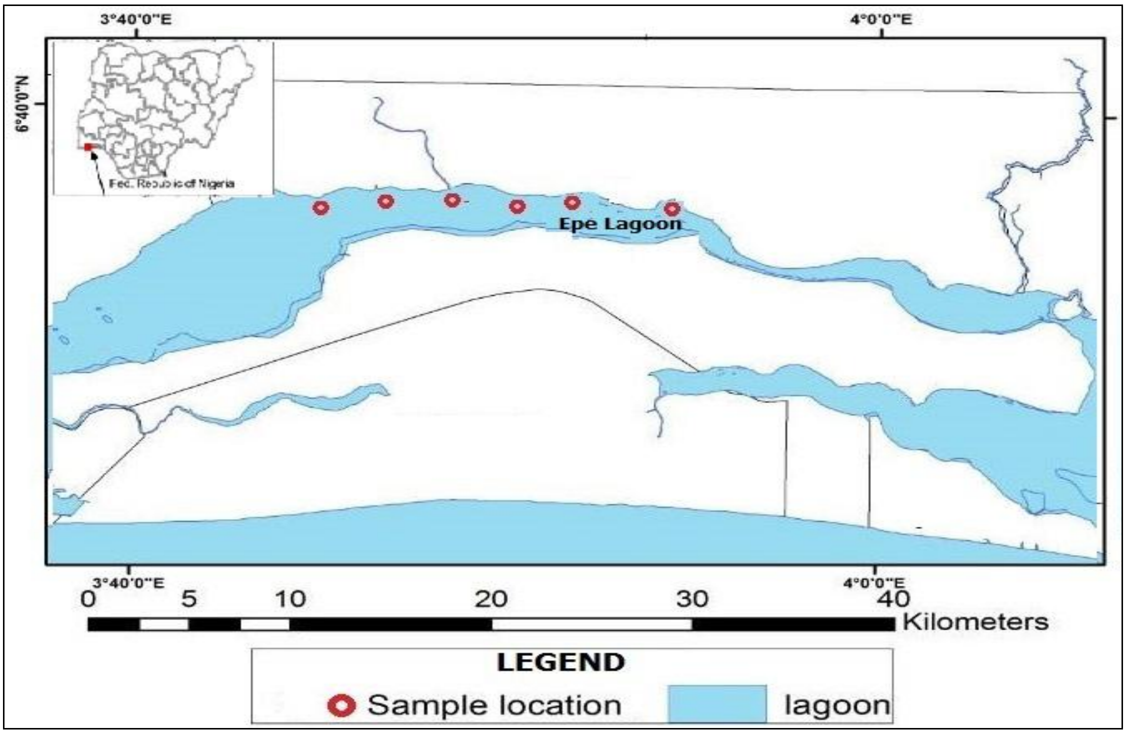

Figure 1: Map showing the study area (Moruf, 2022).

Collection of samples

Water, sediment, and fish samples (*Clarias gariepinus* and *Tilapia mariae*) samples were collected monthly between November 2023 and April 2024, covering the dry season. Water samples (500 mL per sampling point) were collected at a depth of one cm below the water surface in HNO_3 pre-rinsed containers (one L), and five mL of concentrated HNO_3 were added immediately to minimize chemisorption. Sediment samples were collected with the aid of 0.05 m^2 Eckman Grab during low tide at a depth of 10-15 cm. Five water and sediment samples each were randomly collected per sampling station, making 30 samples for the six locations per month. A total of 60 mature fish of each studied species were caught directly from the lagoon. In each case, samples were properly labelled, kept in clean plastic containers, and stored at 20°C before taking to the laboratory for analyses.

Sample preparation

At the laboratory, the sediments were thawed at room temperature for approximately 24 hours, then dried in an oven at 40°C (Gilli et al., 2018). The samples were then disaggregated and sieved through a 200 μm sieve. The sieved samples were subsequently homogenized in a porcelain mortar and re-sieved. Approximately five grams of the samples were put into Teflon tubes, and five mL aqua regia (HCl: HNO_3 in a ratio of 3:1) were added for digestion, following the ISO 11466 digestion method (Pueyo et al., 2001)

Sub-samples of approximately one gram of tissue were weighed on a precision scale with a decimal resolution of 0.001 g, and digested in a mixture of five ml of concentrated nitric acid (TMA, Hiperpure, PanReac, Spain) and three mL of 30% w/v hydrogen peroxide (PanReac, Spain) in a microwave-assisted digestion system (Ethos Plus, Milestone, Sorisole, Italy). Digested samples were transferred to polypropylene sample tubes and diluted to 15 mL with ultrapure water. As described by Dussubieux and Van Zelst (2004), the determination of the trace elements copper, zinc, chromium, lead, and cadmium in all the samples was carried out by ICP-MS. ICP-MS-based multi-element determination was performed using an Agilent 7700x ICP-MS system (Agilent Technologies, Tokyo, Japan) equipped with collision/reaction cell interference reduction technology. The continuous sample introduction system consisted of an autosampler, a Scott double-pass spray chamber (Agilent Technologies, Tokyo, Japan), a glass concentric MicroMist nebuliser (Glass Expansion, West Melbourne, Australia), a quartz torch, and nickel cones (Agilent Technologies, Tokyo, Japan). Elemental concentrations were quantified using a Mass Hunter Work Station Software for ICPMS (version A.8.01.01 Agilent Technologies, Inc. 2012, Tokyo, Japan).

Analytical quality control was guaranteed through the implementation of laboratory quality assurance protocols and laboratory methods, including the use of standard operating procedures, calibrations with standards, and analyses with reagent blanks. Samples were analysed in triplicates, and all chemicals and reagents used were of analytical grade. The limits of detection calculated for the investigated metals in all media were 1.0 × 10-4 mg/L for water and 1.0 × 10-4 mg/kg for sediment and biota. The accuracy of the determination was evaluated by comparison with the analytical recoveries determined in certified reference materials (fish protein DORM-3 National Research Council, Ottawa, Ontario, Canada) analysed following exactly the same procedure as for the samples.

Statistical analysis

The statistical analyses were performed using the Statistical Package for Social Sciences (SPSS) version 20.0. The Duncan multiple range test was used to determine the differences between the concentration levels of each analysed element in water, sediment, and fish samples. A significance level of $P < 0.05$ was considered of statistically significance. The bioaccumulation factor (BAF), which is the ratio of the concentration of metal in organism tissue to the concentration of metal in sediment (bio-sediment accumulation) and water (bio-water accumulation), was calculated. Health risk was estimated based on the Environmental Protection Agency guidelines (EPA, 2005). To assess the potential health risk via the consumption of the fish species, the estimated daily intake (EDI), target hazard quotient (THQ) and target hazard index (THI) were calculated using equations 1, 2, and 3, respectively with the following assumptions:

- The hypothetical body weight for adult Nigerian was 70 kg (Agwu et al., 2018).
- The maximum absorption rate was 100% while bioavailability factor was also 100%.

$$Estimated\ Daily\ Intake, EDI\,(mg/kg/day)\ \frac{CXCRXAFXEF}{Bw}$$

(ATSDR, 2005) Eqn. 1

Where C = Concentration of the contaminant in the exposure pathway (mg/kg);
CR = Consumption Rate; Nigeria aquatic crab taken/day, 0.0366 kg/day = 13.359 kg/y;
AF = Bioavailability factor (100%);
EF = Exposure Factor = 1;
Bw = Body weight (70kg);

$$\text{Target Hazard Quotient, THQ}\ \frac{EDI}{RfD}$$ (Wang et al., 2005) Eqn. 2;

Where EDI = Estimated Daily Intake and RfD = the oral reference dose (mg/kg/day);

$$THI = \Sigma\ THQ_i$$

(Wang et al., 2005) Eqn. 3;

Where THQ is the target hazard quotient of an individual trace metal.

RESULTS AND DISCUSSION

Table 1 presents the distribution of trace metals (chromium, copper, iron, lead, manganese, nickel, and zinc) in water, sediment, and two fish species (*Clarias gariepinus* and *Tilapia mariae*) in the Epe Lagoon. The values are mean concentrations with standard errors and the range of concentrations measured in mg kg^{-1}. The highest mean concentration levels of these trace metals were observed in the sediment, with values as follows: chromium (0.28 ± 0.07), copper (0.87 ± 0.28), iron (34.83 ± 13.67), lead (0.21 ± 0.14), manganese (1.32 ± 0.37), nickel (0.77 ± 0.30), and zinc (0.57 ± 0.15). The elevated concentrations of trace metals in sediment compared to water and fish are attributed to the strong binding and low mobility of metals in sediments, along with environmental and biological processes that favor the accumulation of metals in this part of the ecosystem. According to Moruf et al. (2022), sediment acts as the primary repository for metals, containing up to 99% of all metals found in the aquatic environment under certain conditions. Studying sediment metals provides insight into the long-term pollution status of the aquatic ecosystem.

The trend of trace metal concentrations observed in this study was in the order of iron > zinc > manganese > copper > nickel > chromium > lead, which aligns with the trend reported in the Oshiri and mining areas of Southeastern Nigeria (Igwe et al., 2021). Both *Clarias gariepinus* and *Tilapia mariae* exhibited higher levels of most metal concentrations compared to water, likely due to biological accumulation. Similarly, Lawal-Are et al. (2017) found elevated levels of zinc (0.74 ± 0.13 mg kg^{-1}), copper (1.21 ± 0.03 mg kg^{-1}), chromium (0.28 ± 0.10 mg kg^{-1}), lead (0.26 ± 0.07 mg kg^{-1}), and nickel (0.28 ± 0.03 mg kg^{-1}) in *Clarias amnicola* from the Igbese River. According to Ezemonye et al. (2019), elevated trace metal concentrations in aquatic organisms indicate cumulative exposure through water and/or food.

Table 1: Trace metal distribution in water, sediment and two fish species in Epe Lagoon.

Metal	Water	Sediment	*Clarias gariepinus*	*Tilapia mariae*
Chromium	0.01 ± 0.00 (0.0005-0.0092)	0.28 ± 0.07 (0.0248-0.4593)	0.01 ± 0.00 (0.0016-0.0217)	0.01 ± 0.00 (0.0004-0.0129)
Copper	0.02 ± 0.01 (0.0022-0.035)	0.87 ± 0.28 (0.1089-1.7522)	0.56 ± 0.14 (0.0591-0.8911)	0.36 ± 0.18 (0.005-0.9566)
Iron	0.70 ± 0.27 (0.072-1.7148)	34.83 ± 13.67 (3.5986-85.7378)	14.74 ± 4.5 (1.3634-26.9381)	13.11 ± 6.13 (0.3834-36.3021)
Lead	0.00 ± 0.00 (0.0001-0.0175)	0.21 ± 0.14 (0.0000-0.8759)	0.13 ± 0.09 (0.000-0.56)	0.02 ± 0.01 (0.000-0.0746)
Manganese	0.03 ± 0.01 (0.0057-0.0504)	1.32 ± 0.37 (0.2854-2.5205)	0.05 ± 0.01 (0.0108-0.0957)	0.02 ± 0.01 (0.0001-0.0469)
Nickel	0.02 ± 0.01 (0.0012-0.0412)	0.77 ± 0.30 (0.0607-2.0610)	0.01 ± 0.00 (0.0002-0.024)	0.00 ± 0.00 (0.0000-0.0138)
Zinc	0.57 ± 0.15 (0.1228-1.1032)	28.67 ±7.51 (6.1411-55.1594)	1.32 ± 0.33 (0.241-2.1644)	0.26 ± 0.12 (0.0029-0.6751)

The bioaccumulation factors of trace metals in the sampled fishes are shown as the Bio-water Accumulation Factor (BWAF) and Bio-sediment Accumulation Factor (BSAF) in figures 2 and 3, respectively. All the investigated trace metals were found to bioaccumulate in measurable concentrations in the two studied fish species. Significant differences ($p < 0.05$) were observed only for zinc in both BWAF and BSAF between the two fish species. The recorded BSAF values were less than one for all the trace metals, with copper (0.80) and iron (0.35) being the most bioaccumulated in *Clarias gariepinus* and *Tilapia mariae*, respectively. The high BAF value of copper, despite its low concentration in sediment, reveals its significant biomagnification abilities. These findings for BSAF align with those of Capparelli et al. (2016), who found that BAF values in mudflat fiddler crabs decrease as metal concentration in sediments increases.

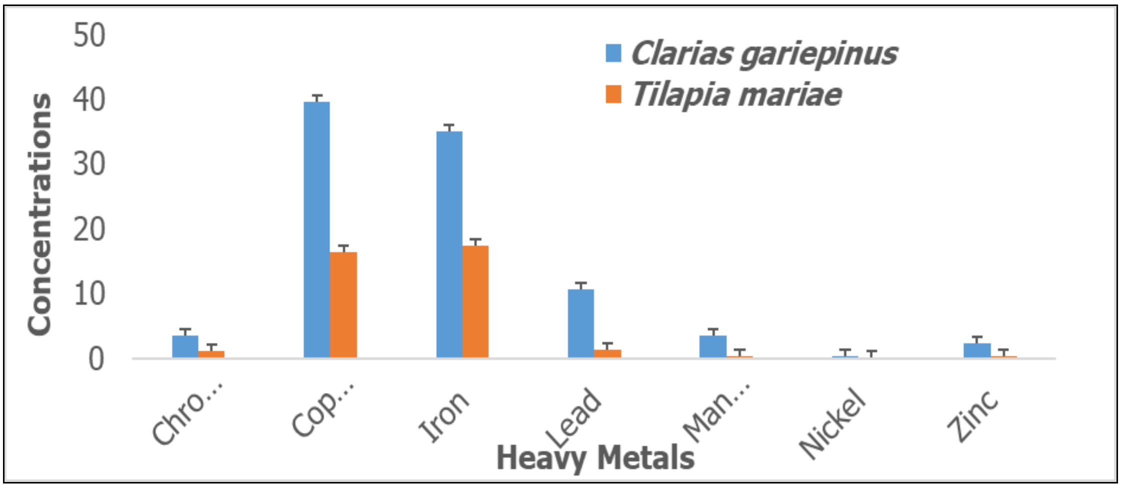

Figure 1: Bio-water accumulation factor of two fish species in Epe Lagoon.

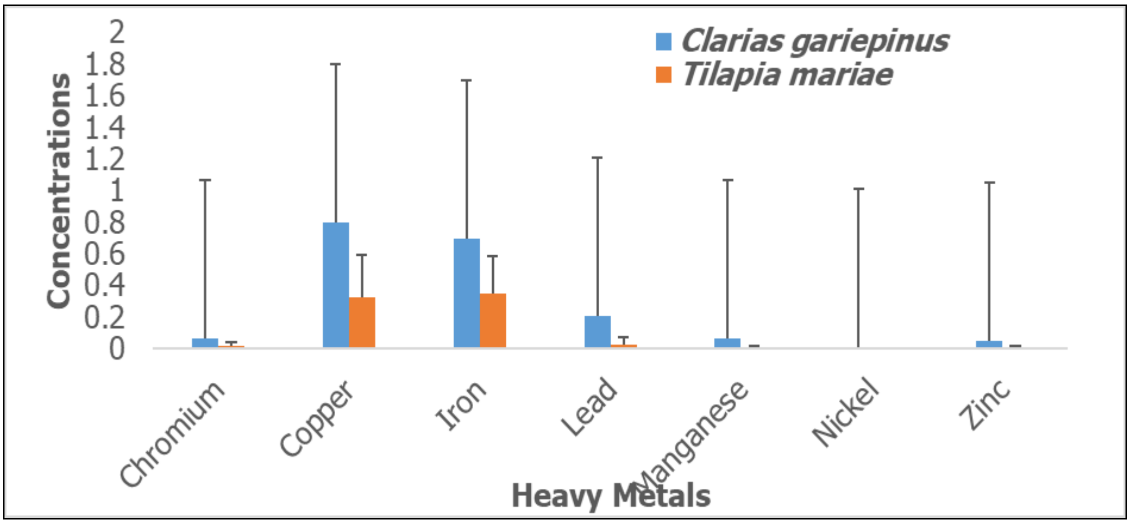

Figure 2: Bio-sediment accumulation factor of two fish species in Epe Lagoon.

The Estimated Daily Intake (EDI) of metals through the consumption of *Clarias gariepinus* and *Tilapia mariae* is presented in table 2. The results, expressed in milligrams per body weight per day, show that the EDI values for the investigated metals in both fish species were lower than their respective oral reference doses (RFD). This finding aligns with the results of Moslen and Miebaka (2017), who reported that the EDI of copper, zinc, iron, chromium, lead, and cadmium was below the reference oral doses for *Clarias amnicola* from an estuarine creek in the Niger Delta. In the present study, the EDI values also fell within the recommended range set by FAO/WHO (2004).

The Target Hazard Quotient (THQ) values for individual metals are presented in table 3. The highest THQ value was observed for iron in both *Clarias gariepinus* (0.02543 ± 0.0005) and *Tilapia mariae* (0.0251 ± 0.0003), while the lowest was for chromium (0.0011 ± 0.00) across species. The THQ trend for both fish species followed the order: iron > lead > copper > zinc > cadmium > chromium. No significant difference was noted in the THQ values between the two species. In contrast, Moslen and Miebaka (2017) reported a THQ order of Pb > Cr > Cd > Cu > Ni, with all values below one. In this study, the Total Hazard Index (THI) for all investigated metals was also below one, specifically 0.0525 for *Clarias gariepinus* and 0.0334 for *Tilapia mariae*, indicating no significant health hazard from consuming these fish species, according to USEPA (2000) guidelines.

Table 2: Estimated Daily Intake (mg/kg/day) of trace metals in two fish species in the Epe Lagoon

Metal	*Clarias gariepinus*	*Tilapia mariae*	Oral reference dose (RfD)
Copper	0.0003 ± 0.0001 (0.0000-0.0005)	0.0002 ± 0.0001 (0.0000-0.0005)	0.04
Zinc	0.0007 ± 0.0002 (0.0001-0.0011)	0.0001 ± 0.0001 (0.0000-0.0004)	0.300
Iron	0.0077 ± 0.0024 (0.0007-0.0141)	0.0068 ± 0.0032 (0.0002-0.0190)	100.000
Chromium	0.0022 ± 0.00 (0.0017-0.0028)	0.0024 ± 0.00 (0.0021-0.0029)	1.500
Lead	0.0001 ± 0.0000 (0.0000-0.0003)	0.0001 ± 0.0000 (0.0000-0.0002)	0.004
Cadmium	0.0010 ± 0.00 (0.0000-0.0018)	0.001 ± 0.00 (0.0000-0.0024)	0.001

Table 3. Target hazard quotient (THQ) and Total Hazard Index (THI) of trace metals via the consumption of two fish species in the Epe Lagoon.

Metal	*Clarias gariepinus*	*Tilapia mariae*	*P*-Value
Copper	0.0073 ± 0.0019 (0.0008-0.0116)	0.0047 ± 0.0023 (0.0001-0.0125)	0.09
Zinc	0.0023 ± 0.0006 (0.0004-0.0038)	0.0005 ± 0.0002 (0.0000-0.0012)	0.08
Iron	0.02543 ± 0.0005 (0.0024-0.0469)	0.025130 ± 0.0003 (0.0007-0.0632)	1.30
Chromium	0.00011 ± 0.00 (0.0001-0.00021)	0.00011 ± 0.00 (0.0001-0.00023)	0.12
Lead	0.0171 ± 0.0122 (0.0000-0.0732)	0.0023 ± 0.0016 (0.0000-0.0098)	0.14
Cadmium	0.0003 ± 0.0001 (0.0002-0.0006)	0.0007 ± 0.0002 (0.0003-0.0017)	0.10
Total Hazard Index	0.0525	0.0334	

CONCLUSIONS

This study revealed that sediments exhibited the highest mean concentrations of trace metals in the order of iron > zinc > manganese > copper > nickel > chromium > lead. Significant differences were observed in zinc levels for both the Bio-water Accumulation Factor (BWAF) and Bio-sediment Accumulation Factor (BSAF) between the two fish species, *Clarias gariepinus* and *Tilapia mariae*. The BSAF values for all trace metals were below one, indicating limited bioaccumulation, with copper and iron being the most bioaccumulated in *Clarias gariepinus* and *Tilapia mariae*, respectively. The Estimated Daily Intake (EDI) of metals for both fish species was below the oral reference dose (RFD), suggesting minimal risk from their consumption. Iron recorded the highest Target Hazard Quotient (THQ) in both fish species, while chromium had the lowest THQ value. No significant differences in THQ values were observed between the two species. These findings highlight the varying bioaccumulation patterns and potential health risks associated with trace metal contamination in the Epe Lagoon area. They underscore the importance of continued monitoring and the implementation of strategies to mitigate pollution, ensuring the health of the ecosystem and the safety of its resources for local communities.

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CULTURE TECHNIQUE OF SHING WITH OTHER HIGH-VALUE FISH SPECIES IN THE SEMI-ARID ZONE OF BANGLADESH

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KEYWORDS: short life cycle fishes, drought prone, utilization, seasonal, ponds.

ABSTRACT

This study was conducted in farmer's ponds within the northern region of Bangladesh to observe the growth and yield of Shing, *Heteropneustes fossilis* under a polyculture system. The highest total production of fish registered among the treatments in pattern-1 was 6,331 kg ha^{-1} with a benefit-cost ratio of 1.52. The best combinations were chosen for multi-location testing (MLT) to verify the previous results. After five months of multi-location testing of the selected combinations with Shing as the main species, the significantly ($P < 0.05$) highest production of Shing (5,828 kg ha^{-1}), total production of fishes (7,352 kg ha^{-1}) and benefit-cost ratio (1.73) were found.

RÉSUMÉ: Technique de culture du Shing avec des espèces de poissons de grande valeur dans la zone semi-aride du Bangladesh.

L'étude a été menée dans des étangs d'agriculteurs dans la région nord du Bangladesh pour observer la croissance et le rendement du Shing, *Heteropneustes fossilis* dans un système de polyculture. La meilleure production totale de poisson enregistrée parmi le modèle de traitement-1 était de 6.331 kg ha^{-1} avec un rapport avantages-coûts de 1,52. Ces meilleures combinaisons ont été choisies pour des tests multi-sites (MLT) afin de vérifier les résultats précédents. La combinaison sélectionnée après cinq mois de tests multi-sites avec Shing comme espèce principale, la production de Shing significativement ($P < 0,05$) la plus élevée (5,828 kg ha^{-1}), la production totale de poissons (7.352 kg ha^{-1}) et le bénéfice -un rapport de coût (1,73) a été trouvé.

REZUMAT: Tehnica de cultivare a speciei de pește pisica fosilă cu specii de pești de mare valoare în zona semi-aridă din Bangladesh.

Studiul a fost efectuat în iazurile fermierilor din regiunea de nord a Bangladeshului pentru a observa creșterea și randamentul pisicii fosile, *Heteropneustes fossilis*, în cadrul unui sistem de policultură. Cea mai bună producție totală de pește înregistrată între tratamente în modelul-1 a fost de 6.331 kg ha^{-1}, cu un raport beneficii/costuri de 1,52. Aceste combinații optime au fost alese pentru testarea în mai multe locații (MLT) pentru a verifica rezultatele anterioare. Combinația selectată, după cinci luni de testare în mai multe locații cu Shing ca specie principală, în mod semnificativ ($P < 0,05$) cea mai mare producție de Shing (5.828 kg ha^{-1}), cu o producția totală de pești (7352 kg ha^{-1}) și un raportul beneficiu-cost (1,73), dintre cele trei locații, a fost găsită în Dimla.

INTRODUCTION

The Northern region (Rangpur District) of Bangladesh is known as a drought and river bank erosion prone area. Most of the districts within this district have been experiencing frequent natural disasters and adverse impacts of climate change. Their ponds and canals are extremely depleted, even major rivers see reduced water volume for up to six-eight months. As a result, the number of seasonal waters (Mou et al., 2024; Hasan et al., 2023) is increasing with approximately 55% ponds being seasonal. 60% of those retain water for four-six months while 40% retain for six-nine months in a year and even more in some instances (Ahamed et al, 2017b). These small water bodies are being used mainly for household activities, but some are still abandoned due to their derelict and marshy nature. In this semi-arid zone, fish farmers are lacking appropriate fish culture techniques and most farmers utilize traditional methods (Ahamed et al., 2017a). The polyculture attempts on short cycle species such as Shing (*Heteropneustes fossilis*), Pabda (*Ompuk Pabda*), Rajpunti (*Barbodes gonionoutus*) and GIFT (*Oreochromis* sp.), etc., might have useful applications in those seasonal waters. The species is not only recognized for its delicious taste and market value but is also highly esteemed for its nutritional and medicinal properties (Ahamed et al., 2020). Bangladesh is ranked fourth position in inland fishery production just after China, India, and Myanmar and fifth position in marine water (FAO, 2018). The fisheries sector is inseparable from the life and lifestyle of the people of Bangladesh since it contributes 4.37% to the national GDP and almost one-fourth (23.37%) to the agricultural GDP (DoF, 2013). About 1.5 million people are directly employed by this sector (DoF, 2012). Within the northern region is where this culture technique would be most effective in increasing fish production as well as income for all types of fish farmers. In this context, culture techniques of these species are to be disseminated in this region. The polyculture technology of shing can help to meet the dietary needs and improve the socio-economic status of the people especially in the Northern region of Bangladesh.

MATERIAL AND METHODS

Experiments were conducted in farmer's ponds located within the Nilphamari District of Bangladesh for five months from mid-July to mid-December of 2017 to observe the growth and yield performance of Shing, *Heteropneustes fossilis* under a polyculture system. Six seasonal ponds (10-15 decimal) were selected for each experiment. The six ponds were divided into three groups and each group was used for a treatment. Ponds were selected with the concern of relevant Subdistrict Fishery Officer (UFO/SUFO). The experimental designs are described as follows in table 1.

Table 1: Polyculture of Shing, (pattern-1) under different stocking densities in farmer's pond.

Treatments	Species wise stocking size (cm)	Stocking density (indi. dec^{-1})			
		Shing	Pabda	Rajpunti	GIFT
T_1	Shing (7-10) Pabda (5-7) Rajpunti (7-8) GIFT (5-6)	400	100	10	05
T_2		500	100	10	05
T_3		600	100	10	05

The ponds were prepared by dewatering and drying. Aquatic weeds were removed manually before lime was applied 1.0 kg decimal^{-1}. After seven days of liming, urea 100 g decimal^{-1} and TSP 75g decimal^{-1} were applied at the initial stage of pond preparation. The hatchery produced fingerlings (5-10 cm) of selected fish and ponds were stocked as per experimental design (Tabs. 1 and 2). Commercially available fish feed (containing 30-35% protein) was fed 15-5% BW day^{-1} to the fish. Length and weight data was collected every two weeks in the morning from 8.00 am to 9.00 am. Samplings were done by cast net. Fish length was measured using a measuring meter scale (cm) and weight was taken by a precision weighing balance (measuring range from 1.0 g to 1.0 kg). Water quality parameters of the experimental ponds were also monitored every two weeks. Water temperature (°C) was measured by a Celsius thermometer, transparency (cm) by secchi disc, water pH by digital pH meter (Hanna Co. Japan), dissolved oxygen (DO) (mg l^{-1}) by digital DO meter (Lutron PDO-519, Taiwan) and ammonia (NH_3) (mg l^{-1}) by ammonia test kit (Hanna Co. Japan). At the end of the experiment the ponds were completely dewatered, all fish were harvested and the different fish species counted. Then the final length-weight of each species was recorded. The parameters such as length gain, weight gain, % weight gain, SGR, FCR, and survival rate (%) and benefit cost ratio (BCR) were calculated and evaluated on the growth and yield of fish.

Exp-2 Dissemination of suitable polyculture patterns of short life cycle fish species in different parts of a semi-arid zone (northern part) of Bangladesh (2018).

Polycultures of *Heteropneustes fossilis* were tested under different treatments in seasonal farmer's ponds in the adjacent areas of FSS, Saidpur from mid-July to mid-December of 2017. Of them, 500 Shing+100 Pabda+10 Rajpunti+5 GIFT (indi. dec.$^{-1}$) from Shing polyculture (pattern-1) were selected due to technically sound, socially acceptable, and economically viable polyculture patterns. These combinations were disseminated in different aquatic ecological zones within the northern region of Bangladesh from May to September of 2018 through a multi-location testing (MLT) program. Multi location testing programs were conducted in different subdistricts of the northern region of Bangladesh to verify the research results of previously tested suitable culture patterns and exchange views among the researcher, and farmers. A total of six seasonal ponds were selected in six different subdistricts of the Rungpur region (Tab. 3). The six ponds were divided into two groups. Each group was considered as one pattern e.g. pattern-I and pattern-II and each pond or subdistrict was considered as one replication. The areas of ponds ranged between 10 and 15 decimal. The on-farm ponds were selected with the concern of relevant Senior Subdistrict Fishery Officer (SUFO/UFO). In preparation for the study selected ponds were drained and aquatic weeds were manually removed. Undesirable fish species were removed using rotenone 25-35 g dec^{-1} ft^{-1} if necessary and ponds were limed 1 kg dec^{-1}. After five days of liming, cow-dung six kg dec^{-1}, urea 100 g dec^{-1} and TSP 75 g dec^{-1} were applied at the initial stage of pond preparation. About 7-10 cm fingerlings of those fish were stocked as per experimental design (Tab. 3). Fish were fed commercially available fish feed 10-5% BW day^{-1} (containing 30-35% protein). Length-weight data and water quality parameters (viz., temperature, pH, DO, CO_2, NH_3, etc.) were collected fortnightly. The experimental design is presented in table 2.

Table 2: Experimental design of pattern-1 in different Subdistrict of Rungpur region.

Culture pattern	Replication (one pond/subdistrict)	Species combination	Stock density (indi. decl^{-1})
Pattern-1	Saidpur, Niphamari+Hatibanda, Lalmonirhat+Dimla, Niphamari	Shing+Pabda+ Rajpunti+GIFT	500+100+10+5

At the end of the experiment the selected ponds were completely dewatered and all the fish were harvested and counted species wise. Then the final length-weight of each species was recorded. The parameters such as length gain, weight gain, % weight gain, SGR, FCR, survival (%) and benefit cost ratio (BCR) were calculated. Data were analysed using MS Excel and one-way analysis of variance (ANOVA) (Duncan, 1993) and SPSS 20 (Chicago, USA) to detect significant differences among the treatments at 5% level. The values were given with means ± SD, and differences were considered significant at subset for alpha = 0.05 ($p \leq 0.05$).

RESULTS AND DISCUSSION

Growth performances of Shing, *Heteropneustes fossillis* under polyculture in farmer's pond

The growth parameters such as weight gain, SGR, survival rate (%), production of Shing and total production of candidate species were studied and presented in table 3. In this experiment, the final weights of Shing were 55.5, 52.3, and 42.2 g in T_1, T_2 and T_3 respectively. The highest weight gain (53.5 g) was found in T_1 and the lowest (40.2 g) was found in T_3. The weight gain of Shing was found to be significantly identical ($P < 0.05$) in T_1 and T_2 but higher than T_3. A more or less similar growth pattern was observed by Mou et al. (2018) who stated the growth of Shing varied between 49.5 and 69.4 g from six months. The growth performances of Shing were found to be inversely related with the stocking density. This might be due to competition for food and space. The SGR of Shing was 2.21, 2.17 and 2.03% day^{-1} respectively in T_1, T_2 and T_3. After analysis, the SGR showed to be significantly ($P < 0.05$) similar in T_1 and T_2 but lower in T_3. The FCR values were higher in T_3 (2.65) and lower in T_1 (2.51). Analytical results showed, the FCR values were directly related with the stocking density. Overall, the FCR values were at acceptable levels and indicated utilization of food soundly, which agrees with Hossain et al. (2018) and Ahamed et al (2018). The survival rate was estimated after harvesting the fish by dewatering the ponds. The survival (%) values of shing were 84, 80 and 73, respectively in T_1, T_2 and T_3. The survival of Shing was found significantly ($P < 0.05$) different among the three treatments and inversely related with the stocking density. The reason for this might again be space and food competition among the individuals. These findings agreed with Hossain et al. (2019). The production of Shing was recorded to be 4,620, 5,080 and 4,599, respectively in T_1, T_2 and T_3. Based on analysis, the production (kg ha^{-1}) of Shing in T_2 showed to be significantly ($P < 0.01$) higher followed by T_1 and T_3. The total fish production (kg ha^{-1}) was 5,896, 6,331 and 5894 in T_1, T_2 and T_3 respectively. The total production was also significantly ($P < 0.05$) higher in T_2 than in T_1 and T_3. The production is directly related with stocking density, survival and individual growth. Individual growth and survival of Shing were both higher in T_1 but T_2 had higher production. The higher production in T_2 was due to having a more dense population than $T_{1,}$ because individual weight and survival of Shing was not found significantly ($P < 0.05$) different between T_1 and T_2. In the present study, the values of survival and individual growth of shing were relatively sensible at impact on the production in T_2 than that of T_1 and T_3. The present findings are supported by the findings of Hasan et al. (2023) who obtained higher production from a higher stocking density but also found that individual growth was inversely related with the stocking density.

Table 3: Growth performances of *Heteropneustes fossillis* under polyculture in farmer's pond; within rows values with different superscripts are significantly different ($P < 0.05$).

Parameters	Treatments		
	T_1	T_2	T_3
Stock. dens. of Shing (indi. dec^{-1})	400	500	600
Culture period (months)	05	05	05
Initial weight (g)	2.0 ± 0.1	2.0 ± 0.1	$2.0 \pm .01$
Final weight (g)	55.5 ± 5.2^{a}	52.3 ± 6.5^{a}	42.2 ± 7.5^{b}
Weight gain (g)	53.5 ± 4.1^{a}	50.3 ± 5.5^{a}	40.2 ± 6.7^{b}
SGR (% day^{-1})	2.21 ± 0.03^{a}	2.17 ± 0.03^{a}	2.03 ± 0.02^{b}
FCR	2.51 ± 0.01^{c}	2.55 ± 0.01^{b}	2.65 ± 0.01^{a}
Survival (%)	84 ± 1.0^{a}	80 ± 2.6^{a}	73 ± 2.6^{ab}
Production of Shing (kg ha^{-1})	4620 ± 30^{b}	$5,080 \pm 75^{a}$	4599 ± 20^{b}
Total production (kg ha^{-1})	$5,896 \pm 87^{b}$	$6,331 \pm 211^{a}$	5894 ± 100^{b}

Physiochemical parameters of the experimental ponds

The water quality parameters viz., temperature (^{o}C), transparency (cm), water pH, DO (mg l^{-1}) and ammonia (mg l^{-1}) of experimental ponds under three different treatments were monitored and presented in table 4. The water temperature varied between 27.8°C and 28.5°C during the experiment and there were no significant differences among the treatments. The range was selected due to being suitable for fish culture as reported by Rahman et al. (2018) and Hossain et al. (2022) who stated that 25.5℃ to 30.0℃ is favorable for fish culture. The values of water transparency were 26.5, 26.6 and 27.1 cm respectively, in T_1, T_2 and T_3. Similar values were reported by Kohinoor and Rahman (2015) who recorded 26.8 to 30.4 cm transparency in successful Koi culture ponds. The mean values of pH were 7.8, 7.7 and 7.6 in treatment T_1, T_2 and T_3 respectively. According to Boyd (1982), the pH values of water ranging from 7.3 to 9.0 indicated that the experimental ponds were suitable for fish culture. The DO concentration ranged from 5.4 to 6.0 mg l^{-1} during the experiment and no significant difference was observed among the treatments. Hasan et al. (2023) reported that dissolved oxygen content for fish culture should be maintained from 5.0 to 8.0 mg l^{-1}. So, it is to be assumed that the dissolved oxygen level was suitable for fish culture in the present study. Ammonia varied from 0.08 to 0.12 mg l^{-1} among the treatments. Shamsuddin et al. (2022), Ahamed et al. (2018), and Huque et al. (2008) stated that ammonia levels varied between 0.16 and 0.24 mg l^{-1} in Shing polyculture ponds within the northern region of Bangladesh. This finding agrees with the findings of the present study. Based on experimental results and the above discussion it can be concluded that the water quality parameters of the present study were ideal for fish culture.

Table 4: Physiochemical parameters of the experimental ponds of H. fossillis polyculture.

Water quality parameters	T_1	T_2	T_3
Water temperature (^oC)	28.0 ± 2.5	27.8 ± 3.0	28.5 ± 2.0
Water transparency (cm)	26.5 ± 1.5	26.6 ± 2.0	27.1 ± 1.0
Water pH	7.8 ± 1.0	7.7 ± 1.5	7.6 ± 1.0
DO (mg l^{-1})	6.0 ± 0.5	5.5 ± 0.6	5.4 ± 0.5
NH_3 (mg l^{-1})	0.08 ± 0.01	0.10 ± 0.01	0.12 ± 0.01
Water temperature (^oC)	28.0 ± 2.5	27.8 ± 3.0	28.5 ± 2.0
Water transparency (cm)	26.5 ± 1.5	26.6 ± 2.0	27.1 ± 1.0

Economic analysis

A simple economic analysis was conducted to estimate the benefit cost ratio (BCR) of Shing polycultures (Tab. 6). The total production (kg ha^{-1}) of fish was recorded as 5,896, 6,331 and 5,894, respectively in T_1, T_2 and T_3. The results were similar to those found by Ahamed et al. (2017a) who reported a production range from 6,981 to 7,793 kg ha^{-1} where the stocking density of Shing ranged from 500 to 700 indi. $dec.^{-1}$ during a five month polyculture. The highest production cost (Tk. ha^{-1}) was recorded in T_3 (1,362,392) and the lowest was T_1 (1,159,508) (Tab. 5). The expenditures in three treatments varied significantly ($P < 0.05$) among themselves. On the basis of analysis, the gross return (Tk. ha^{-1}) was found significantly ($P < 0.05$) highest in T_2 (1,877,570) followed by T_3 (1,736,143) and then T_1 (1,731,253). In the case of gross margin (Tk. ha^{-1}), it was significantly ($P < 0.05$) highest in T_2 (644,570) followed by T_1 (571,828) and T_3 (373,752). Furthermore, significantly ($P < 0.05$) higher BCR were recorded in T_2 (1.52) followed by T_1 (1.48) and then T_3 (1.27). This BCR in the present study aligned with the findings of Ahamed et al. (2017b) who stated that the BCR ranged from 1.32 to 1.69 when the stocking density of Shing ranged from 500 to 700 indi. $dec.^{-1}$ induring a five month polyculture. Previous study results agreed with the findings of the present study and the results indicated that improvement of growth, survival and production of Shing through polyculture was possible in seasonal waters. Alongside the economic aspect species combinations were also considered as an important factor for large scale production. After a discussion of the results and consideration of economic aspectsit can be concluded that T_2 (500 Shing+100 Pabda+10 Rajpunti+5 GIFT indi. $dec.^{-1}$) is the best combination for Shing polycultures in seasonal ponds within the Semi-arid zone of Bangladesh.

During the first year of the study polyculture testing of Shing, *Heteropneustes fossilis*, Tengra and *M. vittatus* were carried out in the seasonal ponds of farmers adjacent to FSS, Saidpur. Different treatments were tested and of them, the 500 Shing+100 Pabda+10 Rajpunti+5 GIFT (indi. $dec.^{-1}$) combination from Shing polyculture and the 500 Tengra+100 Magur+10 Rajpunti+5 GIFT (indi. $dec.^{-1}$) combination from Tengra polyculture were selected for multi-location testing due to having the highest yield and economic viability. For this reason, the two chosen combinations were demonstrated in different subdistrict within the northern region of Bangladesh from May 2018 to September 2018.

Table 5: Benefit and cost analysis of Shing under polyculture in three treatments; within rows values with different superscripts are significantly different ($P < 0.05$).

Item wise expenditure	T_1	T_2	T_3
Pond preparation (Tk. ha^{-1})	25,000	25,000	25,000
Fingerling cost (Tk.ha^{-1})	2,71,500	3,29,000	4,04,583
Lime and fertilizer (Tk.ha^{-1})	12,500	12,500	12,500
Feed costs(Tk.ha^{-1})	8,00,258	8,16,375	8,88,391
Transport, labor etc.(Tk.ha^{-1})	50,000	50,000	50000
Total production costs (Tk. ha^{-1})	11,59,258 ± 4409^c	12,32,875 ± 2281^b	13,80,474 ± 12291^a
Income and output			
Total production (kg ha^{-1})	5,896 ± 87^b	6,331 ± 211^a	5894 ± 100^b
Gross return (Tk. ha^{-1})	17,31,253±1397^b	18,77,570 ± 400^a	17,36,143±3755^b
Gross margin (Tk. ha^{-1})	5,71,828 ± 3402^b	6,44,570 ± 8886^a	3,73,752 ± 2558^c
Benefit cost ratio (BCR)	1.49^b	1.52^a	1.25^c

Polyculture of Shing, *Heteropneustes fossilis* in multi-location testing

After the culture tenure, growth parameters and production of Shing, the total production of cultured fish and the economics of multi-location trials are presented in table 6. The initial weight (g) of Shing was 1.90, 1.87 and 1.92 respectively in Saidpur, Hatibandha, Dimla. The highest final weight of Shing was recorded (59.3 g) in Dimla Subdistrict. The final weight gain (g) of Shing varied in different locations and the value of Dimla (57.38) was found to be higher compared to that of Saidpur (54.33) and Hatibandha Subdistrict (52.33). The highest SGR (2.29) was in Dimla. The survival rate (78%) was significantly ($P < 0.05$) higher in Saidpur followed by Hatibandha and Dimla Subdistrict. Ahamed et al. (2017a) also found similar results in their case studying the adaptability of stinging catfish within the Northern region. The production of Shing (5,828 kg ha-^{1}) and the total production of fish (7,352 kg ha-^{1}) were found to be highest in Dimla Subdistrict followed by Saidpur and then Hatiandha. The gross return (Tk. ha^{-1}) 2,135,000, gross margin (Tk ha^{-1}) 906,000 and benefit cost ratio (1.73) were significantly ($p < 0.05$) higher in Dimla Subdistrict compared to Saidpur and Hatibandha. Ahamed et al. (2018) mention that in case of Vietnamese koi generated the highest return over a period of four months Tk. 7, 26,780/ ha. The lowest net return was found to be Tk. 2, 64,160/ha within T-2 in the semi-arid zone of Bangladesh. This variation could be attributed to different species, culture systems and market prices. Significantly higher ($P < 0.05$) BCR was recorded in T-1 (1.64) with low production cost and comparatively higher net profit than other treatments. According to multi location results, the production of Shing, total production of fish and the BCR were found higher and more satisfactory than that of the 1st year, which might be due to suitable stocking density and appropriate culture periods. Therefore, the combinations including 500 Shing+100 Pabda+10 Rajpunti+5 GIFT indi. dec. $^{-1}$ can be recommended for culture in the northern region of Bangladesh. It is also important to remember that this type of culture pattern is appropriate in seasonal water bodies with no requirement of additional water supply during the culture period ranging from May to October.

Table 6: Growth and yield performances of *Heteropneustes fossilis* under polyculture in multi-location of northern part of Bangladesh; within rows values with different superscripts are significantly different ($P < 0.05$).

Parameters	Multi-location			Average
	Saidpur	Hatibandha	Dimla	
Stock. dens of Shing (indi. dec^{-1})	500	500	500	500
Culture period (months)	5	5	5	5
Initial weight (g)	56.3 ± 0.9^{b}	1.87 ± 0.0	1.92 ± 0.0	1.9 ± 0.3
Final weight (g)	56.3 ± 0.9^{b}	54.2 ± 0.3^{c}	59.30 ± 0.9^{a}	56.6 ± 2.6
Weight gain (g)	54.3 ± 0.9^{b}	52.3 ± 0.3^{c}	57.38 ± 0.9^{a}	54.66 ± 2.6
SGR (% day^{-1})	2.25 ± 0.02^{ab}	2.24 ± 0.02^{b}	2.29 ± 0.02^{a}	2.26 ± 0.3
FCR	2.63 ± 0.01	2.64 ± 0.02	2.62 ± 0.01	2.63 ± 0.1
Survival (%)	78 ± 1.5^{a}	75 ± 1.0^{b}	74 ± 1.5^{b}	75.67 ± 2.1
Production of Shing (kg ha^{-1})	5476 ± 2.0^{b}	5351 ± 3.5^{c}	5,828 ± 3.1^{a}	5552 ± 247
Total production (kg ha^{-1})	7039 ± 70.3^{b}	6766 ± 40^{c}	7352 ± 2.5^{a}	7052 ± 193
Total cost (Tk. ha^{-1})	1221500 ± 15.3	1221650 ± 10	1229000 ± 10	1224050 ± 4287
Gross return (Tk. ha^{-1})	2040850 ± 5.7^{b}	1976375 ± 5.0^{c}	2135000 ± 7.6^{a}	2050742 ± 79773
Gross margin (Tk. ha^{-1})	819350 ± 18.5^{b}	759875 ± 8.1^{c}	906000 ± 10.4^{a}	826692 ± 73482
BCR	1.67^{b}	1.62^{c}	1.73^{a}	1.67

CONCLUSIONS

Polycultures of Shing (*Heteropneustes fossilis*) are economically viable. Considering the growth and survival, Shing was found as the most suitable stocking density for a polyculture system. Fish farmers should follow the combination 500 Shing+100 Pabda+10 Rajpunti+5 GIFT indi. dec.$^{-1}$ for a Shing polyculture. Within the context of fish production, Shing polycultures were found suitable for semi-arid zones. From an economic perspective, Tengra polycultures and Shing polycultures were identical. An appropriate culture period from April to August and overwintered fingerlings were identified as the keys to a successful fish culture in seasonal ponds. Water quality parameters were also found suitable for fish culture. Fish farmers were very much interested in Shing polycultures due to this being a modern technique. The study as a whole explored new culture techniques for the Northern semi-arid region of Bangladesh.

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