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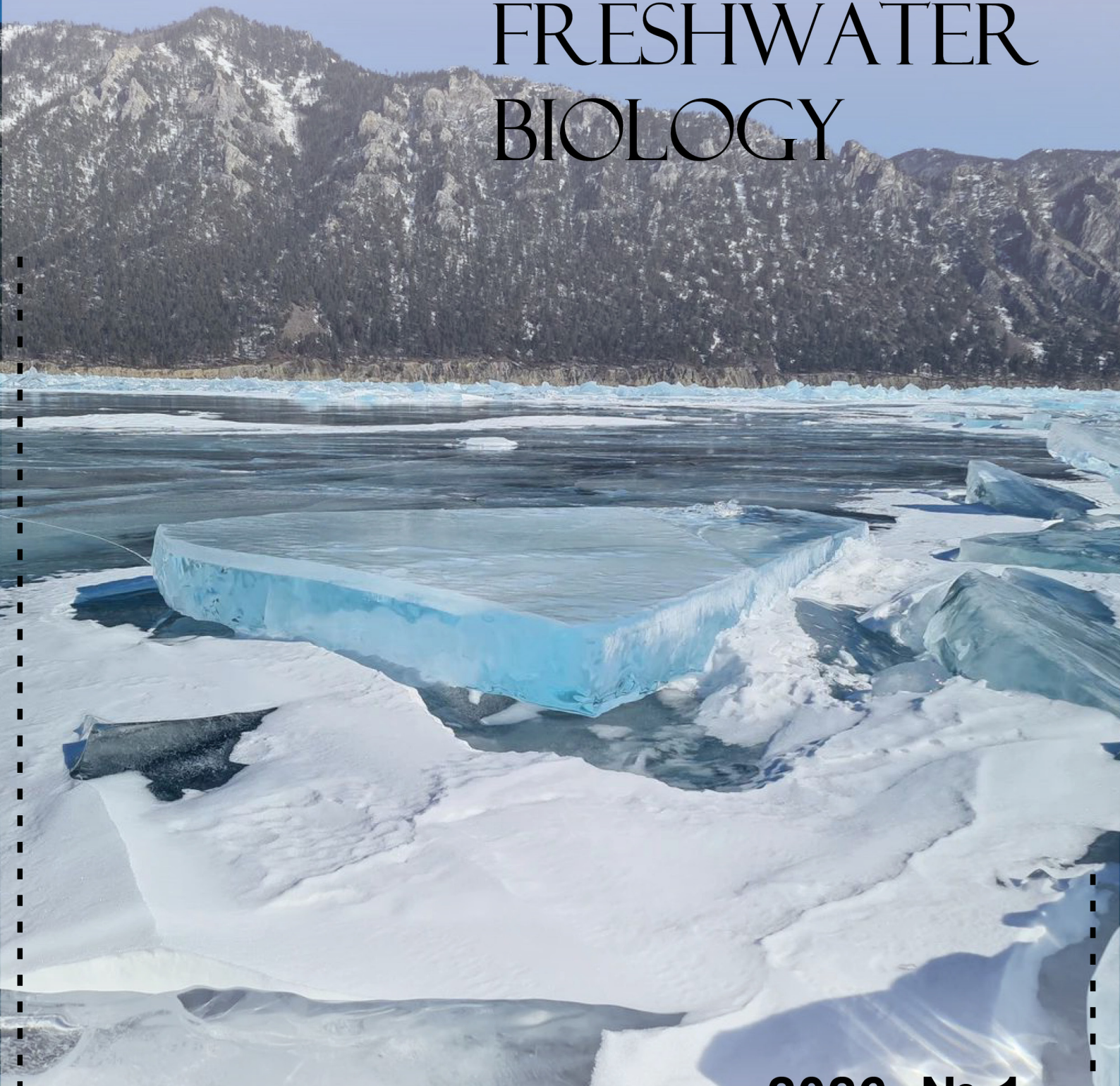

2026, № 1

> abiotic and biotic water components;
> ecosystem-level studies;
> systematics and aquatic ecology;
> paleolimnology and environmental histories;
> laboratory experiments and modeling

New report of *Craspedacusta sowerbii* (Lankester, 1880) medusae in Belgium

Short communication

LIMNOLOGY FRESHWATER BIOLOGY

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ABSTRACT. The jellyfish *Craspedacusta sowerbii* (Lankester, 1880) is known to be an invasive species in freshwater worldwide, except in Antarctica. In Belgium, *C. sowerbii* was first recorded in 1939 by Damas in the River Meuse. Since then, few further Belgian observations of this species have been made. Here, we report the presence and determine the gender of *C. sowerbii* medusae in an artificial pond in Forrières, in southern Belgium. This is the first record of this species in the province of Luxembourg. Our findings suggest that the population consists exclusively of females.

Keywords: *Craspedacusta sowerbii,* freshwater jellyfish, invasive species

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1. Introduction

Craspedacusta sowerbii (Lankester, 1880) (Cnidaria: Limnomedusae: Olindiidae) is a freshwater jellyfish considered to be an invasive species. Native to the Yangtze valley in China, *C. sowerbii* is now found on every continent except Antarctica (Dumont, 1994; Oualid et al., 2019). The life cycle of *C. sowerbii* includes both an attached polyp and swimming medusa (jellyfish) stage. However, most of the studies provided observations of the medusa form only. The polyp form is much more difficult to observe because it is only about a millimetre in size. In Belgium, *C. sowerbii* was first recorded in 1939 by Damas in the River Meuse. In his work, Damas reported finding numerous polyps of *C. sowerbii* attached to stones at the riverbed. He observed the jellyfish only in June and noted that the specimens were exclusively young, non-sexual specimens not exceeding 1.5 mm in size. Since then, there have been few scientific publications mentioning the presence of *C. sowerbii* in Belgium, though specific locations were not always provided (Parent, 1982; Dumont, 1994). Additionally, three sightings have been reported by naturalist groups and in local magazines targeted at the general public. The first concerns an artificial pond located in Anderlecht, a municipality in the city of Brussels (Symoens, 1953). The other two were reported in artificial environments: Lake Eau d'Heure in 2012 in the province of Hainaut and, a year later in 2013, in the Kelchterhoef pond in the province of Limburg (Notre Nature, 2021).

In this work, a new record of *C. sowerbii* from an artificial pond in Southern Belgium is reported. Morphological methods were used to identify and sex the jellyfish.

2. Material and methods

2.1. Study area

During July of 2024, *C. sowerbii* medusae were observed in a small artificial pond constructed in 2006 in the Luxembourg Province. The pond is located in the village of Forrières at 202 m a.s.l. (50°07'58.81"N; 5°16'48.26"E), has a maximum depth of about 1 m and covers an area of around 3,000 square meters (Fig 1A, B). The water temperature, pH and conductivity were measured using a Flintronic multiparameter probe (EZ9908). NO^{2-}, NO^{3-}, GH, KH and Cl_2 values were roughly estimated using JBL test strips for water analysis.

2.2. Observation of live jellyfish

The high turbidity of the water limited visibility to just a few centimetres below the surface, allowing

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observation only of jellyfish near the water's surface (Fig. 1C). A visual inspection of the jellyfish distribution was undertaken throughout the pond. On July 11, four specimens were collected and placed in a 20-litre aquarium for 10 days to photograph and record videos using a Canon M50 digital camera. The aquarium was maintained at room temperature, with no artificial lighting or filtration. Daily, one litre of water was replaced with fresh pond water to provide the necessary plankton for feeding the jellyfish. On the tenth day, the jellyfish were fixed in a 4% formalin solution. A further 17 specimens were then captured and immediately fixed in the same solution.

2.3. Microscopic investigations

One specimen was dissected to isolate the tentacles and gonads. Tentacles were placed on a glass slide, stained for 5 minutes with haematoxylin and washed with fresh water. Gonads were also placed on a glass slide, stained for 5 minutes with haematoxylin, washed with fresh water, stained for 5 minutes with 2% eosin and washed again with fresh water. The tissues were covered with a coverslip and gently squashed so that they could be observed under the light microscope Swift SW380T equipped with a Canon M50 camera. The twenty other specimens were observed without staining under a Bresser Analyth STR stereomicroscope used with transmitted light and equipped with the same camera. The diameter of the jellyfish was measured on individuals fixed in formalin.

3. Results

3.1. Observation of live jellyfish

The jellyfish were observed *in situ* for a period of three weeks. They were visible only on the surface of the water. Nevertheless, we were able to collect them on the bottom of the pond using a net in places where they were not visible on the surface. Their location varied from day to day, but they were observed in all parts of the pond during this period. The high turbidity of the water made it impossible to assess the density of the populations, but we never saw more than three jellyfish per square metre. The average temperature of the pond was 23.5°C, pH 8.2, conductivity was 451 µS. Nitrite, nitrate and chloride ions were undetectable, hardness (GH) was between 125 and 250 ppm and alkalinity (KH) was around 250 ppm. In the aquarium, the jellyfish were active day and night, swimming actively towards the surface and dropping slowly to the bottom.

3.2. Morphology and sex determination

The average diameter of fixed specimens was 15 +/- 3 mm, ranging from 10 to 20 mm. Boothroyd et al. (2002) report that the average shrinkage due to 4% formalin is more or less 10%.

The taxonomic status of the jellyfish was determined according to the descriptions of Jankowski (2001) and Lüskow et al. (2021). The manubrium extends onto the subumbrella (Fig. 2A), four gonads

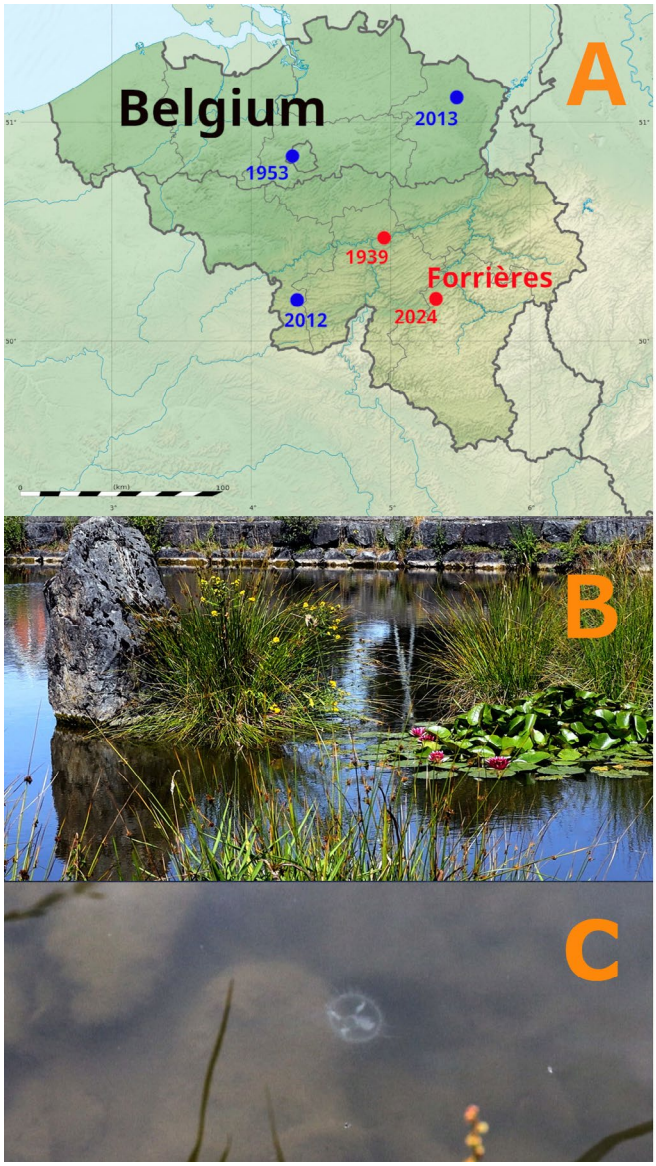

Fig.1. a) Location of Forrières on the map of Belgium. Blue dots : naturalist publications, red dots : scientific sightings. b) General aspect of the pond. c) Mature medusa videotaped during an observation on 25 July 2024.

are suspended along the radial canals (Fig. 2A, B), and four long perradial tentacles are visible in the extension of the radial canals (Fig. 2B). On the surface of the tentacles, the nematocysts are grouped together in plates (papillae), arranged in more or less complete parallel rings (Fig 2C, D).

The gonads of the 20 jellyfish were examined under the transmitted light stereomicroscope to detect oocytes. The gonads are elongated and flattened laterally. They are larger near the edge of the umbrella and smaller near the stomach (Fig. 2E). All gonads of the 20 jellyfish contain oocytes between 50 and 150 µm in size (Fig. 2F). They appear dark and are clearly visible inside the gonad (Fig. 2E). All the jellyfish sampled were therefore female. The ovaries of the smallest jellyfish (10 mm in diameter) contain far fewer oocytes (Fig. 2G). In larger individuals, the number of oocytes appears to be highly variable (from 10 to more than 300 oocytes per gonad, Fig. 2H).

4. Discussion and conclusion

There is very few scientific literature documenting sightings of *C. sowerbii*. in Belgium. This is the first time this jellyfish has been found in Forrières and even in the province of Luxembourg. The origin of this population is unclear, but it is likely related to the introduction of polyps or resistant forms known as podocysts. Under adverse conditions, *C. sowerbii* polyps can encyst, forming a chitinous shell. This podocyst stage can endure complete desiccation for up to 40 years (Bouillon and Boero, 2000). Podocysts can be carried by plants or animals, such as birds or fish (El Mousaoui, 2015). This may explain the presence of *C. sowerbii* in Forrières, where ornamental water lilies are present and where ducks, geese and grey cranes (*Grus grus*) are frequently observed. Additionally, local fishermen regularly bring in fish.

In temperate zones, the jellyfish occurrence generally coincides with the warmest temperatures (DeVries, 1992; Purcell, 2005).The Forrières pond is very shallow and its temperature can easily rise in summer, even in depth. This could explain the appearance of jellyfish. Some researchers therefore suggest that the increase in the number of sightings of the jellyfish form is due to recent climate change (Lundberg and Svensson, 2003; Marchessaux et al., 2022). However, there is currently no evidence of this.

The jellyfish collected in Belgium measured 10–20 mm in diameter (fixed specimens). Considering an estimated 10% shrinkage in 4% formalin, their in vivo size would range from approximately 11 to 22 mm. These dimensions are fully consistent with the typical size range reported for *Craspedacusta sowerbii*, whose medusae generally reach 5–25 mm and occasionally up to 25–30 mm under warm conditions (25–30 °C) (Lüskow et al., 2021). Therefore, the Belgian specimens fall within the expected range for this species and correspond to the medium-sized, sexually mature individuals frequently recorded in lentic environments of temperate regions.

Determining the sex of *Craspedacusta sowerbii* fixed in formalin requires microscope examination of the gonads in sexually mature individuals. All specimens examined from the pond in Forrières were available for sex determination and were of the same sex: female. Our observations are consistent with other studies (Boothroyd et al., 2002). Outside of China, very few reports have documented populations with medusae of both sexes, e.g.: a pond in Southampton Quarry at Richmond, USA (Rice, 1958), Lake Mead, Nevada, USA (Deacon and Haskell, 1967), Haruta-ike, an artificial pond in Chikuma City, Nagano, Japan (Peterson et al., 2022), Lake Santo of Monte Terlago, Italy (Morpurgo et al., 2024).

Several hypotheses may explain the unisexual character of the population observed at Forrières. It may result from a founder effect, where genetic factors determine sex, and a single polyp or podocyst gives rise to medusae of only one sex. In 1924, Payne proposed parthenogenetic development of the eggs of *Craspedacusta ryderi*, but true parthenogenesis, i.e. the development of unfertilized eggs, has never been

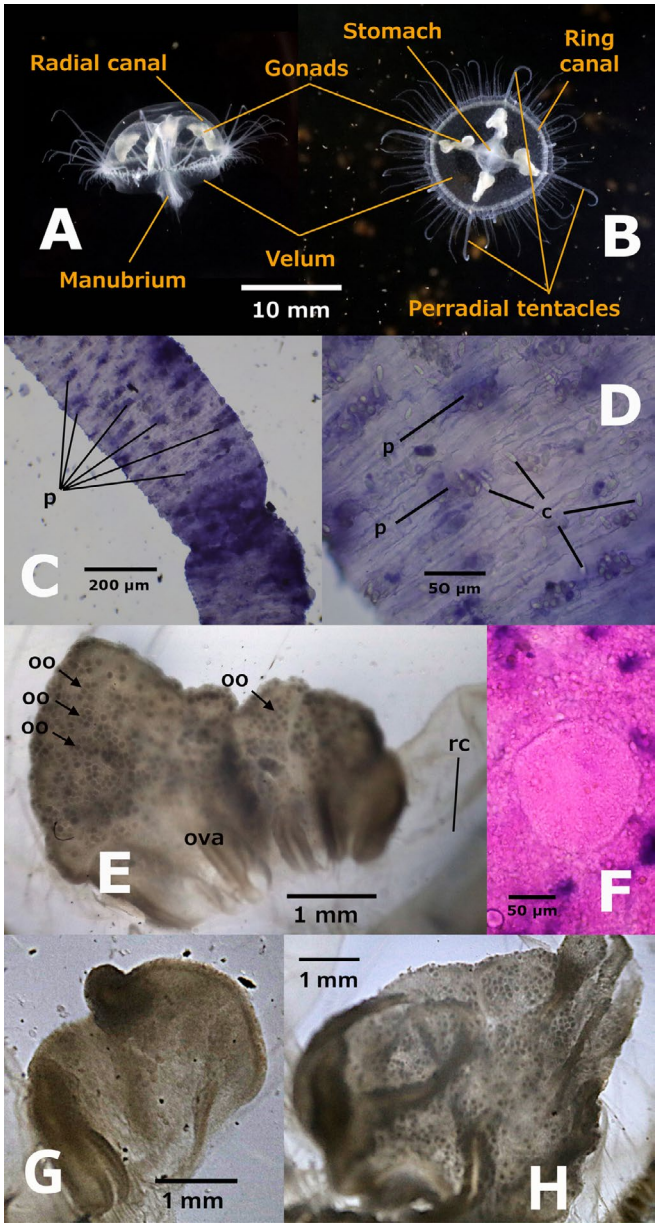

Fig.2. A-B. Side view (A) and top view (B) of the *C. sowerbii* medusae observed in aquarium. C. Tentacle showing the circular arrangement of the nematocyte plates or papillae (p). D. High magnification of papillae (p) showing cnidocytes (c). E. General view of the gonads: ovary (ova) showing oocytes (oo). rc = radial canal. F. High magnification of a large oocyte stained with hematoxylin/eosin. G. Ovary of small individual (10 mm in diameter) with few, sparsely distributed oocytes. H. Ovary of large individual (20 mm in diameter) with numerous oocytes at various stages of maturation.

demonstrated in *C. sowerbii*. In 2000, Carré and Carré reported that the sex of medusae in the genus *Clytia* is temperature-dependent, suggesting that environmental factors can influence sex determination in some hydrozoans. Recent molecular analyses revealed the existence of distinct genetic lineages within the *C. sowerbii* species complex, some of which appear to correlate with the sex of medusae (Schachtl, 2019; Wang, 2022). While environmental factors may modulate the timing and maturation of gonads, they do not satisfactorily explain the widespread unisexual swarms, which are best interpreted as the result of an underlying genetic mechanism; genome-based sex markers are still needed to confirm the exact pathway.

Our report indicates the presence of *C. sowerbii* in a Belgian region where it has not yet been described. It is the first report in Belgium that specify the sex of the *C. sowerbii* jellyfish population observed. It would be useful to survey other ponds in the region to assess the extent of the invaded areas. To do this, polyp forms should be detected.

Acknowledgements

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Conflict of Interest

The authors declare no conflicts of interest.

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Assessing the effects of dietary and waterborne commercial salt on the performance and welfare of common carp

Original Article

LIMNOLOGY FRESHWATER BIOLOGY

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ABSTRACT. This study investigated the effects of salt additives in water and diet on the physiological and health parameters of common carp (*Cyprinus carpio* L.). Fingerlings (~50 g) were divided into seven groups: a control group (G1), three water treatment groups ($G2_{A-C}$; 4, 8, and 12 g L^{-1} salt), and three diet treatment groups ($G3_{A-C}$; 5, 10, and 15 g kg^{-1} dietary salt). All fish were fed a standard diet containing 28% crude protein at 3% body weight per day. Results revealed that G1 had the highest values for lymphocyte and monocyte counts, mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), cholesterol, triglycerides, low-density lipoprotein cholesterol (LDL), albumin, total protein, and several somatic indices. $G2_B$ exhibited significantly higher potassium and sodium levels, while $G3_A$ showed elevated white blood cell (WBC), granulocytes, liver enzyme activities (Alanine aminotransferase activity (ALT), aspartate aminotransferase activity (AST)), creatinine, glucose, chloride, and various somatic indices. $G2_A$ and $G2_B$ groups had increased red blood cells (RBC), hemoglobin, hematocrit, and body weight index. The findings indicate that using 12 g L^{-1} salt in rearing water and 15 g kg^{-1} salt in the diet can enhance growth and health performance in *Cyprinus carpio*. This study provides important insights into salt-induced physiological responses in carp and offers practical recommendations for aquaculture practices.

Keywords: dietary salt, adding to rearing water, *Cyprinus carpio*, health, blood

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1. Introduction

The aquaculture and fisheries sectors play a crucial role in ensuring global food security and nutrition. Further improvement of this contribution requires faster implementation of key reforms in policy, management, innovation, and investment (FAO, 2022). Common salt (NaCl) is readily accessible and safe for humans and fish. It leaves no residue on the flesh of fish, making it a popular choice for aquaculture in many countries. Salt is a basic and economical material frequently used in handling freshwater fish. Its numerous beneficial applications and advantages include the capacity to mitigate handling stress, support osmoregulatory function, and contribute to the prevention and control of diseases, enhance fish health and survival both before and after transportation, reduce the effects of adverse environmental conditions, and promote the welfare of breeding fish throughout and following the spawning period (Kubitza, 2016).

The bulk of aquaculture farmers do not consider the use of salt to reduce fish losses. In fact, salt is frequently overused, administered too late, or used during very small periods or at extremely low and useless dosages. Furthermore, many fish farms lack proper facilities for timely fish handling and treatment. In addition to being a reliable and secure medication for controlling some external parasites, salt was also reported to lower the incidence of bacterial and fungal infections following fish handling by mitigating associated stress responses (Kubitza, 2016).

Due to increased GRs or shortened culture periods, adding growth-inducing chemicals to meals has the potential to be lucrative (Abdel-Tawwab et al.,

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2022; Abdulrahman, 2022; Abedalhammed et al., 2017; Nader and Abdulrahman, 2017). Supplementary additives such as sodium chloride are almost perfect for promoting growth when added to artificial feed. Using salt is not a recent development. Salt is one of the essential mineral elements needed by both animal and plant bodies for normal functioning. It improves the taste of food, controls the body's osmotic pressure, forms acid in the stomach mucous membrane (activating pepsin and salivary gland enzymes), and maintains normal digestive processes (Debnath et al., 2017).

Juvenile common carp reared in freshwater for fish farming may benefit from the addition of sodium chloride to the meal, since it affects body composition. Common carp juveniles raised in freshwater can greatly benefit from increased food consumption and growth performance when 1.5% salt is added to their diet. For young common carp raised in freshwater, the 1.5% diet had the best growth and biological performance (Nasir and Qusey, 2016).

This study aims to investigate the effect of salt addition in common carp as a way of precaution and treatment. This will be achieved by measuring certain physiological and health indices in six experimental groups, one control group, and three groups receiving different levels of salt added to the rearing water and mixed into their diets. There are three main objectives in this article: (a) to determine the effects of salt diets on some physiological aspects; (b) to determine if salt treatment can prolong the health indices; and (c) to examine the effect of salt additives in fish diets on health indices.

2. Material and methods

Fingerlings of common carp were obtained from a fish farm in Hilla, Iraq, and acclimatized to laboratory conditions for 21 days. During this period, the fish were fed a commercial diet with a 28% crude protein content. After acclimation, fish averaging ~51.9 g were separated into seven groups, with each group replicated three times, and placed in 21 tanks, each containing 70 liters (10 fish per tank). All tanks were equipped with air stones connected to an air pump for aeration. Fish were fed the control diet twice per day, with an amount equivalent to 3% of their body weight, and the tank's water was changed daily.

Each tank was fitted with air stones connected to an air pump for aeration. Fish were fed the control diet twice daily. Fish were reared for 10 weeks to study the effect of daily use of normal salt in rearing tanks with the following levels:

G1: the control group without salt;
$G2_A$: daily adding of 4 g L^{-1} rearing water;
$G2_B$: daily adding of 8 g L^{-1} rearing water;
$G2_C$: daily adding of 12 g L^{-1} rearing water;
$G3_A$: daily adding of 5 g kg^{-1} diet;
$G3_B$: daily adding of 10 g kg^{-1} diet;
$G3_C$: daily adding of 15 g kg^{-1} diet.

Table 1 summarizes the chemical composition of aquarium water (including temperature, pH, dissolved oxygen (DO), total dissolved solids (TDS), and total alkalinity), which was assessed at the start, mid-point, and end of the trial under the protocols established by APHA (2017). All parameters remained within accept-

Table 1. The chemical composition of aquarium water (mg L^{-1})

Water parameters	Time	Control	4 g salt 1L rearing water	8 g salt 1L rearing water	12 g salt 1L rearing water	5 g salt kg diet	10 g salt kg diet	15 g salt kg diet
Temperature	Start	25 ± 0.35	25 ± 0.35	25 ± 0.35	25 ± 0.35	25 ± 0.35	25 ± 0.35	25 ± 0.35
	Mid	24.37 ± 0.34	25.05 ± 0.35	24.95 ± 0.35	25.27 ± 0.36	25.17 ± 0.35	25.04 ± 0.35	24.83 ± 0.35
	End	24.18 ± 0.33	24.99 ± 0.35	25.94 ± 0.36	25.92 ± 0.36	24.91 ± 0.35	24.87 ± 0.35	25.18 ± 0.35
DO	Start	4.23 ± 0.05	4.54 ± 0.06	4.31 ± 0.06	4.44 ± 0.06	4.05 ± 0.05	4.93 ± 0.07	4.59 ± 0.06
	Mid	4.33 ± 0.06	3.19 ± 0.04	3.88 ± 0.05	4.07 ± 0.05	4.13 ± 0.05	4.05 ± 0.05	3.87 ± 0.05
	End	4.27 ± 0.06	4.12 ± 0.05	4.23 ± 0.05	3.99 ± 0.05	4.17 ± 0.05	4.28 ± 0.06	3.23 ± 0.04
pH	Start	7.1 ± 0.09	7.11 ± 0.09	7.18 ± 0.09	7.94 ± 0.11	7.92 ± 0.11	7.17 ± 0.09	7.11 ± 0.09
	Mid	7.65 ± 0.1	8.62 ± 0.12	8.07 ± 0.11	7.99 ± 0.11	7.23 ± 0.09	7.19 ± 0.09	7.2 ± 0.09
	End	7.67 ± 0.1	8.26 ± 0.12	8.26 ± 0.12	8.27 ± 0.12	7.27 ± 0.1	7.79 ± 0.11	7.87 ± 0.11
TDS	Start	212.18 ± 2.99	215.27 ± 3.04	211.15 ± 2.98	213.21 ± 3.01	216.3 ± 3.06	218.36 ± 3.09	214.24 ± 3.02
	Mid	206 ± 2.91	209 ± 2.95	205 ± 2.89	207 ± 2.92	210 ± 2.96	212 ± 2.99	208 ± 2.94
	End	214.24 ± 3.02	217.36 ± 3.07	213.2 ± 3.01	215.28 ± 3.04	218.4 ± 3.09	220.48 ± 3.11	216.32 ± 3.06
Total Hardness	Start	188.9 ± 2.67	195.7 ± 2.76	189.82 ± 2.68	191.58 ± 2.7	197.76 ± 2.8	196.83 ± 2.78	193.84 ± 2.74
	Mid	183.4 ± 2.59	190 ± 2.68	184.3 ± 2.6	186 ± 2.63	192 ± 2.71	191.1 ± 2.7	188.2 ± 2.65
	End	190.73 ± 2.69	197.6 ± 2.79	191.67 ± 2.7	193.44 ± 2.73	199.68 ± 2.82	198.74 ± 2.81	195.72 ± 2.77
Total Alkalinity	Start	133 ± 1.88	144.2 ± 2.03	140.1 ± 1.97	140 ± 1.97	128.2 ± 1.81	127.3 ± 1.8	127.9 ± 1.81
	Mid	136.99 ± 1.93	148.52 ± 2.09	144.3 ± 2.03	144.2 ± 2.03	132.04 ± 1.86	131.11 ± 1.85	131.73 ± 1.86
	End	142.46 ± 2.01	154.46 ± 2.18	150.07 ± 2.12	149.96 ± 2.11	137.32 ± 1.94	136.36 ± 1.92	137 ± 1.93
Turbidity	Start	0.51 ± 0.01	1.23 ± 0.01	2.47 ± 0.03	2.78 ± 0.03	1.13 ± 0.01	1.44 ± 0.02	1.64 ± 0.01
	Mid	0.5 ± 0.01	1.2 ± 0.01	2.4 ± 0.03	2.7 ± 0.03	1.1 ± 0.01	1.4 ± 0.02	1.6 ± 0.02
	End	0.52 ± 0.01	1.26 ± 0.02	2.52 ± 0.03	2.83 ± 0.03	1.15 ± 0.01	1.47 ± 0.02	1.68 ± 0.02

Water parameters	Time	Control	4 g salt 1L rearing water	8 g salt 1L rearing water	12 g salt 1L rearing water	5 g salt kg diet	10 g salt kg diet	15 g salt kg diet
Sulphates (SO4-)	Start	15.4 ± 0.21	15.8 ± 0.22	16.3 ± 0.23	16.1 ± 0.22	15.9 ± 0.22	17.1 ± 0.24	16 ± 0.22
	Mid	15.86 ± 0.22	16.27 ± 0.22	16.78 ± 0.23	16.58 ± 0.23	16.37 ± 0.23	17.61 ± 0.24	16.48 ± 0.23
	End	16.01 ± 0.22	16.43 ± 0.23	16.95 ± 0.24	16.74 ± 0.23	16.53 ± 0.23	17.78 ± 0.25	16.64 ± 0.23
Nitrates (NO_3^-)	Start	33 ± 0.46	41.9 ± 0.59	44.11 ± 0.62	45.5 ± 0.64	42.1 ± 0.59	44.15 ± 0.62	46.5 ± 0.65
	Mid	34.65 ± 0.48	43.99 ± 0.61	46.31 ± 0.65	47.77 ± 0.67	44.2 ± 0.62	46.35 ± 0.65	48.82 ± 0.68
	End	33.99 ± 0.48	43.15 ± 0.61	45.43 ± 0.64	46.86 ± 0.66	43.36 ± 0.61	45.47 ± 0.63	47.89 ± 0.67
Phosphates (PO_4^-)	Start	0.3 ± 0.01	0.37 ± 0.01	0.38 ± 0.01	0.36 ± 0.01	0.32 ± 0.01	0.35 ± 0.01	0.36 ± 0.01
	Mid	0.3 ± 0.01	0.36 ± 0.01	0.37 ± 0.01	0.35 ± 0.01	0.32 ± 0.01	0.34 ± 0.01	0.35 ± 0.01
	End	0.31 ± 0.01	0.37 ± 0.01	0.38 ± 0.01	0.36 ± 0.01	0.33 ± 0.01	0.35 ± 0.01	0.36 ± 0.01
Chlorides (Cl^-)	Start	20 ± 0.28	22.3 ± 0.31	23.5 ± 0.33	24 ± 0.33	22.8 ± 0.32	24.2 ± 0.33	23.8 ± 0.33
	Mid	20.6 ± 0.28	22.96 ± 0.32	24.2 ± 0.34	24.72 ± 0.34	23.48 ± 0.33	24.92 ± 0.35	24.51 ± 0.34
	End	21 ± 0.29	23.41 ± 0.32	24.67 ± 0.35	25.2 ± 0.35	23.94 ± 0.33	25.41 ± 0.36	24.99 ± 0.35

able thresholds for carp culture, indicating stable environmental conditions throughout the experiment. All physicochemical properties of water were assessed at the Environment Directorate laboratory in Sulaimani.

Physiological and health indicators were assessed to investigate the impact of daily salt usage. At the end of the experiment, three fish per tank were randomly selected, sedated using buffered clove powder (2.5 g L^{-1}), and sampled to take blood from the caudal vein. Fish weight and length were measured, and the fish were subsequently dissected. The liver, spleen, gills, kidney, and viscera were removed and weighed.

Condition factor (CF), hepatosomatic index (HSI), gill somatic index (GSI), visceral somatic index (VSI), spleen somatic index (SSI), and kidney somatic index (KSI) were calculated according to (Hama et al., 2025).

The blood samples obtained were divided into two sets of Eppendorf tubes. The first set contained sodium heparin at a concentration of 20 U L^{-1} as an anticoagulant. It was used to measure various blood parameters, including white blood cells (WBCs), granulocytes (%), lymphocytes (%), monocytes (%), RBCs (counted as 10^{12} L^{-1}), hemoglobin (HGB) (g dL^{-1}), hematocrit test (HCT), MCH, mean corpuscular hemoglobin concentration (MCHC) (g dL^{-1}), and platelets (counted as 10^3 uL^{-1}). The second set was not treated with any coagulant and was kept at 4°C until it clotted. After clotting, the second set was centrifuged at room temperature at 5,000 RPM for 20 minutes to obtain serum for measuring biochemical parameters.

Serum biochemical analyses were performed using an automatic chemical analyzer with commercial kits from Spinreact, S.A. (Gerona, Spain). The parameters measured included glucose (GL) (mmol L^{-1}), total protein (TP) (g dL^{-1}), albumin (ALB) (g dL^{-1}), total cholesterol (mmol L^{-1}), low-density lipoprotein cholesterol (LDL, mmol L^{-1}), high-density lipoprotein cholesterol (HDL, mmol L^{-1}), triglycerides (mmol L^{-1}), creatinine (µmol L^{-1}), aspartate aminotransferase (AST, U L^{-1}), alanine aminotransferase (ALT, U L^{-1}), creatine kinase isoenzyme (CKI, U L^{-1}), C-reactive protein (CRP), and electrolytes (sodium (Na), potassium (K), calcium (Ca), and chloride (Cl)). Scheme 1 summarizes the experimental design and different measurements.

3. Results

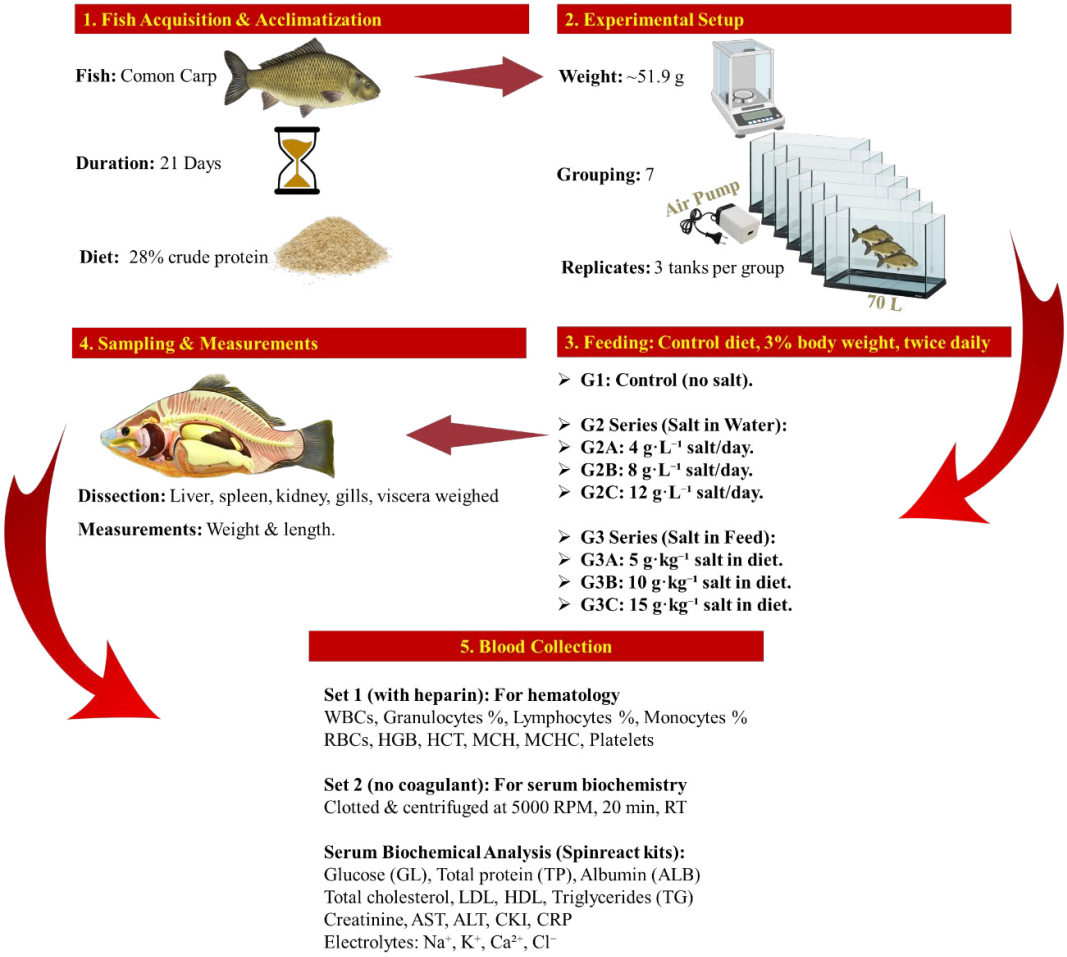
Fig. 1a shows no significant differences in the initial weight of common carp. However, by the end of the study, the $G2_B$, $G2_C$, $G3_A$, and $G3_B$ groups had significantly higher weights compared to the other groups. Furthermore, the daily growth rate (GR) and feed efficiency ratio (FER) were significantly higher in all groups compared to the control (G1) and $G2_A$ groups (Fig. 1g and Fig. 1e, respectively). No significant variations were noted in specific GR (Fig. 1f). The data in (Fig. 1h) indicate a clear and statistically significant drop in feed conversion ratio (FCR) ($p < 0.05$) for the $G2_B$, $G2_C$, $G3_A$, $G3_B$, and $G3_C$ groups compared to the control (G1) and low-salt water group ($G2_A$). This suggests that adding salt, whether through water or diet, at moderate levels can enhance how efficiently common carp convert feed into body mass.

There are significant differences in the RBC, HGB, and HCT levels of the G2A and G2B groups compared to the other groups (Fig. 2a, Fig. 2b, and Fig. 2c, respectively). Notably, the G1 demonstrated significantly higher MCH, MCHC, and MCV levels than the treatment groups (Fig. 2d, Fig. 2e, and Fig. 2f, respectively). Moreover, the platelet levels displayed a significant increase in the treatment groups that received salt additives in their fish diets.

Data in Fig. 3a and Fig. 3b revealed a significant increase in the WBC levels and granulocytes in the $G3_c$ group after adding 15 g kg^{-1} diet. The lymphocyte and monocyte levels in the G1 increased significantly compared to other groups (Fig. 3c and Fig. 3d).

Cholesterol, triglyceride, and LDL levels in the G1 group increased significantly compared to the additive groups (Fig. 4a, Fig. 4b, and Fig. 4c, respectively). Additionally, the HDL level was significantly higher in the $G3_C$ group when salt was added to the diets (Fig. 4d).

According to Fig. 5, the results indicated a significant increase in ALT, AST, and CKI levels in the $G3_C$ group that received a 15 g kg^{-1} diet compared to other groups (Fig. 5c, Fig. 5d, and Fig. 5e, respectively). The addition of salt to water in the $G4_A$, $G4_B$, and $G4_C$ groups led to a significant increase in globulin levels (Fig. 5a). However, only the G1 group showed a con-



Scheme 1. Experimental design and measurements

siderable increase in ALB levels (Fig. 5b). The level of TP showed a significant increase in the G1 group compared to other groups (Fig. 5f). GL, creatinine, and CRP were significantly higher in the $G3_C$ with a 15 g kg^{-1} (Fig. 5g, Fig. 5h, and Fig. 5i, respectively).

Condition factor and intestine weight index were significantly higher in the G1 group compared to the treatment groups (Fig. 6a and Fig. 6c), while the intestine length index increased in all treatment groups compared to the G1 group (Fig. 6b), and intestine length index (To fish length) increased in $G3_C$ compared to other treatments, as seen in (Fig. 6d). There is also a significant increase in HSI of G1 compared to other groups (Fig. 6e). The spleen somatic, kidney somatic, and gill somatic indices were higher in $G3_C$ (Fig. 6f, Fig. 6g, and Fig. 6h, respectively).

The potassium (Na) level increased significantly in $G4_C$ with 14 g L^{-1} water, as well as chloride (Cl) in $G3_C$, compared to other groups (Fig. 7a and Fig. 7d). K also increased in $G4_B$ compared to different groups, as well as Ca in the water additive's groups $G4_A$, $G4_B$, and $G4_C$ as shown in Fig. 7b and Fig. 7c.

Fig. 8 conclude that the weight level without head index revealed a significant increase in $G4_A$ and $G4_B$ compared to other groups (Fig. 8a), while G1 only displayed a significant increase in the weight level without head and visceral compared to other treatment groups (Fig. 8b).

4. Discussion

This study examined how different concentrations of sodium chloride (NaCl), delivered either through rearing water or the diet, affect the physiology and biochemistry of common carp (*Cyprinus carpio*) over 10 weeks. The results demonstrate that moderate levels of salt, specifically 8 and 14 g L^{-1} in water and 5 and 10 g kg^{-1} in the diet, can improve growth and metabolic performance. However, high salt levels, particularly in the diet at 15 g kg^{-1}, led to signs of physiological stress, as reflected in blood parameters and organ health.

Scientists cannot agree on the proper amount of NaCl in fish diets. Identifying Cl^- and Na^+ deficiencies in fish is challenging, since these ions are abundant in food and water. Consequently, metabolic deficiencies are rarely noticed. The study indicated that, although the likelihood of a deficit is minimal, excessive levels might have a detrimental impact on fish performance, resulting in aberrant behavior and shortened lifespans in the tanks (NRC, 2011).

This study found no significant differences in specific GR, and significant differences in FCR ($p < 0.05$) were observed between the control group (G1) and group $G4_A$ compared to the other groups. In contrast, *Labeo rohita* displayed the lowest FCR and the highest specific GR per day when supplemented with choline (Das et al., 2022). Higher salt concentrations in salted

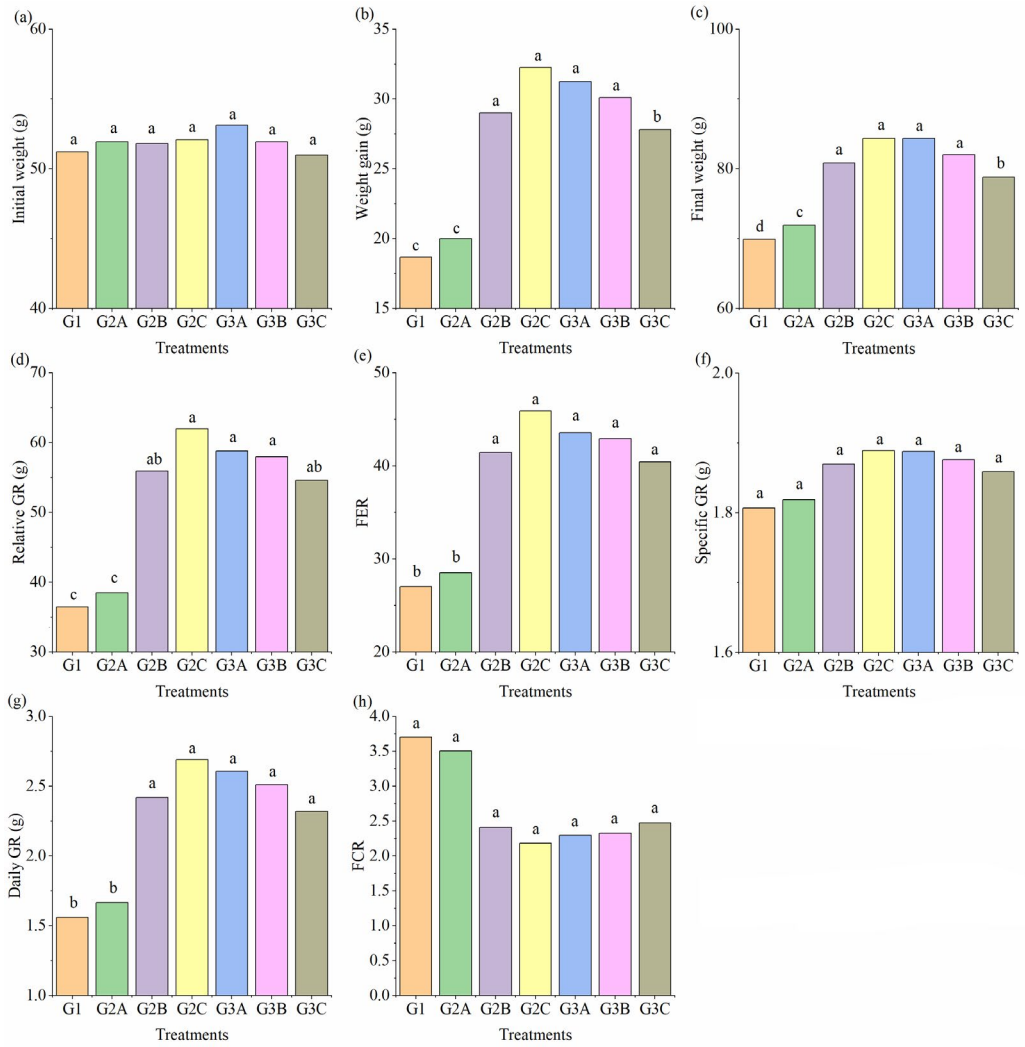


Fig.1. Evaluation of the common carp performance after salt supplementation in water and diet. Columns represent mean data, and bars above the columns represent data variability SDs (n = 9). Columns with different letters indicate statistical differences at the $p < 0.05$ level.

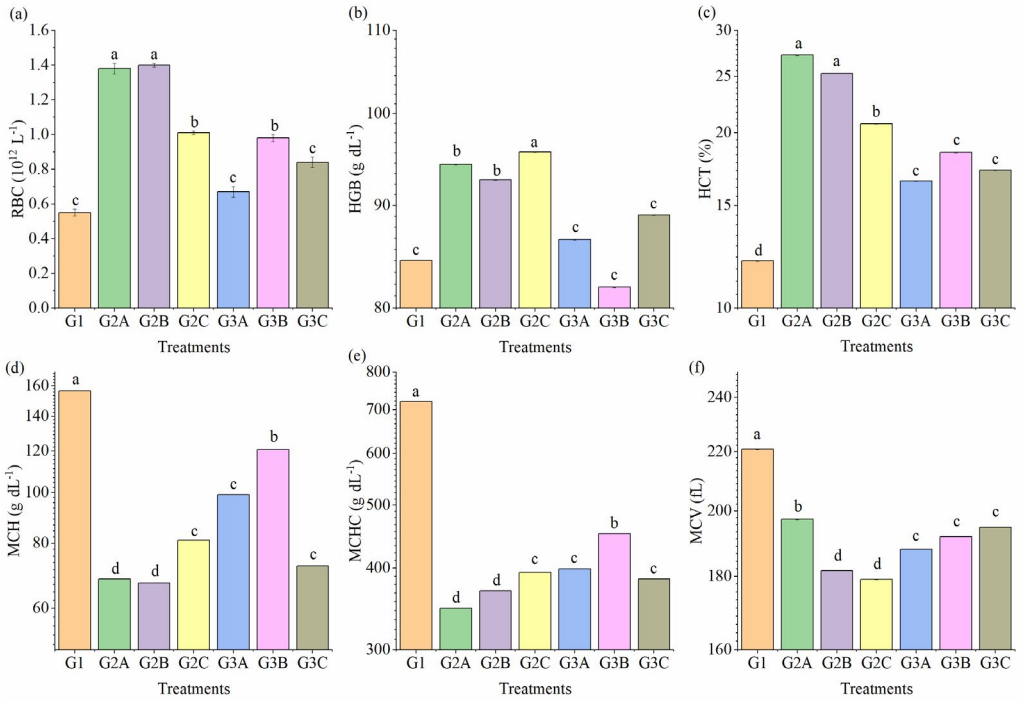


Fig.2. Evaluation of the common carp blood profile after salt supplementation in water and diet. Columns represent mean data, and bars above the columns represent data variability SDs (n = 9). Columns with different letters indicate statistical differences at the $p < 0.05$ level. Note that the y-axes for most of the panels are on a logarithmic scale, and some of the standard deviations are not visible because of small values.

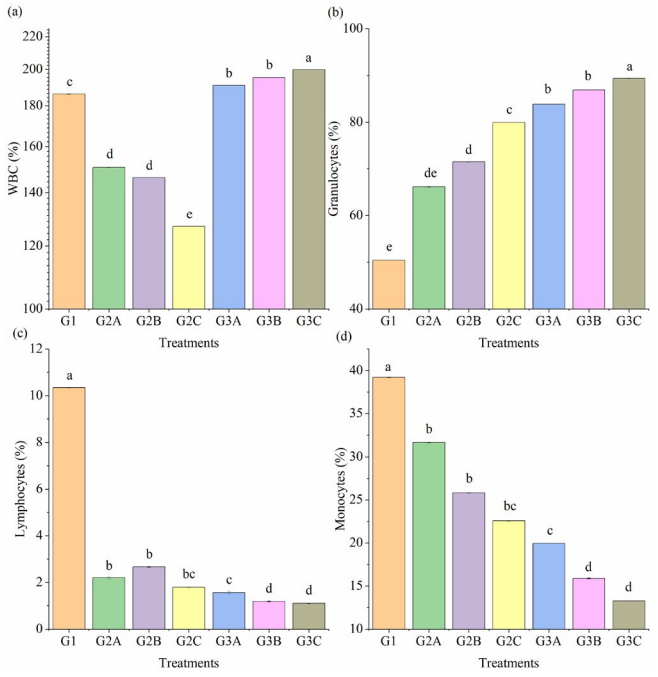


Fig.3. Evaluation of the common carp WBC counts after salt supplementation in water and diet. Columns represent mean data, and bars above the columns represent data variability SDs (n = 9). Columns with different letters indicate statistical differences at the $p < 0.05$ level. Note that some of the standard deviations are not visible because of small values.

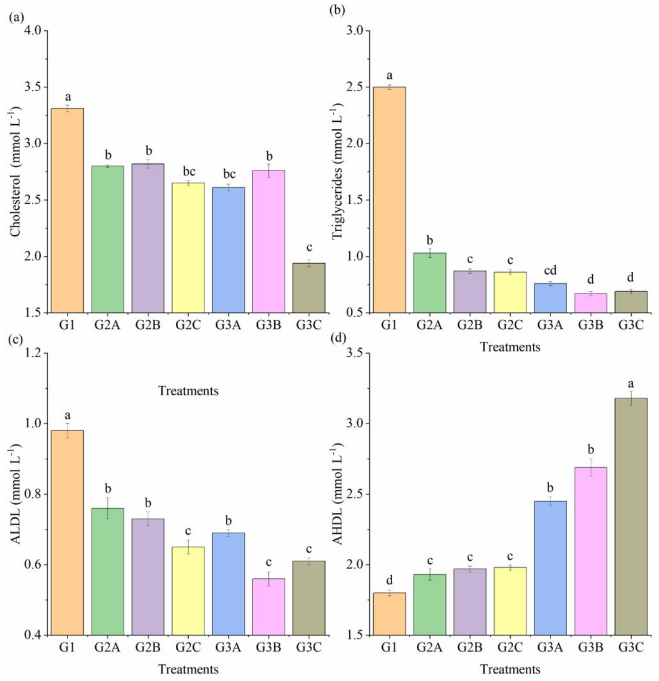


Fig.4. Evaluation of the common carp lipid profile after salt supplementation in water and diet. Columns represent mean data, and bars above the columns represent data variability SDs (n = 9). Columns with different letters indicate statistical differences at the $p < 0.05$ level.

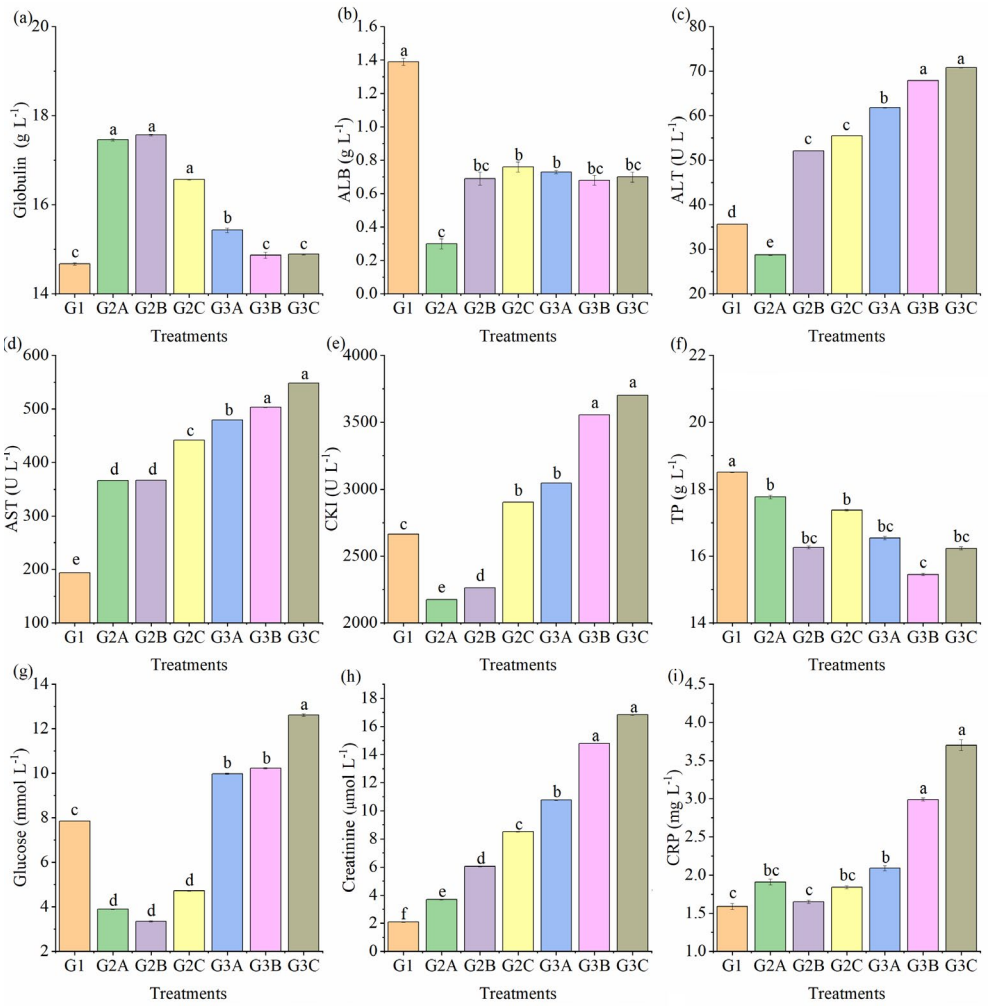


Fig.5. Evaluation of the common carp blood biochemical parameters after salt supplementation in water and diet. Columns represent mean data, and bars above the columns represent data variability SDs (n = 9). Columns with different letters indicate statistical differences at the $p < 0.05$ level.

Note that some of the standard deviations are not visible because of small values.

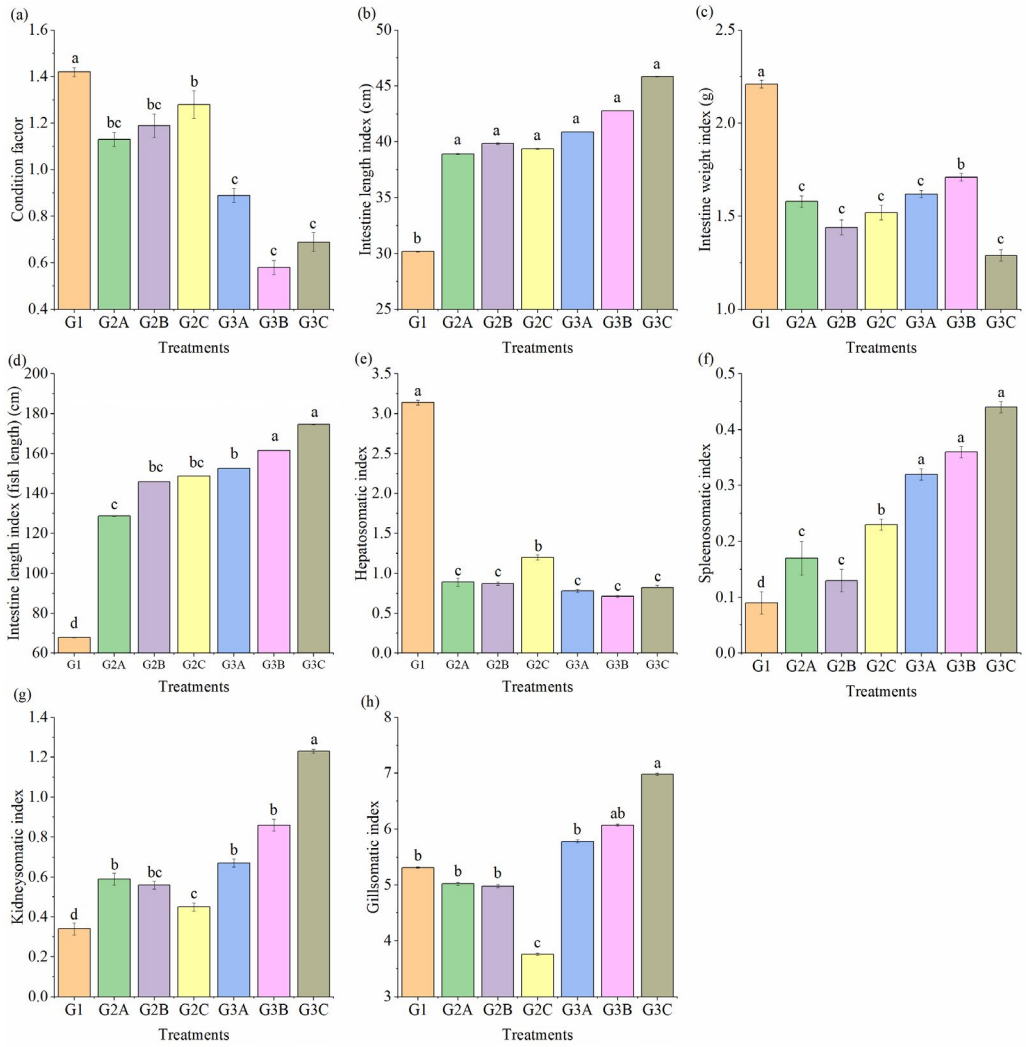


Fig.6. Evaluation of the common carp health indices after salt supplementation in water and diet. Columns represent mean data, and bars above the columns represent data variability SDs (n = 9). Columns with different letters indicate statistical differences at the $p < 0.05$ level. Note that some standard deviations are not visible because of small values.

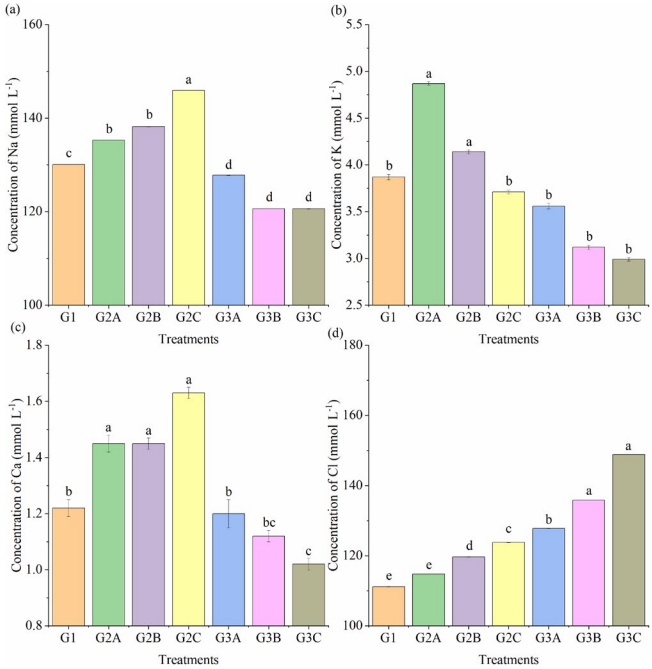


Fig.7. Evaluation of the common carp alkali metal level after salt supplementation in water and diet. Columns represent mean data, and bars above the columns represent data variability SDs (n = 9). Columns with different letters indicate statistical differences at the $p < 0.05$ level. Note that the y-axes for most of the panels are on a logarithmic scale, and some of the standard deviations are not visible because of small values.

viscera meal mixtures adversely affected the palatability and digestibility of the diets, reducing fish growth and diet utilization (He et al., 2023). This is diminished due to reduced palatability resulting from an imbalance of essential nutrient substances (Hasan et al., 2019; Pratoomyot et al., 2011).

4.1. Growth and feed efficiency

Most salt-treated groups, except $G4_A$, showed significantly better growth compared to the control group (G1). Fish in $G4_B$, $G4_C$, $G3_A$, and $G3_B$ gained more weight, suggesting that adding moderate salt can improve nutrient absorption and promote growth. This suggests that the presence of potassium chloride reduced the energy expenditures for osmotic regulation in fish that were not fed on it, as was the case in the treatment control (Albadran et al., 2022). Enhanced daily GR and FER across all treated groups (except $G4_A$) further support the idea that salt helps reduce the energy fish expend on osmoregulation, allowing more energy to be directed toward growth. However, the lack of changes in specific GR indicates that growth improvements may not have been consistent on an individual basis, due to differences in how fish responded to salt or absorbed nutrients. This discrepancy might be attributed to variations in feeding behavior or metabolic partitioning (V. Kumar et al., 2011).

According to Debnath et al. (2017), adding salt to the feed can significantly enhance the growth of tilapia fry. Among the different salt levels tested, the group with 1.5% salt showed the best performance in terms of weight, length, specific GR, and survival rate. The optimum salt level for incorporating in *Oreochromis niloticus* diets is 1.5%. Both 1% and 1.5% salt inclusion levels resulted in better feed utilization. However, increasing the salt level beyond a certain optimum level can render the feed unsuitable for consumption and negatively affect growth (Mubarik et al., 2019; Muhsan and Al-Shawi, 2017).

The daily GR and FER were significantly higher in all groups compared to the G1 and $G4_A$ groups. The study of Pratoomyot et al., (2011) completely disagrees with our finding, where dietary salted viscera meal mixtures did not significantly influence FER or PER in Atlantic salmon. Nile tilapia fingerling PER decreased significantly ($p < 0.05$) with increasing NaCl levels (De Aguiar et al., 2020). These findings were the opposite of the results in our experiment.

4.2. Blood cell responses

Over the last century, it has been reported that brackish water or seawater plays a significant role in various biological and physical traits. Recent studies show that blood parameters are reliable indicators of fish health and welfare, and monitoring these parameters provides valuable insights into fish health status (Acar et al., 2019; Fazio, 2019).

According to the present study's findings, RBC, HGB, and HCT in the $G4_A$ and $G4_B$ groups were significantly increased above the other groups. Buyukates et al. (2023) found that acclimating fish to seawater significantly increased hematological parameters ($P < 0.05$). The G1 group also exhibited a markedly higher MCH, MCHC, and MCV levels than the treatment groups, Buyukates et al. (2023) showed a significant decline ($P < 0.05$) in MCH, MCHC, and MCV of rainbow trout during the gradual transfer from freshwater to seawater. Additionally, the platelet levels showed a significant increase in the treatment groups that received salt additives in their fish diets. Leukocyte count (WBC) is essential for evaluating immune status in vertebrates and is composed of different cell types, including lymphocytes, monocytes, neutrophils, eosinophils, and basophils (Fazio, 2019). The data demonstrated an elevation in WBC and granulocyte levels accompanied by a significant reduction in lymphocyte and monocyte ranges in the treated groups.

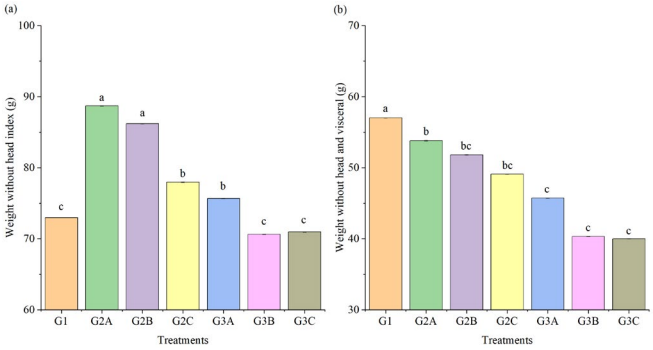


Fig.8. Evaluation of the common carp meat indices after salt supplementation in water and diet. Columns represent mean data, and bars above the columns represent data variability SDs (n = 9). Columns with different letters indicate statistical differences at the $p < 0.05$ level. Note that the y-axes for most of the panels are on a logarithmic scale, and some of the standard deviations are not visible because of small values.

The highest salt-fed group ($G3_C$) showed increased WBCs and granulocytes, which often signals an immune system response to stress or inflammation. Meanwhile, the control group had significantly higher lymphocyte and monocyte levels, which could indicate a compensatory immune response in less optimal conditions (Hrubec et al., 2000). These findings suggest that salt can influence the immune system; moderate levels may support immune function, while high levels can trigger stress-related immune responses. Salt, in moderate levels, is known to modulate immune parameters in freshwater fish, potentially through improved osmoregulation and reduced cortisol levels (Emeish, 2019). The common carp can withstand salinities of up to 6 ppt without compromising their ability to survive or their osmoregulatory, immunological, or stress responses (Emeish, 2019).

4.3. Lipid metabolism and liver function

Salt supplementation also had a noticeable effect on lipid metabolism. Control fish had the highest levels of cholesterol, triglycerides, and LDL, which are often associated with fat buildup and metabolic stress.

In contrast, fish in the high-salt diet group ($G3_C$) had increased levels of HDL, which is generally considered beneficial. These shifts suggest that salt can positively influence fat metabolism when used in moderation. Choline supplementation reduced cholesterol and triglycerides in fish (He et al., 2023). The reduction in triglyceride levels can be attributed to the role of choline in preventing the accumulation of excess lipids, which are associated with the development of fatty liver (NRC, 2011). Decreased cholesterol levels may result from a reduction in hepatic lipid content, thereby mitigating the risk of liver dysfunction in fish receiving choline supplementation (Li et al., 2014; Luo et al., 2016).

Elevated liver enzymes (ALT and AST) and (CKI) in the $G3_C$ group point to potential liver and muscle stress, which could reflect liver and muscle stress or damage due to excessive dietary salt (Hoseini et al., 2018). These enzymes are sensitive biomarkers of hepatic and muscular injury (Palanivelu et al., 2005), and their increase in high-salt diet groups aligns with Nassar et al. (2021) findings in carp under osmotic stress.

Our results indicated a significant increase in ALT, AST, and CKI levels in the $G3_C$ group compared to other groups. In contrast with our finding, fish under choline supplementation, AST, ALT, and cholesterol displayed a drastic reduction (He et al., 2023). The decreased levels of ALT and AST in the bloodstream suggest the presence of a defatted liver, which is attributed to exposure to choline chloride as well as its associated lipotropic metabolites (Das et al., 2022). Conversely, elevated ALT and AST activity in plasma was observed in the common carp following treatment with diazinon (Banaei et al., 2008; Takeuchi-Yorimoto et al., 2013). Additionally, elevated levels of ALT and AST were observed in the liver and muscle of *L. rohita* following exposure to endosulfan. These levels returned to normal after treatment with choline and its metabolites (N. Kumar et al., 2012). The addition of salt to water in the $G4_A$, $G4_B$, and $G4_C$ groups led to a significant increase in globulin levels. Unexpectedly, only the control group showed a significant increase in ALB levels. Nevertheless, the elevation in ALB and GL in seawater trout, in comparison with freshwater samples, was statistically significant ($P < 0.05$). Moreover, the rise in ALB was observed until the end of the experiment (Buyukates et al., 2023).

4.4. Protein levels, inflammation, and kidney function

The control group had the highest TP and ALB, while fish exposed to salt, the higher TP activity was observed during the breeding season with choline (Das et al., 2022). Additionally, seawater-acclimated fish showed higher serum TP levels than those from freshwater samples; however, this increase was not statistically significant ($P < 0.05$) (Buyukates et al., 2023). It had increased globulin levels, which may relate to enhanced immune activity. Notably, fish in the $G3_C$ group also showed higher GL, creatinine, and CRP levels, indicating possible kidney strain and systemic inflammation. As a result of increasing salinity in the environment, an increase in plasma GL levels is anticipated (Emeish, 2019), and elevated serum GL levels were observed when fish were transferred to seawater conditions. These results are consistent with previous studies that linked high salt intake to stress and reduced kidney function in freshwater species (Sampaio and Bianchini, 2002).

4.5. Organ health and intestinal changes

The control group also had higher condition factors and liver size (HSI) due to increased fat storage and lower feed utilization efficiency. The HSI is an indicator of the systemic health status of fish, reflecting hepatic energy stores and metabolic functions (Pyle et al., 2005). In contrast, fish in the $G3_C$ group had larger spleen somatic, kidney somatic, and gill somatic indices, potentially as a result of increased immune activation and osmoregulatory demand (Aalamifar et al., 2020). Interestingly, all salt-treated groups exhibited increased intestinal length, particularly in $G3_C$, indicating an adaptive response that enhances digestion and nutrient absorption in response to the altered environment (Ural and Sağlam, 2005).

4.6. Mineral balance and ion regulation

As expected, salt treatments affected the balance of ions in the fish blood. Na levels were significantly higher in $G4_C$; potassium levels were significantly higher in $G4_A$, and Cl levels were the highest in $G3_C$. These changes indicate the active regulation of ions and mineral uptake, particularly in groups exposed to salt through water. Katuli et al. (2014) found that exposure to diazinon affected the regulation of plasma sodium, chloride, and potassium in Caspian roach (*Rutilus caspicus*) after they were exposed to saltwater. The $Na+$ level in plasma, muscles, and erythrocytes of goldfish decreases in the total water content, which indicates the development of initial signs of tissue dehydration (Andreeva et al., 2022). The increase in calcium in water-treated groups may be related to improved mineral absorption or changes in gill function (Barton and Iwama, 1991). However, excessive ion accumulation, especially at high salt levels, could disrupt cellular balance if not properly regulated. Fish must actively use energy to enter ions via their gills and digestive system against the concentration gradient in freshwater, which is a hypotonic environment (Hallali et al., 2018). The findings of the current experiment are consistent with other research on euryhaline species that demonstrated the benefits of salt-enriched diets for both food efficiency and development (Salman, 2009).

Additionally, the level of K increased in $G4_A$ compared to different groups, as well as Ca in the water treated groups $G4_A$, $G4_B$, and $G4_C$. *L. rohita* revealed the maximum concentration of Ca among the studied species (Das et al., 2022). Conversely, a decreasing trend in Ca content was also observed in *Anabas testudineus* following anthracene exposure, likely due to increased Ca elimination (Dey et al., 2019). Sulfhydryl concentra-

tion decreased at higher NaCl concentrations, possibly due to increased protein solubility and unfolding under elevated salt conditions (He et al., 2023).

4.7. Body composition and somatic development

Fish in the $G4_A$ and $G4_B$ groups had higher "weight without head" indices, indicating that moderate salt in water may support lean body growth. Meanwhile, the G1 only displayed a significant increase in weight without head and visceral compared to other treatment groups. HSI and visceral are commonly employed as biological indicators (Ha et al., 2021). These observations support the role of controlled salinity in directing energy toward muscle development rather than fat accumulation.

5. Conclusion

In conclusion, we studied the effects of different salt additives in fish diets and water on the hematological and biochemical parameters, and organ indices of common carp in Sulaimaniyah province, Iraq. Overall, the results of the present experiment suggested that different additives of salt in fish diets and water can have significant effects on the growth, blood-related and biochemical parameters, and organ indices of common carp. These findings may be beneficial for fish farmers in identifying the most suitable and effective salt additives for both fish diets and rearing water. Furthermore, this study demonstrated that the application of 14 g L^{-1} of salt in rearing water and 15 g kg^{-1} of salt in the diet represents the optimal concentrations for enhancing fish growth and health in practical applications. Further investigation is required to elucidate the mechanisms behind the observed effects and to enhance the application of salt additives in aquaculture practices.

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Availability of data and materials

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Conflict of Interest

The authors declare no conflicts of interest.

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Original Article

LIMNOLOGY FRESHWATER BIOLOGY

Influence of sex and body size on selected morphometric traits of *Metopograpsus frontalis* (Miers, 1880) from Southern Vietnam

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ABSTRACT. A total of 167 specimens of *Metopograpsus frontalis* (105 males and 62 females) were collected monthly from intertidal mudflats in Ganh Hao, Ca Mau Province, Southern Vietnam, from July to October 2025. Selected morphometric traits, including carapace width from left to right ($CW_{L–R}$), carapace width from right to left ($CW_{R–L}$), manus width (MW2, MW3), pollex width (PW5), and dactyl width (DW7), were measured with a 0.01-mm vernier caliper. Generalized linear models were fitted to evaluate the effects of sex and body size, using Gaussian models for CW and Gamma models with a log link for MW, PW, and DW. We found no significant sex-related differences in $CW_{L–R}$ and $CW_{R–L}$ ($p > 0.05$), whereas MW2, MW3, PW5, and DW7 were significantly larger in males ($p < 0.001$). Sex coefficients ($\beta = 0.156–0.257$) corresponded to rate ratios (RR) of 1.17–1.29, indicating that male claws were 17–29% larger after adjusting for $CW_{L–R}$ and $CW_{R–L}$. Both CW variables had strong positive effects ($\beta = 0.054–0.070$; RR = 1.06–1.07; and $p < 0.001$). All Gamma models showed $\chi^2/df < 1$, confirming good model fit without overdispersion. These results provide quantitative evidence of sex-related variation in morphometric traits of *M. frontalis* and establish a morphometric baseline for future ecological and evolutionary studies of mangrove crabs in the Mekong Delta.

Keywords: *Metopograpsus frontalis*, Mekong Delta, morphometric traits, Generalized Linear Model, sex-related differences

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1. Introduction

Intertidal crabs are key components of macrobenthic communities in tropical and subtropical estuaries, functioning as ecosystem engineers and major trophic intermediates. Through bioturbation and feeding, they modify sediment composition (Botto and Iribarne, 1999; Escapa et al., 2004), regulate ecosystem productivity (Koch and Wolff, 2002; Werry and Lee, 2005), influence vegetation structure (Bosire et al., 2005) and faunal composition (Dye and Lasiak, 1986; Botto et al., 2000), and mediate energy flow across trophic levels (Wolff et al., 2000). Among estuarine brachyurans, the superfamilies Grapsoidea and Ocypodoidea dominate tropical mudflats (Lee, 2008; Nagelkerken et al., 2008). In Vietnam, the family Grapsidae is diverse and widespread, encompassing genera such as Sesarma, Metaplax, Neoeposesarma, and Metopograpsus (Nhuong, 2003).

Crab morphology reflects the combined effects of genetic, ecological, and sexual selection pressures (Parsons, 1992; Voje, 2016). For example, fiddler crabs (subfamily Gelasiminae), which are abundant in tropical and subtropical estuaries, rely heavily on visual communication strategies such as claw waving and body posturing (Zeil and Hemmi, 2006; How et al., 2008). In these species, sex-related differences are extreme, with males possessing one enlarged chela while females have two smaller claws of similar size. Such morphological divergence has received considerable attention in behavioral and ecological studies (Valiela et al., 1974; Oliveira and Custodio, 1998; Allen and Levinton, 2007).

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Within the family Grapsidae, *Metopograpsus frontalis* (Miers, 1880) is an ecologically important mangrove crab species. It is an opportunistic omnivore with strong predatory capabilities and plays a crucial role in estuarine food webs (Poon et al., 2010). Morphologically, the chelae and walking legs of *M. frontalis* contain low calcium levels but are enriched with halogens such as chlorine and bromine, while the carapace is highly calcified (Cribb et al., 2009). Previous research has addressed various aspects of this species, including taxonomy and phylogenetic relationships (Guan et al., 2018), molecular identification (Paransa et al., 2024), feeding ecology (Poon et al., 2010), and population structure (Poon, 2004; Vermeiren et al., 2021).

However, in the Mekong Delta, particularly in Ca Mau Province, information on *M. frontalis* remains limited, especially regarding its morphological characteristics and how these vary with sex and body size. The findings of this study provide baseline morphometric data that may support future ecological, evolutionary, and population-level assessments in the mangrove ecosystems of Southern Vietnam.

2. Materials and methods

2.1. Sample collection and analysis

Specimens of *Metopograpsus frontalis* (Fig. 1) were collected manually during daylight hours from intertidal mudflats along riverbanks at low tide. Sampling was conducted monthly from July to October 2025 in the Ganh Hao area, Ca Mau Province, Southern Vietnam (Fig. 2). Approximately 30 individuals were collected per month. Crabs were captured by hand or using baited traps and fixed in 70% ethanol for transport to the laboratory.

In the laboratory, specimens were identified based on morphological descriptions provided by Miers (1880). The following morphometric parameters were measured using a vernier caliper with a precision of 0.01 mm: carapace width from left to right ($CW_{L–R}$), carapace width from right to left ($CW_{R–L}$), manus width (MW2 and MW3), pollex width (PW5), and dactyl width (DW7) (Fig. 3).

Each specimen was sexed by examination of abdominal morphology: males possess a narrow, triangular abdomen, whereas females have a broader, rounded abdomen. Morphological features characteristic of *M. frontalis* include a smooth and glossy carapace, dark brown coloration, and two chelae of similar size, with males typically having larger and more robust claws than females.

2.2. Data analysis

Before model fitting, the normality of each morphometric variable was assessed using the Shapiro–Wilk test. $CW_{L–R}$ and $CW_{R–L}$ were normally distributed ($p > 0.05$), whereas MW2, MW3, PW5, and DW7 deviated from normality ($p < 0.05$). Consequently, CW variables were analyzed using generalized linear models (GLMs) with a Gaussian distribution, while MW, PW, and DW variables were analyzed using GLMs with a Gamma distribution and a log-link function.

Separate models were fitted for each dependent variable using the general structure: $Y \sim Sex + CW_{L–R}/CW_{R–L}$, where Y represents MW2, MW3, PW5, or DW7, and Sex is a categorical factor (male or female). Covariates were mean-centered before analysis. Model outputs included: regression coefficients ($\beta \pm SE$), Wald z-values, p-values, rate ratios ($RR = e^{\beta}$), and fit indices (AIC, BIC, Deviance, χ^2/df). Significant p-values (< 0.05) for Sex indicated sex-related differences in trait size. Positive β(CW) values signified that the trait increased with body size. For Gamma models, $\chi^2/df < 1$ denotes adequate fit and no overdispersion.

Statistical analyses were performed using jamovi v2.6.44 (The jamovi project, 2024), with the GAMLj module. Descriptive values ($Mean \pm SE$) were obtained from the Estimated Marginal Means (EMMs) derived from GLMs rather than raw data averages for interpretability.

3. Results

Descriptive statistics and normality

The Shapiro–Wilk test indicated that both carapace-width variables, $CW_{L–R}$ and $CW_{R–L}$, followed a normal distribution ($p = 0.517$ and $p = 0.854$, respectively), whereas MW2, MW3, PW5, and DW7 deviated significantly from normality ($p < 0.01$). Accordingly, Gaussian GLMs were applied for $CW_{L–R}$ and $CW_{R–L}$, and Gamma–log GLMs were used for MW2, MW3, PW5, and DW7 (measurement scheme in Fig. 3).

Fig.1. *Metopograpsus frontalis* (A: Dorsal view; B: Frontal view).

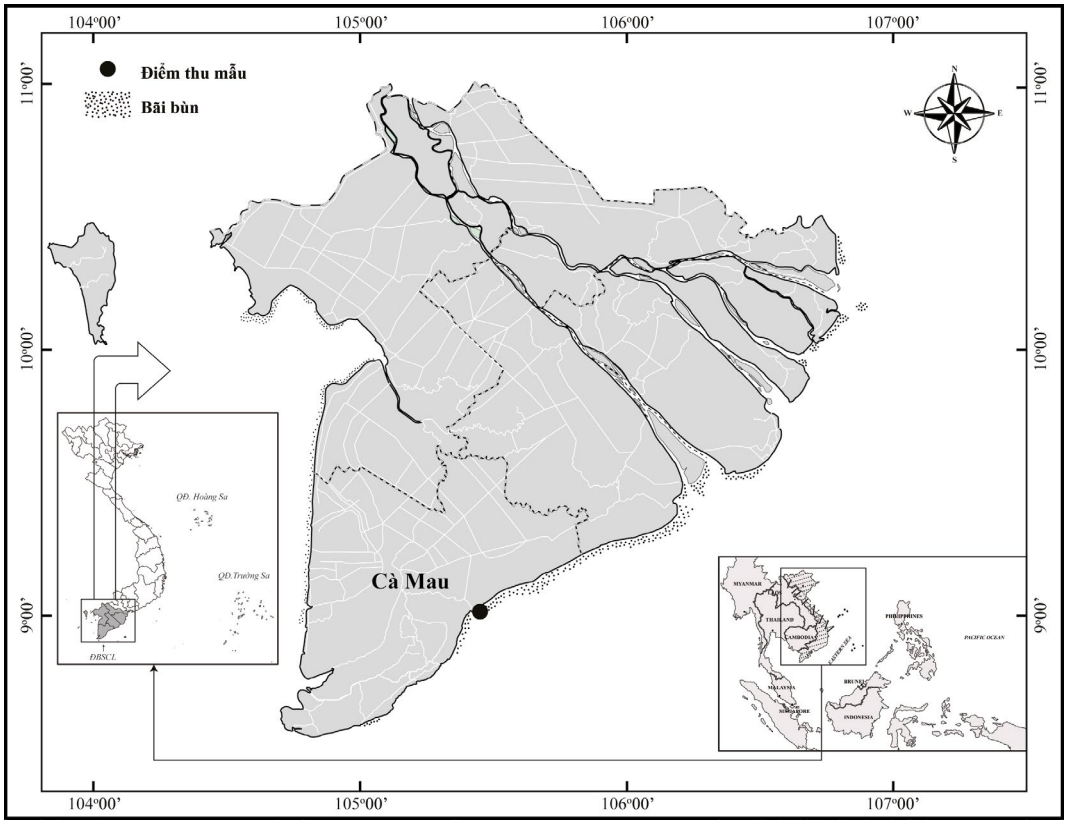

Fig.2. Sampling map in Ca Mau, Vietnam (● Sampling sites: Ganh Hao, Ca Mau; source: Dinh (2018)).

Carapace width ($CW_{L–R}$ and $CW_{R–L}$)

No significant sex-related differences were detected in either $CW_{L–R}$ ($\beta = 0.280 \pm 0.526$, $p = 0.594$) or $CW_{R–L}$ ($\beta = 0.175 \pm 0.515$, $p = 0.734$). Estimated marginal means (EMMs) were nearly identical between males and females (Table 1), indicating comparable overall body size. The overlapping confidence intervals and p-values > 0.05 confirm the absence of sex effects on carapace width.

Functional morphometric traits (MW2, MW3, PW5, and DW7)

Gamma–log GLMs showed strong effects of sex on all cheliped-related traits after accounting for carapace width. We fitted two models for each trait with $CW_{L–R}$ and $CW_{R–L}$ entered separately as covariates; conclusions were consistent (Table 2).

Sex coefficients varied, $\beta = 0.156–0.257$ (all $p < 0.001$), corresponding to RR = 1.17–1.29, i.e., male traits were 17–29% larger than female traits at a given carapace width. Both CW covariates positively affected all traits ($\beta = 0.054–0.070$; RR ≈ 1.06–1.07; and $p < 0.001$), indicating a ~6–7% increase in trait size per 1-mm increase in $CW_{L–R}$ and $CW_{R–L}$. All Gamma models showed $\chi^2/df < 1$, indicating a good fit without overdispersion.

Mean ± SE values (EMMs) for each sex and trait are summarized below (Table 3). These results demonstrate a clear and consistent pattern of sexual differentiation in chela components, whereas the carapace width remains similar between sexes.

Table 1. Estimated marginal means of carapace width (mm) by sex from Gaussian GLMs

Parameter	Sex	n	Mean ± SE	p
$CW_{L–R}$ (mm)	Female	62	22.10 ± 0.38	0.594
	Male	105	22.38 ± 0.35	
$CW_{R–L}$ (mm)	Female	62	22.04 ± 0.38	0.734
	Male	105	22.22 ± 0.33	

Note: Carapace width from left to right ($CW_{L–R}$), carapace width from right to left ($CW_{R–L}$)

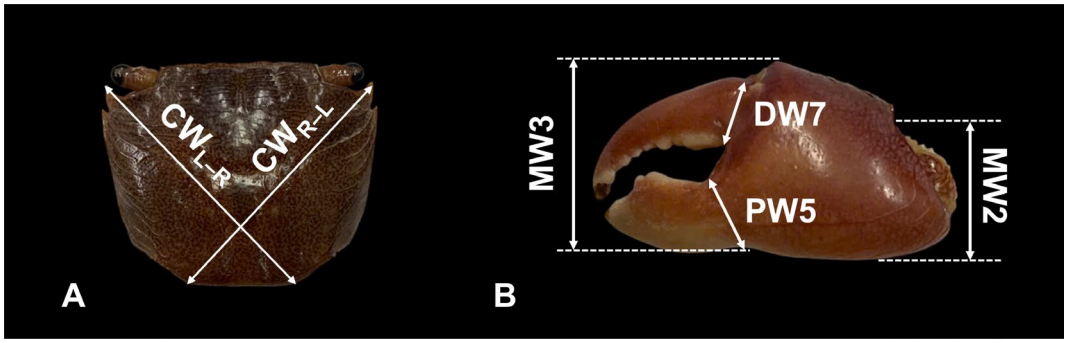

Fig.3. Illustration of morphological measurement parameters of *Metopograpsus frontalis* (A: carapace width from left to right ($CW_{L–R}$), carapace width from right to left ($CW_{R–L}$); B: manus width (MW2, MW3), pollex width (PW5), and dactyl width (DW7).

Table 2. Gamma–log GLM results for effects of sex and carapace width on functional traits

Parameter	Controlled variable	β (sex) ± SE	z	p	RR	Sex effect	β	p	AIC	BIC
MW2	$CW_{L–R}$	0.156 ± 0.039	3.99	< 0.001	1.17	♂ 17 % larger	0.054	< 0.001	550.98	563.45
	$CW_{R–L}$	0.162 ± 0.039	4.12	< 0.001	1.18	♂ 18 % larger	0.055	< 0.001	551.26	563.74
MW3	$CW_{L–R}$	0.230 ± 0.031	7.44	< 0.001	1.26	♂ 26 % larger	0.065	< 0.001	557.52	569.99
	$CW_{R–L}$	0.237 ± 0.031	7.69	< 0.001	1.27	♂ 27 % larger	0.067	< 0.001	554.38	566.85
PW5	$CW_{L–R}$	0.251 ± 0.035	7.27	< 0.001	1.29	♂ 29 % larger	0.059	< 0.001	312.35	324.82
	$CW_{R–L}$	0.257 ± 0.034	7.49	< 0.001	1.29	♂ 29 % larger	0.060	< 0.001	309.86	322.33
DW7	$CW_{L–R}$	0.203 ± 0.038	5.34	< 0.001	1.23	♂ 23 % larger	0.068	< 0.001	312.19	324.67
	$CW_{R–L}$	0.211 ± 0.038	5.62	< 0.001	1.24	♂ 24 % larger	0.070	< 0.001	308.41	320.89

Note: Carapace width from left to right ($CW_{L–R}$), carapace width from right to left ($CW_{R–L}$); manus width (MW2, MW3), pollex width (PW5), and dactyl width (DW7)

4. Discussion

The present analysis provides quantitative evidence of sex-related morphometric differences in *Metopograpsus frontalis* from Southern Vietnam. Although overall body size ($CW_{L–R}$ and $CW_{R–L}$) did not differ significantly between sexes, all functional claw components (MW2, MW3, PW5, and DW7) were markedly larger in males. The rate ratios (RR = 1.17–1.29) demonstrate that, after controlling for carapace width, males possess 17–29% larger claw dimensions than females. This pattern remained consistent regardless of the carapace-width covariate used ($CW_{L–R}$ or $CW_{R–L}$), confirming the robustness of the observed sex effect.

The strong positive allometric slopes for both CW variables (β = 0.054–0.070; RR ≈ 1.06–1.07) indicate that each 1-mm increase in the carapace width corresponds to a proportional 6–7% increase in claw trait size, emphasizing the importance of size scaling in the species. However, the absence of a sexual difference in the carapace width suggests that dimorphism is localized to the chelae rather than generalized body enlargement. This pattern of functional rather than overall dimorphism was reported in several grapsoid and ocypodoid crabs (Chatterjee and Chakraborty, 2015; Vermeiren et al., 2021).

In *M. frontalis*, enlarged male claws likely provide advantages during courtship, territorial defense, and intraspecific competition, consistent with findings in other species (Aldea et al., 2025). Females, by contrast, display smaller, more slender claws, possibly reflecting an energetic trade-off favoring reproduction, feeding efficiency, and burrow maintenance. The magnitude of sexual disparity (17–29%) observed here parallels reports for M. latifrons and Parasesarma longicristatum, where male chelae exceeded those of females by approximately 15–33% (Vermeiren et al., 2021; Aldea et al., 2025).

Overall, the results confirm that *M. frontalis* exhibits sex-linked allometric differentiation, characterized by disproportionate investment in cheliped growth relative to carapace size. Such differentiation likely reflects the combined influence of sexual selection and functional specialization, allowing males to enhance their signaling and competitive performance, while females prioritize their reproductive roles. This adaptive divergence highlights the ecological and evolutionary significance of claw morphology in mangrove crabs inhabiting dynamic estuarine environments.

Table 3. Estimated marginal means of functional traits (mm) by sex from Gamma–log GLMs

Parameter	Sex	Mean ± SE
MW2	Female	4.57 ± 0.14
	Male	5.34 ± 0.13
MW3	Female	5.66 ± 0.14
	Male	7.13 ± 0.13
PW5	Female	2.45 ± 0.07
	Male	3.16 ± 0.07
DW7	Female	2.23 ± 0.07
	Male	2.75 ± 0.06

Note: Manus width (MW2, MW3), pollex width (PW5), and dactyl width (DW7)

5. Conclusion

This study provides quantitative evidence of sex-related differences in *Metopograpsus frontalis* from the Ganh Hao estuary, Ca Mau Province. Although males and females exhibit similar carapace widths, males possess significantly larger functional morphometric traits (MW2, MW3, PW5, and DW7), being 17–29% greater after controlling for body size. The positive effect of CW_{L-R} and CW_{R-L} on all traits confirms that growth scaling influences these differences but does not entirely explain. The results provide baseline morphometric data that will support future ecological and evolutionary studies on sex-related variation in mangrove crabs of Southern Vietnam.

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Conflict of interest

The authors declare no conflicts of interest.

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Original Article

LIMNOLOGY FRESHWATER BIOLOGY

Nanozinc as an emerging nanopollutant: sublethal effects on the invasive freshwater bivalve *Limnoperna fortunei* (Dunker, 1857)

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ABSTRACT. Zinc oxide nanoparticles (ZnONP) are among the most widely produced nanomaterials worldwide given their unique properties, despite their nanotoxicity has been poorly addressed. In parallel, environmental concern has risen because of increasing amounts of them reaching the environmental matrices, whereas the aquatic ones are one of the main final sinks. As key non-target species, we aimed to expose the invasive freshwater bivalve *Limnoperna fortunei* to sublethal concentrations of ZnONP (0, 0.025, 0.25, and 2.5 mg/L) to evaluate tissue damage and oxidative stress-related markers in the soft tissue. After a 96 h-exposure, the alkaline phosphatase enzyme activity increased after 0.025 mg/L, and the alanine aminotransferase activity decreased at 2.5 mg/L. Aspartate aminotransferase enzyme activity also decreased at 0.25 and 2.5 mg/L. In terms of oxidative stress, only superoxide dismutase activity increased after exposure to the lowest nanozinc concentration. We concluded that nanozinc may pose a threat to the aquatic biota in a context that lacks proper regulation and control for nanopollutants, and that the toxicity mechanisms in this species are mainly related to tissue damage, even at environmentally relevant concentrations, as the lowest one tested.

Keywords: biomarkers, oxidative stress, tissue damage, zinc oxide nanoparticles

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1. Introduction

Nanotechnology is a field of study under expansion that involves the synthesis and manipulation of materials (nanomaterials, NM) at a scale of 1 to 100 nm. Among the wide variety of NM, metallic-based ones have been of particular interest due to their unique properties in terms of catalysis, optics, and antimicrobial action (Pal and Pareek, 2025). Zinc oxide nanoparticles (ZnONP) are one of the most applied NM worldwide, after silica- and titanium-based NM. For example, they are extensively used in solar cells, optoelectronic devices, biomedicine, antibacterial materials, and personal care products (Wu et al., 2025). Their global annual production reaches up to 36,000 tons, and the predicted environmental concentrations are in the low range of 0.17 μg/L (Ale et al., 2024). However, through their multiple applications, the NM are released into the environment in increasing amounts. In this sense, it was proven that NM are released during all their life cycle; therefore, rising concern about their environmental fate, and interaction with non-target biota is in need to be addressed (Santos-Rasera et al., 2022). Particularly, when reaching the aquatic environments, ZnONP suffer from multiple transformations, which will depend on other factors (e.g., temperature, UV irradiation, or presence of organic matter), such as dissolution (and Zn ion release), agglomeration, and further sedimentation. The latter may enhance the bioavailability for benthic organisms; however, only a few studies were conducted on this animal group (Yung et al., 2014).

There is some robust evidence that ZnONP exert deleterious effects on aquatic biota (Abdel-Halim et al., 2020; Marisa et al., 2016a; Saidani et al., 2019; Zhao et al., 2016); however, these particles were studied to a lesser extent in terms of ecotoxicology (in comparison with, for example, Ag- or Ti-based NM) (Gutierrez et al., 2021). Mollusks were considered particularly sensitive to nanopollutants given both their filter-feeding and sessile habits (Skawina et al., 2024). Furthermore,

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according to the available bibliography, freshwater mollusks were even less studied in comparison with the marine ones, altough the toxicity mechanisms of any NM were proved to differ according to the ionic strength of the media. In other words, among other environmental factors, the ultimate nanotoxicity of freshwater environments differs extensively from the marine ones (Petosa et al., 2010; Yung et al., 2014). Therefore, there is currently a need for further research on the ecotoxicology of nanozinc, with emphasis on freshwater biota.

Oxidative stress is the most reported toxicity mechanism from nanozinc (and most NM) exposure. For example, the freshwater mussel *Elliptio complanata* showed altered antioxidant enzyme activities and lipid peroxidation in the soft tissue (apart from weight loss) after exposure to ZnONP (Gagné et al., 2013). Similarly, the freshwater snail *Lymnaea luteola* showed altered antioxidant enzyme activities and DNA damage in the digestive gland (Ali et al., 2012). Finaly, DNA damage, altered antioxidant enzyme activities, oxidative damage in lipids and proteins, and bioaccumulation were evidenced in the *Limnoperna fortunei* bivalve exposed to high concentrations of ZnONP (Girardello et al., 2021). Other less-studied biomarkers in the nanotoxicology field are tissue damage-related enzymes. For example, serum from fish exposed to ZnONP had low transaminase activity (Rasheed et al., 2023), but the tissue of the freshwater snail *Biomphalaria alexandrina* showed increased activity (Fahmy et al., 2014).

The golden mussel *Limnoperna fortunei* (Dunker, 1857) is native to Southeast Asia, being highly recognized for invading the aquatic ecosystems worldwide (Barbosa, 2014). Their wide availability, easy adaptation to laboratory conditions, and sensitivity have made them promising sentinel species for assessing early warning responses (Vereycken and Aldridge, 2023). This work includes an independent preliminary study that was conducted prior to another recently published work that has also employed *L. fortunei* as test species and nanozinc exposures (Ale et al., 2025). In order to deepen the understanding of this emerging nanopollutant, the present study aims to evaluate the sublethal effects of ZnONP in terms of oxidative stress and tissue damage in this freshwater bivalve species.

2. Materials and methods

2.1. Zinc oxide nanoparticles (ZnONP)

The particles were purchased from Sigma-Aldrich® (Product number 721077), guaranteeing their purity and stability. According to the Certificate of Analysis, the particles resulted correctly dispersed in the media (H_2O), pH 8.9, and their size was reported as 40 nm. The stock concentration was 19% wt.

The complete particle characterization has been reported in Ale et al. (2025). The transmission electron microscopy (TEM) analysis revealed that the ZnONP had an average primary diameter of approximately 27 nm with some degree of agglomeration. On the other hand, analysis by dynamic light scattering (DLS) confirmed the ZnONP aggregation in aqueous suspension by showing large agglomerates (630 ± 207 nm). Finally, the zeta potential was reported as -26.09 ± 0.79 mV, suggesting moderate colloidal stability.

2.2. Bivalves and exposure conditions

In March 2024 (summer season), the bivalves (adults, $n = 25$) were manually collected from a dock on the shore of Río Santa Fe (a secondary channel of the Middle Paraná River, 31°38'34.90" S; 60°41'6.22" W). This bioassay was conducted independently and in parallel to the study reported in Ale et al. (2025), using organisms collected at different time points. All experimental procedures, from bivalve collection to biomarker determination, were performed separately. The acclimatation procedure was described by Cazenave et al. (2025). After the bivalves were transferred to the laboratory (in plastic containers with natural river water), they were carefully separated and brushed to remove the biofilm. For the first 24 h, they were kept in a medium composed of half natural river water and the other half with dechlorinated tap water, always with oxygenation, at 27 ± 1ºC by employing incubators. The temperature was based on the mean temperature in their habitat in summer (Iriondo and Paira, 2007). Then, the bivalves were kept in dechlorinated tap water for 48 h, with a 16/8 h photoperiod (light/darkness) and algae-based food (*Tetradesmus obliquus* algae culture) *ad libitum* until 24 h before starting the final experiments. When an individual was attached and showed signs of valve activity in response to physical stimuli, it was considered healthy. At the beginning of the experiment, the organisms' mean length and weight were 29.33 ± 2.31 mm and 1.83 ± 0.42 g, respectively (and they were also weighed at the end of it).

2.3. Experimental design

The lowest concentration of ZnONP tested was chosen based on the previously reported Zn environmental concentration for urban surface and municipal effluents (0.010-0.050 mg/L) (Clara et al., 2012; Gagnon et al., 2014). For the higher concentrations, it was contemplated that non-lethal nanozinc concentrations for the test species (*L. fortunei*) were in the range of 1-50 mg/L (Girardello et al., 2021).

The experiment involved 250 mL aquaria with 0 (control), 0.025, 0.25, or 2.5 mg/L of ZnONP, and the exposure period was 96 hours. The conditions remained similar to the acclimatation procedure (dechlorinated tap water with constant aeration, 27 ± 1ºC, and a 16/8 h light/darkness photoperiod). The experimental unit was defined as an aquarium with five organisms, which was replicated three times per treatment. The particle administration was performed directly into the aquarium, before the corresponding dissolution with ultrapure water. Treatments were renewed every 24 hours, so ZnONP dissolutions were prepared daily from the stock to avoid particle transformation and/or agglomeration. The bivalves were not fed during the bioassay. The water conditions were always monitored, which remained similar before and after the renewal

procedure: pH = 7.66 ± 0.15 and conductivity = 165 ± 10 µS/cm. No mortality was recorded during the trial, except for the highest ZnONP concentration (2.5 mg/L), which was < 10%. At the end, the soft tissue of each organism was extracted and stored at -80ºC until the biomarker determinations.

2.4. Sublethal biomarker assessment

The soft tissue of each bivalve was homogenized to determine enzyme tissue damage according to Bacchetta et al. (2011) and oxidative stress markers following the technique proposed by Reglero et al. (2009) (n = 5 per biomarker type). Enzyme activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) were measured through the methodology proposed by Reitman and Frankel (1957) with commercial kits (Wiener Lab®).

Oxidative stress was determined by measuring the antioxidant activities of superoxide dismutase (SOD) by the technique proposed by Misra and Fridovich (1972); catalase (CAT)–by following the procedure according to Beutler (1982), and glutathione S-transferase–by employing the technique suggested by Habig et al. (1974). Finally, lipid peroxidation levels (LPO) were calculated by the thiobarbituric acid reactive substances (TBARS) assay according to Yagi (1976). Each sample was measured in triplicate, and all the results were expressed in terms of protein content (Bradford, 1976).

2.5. Statistics

Data are reported as mean ± standard error. The Shapiro-Wilks and Levene's tests were applied to evaluate normality and homogeneity of variance, respectively. Variables without a normal distribution were transformed using log10 and tested again before parametric analysis. For statistical data comparisons among the treatments, 1-way ANOVA followed by Tukey post-test was used for normally distributed data, and the Kruskal-Wallis test–for non-normally distributed data. All statistical analysis was performed using the InfoStat software (Universidad Nacional de Córdoba, Argentina).

3. Results and discussion

At the end of the experiment (after 96 h), the bivalves were weighed again, and at the highest ZnONP concentration (2.5 mg/L), a significant increase in the weight was evidenced in comparison with the remaining treatments ($p = 0.0388$) (Table 1). Furthermore, at dissection time (when the mollusk valves were removed), the soft tissue of them was found to be light white compared to the other treatments (observational feature).

Additionally, we observed a white mucus secretion in the case of the highest exposure, which started after 48 h of exposure and became more obvious after 72 h (observational feature) (Fig. 1).

Interestingly, similar visualizations were made in this species but with exposure to titanium dioxide nanoparticles (TiO_2NP) (Manske Nunes et al., 2018). After microscopy analysis, the authors corroborated the presence of Ti in the mucus and explained that its production is a defense mechanism against the toxic effects caused by the nanopollutant. The white mucus secretion observed and the augmented weight of the exposed bivalves at 2.5 mg/L could confirm that the particles were actually captured by the organisms and potentially accumulated in the tissue. In this regard, Rojas Molina et al. (2010) explained that *L. fortunei* is capable of selectively ingest and retain particles spanning a broad size range (approximately 1-1000 µm), while smaller particles (≤ 1 µm) are typically aggregated and expelled as pseudofeces (Frau et al., 2016). However, when the particles lose stability and suffer from physical and chemical transformations such as ion dissolution, the released Zn ions may play a crucial role in metal bioaccumulation, as there is robust evidence that nanozinc can bioaccumulate in the tissue of freshwater and marine bivalves. For example, high levels of total Zn were found in *Dreissena bugensis* exposed to 50 µg ZnONP/L for 96 h (Auclair et al., 2020), and similar results were obtained for the *Unio tumidus*, *Xenostrobus secures*, and *Mytilus galloprovincialis* marine mussels exposed to ZnONP (Falfushynska et al., 2015; Hanna et al., 2013; Lai et al., 2023).

Table 1. Final weight of *L. fortunei* (whole organism) exposed to 0, 0.025, 0.25, and 2.5 mg ZnONP/L. The values are expressed as means ± SE. Means not sharing the same superscript (A or B) are significantly different at $p < 0.05$.

Treatment (mg ZnONP/L)	Final weight (t = 96 h) (g)
Control	1.48 ± 0.09 ^{A}
0.025	1.87 ± 0.13 ^{AB}
0.25	1.82 ± 0.20 ^{AB}
2.5	2.15 ± 0.16 ^{B}

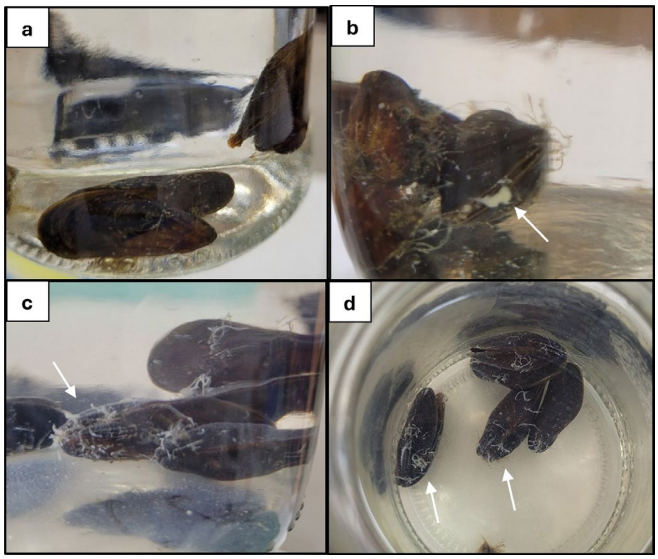

Fig.1. Individuals of *L. fortunei* belonging to (a) the control group (after 72 h) and exposed to 2.5 mg ZnONP/L for (b) 48 h and (c, d) 72 h. White arrows show the light white mucus secretion of the bivalves (the remaining treatments are not shown, as they visually resembled the control group).

Fig. 2 shows the tissue-damage-related enzyme activities in the soft tissue of *L. fortunei* exposed to ZnONP. Both ALT and AST enzyme activities decreased after the highest ZnONP exposure (2.5 mg/L) ($p = 0.0409$ and $p = 0.0295$, respectively), meanwhile ALP increased after the lowest concentration tested (0.025 mg/L) ($p = 0.0331$).

Enzyme tissue damage-related biomarkers showed to be particularly sensitive to nanozinc exposure. However, it is challenging to discuss our findings, as, to the best of our knowledge, ZnONP-related ecotoxicity has not been addressed in the soft tissue of bivalves. Our recent study (Ale et al., 2025) reported comparative nanozinc toxicity in terms of different temperatures, and for the one similar to the employed in this study (27 ºC), similar tendencies for AST decreased enzyme activity were also evidenced at all the concentrations tested. Conversely, discrepant results were found with ALT activity; while no effects were observed in the other study, which employed a lower ZnONP concentration. Here, we evidence an inhibition of the enzyme activity in soft tissue of bivalves exposed to the highest concentration (2.5 mg ZnONP/L). In this regard, the augmented particle (and metal) concentration could have exceeded the enzyme capacity to avoid tissue damage, or even the active site of the enzyme could also have been affected, causing its activity inhibition. For ALP, here we evidence an increased enzyme activity at the lowest concentration tested (0.025 mg/L); however, in Ale et al. (2025) its activity has been found to be decreased, suggesting that ecotoxicity of ZnONP is clearly affected by the biological responses of the test organisms. Although all organisms were collected from the same sampling site (but during different collection events, specifically in different months during the summer of 2024), complex variations in their habitat could be attributed to their discrepant sensitivity, as these organisms are considered tolerant to changes in river flow- and climate-related variables. In this regard, further studies of nanopollutant exposures to freshwater bivalves are highly necessary.

Other studies of mollusks exposed to different stressors like metals and even nanoplastics identified alterations of these enzyme activities (Brandts et al., 2022; Kokhan et al., 2024; Li et al., 2012; Narvia and Rantamäki, 1997). In case of freshwater species, increased enzyme activities of transaminases (ALT and AST) and ALP were found in serum of *Cyprinus carpio* injected with ZnONP, and it was explained that the particles caused dysfunction in the kidney of fish (Rasheed et al., 2023). In terms of mollusks, an interesting study made on the freshwater snail *Biomphalaria alexandrina* exposed to 7.35 mg ZnONP/L evidenced transaminases and ALP increased both in hemolymph and soft tissue of the organisms. Such results were related to muscle damage, intestinal and hepatopancreatic injuries, and toxic hepatitis in the snail (Fahmy et al., 2014). This study clearly indicates damage in the soft tissue of *L. fortunei* after the highest nanozinc concentrations tested (0.25 and 2.5 mg/L).

Table 2 shows oxidative stress-related biomarkers. Only SOD activity was increased with exposure to the lowest ZnONP concentration (0.025 mg/L) ($p = 0.0394$), while no differences were found for the remaining treatments, neither for the other antioxidant enzymes nor peroxidation levels in comparison with the control group. In this context, we highlight the need for studies under realistic conditions, as the actual nanotoxicity could be underestimated.

Nanoparticles were closely associated with cytotoxicity by increasing intracellular reactive oxygen species (ROS) and the levels of the proinflammatory mediator; thus, the homeostatic redox state of the test organism becomes disrupted upon ROS induction by NP (Khanna et al., 2015). Their related biomarkers were widely reported throughout the available literature in terms of nanotoxicity in aquatic organisms (Ale et al., 2024; 2021; Cazenave et al., 2019; Gutierrez et al., 2021). In the case of ZnONP, it was explained that their metabolisms in cells generate ROS overproduction, leading to oxidative damage (Zhao et al., 2016). However, the LPO levels analyzed in soft tissue of *L. fortunei* showed no changes in comparison with the control group. Among the available literature, augmented LPO levels with ZnONP exposure were found at much higher concentrations than those tested in this study. For example, in a tissue of the freshwater snails *Lymnaea luteola* and *Biomphalaria alexandrina*, increased LPO levels (measured by malondialdehyde content, MDA) were evidenced at 7-32 mg ZnONP/L (Ali et al., 2012; Fahmy et al., 2014). Accordingly, Zhao et al. (2016) exposed the *Danio rerio* fish to increasing ZnONP concentration and found increased MDA levels only at higher concentrations (from 10 mg/L onwards,

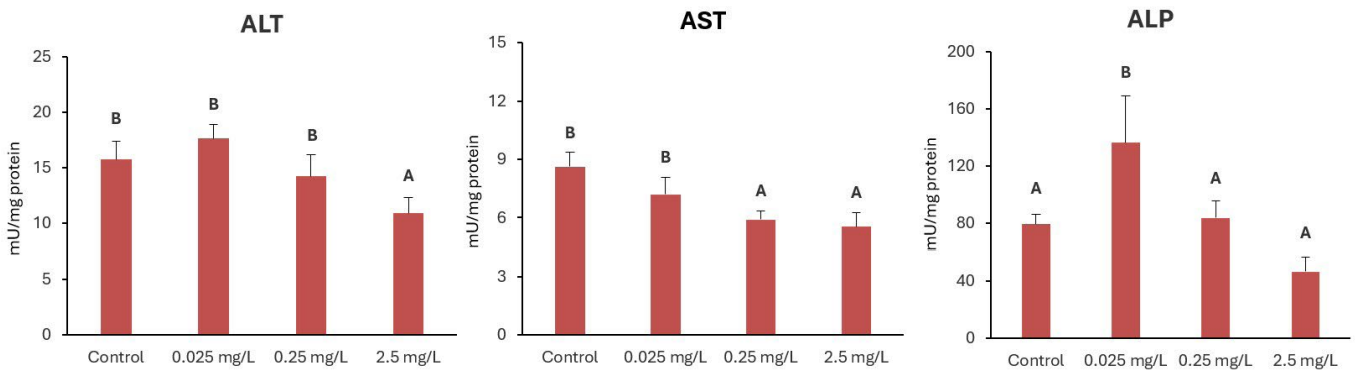

Fig.2. Tissue damage-related enzyme activities: alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) in soft tissue of *L. fortunei* exposed to 0, 0.025, 0.25, and 2.5 mg ZnONP/L for 96 h. The values are expressed as means ± SE. Means not sharing the same superscript (A or B) are significantly different at $p < 0.05$.

Table 2. Oxidative stress-related enzyme activities: superoxide dismutase (SOD), catalase (CAT), and glutathione S-transferase (GST), and lipid peroxidation levels (LPO) in soft tissue of *L. fortunei* exposed to 0, 0.025, 0.25, and 2.5 mg ZnONP/L for 96 h. The values are expressed as means ± SE. Means not sharing the same superscript (A or B) are significantly different at $p < 0.05$.

Treatment (mg ZnONP/L)	SOD (U/mg protein)	CAT (U/mg protein)	GST (mU/mg protein)	LPO (nmol TBARS/mg protein)
Control	348.3 ± 54.4 ^{A}	6.7 ± 0.7	72.0 ± 13.4	0.6 ± 0.2
0.025	608.3 ± 62.8 ^{B}	6.6 ± 0.9	98.3 ± 15.6	1.0 ± 0.1
0.25	446.1 ± 48.6 ^{AB}	6.6 ± 0.9	89.9 ± 8.9	0.9 ± 0.2
2.5	551.9 ± 32.7 ^{AB}	7.0 ± 0.7	90.3 ± 6.0	0.9 ± 0.1

up to 120 mg/L). Therefore, our tested concentrations may not generate enough oxidative damage to be evidenced after 96 h of exposure.

Concerning the measured antioxidant enzyme activities, only SOD was evidenced to differ from the control values (Table 2). Interestingly, the increase in its activity was only at the lowest ZnONP concentration assayed (0.025 mg/L), suggesting that the exerted nanotoxicity may not be concentration-dependent in this case. Therefore, studies that employ only high NP concentrations and do not consider environmentally relevant exposure conditions may underestimate the actual ecotoxicological scenario. In our previous work, we have also observed SOD activation with *L. fortunei* exposure to low ZnONP concentrations (Ale et al., 2025). One explanation for this result could be related to the lower dispersion of the NP at higher concentrations (and reduced bioavailability), as it was already proved for AgNP in the marine mussel *Saccostrea glomerata* (Carrazco-Quevedo et al., 2019). The importance of SOD in maintaining the homeostatic redox state lies in being the first one to handle oxyradicals, particularly, for catalyzing the dismutation of the superoxide radical O^{2-} to hydrogen peroxide (Zhao et al., 2016). Other studies of mussels also found increased SOD activities. In this regard, Falfushynska et al. (2015) exposed the freshwater mussel *Unio tumidus* to ZnONP for 14 days (concentration reported as 3.1 μM) and explained that SOD augmented activity was due to the ROS overproduction. Furthermore, under environmentally relevant nanozinc concentrations (1 and 10 μg/L), SOD activity increase was evidenced in gills and digestive gland of marine mussels *Ruditapes philippinarum* and *Xenostrobus secures* (Lai et al., 2023; Marisa et al., 2016b).

4. Conclusions

Nanotoxicity is induced in complex ways that are in urgent need of further investigation. The ultimate toxicology on test organisms will depend on the particle type, poorly assessed ZnONP (when compared to others like Ag- and Ti-based), the released media (freshwater or marine), intrinsic properties, and biology of the test organism, among others. The latter results are particularly important, as bivalves are not only filtering-feeding organisms but also vulnerable to further agglomeration and sedimentation of the NM due to their sessile habits. We conclude that *L. fortunei* was sensitive to the tested ZnONP, which could be considered low (in comparison to the available bibliography), even at the lowest concentration that is regarded as environmentally relevant. Finally, we highlight the urgent need for further studies assessing the toxicological implications associated with this nanopollutant, with emphasis on freshwater mollusks and realistic exposure conditions.

Conflict of interests

The authors declare no conflicts of interest.

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Author contributions

Conceptualization: A.A.; Methodology: all authors; Formal analysis: A.A.; Investigation: all authors; Writing - original draft preparation: A.A.; Writing - review and editing: all authors; Funding acquisition: A.A., F.R.M.; Resources: A.A., F.R.M., L.M.; Supervision: A.A., F.R.M.

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Morphometric analysis of selected claw traits in *Tubuca forcipata* (Ocypodidae) from Southern Vietnam

Original Article

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ABSTRACT. A total of 233 specimens of the fiddler crab *Tubuca forcipata* (189 males and 44 females) were collected monthly from intertidal mudflats in Dam Doi and Hoa Binh, Ca Mau Province, Southern Vietnam, from July to October 2025. Selected morphometric traits, comprising the carapace width left-to-right ($CW_{L–R}$), the carapace width right-to-left ($CW_{R–L}$), the manus widths (*MW2* and *MW3*), the pollex width (*PW5*), and the dactyl width (*DW7*), were measured to 0.01 mm. Generalized linear models with a gamma distribution and log link were used for trait comparisons; cheliped traits were analyzed with sex and the carapace width ($CW_{L–R}$ or $CW_{R–L}$) as predictors. The carapace width did not differ by sex ($p > 0.05$), whereas *MW2*, *MW3*, *PW5*, and *DW7* were significantly larger in males after adjusting for the carapace width ($\beta_{sex} = 0.156–0.257$; rate ratios (RR) = 1.17–1.29; $p < 0.001$). The carapace width had strong positive effects on all cheliped traits ($\beta_{CW} = 0.054–0.070$; RR ≈ 1.06–1.07; $p < 0.001$). Model diagnostics showed $\chi^2/df < 1$, indicating no overdispersion. These results demonstrate pronounced sex-linked functional dimorphism in the chelae of *T. forcipata* despite similar overall body size, providing a quantitative morphometric baseline for ecological and evolutionary studies of mangrove crabs in the Mekong Delta.

Keywords: dactyl width, manus width, Mekong Delta, pollex width, *Tubuca forcipata*

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1. Introduction

Fiddler crabs (family Ocypodidae) are among the most ecologically influential organisms in tropical and subtropical intertidal ecosystems, acting as key bioturbators and bioindicators of habitat condition (Rosenberg, 2020). They typically occupy distinct intertidal zones within mangrove forests, exhibiting strong habitat preferences and high adaptability to microenvironmental variations (Zolkhiflee et al., 2021). In Vietnam, the diversity and distribution of fiddler crabs, including species of the genus *Tubuca*, were extensively recorded along coastal regions (Shih et al., 2022). Within this context, the Mekong Delta, particularly Ca Mau Province, represents an ecologically important area where populations of *Tubuca* species have recently attracted increasing scientific interest (Tran et al., 2025b).

The species *Tubuca forcipata* (Adam & White, 1849) constitutes an integral component of mangrove faunas throughout Southeast Asia (Auliaputri et al., 2023). Accurate identification of this species, previously classified within the genus *Uca*, often requires a combination of morphological and molecular approaches to resolve its phylogenetic relationships (Andriyono et al., 2019). Fiddler crabs, in general, have a complex and dynamic taxonomic history that continues to be revised through modern morphometric and phylogenetic analyses (Rosenberg, 2001). Recent systematic updates have reclassified several species from *Uca* (sensu lato) into *Tubuca*, reflecting clearer evolutionary lineages (Rosenberg, 2019).

A defining feature of *Tubuca* species is their marked sexual dimorphism, first described in classical works (Crane, 1975). Males possess a characteristically enlarged major claw, while females have two smaller symmetrical claws (Tran and Dinh, 2025). The male claw, a hallmark of sexual selection, exhibits substantial variation in shape and mechanical structure among species (Swanson et al., 2013). Even subtle differences

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in claw morphology can serve as diagnostic features for distinguishing closely related or cryptic taxa within the genus (Shih et al., 2018).

Ecologically, fiddler crabs contribute to sediment aeration, nutrient cycling, and overall mangrove ecosystem functioning through their burrowing and mound-building activities (Pardo et al., 2020). Consequently, the composition and diversity of fiddler crab assemblages, including *T. forcipata*, are often regarded as reliable indicators of mangrove ecosystem health and resilience (Ashton and Macintosh, 2024).

Most previous studies on fiddler crabs focused on aspects of reproductive biology, population dynamics, and environmental influences, including gonadal development (Castiglioni et al., 2007), breeding seasons (Andriyono et al., 2019; Cobo and Fransozo, 2003), sex ratios, and maturity sizes (Oshiro, 1999; Silva and Oshiro, 2002). Furthermore, recent phylogeographic analyses have enhanced understanding of the historical dispersal and isolation of *Tubuca* species across Southeast Asia (Shih et al., 2022).

In Vietnam, emerging research has begun to document morphological variations among *Tubuca* species in the Mekong Delta, for instance, interprovincial differences in *T. paradussumieri* (Tran et al., 2025a) and claw and leg morphometrics in *T. rhizophorae* (Tran and Dinh, 2025). Dao et al. (2024) further reported significant sexual and spatial differences in *T. rhizophorae* populations from Bac Lieu and Ca Mau. However, detailed morphometric data for *T. forcipata* in Ca Mau remain scarce, creating a critical knowledge gap regarding its sexual dimorphism, allometric scaling, and ecological role within mangrove ecosystems of the Mekong Delta.

In this study, we test the hypothesis that males of *T. forcipata* possess disproportionately larger cheliped components (manus, pollex, and dactyl) than females of equivalent body size. Specifically, we predict that: (i) the carapace width left-to-right ($CW_{L–R}$), the carapace width right-to-left ($CW_{R–L}$) (does not differ significantly between sexes, indicating comparable overall body size; and (ii) functional claw traits (*MW2*, *MW3*, *PW5*, and *DW7*) are significantly larger in males after accounting for the carapace width, reflecting sex-linked allometric investment in chela development. By quantitatively assessing these relationships using generalized linear models (GLMs), this study aims to clarify the extent and scaling of sexual dimorphism in *T. forcipata*, providing essential baseline data for future ecological and evolutionary investigations of mangrove crabs in Southern Vietnam.

2. Materials and methods

2.1. Sample collection and morphometric measurements

Specimens of *T. forcipata* (Fig. 1) were collected manually from intertidal mudflats along the Dam Doi and Hoa Binh coastlines, Ca Mau Province, Southern Vietnam, during low tide from July to October 2025 (Fig. 2). Sampling was conducted monthly, yielding a total of 233 individuals (189 males and 44 females). Crabs were captured by hand or using baited traps, then preserved in 70% ethanol for transport to the laboratory.

In the laboratory, individuals were identified based on external morphological characteristics consistent with the descriptions of *Tubuca* species in the Indo-Pacific region. Each specimen was sexed by observing the abdominal shape—males possess a narrow, triangular abdomen, whereas females have a broad, rounded one.

Morphometric characteristics were analyzed following the procedures described by Dao et al. (2024), including the carapace width from left to right ($CW_{L–R}$), the carapace width from right to left ($CW_{R–L}$), the manus width (*MW2* and *MW3*), the pollex width (*PW5*), and the dactyl width (*DW7*) (Fig. 3). All measurements were taken using a digital caliper (Model: MOORMW-110-15DIP) with a precision of 0.01 mm.

2.2. Data analysis

All statistical analyses were conducted using jamovi v2.6.44 (the jamovi project, 2024) with the GAMLj module. Prior to model fitting, the normality of each variable was assessed using the Shapiro–Wilk test. Results indicated that both $CW_{L–R}$ and $CW_{R–L}$ slightly deviated from normality ($p < 0.05$), whereas all other variables (*MW2*, *MW3*, *PW5*, and *DW7*) were strongly right-skewed. Consequently, all variables were analyzed using generalized linear models (GLMs) with a gamma distribution and log-link function, which appropriately handle skewed continuous data.

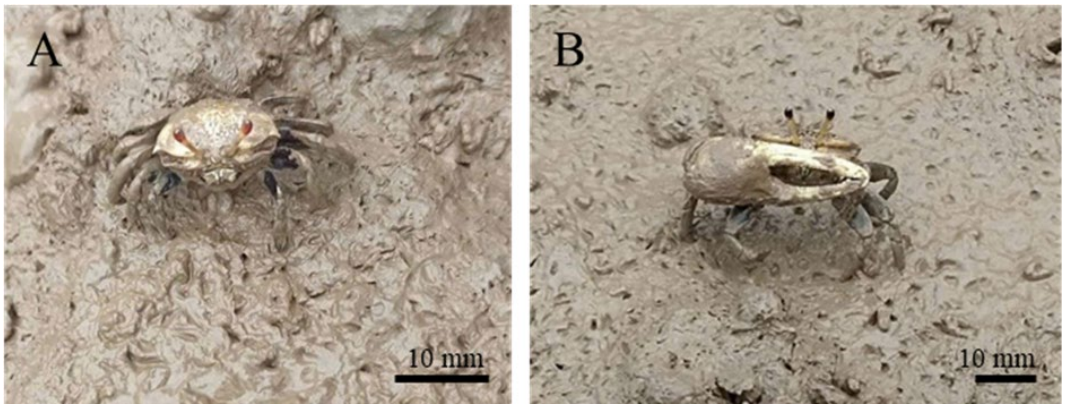

Fig.1. *Tubuca forcipata* (A: female; B: male).

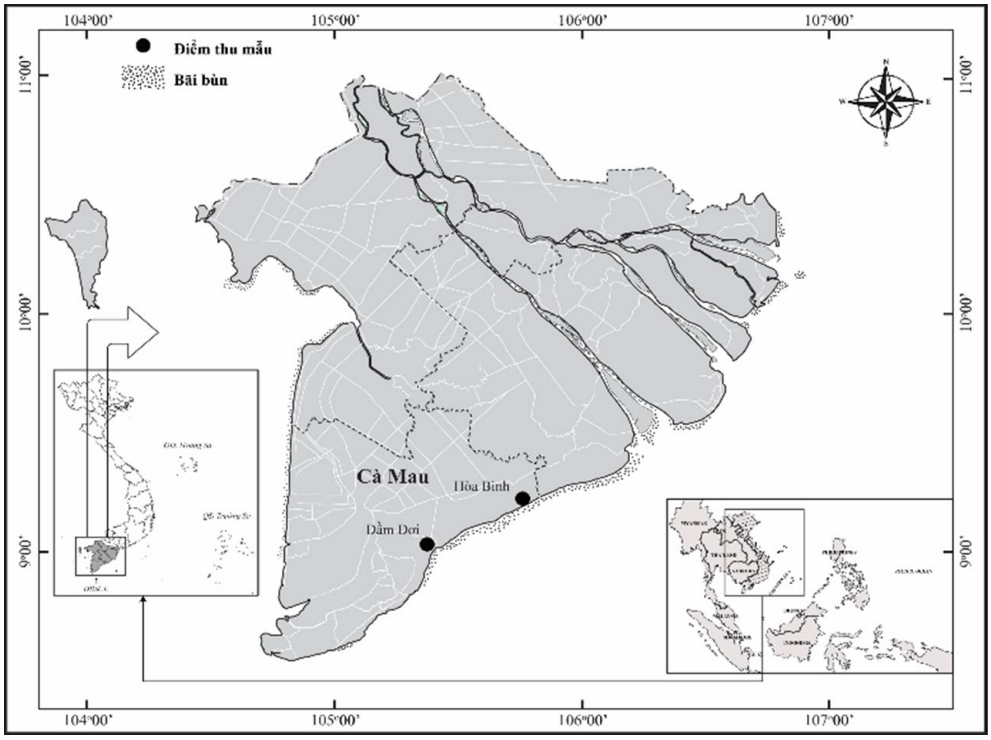

Fig.2. Sampling sites map in Cau Mau (modified from Dinh (2018)).

Separate GLMs were fitted for each response variable following the general structure: Y ~ Sex + $CW_{L–R}/CW_{R–L}$, where Y denotes each morphometric trait (*MW2*, *MW3*, *PW5*, and *DW7*), and Sex is a categorical factor with two levels (male and female). Carapace-width covariates were mean-centered before analysis to enhance model interpretability. For each model, parameter estimates (β ± SE), Wald z-values, p-values, and rate ratios ($RR = e^{\beta}$) were extracted. Significance was set at $p < 0.05$. Positive β(*CW*) coefficients indicate that the trait increases with body size. To assess model adequacy, the Akaike information criterion (AIC), the Bayesian information criterion (BIC), deviance, and the chi-squared-to-df ratio (χ^2/df) were examined; values of $\chi^2/df < 1$ were interpreted as evidence of good model fit without overdispersion. Estimated marginal means (EMMs) for each sex were obtained from the fitted GLMs to provide adjusted means and confidence intervals on the response scale. These adjusted values are reported in tables and figures of the Results to facilitate interpretation of sex-related differences while accounting for variation in body size.

3. Results

Descriptive statistics and normality

A total of 233 individuals of *T. forcipata* (189 males and 44 females) were analyzed. Descriptive statistics for all morphometric variables are summarized in Table 1. Mean carapace widths were 18.74 ± 0.22 mm ($CW_{L–R}$) and 18.44 ± 0.22 mm ($CW_{R–L}$). The Shapiro–Wilk test indicated significant deviations from normality for most variables ($p < 0.05$), except for MW(3) ($p = 0.0844$). Because both carapace-width variables also deviated slightly from normality ($CW_{L–R}$, $p = 0.0179$; $CW_{R–L}$, $p = 0.0441$), all traits were analyzed using gamma–log GLMs to ensure consistent treatment of skewed data.

Carapace width ($CW_{L–R}$ and $CW_{R–L}$)

No significant sex-related differences were detected for either $CW_{L–R}$ or $CW_{R–L}$ ($p > 0.05$). Estimated marginal means (EMMs) were nearly identical between males and females (Table 1), confirming the first hypothesis that overall body size does not differ significantly between sexes.

Fig.3. Illustration of morphometric measurements in *Tubuca forcipata* (carapace width (*CW*), manus width (*MW2*, *MW3*), pollex length (*PW5*), and dactyl length (*DW7*)).

Functional morphometric traits (MW2, MW3, PW5, and DW7)

When the carapace width was included as a covariate, all cheliped traits (*MW2*, *MW3*, *PW5*, and *DW7*) were significantly affected by sex (all $p < 0.001$), supporting the second hypothesis. Males exhibited markedly larger adjusted means for each trait compared with females (Table 3).

Across all gamma–log models, sex coefficients ranged from $\beta = 0.156$ to 0.257, corresponding to rate ratios (RR) = 1.17–1.29, which indicated that male chela components were 17–29% larger than those of females at equivalent carapace widths. Both *CW* covariates had significant positive effects ($\beta = 0.054$–0.070; $p < 0.001$; RR ≈ 1.06–1.07), indicating that a 1-mm increase in the carapace width resulted in an approximately 6–7% increase in cheliped size.

Model diagnostics confirmed a good fit, with all $\chi^2/df < 1$ and no indication of overdispersion. The inclusion of $CW(_{L–R})$ or $CW(_{R–L})$ as covariates yielded consistent results, demonstrating the robustness of these effects (Table 2).

The adjusted means (EMMs) for each trait by sex (Table 3) clearly illustrate the magnitude of sexual dimorphism. Males averaged 5.34 ± 0.13 mm for *MW2*, 7.13 ± 0.13 mm for *MW3*, 3.16 ± 0.07 mm for *PW5*, and 2.75 ± 0.06 mm for *DW7*. Corresponding female means were 4.57 ± 0.14 mm, 5.66 ± 0.14 mm, 2.45 ± 0.07 mm, and 2.23 ± 0.07 mm, respectively. These results confirm a consistent pattern of functional dimorphism in chela components, while overall body size (as measured by carapace width) remains statistically equivalent between sexes. These findings demonstrate that *T. forcipata* exhibits sex-linked functional dimorphism localized to the chelae, rather than generalized body enlargement.

Table 1. Estimated marginal means of carapace width (mm) by sex from GLMs

Parameter	Sex	n	Mean ± SE	p
$CW_{L–R}$ (mm)	Female	44	18.10 ± 0.48	0.155
	Male	189	18.89 ± 0.24	
$CW_{R–L}$ (mm)	Female	44	17.89 ± 0.50	0.233
	Male	189	18.57 ± 0.25	

Note: Carapace width from left to right ($CW_{L–R}$); carapace width from right to left ($CW_{R–L}$).

4. Discussion

This study provides quantitative evidence of sex-related morphometric differentiation in *T. forcipata* from the Dam Doi and Hoa Binh estuaries, Ca Mau Province. The two tested hypotheses were fully supported: the carapace width did not differ significantly between sexes, whereas all functional cheliped traits, including the manus width (*MW2* and *MW3*), the pollex width (*PW5*), and the dactyl width (*DW7*), were significantly larger in males after adjusting for body size. These findings confirm that sexual dimorphism in *T. forcipata* is primarily functional rather than structural, reflecting differential allocation of growth to the claws rather than general body enlargement.

The present analysis also revealed that neither the carapace width nor the morphometric traits of the minor chela (*MW2*, *MW3*, *PW5*, and *DW7*) differed significantly between males and females, even after controlling for body size. This outcome indicates that the minor chela in both sexes develops proportionally, consistent with its shared role in sediment manipulation and feeding rather than in reproductive signaling or territorial competition (Crane, 1975; Zeil and Hemmi, 2006).

In most fiddler crabs, the major chela, which is found only in males, is highly exaggerated and acts as a sexually selected structure for waving displays, visual communication, and combat (Crane, 1975; Swanson et al., 2013). In contrast, the minor chela serves as the principal feeding appendage and remains morphologically similar between sexes. The morphological stability of this structure in *T. forcipata* supports its interpretation as a functional trait that is shaped predominantly by natural rather than sexual selection. Comparable results were reported for other *Tubuca* species, such as *T. rhizophorae* and *T. paradussumieri*, in which the minor chela shows minimal sexual variation, whereas the major claw exhibits marked dimorphism under strong sexual selection (Dao et al., 2024; Tran and Dinh, 2025; Tran et al., 2025a).

Table 2. Gamma–log GLM results for effects of sex and carapace width on functional traits

Parameter	Controlled variable	β (sex) ± SE	z	p	RR	Sex effect	β	p	AIC
MW2	$CW_{L–R}$	-0.049 ± 0.038	-1.28	0.2003	0.95	♂ 5% smaller	0.041	< 0.001	219.47
	$CW_{R–L}$	-0.057 ± 0.039	-1.46	0.1442	0.94	♂ 6% smaller	0.036	< 0.001	233.84
MW3	$CW_{L–R}$	-0.030 ± 0.023	-1.30	0.1936	0.97	♂ 3% smaller	0.052	< 0.001	95.15
	$CW_{R–L}$	-0.038 ± 0.027	-1.40	0.1601	0.96	♂ 4% smaller	0.045	< 0.001	150.93
PW5	$CW_{L–R}$	-0.014 ± 0.121	-0.11	0.910	0.99	♂ ≈ ♀	0.060	< 0.001	185.50
	$CW_{R–L}$	-0.009 ± 0.124	-0.07	0.944	0.99	♂ ≈ ♀	0.045	0.0015	202.78
DW7	$CW_{L–R}$	-0.062 ± 0.045	-1.38	0.167	0.94	♂ 6% smaller	0.059	< 0.001	-75.49
	$CW_{R–L}$	-0.067 ± 0.048	-1.41	0.158	0.94	♂ 6% smaller	0.050	< 0.001	-53.52

Note: Carapace width from left to right ($CW_{L–R}$) and carapace width from right to left ($CW_{R–L}$); manus width (*MW2* and *MW3*), pollex width (*PW5*), and dactyl width (*DW7*).

The strong positive association between carapace width and all minor-chela dimensions in this study reflects isometric to slightly positive allometric growth, indicating that as individuals grow, the feeding appendages scale proportionally with body size. Such relationships are typical of *Tubuca* species and are linked to their benthic lifestyle, where coordinated development of the body and appendages facilitates sediment processing and burrow maintenance (Pardo et al., 2020; Zolkhiflee et al., 2021).

Overall, the results suggest that sexual dimorphism in *T. forcipata* is localized to the major chela and is absent in the minor chela, which remains functionally conserved across sexes. This pattern reflects an evolutionary trade-off between maintaining feeding efficiency and developing energetically costly display structures. The minor chela thus represents a morphologically stable component, which is constrained by ecological function, while the major claw evolves more dynamically under the pressures of sexual selection. These findings add new quantitative insight into structure-specific sexual dimorphism within *Tubuca*, emphasizing that morphological divergence in fiddler crabs arises from a balance between sexual and ecological selection forces.

5. Conclusion

This study on *T. forcipata* from Ca Mau Province, Mekong Delta, found no significant sex-related differences in the carapace width ($CW_{L–R}$, $CW_{R–L}$) or in minor chela traits (*MW2*, *MW3*, *PW5*, and *DW7*) after accounting for body size, with male characteristics only slightly larger (3–6%, $p = 0.14–0.91$). The carapace width strongly predicted chela trait sizes ($\beta = 0.036–0.060$; $p < 0.001$), indicating proportional scaling between body and appendage growth. Sexual dimorphism in this species is thus limited to the major chela, while the minor chela remains functionally conserved across sexes. These findings provide essential morphometric data for future ecological and evolutionary studies of fiddler crabs in the Mekong Delta.

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Conflict of Interest

The authors declare no conflicts of interest.

Table 3. Estimated marginal means of functional traits (mm) by sex from Gamma–log GLMs

Parameter	Sex	Mean ± SE
MW2	Female	1.62 ± 0.06
	Male	1.70 ± 0.03
MW3	Female	2.06 ± 0.04
	Male	2.13 ± 0.02
PW5	Female	0.91 ± 0.10
	Male	0.92 ± 0.05
DW7	Female	0.76 ± 0.03
	Male	0.81 ± 0.02

Note: Manus width (*MW2* and *MW3*), pollex width (*PW5*), and dactyl width (*DW7*)

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Original Article

LIMNOLOGY FRESHWATER BIOLOGY

Variability of *Coregonus peled* (Gmelin, 1789) fecundity in the middle part of the Ob River (Western Siberia, Russia) and the influence of environmental temperature on it

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ABSTRACT. The peled, *Coregonus peled*, is a widespread species of the Coregonidae in Northern Eurasia. Its numbers remain relatively high throughout the Ob River basin. This species is environmentally plastic. Its fecundity varies widely, both across different water bodies and within the same water body during different observation periods. Hydrological conditions influence the reproductive capacity of peled, with higher fecundity in high-water years. This study aimed to analyze the fecundity of peled migrating upstream to the middle part of the Ob River for natural reproduction and detection the relationship between its fluctuations and environmental temperature. We identified that peled, which comes to spawn in the middle part of the Ob River, reaches sexual maturity at the age of three years. However, individuals aged five to six years form the basis of the spawning stock, comprising up to 70% of the total. The reproductive capacity of peled is highly variable. Individual absolute fecundity ranges from 11.3 to 58.0 thousand eggs, increasing with age, standard length, total weight, body weight, and Fulton and Clark indexes. Individual absolute fecundity ranges from 36 to 103 eggs per gram of body weight and correlates with total weight and Fulton indexes. Peled reproductive capacity indicators vary significantly between years. Overall, the individual relative fecundity of this species in age groups of 5+ years and older has decreased by 41–47% compared to the mid-20th century. There was a relationship between the reproductive capacity of peled migrating to the middle part of the Ob River for spawning and the environmental temperature in the lower part of the Ob River, where fish were feeding before the spawning migration begins: relatively high July temperatures are associated with lower individual absolute fecundity indicators for the most numerous age groups. Thus, global warming may have a negative impact on the reproductive capacity of peled in the Ob River basin.

Keywords: Peled, *Coregonus peled*, fecundity, climate change, Ob River, Western Siberia

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1. Introduction

The peled, *Coregonus peled*, is a widespread species of the Coregonidae family in Northern Eurasia (Reshetnikov et al., 1989; Froese and Pauly, 2025). It is one of the few whitefish species, whose numbers are still relatively high in all areas of the Ob basin (Matkovsky and Krokhalevsky, 2010; Krokhalevsky and Tunev, 2014; Tunev, 2015; Babkin et al., 2018; Tsapenkov et al., 2023). Peled is an object of commercial fishery, including aquaculture purposes: juveniles of the species that were obtained from wild fish are widely used for pasture-based fish farming in Western Siberia (Zlokazov et al., 1983; Mukhachev, 2008; Interesova et al., 2014; Egorov et al., 2020).

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The peled inhabiting the Ob River has a complex life cycle. After wintering in the Gulf of Ob, it migrates upstream to the lower part of the Ob River, where it feeds in shallow and warm floodplain areas (sors). In autumn, mature individuals migrate to spawning grounds in the Ural tributaries of the Ob (the Severnaya Sosva, Voikar, and Shchuchya) and in the middle part of the Ob River. After spawning, the peled migrate back to the lower part of the Ob River (Popov, 2007).

This species is environmentally plastic (Krokhalevsky, 1981; Reshetnikov et al., 1989; Matkovsky and Krokhalevsky, 2010). This also applies to fecundity, which varies widely: the minimum value of absolute individual fecundity of the species in natural waters is more than 51 times lower than the maximum (Reshetnikov et al., 1989). Moreover, this indicator varies greatly not only in different water bodies but also from year to year in the same section of the basin (Iogansen and Petkevich, 1958; Krokhalevsky, 1980; Tunev, 2016). Hydrological regime is known to influence the reproductive capacity of peled: in high-water years it is higher (Moskalenko, 1956; Krokhalevsky, 1980; Bogdanov and Agafonov, 2001; Tunev, 2016; Bogdanov et al., 2024). At the same time, climate change was hypothesized to impact the natural reproduction of whitefish species (Matkovsky, 2019; Kirpotin et al., 2021; Matkovskii and Krasnoperova, 2022), as excessive water warming and decreased oxygen concentrations in summer may worsen feeding conditions for spawners in the Lower Ob floodplain system during the period of trophoplasmic growth of oocytes. This study aimed to analyze the fecundity of peled ascending to the middle part of the Ob River for reproduction and to detection the relationship between its fluctuations and environmental temperature.

2. Materials and methods

For this study, we used data from capturing peled spawners during their spawning migration in the middle part of the Ob River in the Aleksandrovsky District of Tomsk Oblast in September 2019 and 2020 (Fig. 1).

Fish were caught using 100-meter-long drift nets with a mesh size of 40–50 mm. Egg samples weighing 2.5–3.0 g were collected from females of various ages at maturity stage IV, and the samples were fixed in a 2% formalin solution. The standard length and weight of the fish were measured. The gonads were weighed, and scales were collected for age detection. Sample processing and age detection were performed in the laboratory by a single operator. The total number of samples was 45 in 2019 and 49 in 2020. According to generally accepted methods (Pravdin, 1966; Petlina, 1987), individual absolute (IAF) and relative (IRF) fecundity were assessed, and gonadosomatic index (GSI) reflecting the ratio of gonad weight to body weight without entrails was expressed as a percentage. To assess the influence of environmental temperature of the lower part of the Ob River before the start of the spawning migration on the fecundity of peled ascending to the spawning grounds within the middle part of the Ob River for reproduction, air temperature data in the Salekhard area from open sources were used (Reference and information portal…, 2025). Air temperature was chosen for analysis as an indicator that more accurately reflects the environmental temperature in floodplain shallows (feeding grounds of peled) than water temperature according to data from Roshydromet (Russian Federal Service for Hydrometeorology and Environmental Monitoring) gauging stations, since the latter is measured in the riverbed, along the current.

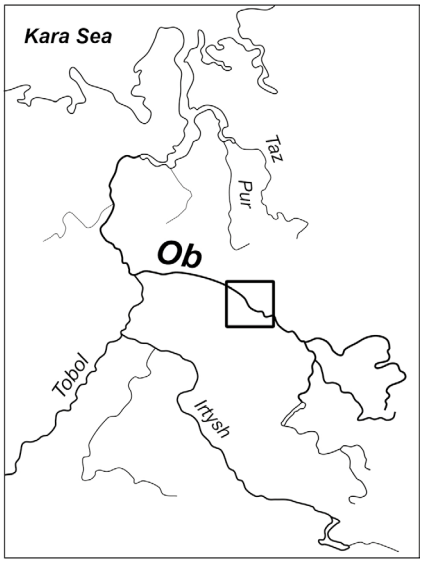

Fig.1. Material collection area (indicated by the dotted line).

To identify differences in the peled reproductive capacity and size characteristics across the years of observation, a t-test was used. The normality of the distribution was assessed using the Shapiro-Wilk test. Pearson correlation coefficients (r) were detected to assess the relationship between IAF, IRF, and GSI of peled with age and size characteristics. Regression equations were calculated to analyze the relationship between IAF and environmental temperature. The statistical significance of differences and relationships was assessed at $\alpha = 0.05$. All calculations were performed using Past software (Hammer et al., 2001).

3. Results and discussion

Ten-year-old fish are currently known among peled spawners that migrate upstream to the middle part of the Ob River to spawn, with the bulk of catches (approximately 70%) comprising fish aged 4+ to 5+ (Tsapenkov et al., 2023). Our samples included females aged 2+ to 7+, which may be due to the selectivity of the net fishing gear used for this study. However, fish aged 4+ to 5+ also predominated, comprising 60.0–69.4% of the sample (Fig. 2).

The size characteristics of female peled of different ages differed little in 2019 and 2020, while the Clark indexes were generally statistically significantly (t-test: $t = 5.28$, $p < 0.001$) higher in 2020 (Table 1).

According to our data, IAF of peled varied from 11.3 to 58.0 thousand eggs (in 2019 it averaged 24.4, and in 2020–31.4 thousand). IRF ranged from 36 to 103 eggs per gram of body weight (in 2019 it averaged 68, and in 2020–84). GSI ranged from 14.7 to 35.1 (in 2019 it averaged 22.2, and in 2020–24.6) (Table 2).

Table 1. Size characteristics of female peled in the middle part of the Ob River.

Age	2019			2020		
	Standard length, cm	Total weight, g	Clark indexes, %	Standard length, cm	Total weight, g	Clark indexes, %
2+	29.5 ± 0.9 27.7–30.4	337 ± 12.9 311–353	1.04 ± 0.07 0.97–1.18	28.2 ± 0.9 26.8–29.8	305 ± 28.0 253–349	1.07 ± 0.02 1.04–1.10
3+	30.4 ± 0.3 29.3–31.4	374 ± 3.3 355–386	1.05 ± 0.03 0.95–1.20	29.8 ± 0.9 27.5–32.4	391 ± 40.5 292–498	1.15 ± 0.04 1.06–1.24
4+	31.5 ± 0.5 29.4–35.8	415 ± 9.4 339–484	1.06 ± 0.03 0.83–1.20	30.9 ± 0.3 27.9–34.8	455 ± 16.0 331–648	1.21 ± 0.02 1.02–1.36
5+	32.2 ± 0.2 30.7–33.8	469 ± 8.3 402–514	1.12 ± 0.02 0.93–1.26	32.1 ± 0.4 30.1–34.4	503 ± 13.1 437–552	1.21 ± 0.03 1.01–1.40
6+	34.2 ± 0.4 32.4–35.6	581 ± 11.6 543–612	1.27 ± 0.13 1.06–2.04	33.4 ± 0.5 32.3–34.5	549 ± 25.2 504–621	1.16 ± 0.04 1.08–1.25
7+	–	–	–	35.6 ± 0.6 34.5–36.5	720 ± 9.7 709–739	1.26 ± 0.05 1.20–1.37

In 2020, all analyzed peled reproductive capacity indicators were statistically significantly ($p < 0.001$) higher than in 2019 (t-test: IAF $t = 4.31$; IRF $t = 6.57$; and GSI $t = 3.81$).

Overall, according to our data, IAF of peled statistically significantly ($p < 0.001$) increases with standard length ($r = 0.63$), total weight ($r = 0.83$), body weight ($r = 0.78$), age ($r = 0.63$), Fulton indexes ($r = 0.50$), and Clark indexes ($r = 0.44$) (Fig. 3).

IRF and GSI correlate with the total weight ($r = 0.24$, $p = 0.021$ and $r = 0.21$, $p = 0.043$, respectively) and with Fulton indexes ($r = 0.37$, $p < 0.001$ and $r = 0.39$, $p < 0.001$, respectively). At the same time, IAF is interconnected with IRF and GSI ($r = 0.71$, $p < 0.001$ and $r = 0.55$, $p < 0.001$). The dependence of IAF on the total weight of female peled from the middle part of the Ob River is expressed by the following regression equation: $y = 0.0959x - 6.8404$ ($R^2 = 0.69$). A slightly higher angular coefficient of the equation is noteworthy compared to that for peled in the Taz River (Tunev, 2016), which may indicate a closer relationship between fecundity and weight of fish in the middle part of the Ob River. The body weight of female peled, both total and without entrails, depends on age ($r = 0.81$ and $r = 0.80$, respectively, in both cases $p < 0.001$) (Fig. 4).

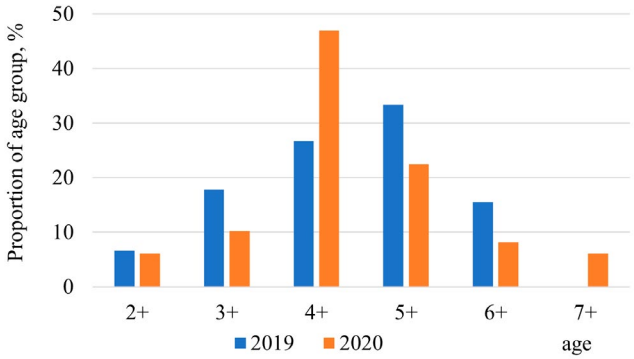

Fig.2. Age composition of female peled in the middle part of the Ob River.

Table 2. Reproductive capacity indicators of peled in the middle part of the Ob River.

Age	2019			2020		
	IAF, thousand eggs	IRF, eggs/g	GSI, %	IAF, thousand eggs	IRF, eggs/g	GSI, %
2+	17.6 ± 3.3 12.7–24.0	66 ± 11 51–88	22.7 ± 2.6 17.5–26.0	19.4 ± 3.0 14.8–25.0	79 ± 6 72–91	21.5 ± 1.2 19.5–23.6
3+	19.0 ± 1.0 15.6–24.8	64 ± 3 56–81	21.6 ± 0.5 19.6–23.4	26.2 ± 3.1 14.8–31.7	84 ± 6 65–97	25.0 ± 2.1 18.3–29.8
4+	21.5 ± 1.4 11.3–26.4	65 ± 3 36–80	20.9 ± 0.8 16.4–26.2	30.0 ± 1.4 19.1–44.7	84 ± 3 64–108	24.7 ± 0.6 20.4–30.3
5+	26.2 ± 1.3 15.3–33.8	70 ± 2 45–88	22.3 ± 1.1 14.7–30.6	33.4 ± 1.6 22.7–41.9	84 ± 4 66–105	24.5 ± 0.7 21.1–28.3
6+	34.8 ± 1.6 27.2–39.1	76 ± 5 56–88	24.7 ± 1.8 16.4–31.3	35.5 ± 2.6 29.5–40.5	82 ± 5 74–95	24.9 ± 1.9 21.6–29.7
7+	–	–	–	49.8 ± 4.8 41.3–58.0	88 ± 9 71–103	26.7 ± 4.2 22.1–35.1

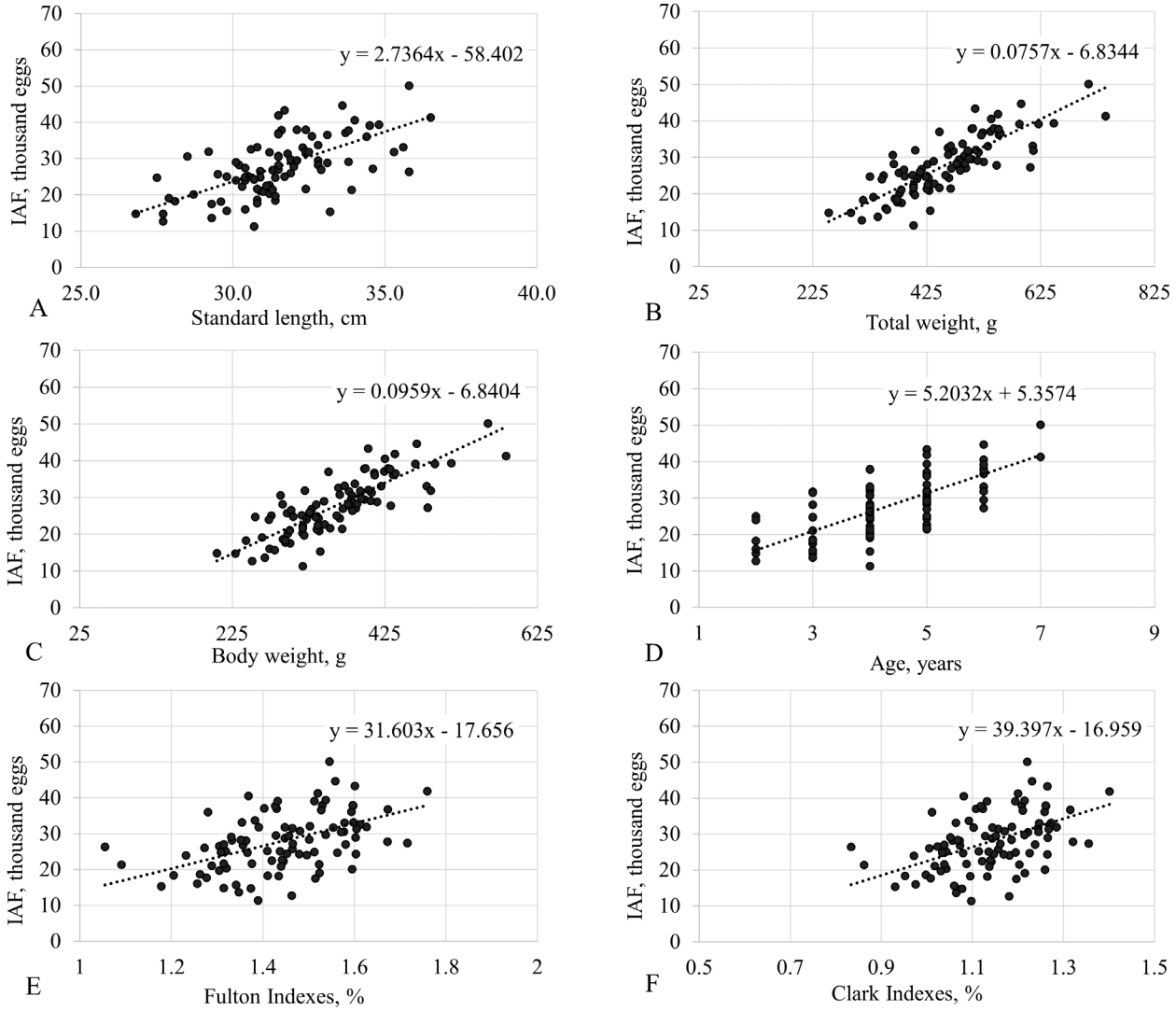

Fig.3. The dependence of individual absolute fecundity of the peled in the middle part of the Ob River on the standard length (A), total weight (B), body weight (C), age (D), Fulton indexes (E), and Clark indexes (F).

A comparison of our data on peled fecundity and information from the mid-20th century (Iogansen and Petkevich, 1958) demonstrated that currently the average IAF of older age groups (5+ and older) has decreased by 41–47%. However, even in the mid-20th century, there were years when IAF was relatively low (Fig. 5).

An analysis of the relationship between IAF of peled ascending for reproduction to the middle part of the Ob River and the environmental temperature in the lower part of the Ob River, where fish was feeding before the start of the spawning migration, reveals a negative effect of high temperature on fecundity. The average IAF of the most numerous age groups in years when the environmental temperature in July exceeded the long-term average (for the period 1950 and 2025) was 43–52% lower than in years when the environmental temperature was below the long-term average (Table 3).

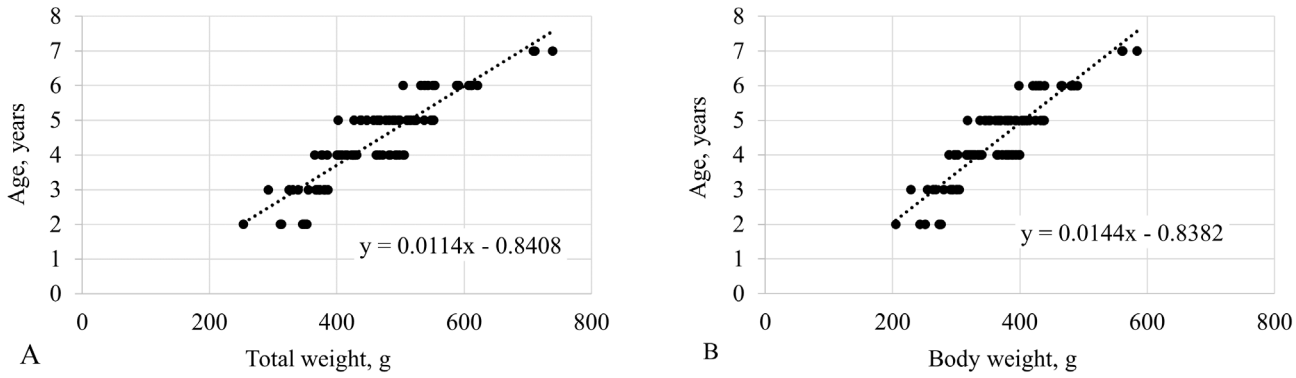

Fig.4. Dependence of total weight (A) and body weight (B) of female peled in the middle part of the Ob River on age.

Table 3. Individual absolute fecundity of peled in the middle part of the Ob River in different years.

Year	Data source	IAF at age, thousand eggs			Average temperature in June, ˚C	Average temperature in July, ˚C
		4+	5+	6+		
2019	Our data	21.47	26.20	34.79	8.1	16.9
2020	Our data	30.03	33.37	35.54	9.9	15.5
1947	Iogansen and Petkevich (1958)	46.39	65.66	91.37	8.0	11.0
1952	Iogansen and Petkevich (1958)	29.24	35.05	64.36	7.6	15.2
1953	Iogansen and Petkevich (1958)	30.10	–	41.74	10.8	16.3

The dependence of IAF of the peled in the middle part of the Ob River on the environmental temperature in July in the lower part of the Ob River can be described by the following equations: $y = -0.25x + 22.72$ ($R^2 = 0.93$) for five-year-olds; $y = -0.15x + 20.46$ ($R^2 = 0.99$) for six-year-olds; and $y = -0.09x + 19.72$ ($R^2 = 0.86$) for seven-year-olds fish. Taking into account that the environmental temperature in summer tends to increase (Fig. 6), a decrease in the reproductive performance of female peled in the spawning stock that migrates to the middle part of the Ob River for reproduction can be expected.

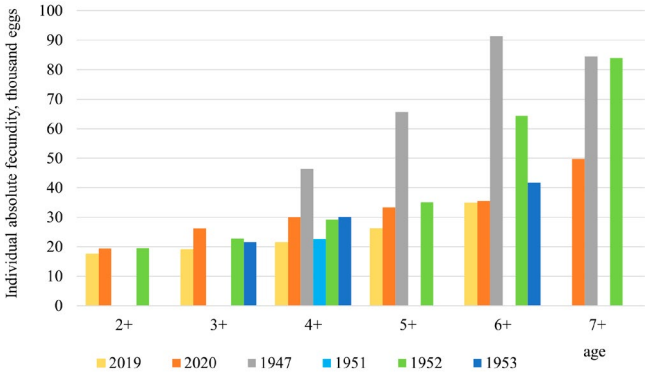

Fig.5. Individual absolute fecundity of peled in the middle part of the Ob River at different observation periods.

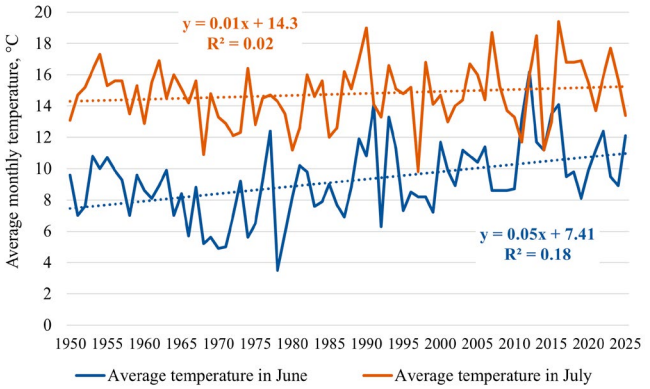

Fig.6. Long-term dynamics of average air temperature in June and July in Salekhard (according to the reference and information portal “Weather and climate”).

4. Conclusions

Peled that are spawning in the middle part of the Ob River begin to reach sexual maturity at three years old. However, most of the spawning stock consists of five- to six-year-old fish, which together account for up to 70% of the spawners. The reproductive capacity of peled in the middle part of the Ob River is highly variable. Individual absolute fecundity ranges from 11.3 to 58.0 thousand eggs, increasing with standard length, total weight, body weight, age, and Fulton and Clark indexes. Individual relative fecundity and gonadosomatic index correlate with total weight and Fulton indexes. The reproductive capacity of peled varies significantly from year to year. Overall, the individual absolute fecundity of this species in age groups 5+ and older has currently decreased by 41–47% compared to the mid-20th century. There is a relationship observed between the reproductive capacity of peled migrating to the middle part of the Ob River for spawning and the environmental temperature in the lower part of the Ob River, where fish were feeding before the spawning migration begins: relatively high July temperatures are associated with lower individual absolute fecundity for the most numerous age groups of peled. Thus, global warming may have a negative impact on the reproductive capacity of peled in the Ob River basin.

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Conflict of interest

The authors declare no conflicts of interest.

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Оригинальная статья

Изменчивость плодовитости пеляди *Coregonus peled* (Gmelin, 1789) Средней Оби (Западная Сибирь) и влияние на нее температуры окружающей среды

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АННОТАЦИЯ. Пелядь *Coregonus peled* – широко распространенный представитель сем. Coregonidae в Северной Евразии, численность которого до сих пор относительно высока на всех участках бассейна Оби. Вид отличается экологической пластичностью, его плодовитость варьирует в очень широких пределах, различаясь как в разных водных объектах, так и в одном и том же в разные периоды наблюдений. Известно, что на воспроизводительную способность данного вида оказывает влияние гидрологический режим – в многоводные годы она выше. Целью данной работы был анализ плодовитости пеляди, поднимающейся для естественного воспроизводства в пределы Средней Оби, и поиск связи ее колебаний с температурой среды. Выявлено, что половая зрелость среднеобского стада пеляди начинает наступать в трехлетнем возрасте, однако его основу составляют пяти–шестилетние экземпляры, суммарно составляющие до 70 % производителей. Показатели воспроизводительной способности вида весьма изменчивы. Индивидуальная абсолютная плодовитость составляет от 11,3 до 58,0 тыс. икринок, увеличиваясь с возрастом, промысловой длиной, общей массой, массой тела без внутренностей, упитанностью по Фультону и по Кларк. Индивидуальная относительная плодовитость колеблется от 36 до 103 икринок на грамм массы тела и коррелирует с общей массой рыб и упитанностью по Фультону. При этом показатели воспроизводительной способности пеляди существенно различаются в разные годы. В целом в настоящее время индивидуальная абсолютная плодовитость данного вида в возрастных группах 5+ и старше по сравнению с серединой XX века снизилось на 41–47 %. Отмечена связь воспроизводительной способности пеляди, поднимающейся на нерест в Среднюю Обь, с температурой среды в Нижней Оби, где происходит нагул производителей перед началом нерестовой миграции – относительно высокие температуры июля ассоциированы с более низкими показателями ИАП наиболее многочисленных возрастных групп. Таким образом, потепление климата может отрицательно сказаться на воспроизводительной способности пеляди в бассейне Оби.

Ключевые слова: пелядь, *Coregonus peled*, плодовитость, изменения климата, Обь, Западная Сибирь

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1. Введение

Пелядь *Coregonus peled* (Gmelin, 1788) – широко распространенный представитель сем. Coregonidae в Северной Евразии (Решетников и др., 1989; Froese and Pauly, 2025). Это один из немногих сиговых видов рыб, численность которого до сих пор относительно высока на всех участках бассейна Оби (Матковский и Крохалевский, 2010; Крохалевский и Тунёв, 2014; Тунев, 2015; Бабкин и др., 2018; Цапенков и др., 2023). Пелядь служит объектом промысла, в том числе для целей аквакультуры – молодь вида, получаемую от диких производителей, широко используют для пастбищного рыбоводства в Западной Сибири (Злоказов и др., 1983; Мухачев, 2008; Интересова и др., 2014; Егоров и др., 2020).

У пеляди, обитающей в Оби, сложный жизненный цикл. После зимовки в Обской губе, она поднимается в пределы Нижней Оби, где нагулива-

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ется на мелководных прогреваемых участках поймы (сорах). Осенью половозрелые особи совершают миграцию на нерестилища в уральские притоки Оби (Северную Сосьву, Войкар, Щучью) и в ее среднее течение. После нереста пелядь вновь скатывается в Нижнюю Обь (Попов, 2007).

Пелядь отличается экологической пластичностью (Крохалевский, 1981; Решетников и др., 1989; Матковский и Крохалевский, 2010). Это относится и к плодовитости, варьирующей в очень широких пределах: минимальное значение абсолютной индивидуальной плодовитости вида в естественных водоемах меньше максимального более чем в 51 раз (Решетников и др., 1989). Более того, этот показатель сильно различается не только в разных водных объектах, но и год от года на одном и том же участке бассейна (Иоганзен и Петкевич, 1958; Крохалевский, 1980; Тунев, 2016). Известно, что на воспроизводительную способность пеляди оказывает влияние гидрологический режим – в многоводные годы она выше (Москаленко, 1956; Крохалевский, 1980; Богданов и Агафонов, 2001; Тунев, 2016; Богданов и др., 2024). Вместе с тем, высказаны предположения о влиянии на состояние естественного воспроизводства сиговых видов рыб происходящего изменения климата (Матковский, 2019; Kirpotin et al., 2021; Матковский и Красноперова, 2024), поскольку излишний прогрев воды и снижение концентрации кислорода в летнее время могут ухудшать условия нагула производителей в пойменной системе Нижней Оби в период трофоплазматического роста ооцитов. Целью данной работы был анализ плодовитости пеляди, поднимающейся для естественного воспроизводства в пределы Средней Оби, и поиск связи ее колебаний с температурой среды.

2. Материалы и методы исследования

Материалом для исследования послужили данные, собранные во время отлова производителей пеляди для целей воспроизводства во время ее нерестовой миграции на р. Оби в Александровском районе Томской области (Рис. 1) в сентябре 2019 и 2020 гг.

Лов рыб проводили плавными сетями длиной 100 м с размером ячеи 40–50 мм. Навеску икры массой 2,5–3,0 г брали у разновозрастных самок на IV стадии зрелости, пробы фиксировали 2%-ным раствором формалина. Измеряли промысловую длину рыб, проводили взвешивание рыб и гонад, собирали чешую для определения возраста. Обработка проб и определение возраста проведены в лабораторных условиях одним оператором. Общее количество проб составило в 2019 г. 45, а в 2020 г. – 49 экз. Согласно общепринятым методикам (Правдин, 1966; Петлина, 1987), оценивали индивидуальную абсолютную (ИАП) и относительную (ИОП) плодовитости, а также гонадосоматический индекс (ГСИ) как отношение массы гонад к массе тела рыбы без внутренностей, выраженное в процентах. Для оценки влияния температуры среды в местах нагула производителей в Нижней Оби перед началом нерестовой миграции на плодовитость пеляди, поднимающейся

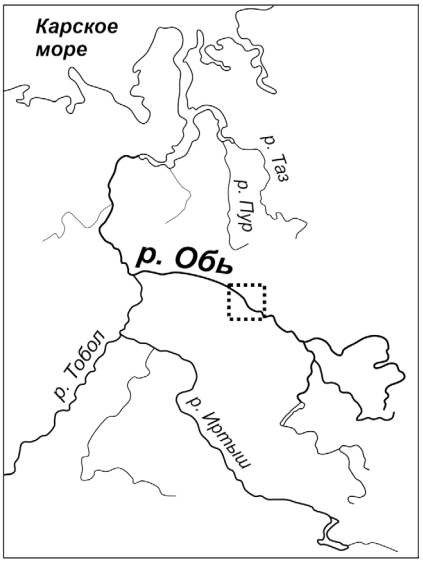

Рис.1. Район сбора материала (обозначен пунктирной линией).

для воспроизводства на нерестилища в пределы Средней Оби, использовали данные о температуре воздуха в районе г. Салехард из открытых источников (Справочно-информационный портал…, 2025). Температура воздуха выбрана для анализа как показатель, более точно отражающий температуру среды в мелководных сорах (местах нагула производителей пеляди), чем температура воды по данным гидропостов Росгидромет, поскольку последнюю измеряют в русле реки, на течении.

Для выявления различий показателей воспроизводительной способности пеляди в годы наблюдений и ее размерных характеристик применяли t-критерий (t-test). Нормальность распределения при этом оценивали с использованием критерия Шапиро-Уилка (Shapiro-Wilk W). Для оценки связи ИАП, ИОП и ГСИ пеляди с возрастом и размерными характеристиками, были определены коэффициенты корреляции Пирсона (r). Для анализа связи ИАП и температуры среды рассчитаны регрессионные уравнения. Статистическую значимость различий и взаимосвязей оценивали на уровне $\alpha = 0{,}05$. Все расчеты проведены с использованием Пакета программ Past (Hammer et al., 2001).

3. Результаты и обсуждение

В составе поднимающихся на нерест в Среднюю Обь производителей пеляди в настоящее время известны десятилетние экземпляры, при этом основу уловов (около 70 %) составляют рыбы в возрасте 4+–5+ (Цапенков и др., 2023). В наших сборах отмечены самки в возрасте 2+–7+, что может быть связано с селективностью использованных для целей данной работы сетными орудиями лова, но преобладают также рыбы в возрасте 4+–5+, составляющие 60,0–69,4 % выборки (Рис. 2).

Размерные характеристики разновозрастных самок пеляди мало отличались в 2019 и 2020 гг., при этом упитанность по Кларк в целом была статистически значимо (t-test: $t = 5{,}28$, $p < 0{,}001$) выше в 2020 г. (Таблица 1).

Таблица 1. Размерные характеристики самок пеляди в Средней Оби.

Возраст, лет	2019 г			2020 г		
	Промысловая длина, см	Масса, г	Упитанность по Кларк	Промысловая длина, см	Масса, г	Упитанность по Кларк
2+	29,5 ± 0,9 27,7–30,4	337 ± 12,9 311–353	1,04 ± 0,07 0,97–1,18	28,2 ± 0,9 26,8–29,8	305 ± 28,0 253–349	1,07 ± 0,02 1,04–1,10
3+	30,4 ± 0,3 29,3–31,4	374 ± 3,3 355–386	1,05 ± 0,03 0,95–1,20	29,8 ± 0,9 27,5–32,4	391 ± 40,5 292–498	1,15 ± 0,04 1,06–1,24
4+	31,5 ± 0,5 29,4–35,8	415 ± 9,4 339–484	1,06 ± 0,03 0,83–1,20	30,9 ± 0,3 27,9–34,8	455 ± 16,0 331–648	1,21 ± 0,02 1,02–1,36
5+	32,2 ± 0,2 30,7–33,8	469 ± 8,3 402–514	1,12 ± 0,02 0,93–1,26	32,1 ± 0,4 30,1–34,4	503 ± 13,1 437–552	1,21 ± 0,03 1,01–1,40
6+	34,2 ± 0,4 32,4–35,6	581 ± 11,6 543–612	1,27 ± 0,13 1,06–2,04	33,4 ± 0,5 32,3–34,5	549 ± 25,2 504–621	1,16 ± 0,04 1,08–1,25
7+	–	–	–	35,6 ± 0,6 34,5–36,5	720 ± 9,7 709–739	1,26 ± 0,05 1,20–1,37

ИАП пеляди по нашим данным варьировала от 11,3 до 58,0 тыс. икринок (в среднем в 2019 г. 24,4, а в 2020 г. – 31,4 тыс.). ИОП колебалась от 36 до 103 икринок на грамм массы тела (в среднем в 2019 г. 68, а в 2020 г. – 84). ГСИ составлял от 14,7 до 35,1 (в среднем в 2019 г. 22,2, а в 2020 г. – 24,6) (Таблица 2).

В 2020 г. все анализируемые показатели воспроизводительной способности пеляди были статистически значимо ($p < 0,001$) выше, чем в 2019 г. (t-test: ИАП $t = 4,31$; ИОП $t = 6,57$; ГСИ $t = 3,81$).

В целом по нашим данным ИАП пеляди статистически значимо ($p < 0,001$) увеличивается с промысловой длиной ($r = 0,63$), общей массой ($r = 0,83$), массой тела без внутренностей ($r = 0,78$), возрастом ($r = 0,63$), упитанностью по Фультону ($r = 0,50$) и по Кларк ($r = 0,44$) рыб (Рис. 3).

ИОП и ГСИ коррелирует с общей массой рыб ($r = 0,24$, $p = 0,021$; $r = 0,21$, $p = 0,043$ соответственно) и упитанностью по Фультону ($r = 0,37$, $p < 0,001$; $r = 0,39$, $p < 0,001$ соответственно). При этом показатели ИАП, ИОП и ГСИ связаны между собой ($r = 0,71$, $p < 0,001$; $r = 0,55$, $p < 0,001$). Зависимость ИАП от массы тела самок пеляди Средней Оби выражается регрессионным уравнением $y = 0,10x–6,84$ ($R^2 = 0,69$). Обращает на себя внимание несколько более высокий угловой коэффициент уравнения по сравнению с таковым для пеляди р. Таз (Тунев, 2016), что может свидетельствовать о более тесной связи плодовитости с массой тела у среднеобских производителей. Масса тела самок пеляди как общая, так и без внутренностей, зависит от возраста ($r = 0,81$ и $r = 0,80$ соответственно, в обоих случаях $p < 0,001$) (Рис. 4).

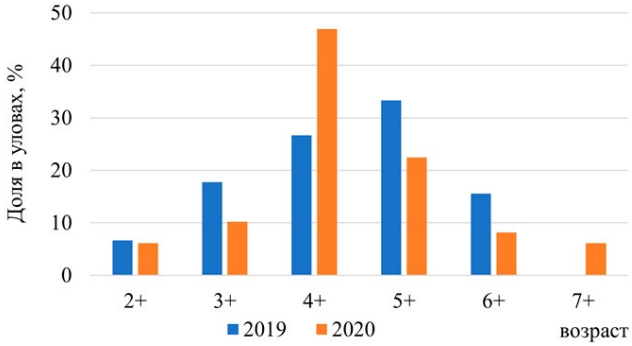

Рис.2. Возрастной состав самок пеляди в Средней Оби.

Таблица 2. Показатели воспроизводительной способности пеляди Средней Оби.

Возраст. лет	2019 г			2020 г		
	ИАП, тыс. шт.	ИОП, шт./г	ГСИ, %	ИАП, тыс. шт.	ИОП, шт./г	ГСИ, %
2+	17,6 ± 3,3 12,7–24,0	66 ± 11 51–88	22,7 ± 2,6 17,5–26,0	19,4 ± 3,0 14,8–25,0	79 ± 6 72–91	21,5 ± 1,2 19,5–23,6
3+	19,0 ± 1,0 15,6–24,8	64 ± 3 56–81	21,6 ± 0,5 19,6–23,4	26,2 ± 3,1 14,8–31,7	84 ± 6 65–97	25,0 ± 2,1 18,3–29,8
4+	21,5 ± 1,4 11,3–26,4	65 ± 3 36–80	20,9 ± 0,8 16,4–26,2	30,0 ± 1,4 19,1–44,7	84 ± 3 64–108	24,7 ± 0,6 20,4–30,3
5+	26,2 ± 1,3 15,3–33,8	70 ± 2 45–88	22,3 ± 1,1 14,7–30,6	33,4 ± 1,6 22,7–41,9	84 ± 4 66–105	24,5 ± 0,7 21,1–28,3
6+	34,8 ± 1,6 27,2–39,1	76 ± 5 56–88	24,7 ± 1,8 16,4–31,3	35,5 ± 2,6 29,5–40,5	82 ± 5 74–95	24,9 ± 1,9 21,6–29,7
7+	–	–	–	49,8 ± 4,8 41,3–58,0	88 ± 9 71–103	26,7 ± 4,2 22,1–35,1

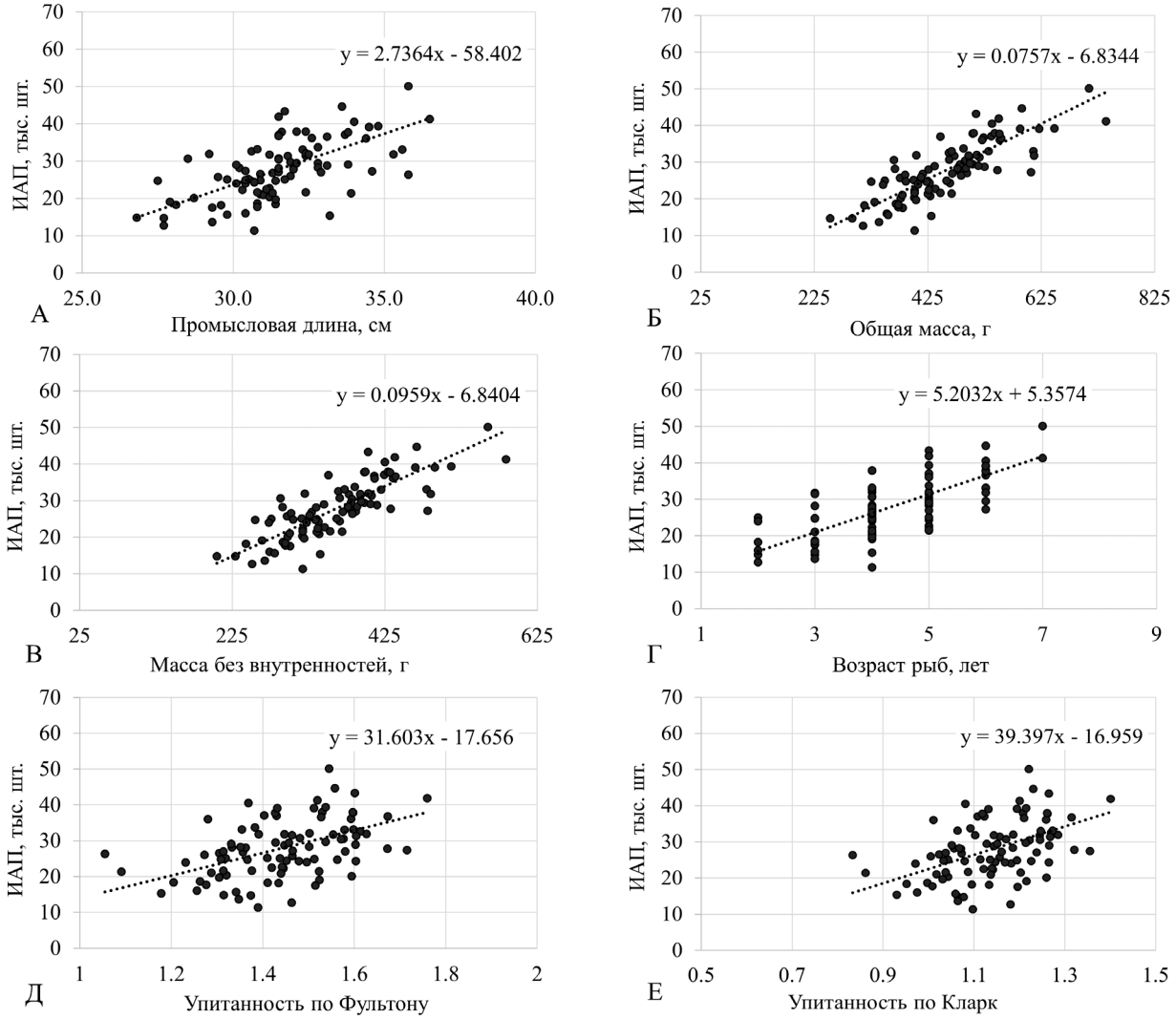

Рис.3. Зависимость индивидуальной абсолютной плодовитости пеляди Средней Оби от промысловой длины (А), общей массы (Б), массы без внутренностей (В), возраста (Г), упитанности по Фультону (Д) и по Кларк (Е).

Сравнение наших данных по плодовитости пеляди и сведений середины XX века (Иоганзен и Петкевич, 1958) показало, что в старших возрастных группах, от 5+, в среднем ИАП вида в настоящее время снизилась на 41–47 %. Однако при этом и в середине прошлого века были годы, когда показатели ИАП были относительно низки (Рис. 5).

Анализ связи ИАП пеляди, поднимающейся для воспроизводства в Среднюю Обь, с температурой среды в Нижней Оби, где происходит нагул производителей перед началом нерестовой миграции, обнаруживает отрицательное влияние высокой температуры на плодовитость. Средняя ИАП наиболее многочисленных возрастных групп нерестового стада пеляди в 1947 году, когда температура среды в июле, наиболее жарком месяце, была ниже среднемноголетних значений (14,6°С за период 1940–2025 гг.), на 43–52 % выше чем в годы, когда температура среды была выше среднемноголетней (Таблица 3).

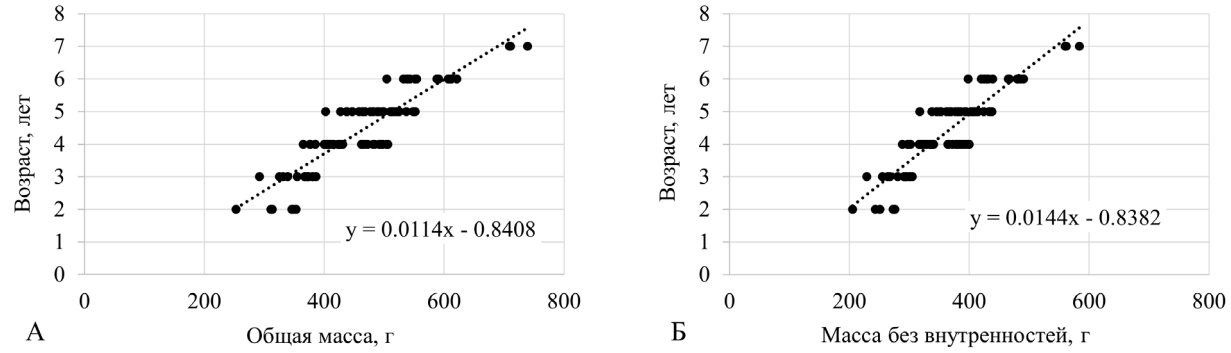

Рис.4. Зависимость общей массы (А) и массы тела без внутренностей (Б) самок пеляди в Средней Оби от возраста.

Таблица 3. Индивидуальная абсолютная плодовитость пеляди Средней Оби в разные годы.

Год	Источник данных	ИАП в возрасте, тыс. шт.			Средняя температура в июне, ˚С	Средняя температура в июле, ˚С
		4+	5+	6+		
2019	Наши данные	21,47	26,20	34,79	8,1	16,9
2020	Наши данные	30,03	33,37	35,54	9,9	15,5
1947	Иоганзен и Петкевич (1958)	46,39	65,66	91,37	8,0	11,0
1952	Иоганзен и Петкевич (1958)	29,24	35,05	64,36	7,6	15,2
1953	Иоганзен и Петкевич (1958)	30,10	–	41,74	10,8	16,3

Зависимость ИАП пеляди Средней Оби от температуры среды в июле можно описать уравнением $y = –0,25x + 22,72$ ($R^2 = 0,93$) для пятилетних, $y = -0,15x + 20,46$ ($R^2 = 0,99$) для шестилетних, и $y = -0,09x + 19,72$ ($R^2 = 0,86$) для семилетних особей. Учитывая, что наблюдается устойчивое повышение температуры среды в летний период (Рис. 6), можно ожидать снижение репродуктивных показателей самок пеляди в нерестовом стаде, поднимающемся для воспроизводства в Среднюю Обь.

4. Заключение

Половая зрелость самок пеляди, поднимающейся на нерест в пределы Средней Оби, начинает наступать в трехлетнем возрасте, однако основу нерестового стада составляют пяти–шестилетние экземпляры, суммарно составляющие до 70 % производителей. Показатели воспроизводительной способности вида весьма изменчивы. ИАП увеличивается с возрастом, промысловой длиной, общей массой, массой тела без внутренностей, упитанностью по Фультону и по Кларк. ИОП и ГСИ коррелируют с общей массой рыб и упитанностью по Фультону. При этом показатели воспроизводительной способности пеляди существенно различаются в разные годы. В целом в настоящее время ИАП данного вида в возрастных группах 5+ и старше по сравнению с серединой XX века снизилось. Отмечена связь воспроизводительной способности пеляди, поднимающейся на нерест в Среднюю Обь, с температурой среды в Нижней Оби, где происходит нагул производителей перед началом нерестовой миграции – относительно высокие температуры июля ассоциированы с более низкими показателями ИАП наиболее многочисленных возрастных групп. Таким образом, потепление климата может отрицательно сказаться на воспроизводительной способности пеляди в бассейне Оби.

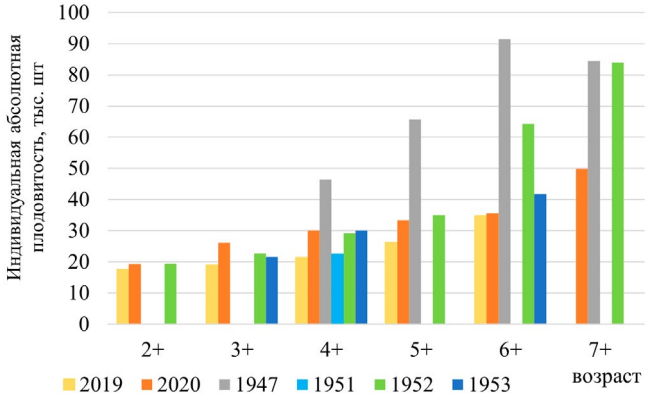

Рис.5. Индивидуальная абсолютная плодовитость пеляди в Средней Оби в разные годы.

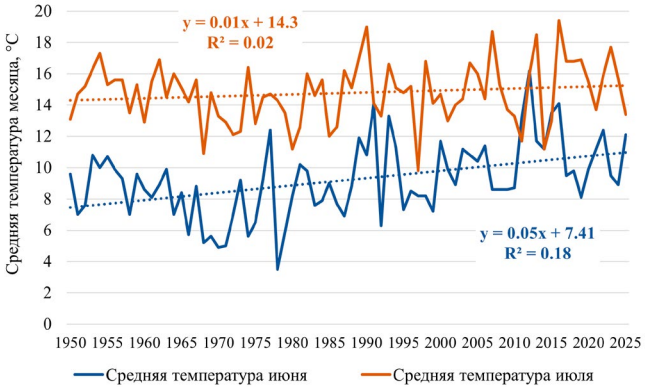

Рис.6. Многолетняя динамика средней температуры воздуха в июне и июле в г. Салехард (по данным Справочно-информационного портала «Погода и Климат»).

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Конфликт интересов

Авторы заявляют об отсутствии конфликта интересов.

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Trophic structure and functional feeding groups of macroinvertebrates in a section of the Gallinazo tropical stream

Original Article
LIMNOLOGY FRESHWATER BIOLOGY

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ABSTRACT. Aquatic macroinvertebrates play crucial roles in ecosystem processes such as grazing, decomposition, and mineralization. Classifying these organisms into functional feeding groups (FFGs) enables a more comprehensive understanding of energy and matter flow through trait-based approaches, complementing traditional taxonomic methods. The study analyzed the trophic structure and FFG variation of aquatic macroinvertebrates in a section of a tropical stream, located in the municipality of Aguachica, Colombia. Sampling was carried out during the rainy season in May and the dry season in July 2024, during which the composition and abundance of macroinvertebrate feeding groups were analyzed. Physical and chemical parameters were measured both in situ and ex situ at strategically selected stations based on accessibility and location. Macroinvertebrates were collected using Surber and D-type nets and identified to the family level. They were then assigned to functional feeding groups. Ecological indices and multivariate statistical analyses were applied to evaluate ecosystem attributes. Results revealed seasonal variations in water parameters, including lower dissolved oxygen and higher chemical oxygen demand during the dry season, suggesting potential organic pollution. Macroinvertebrate richness was higher during the dry season, with *Caenis* sp. and *Tanypodinae* sp. being dominant, while *Drepanotrema* sp. and *Physa* sp. were more abundant during the rainy season, reflecting eutrophic conditions. The P/R index indicated heterotrophic dominance, and low CPOM/FPOM ratios pointed to limited riparian cover. These findings underscore the importance of implementing conservation strategies to mitigate anthropogenic pressures and preserve the ecological integrity of freshwater ecosystems.

Keywords: neotropical streams, predators, FFG, trophic guilds, disturbance, shredders

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1. Introduction

Aquatic ecosystems are essential to the socioeconomic development of human populations, as communities rely on the ecological health and services provided by these systems (Nuñez and Fragoso-Castilla, 2019; Cabrera et al., 2021). Freshwater ecosystems, in particular, are among the most exploited globally due to their high functionality and their direct interaction with anthropogenic activities (Reid et al., 2019; Bănăduc et al., 2024). However, the ecological condition of these water bodies is increasingly affected by land-use changes driven by urban development and agricultural expansion (Forrest et al., 2015; Pascual et al., 2022). Numerous studies have documented the effects of such disturbances on the structural integrity of aquatic ecosystems, particularly in relation to changes in biological communities (Beckmann et al., 2019; Li et al., 2019; Reid et al., 2019).

The use of biological communities to assess the health of freshwater systems has gained reliability in recent decades, as these organisms integrate the cumulative effects of anthropogenic disturbances (Rizo-

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Patrón et al., 2013; Ding et al., 2017; Fierro et al., 2017). Aquatic macroinvertebrates are particularly useful bioindicators due to their varying tolerance levels to environmental stressors and their ability to respond to a wide range of physical and chemical changes in water bodies (Ertaş and Yorulmaz, 2022; Assie et al., 2025), Additionally, their cost-effective sampling methods make them ideal for long-term ecological monitoring (Agboola et al., 2020; Ko et al., 2020). Nevertheless, the taxonomic approach has limitations, especially in regions with high species diversity, as identification to the species level can be challenging and time-consuming (Juvigny-Khenafou et al., 2021). For more than 30 years, multiple studies have addressed this limitation by shifting the focus to food functional ranges of groups, as they reduce the taxonomic effort required for comparison with other methods (Cummins et al., 2005; Ndatimana et al., 2023).

Accurate determination of ecosystem attributes is a complex process that requires extensive data collection along spatial and temporal gradients (Merritt et al., 2002). It is, therefore, that the functional analysis of communities, in particular through FFGs, represents a key tool in community ecology, allowing us to understand how species mediate fundamental ecological processes such as nutrient cycling, primary productivity, and decomposition (Abdul and Rawi, 2019; Doong et al., 2021). The FFG approach requires less taxonomic effort while providing information on the functional structure of macroinvertebrate communities, thus aligning with the principles of functional ecology, which seeks to identify general patterns and mechanisms applicable to multiple ecosystems (Assie et al., 2025). Thus, the aim of this study was to analyse the trophic structure and variation of aquatic macroinvertebrate FFGs in a section of a tropical stream. The study of FFGs not only allows us to assess the ecological integrity of specific systems such as the Gallinazo stream but also contributes to the understanding of the functional responses of tropical lotic ecosystems to gradients of anthropogenic disturbance.

2. Material and methods

2.1. Study area

The present study was carried out in the municipality of Aguachica, located in the department of Cesar, Colombia, at coordinates 8.30667° latitude and -73.6153° longitude, at an altitude of 179 meters above sea level. This region has a mean annual temperature of 28 °C and an average annual rainfall of 1687.13 mm (Alcaldía de Aguachica, 2024). The study focused on a 546.63-meter-long section of the Gallinazo stream, located downstream from the nearest urban settlement, approximately 260 meters away (Fig. 1A). The area has two types of vegetation cover: weedy grasslands (27.29%) and secondary vegetation (72.71%), as well as hydrological soil groups, sandy soils (15.1%), moderately coarse-to-fine textures (56.6%), and moderately fine textures (28.3%) (Saldaña-Escorcia et al., 2022). This area was selected because it receives point source discharges of domestic wastewater, as well as diffuse

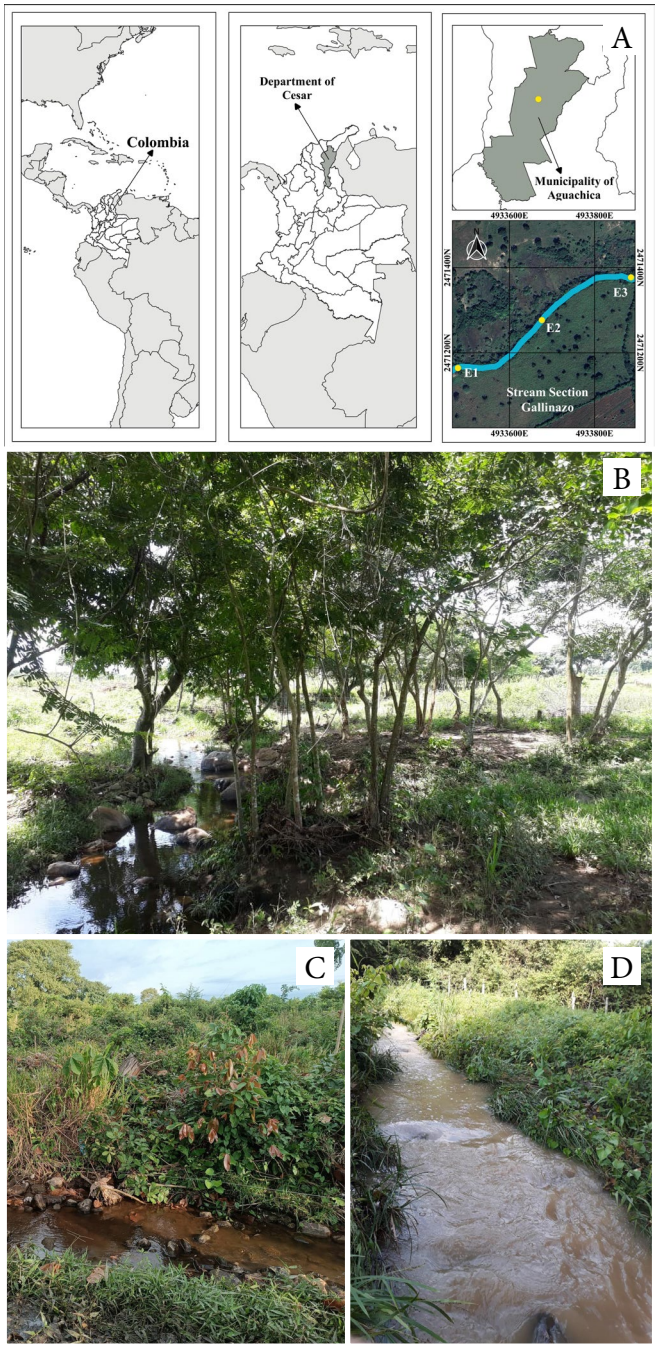

Fig.1. A. Location of the Gallinazo stream section and sampling sites. **B-D** Display a representative image of each sampled site type.

inputs from agricultural activities (Saldaña-Escorcia et al., 2024). Likewise, this section of the stream receives water inputs from a marshy wetland on the left bank, which is directly influenced by livestock farming, factors that are relevant for evaluating changes in water quality and the structure of functional trophic groups.

2.2. Sampling design

Sampling was performed during two distinct climatic periods, with one sampling campaign conducted in each: the rainy season in May and the dry season in July 2024. Three sampling stations were established along the selected stretch in order to capture spatial variability associated with the disturbance gradient. The

first station (E1, Fig. 1B) is at an altitude of 160.7 m; the second (E2, Fig. 1C) is at an altitude of 152.6 m; and the third station (E3, Fig. 1D) is at an altitude of 149.8 m. At E1, the riparian vegetation is moderately preserved, dominated by medium and large trees that provide shade to the stream section, with low human activity. E2 shows high human intervention, with the presence of livestock (cattle, horses, and buffalo) as well as corn crops (Saldaña-Escorcia et al., 2024). The riparian vegetation at this station consists mainly of shrubs, due to human-induced deforestation. At the last station, medium and low-height trees predominate, forming a low canopy over the stream section; this station has high anthropic activity due to its proximity to farm properties. The coordinates of the sampling sites, as well as some environmental and structural characteristics, are presented in Table 1.

2.3. Physical and chemical parameters

A range of physical and chemical parameters (each measured in triplicate) was assessed in situ using specialized field equipment. Temperature (°C) and dissolved oxygen (DO) levels were measured using the HI 98193 probe, while electrical conductivity (μS/cm) was determined using the HI 9835 probe. The pH was recorded using the HI 98191 field meter. For ex situ analysis, water samples were collected in 1000 mL polyethylene bottles and stored at approximately ± 4 °C until further laboratory analysis, following the guidelines of the IDEAM (2020), manual for the collection and preservation of water and sediment samples for water quality monitoring, using standard methods established for the analysis of drinking water and wastewater, according to the recommendations of APHA, AWWA and WEF (Ann and Franson, 1992). In this context, the standard method 2130 B was used to measure turbidity (NTU), the SM 4500-PE method–for the determination of phosphates (mg PO_4^{-3}/L), and the SM 4500-NO_3 B method–to quantify nitrates (mg N-NO_3^-/L). Moreover, the SM 2540 D method was applied to measure total suspended solids (mg/L), while chemical oxygen demand (COD) was determined by the SM 5220 method. Finally, the five-day biochemical oxygen demand (BOD_5) was assessed by the SM 5219 standard method.

2.4. Sampling and identification of macroinvertebrates

Macroinvertebrates were collected using a Surber sampler (500 μm mesh) for stony substrates and a D-frame net (500 μm mesh) for marginal vegetation. The D-net was used to sweep against the current for five minutes at each station, repeated ten times per site (Mena-Rivera et al., 2018; Vargas-Tierras et al., 2023). Samples were transferred to plastic trays, then to sterile, airtight bags, and preserved in 96% ethanol. In the Water Laboratory at Universidad Popular del Cesar, Aguachica campus, organisms were sorted, counted, and identified under a stereoscope. Taxonomic identification was carried out to the genus level using standard keys for Neotropical macroinvertebrates developed by Bejarano (2006), De Carvalho et al. (2005), Domínguez and Fernández (2010), Heckman (2006), Neiss et al. (2018), Passos et al. (2018), Roldán (1988) and Ronderos et al. (2018). Identification to family was deemed sufficient based on literature suggesting that family-level identification provides robust data for water quality assessments (Chará-Serna et al., 2015; Goncharov et al., 2020; Hotor and Kwaku Atsu, 2021; Mendoza-Ramírez et al., 2022), especially in regions lacking species-level taxonomic keys (Cabrera et al., 2021).

2.5. Assignment of traits according to feeding habits

Functional traits were assigned according to Ramírez and Gutiérrez-Fonseca (2014), Damanik-Ambarita et al. (2016), Wehrtmann et al. (2019), and Yang et al. (2020). Taxa were grouped into functional feeding groups following Ramírez and Gutiérrez-Fonseca (2014) and Coccia et al. (2022); these being Pr = Predators, CG = Collector-collectors, CG-Ft = Collector-filtrators, Collector-detritivores (CG-Hb), Sh = Shredders, and SC = Scrapers. Some families were assigned to multiple FFGs to reflect feeding versatility (Cabrera-García et al., 2023). Functional group assignment was not possible for four taxa (Thaumasia, Heleobia, Aropyrgus, and Chlamydotheca) due to limited ecological information.

Table 1. Environmental and structural characteristics recorded at the sampling sites.

Variable	E1	E2	E3
Coordinates (Lat, Long)	8°17′59.60″N – 73°36′19.30″O	8°17′56.10″N – 73°36′26.40″O	8°17′52.10″N – 73°36′33.50″O
Altitude (m above sea level)	160.7	152.6	149.8
Flow velocity (m/s)	0.18 ± 0.06	0.22 ± 0.01	0.10 ± 0.05
Average depth (cm)	21 ± 0.01	36 ± 0.05	51 ± 0.03
Channel width (m)	1.8 ± 0.5	2.4 ± 1.2	3.1 ± 0.3
Structural substrate (available habitat)	Little leaf litter and exposed roots	Abundant leaf litter, fragments of thin branches, and exposed roots	Scattered leaf litter and exposed roots
Presence of logs or submerged wood	Low	Moderate	Moderate

2.6. Indicators of stream ecosystem attributes

FFG-based indices were used to characterize ecosystem attributes following criteria established by Cummins et al. (2005) and Yaagoubi et al. (2023), as presented in Table 2.

2.7. Data treatment

For the analysis of the physical and chemical data, descriptive statistics were used to calculate the mean and standard deviations (SD ± mean) for each parameter and at each sampling station. Additionally, a principal component analysis (PCA) under the Bray-Curtis index was performed to identify distribution patterns and key variables influencing the results. Hierarchical clustering was performed based on Euclidean distance complemented with the Simproff test ($p < 0.05$), and differences between stations, as well as between sampling periods, were evaluated using a permuted multivariate analysis of variance (PERMANOVA) with 9999 permutations, using Euclidean distance for environmental variables; Rv4 software was used. For macroinvertebrate structure, genus richness was estimated, and graphical analyses were performed to determine the relative abundance and percentage proportion of biomass by taxon and period, taking into account the classification of functional groups.

3. Results

3.1. Physical and chemical parameters

Analysis of the water's physical and chemical parameters revealed notable seasonal variations, indicating shifts in environmental conditions between the rainy and dry seasons (Table 3). Dissolved oxygen (DO) ranged from 3.3 to 6.08 mg/L, with a mean of 5.07 ± 1.03 mg/L. The lowest value was recorded during the dry season (station E1M2), falling below the seasonal average, while all rainy season measurements exceeded this average.

Turbidity ranged from 6 to 10.5 NTU, with a mean of 8.56 ± 1.75 NTU. The lowest turbidity was observed at station E2M2 during the dry season, and the same station also recorded the minimum in the rainy season. The maximum value occurred at station E1M1. A coefficient of variation of 20.14% indicated moderate variability. A negative correlation was observed between turbidity and DO (Fig. 2), suggesting that higher turbidity can reduce sunlight penetration, impair aquatic photosynthesis, and reduce oxygen production. Turbidity may also signal the presence of organic pollutants consuming oxygen during decomposition.

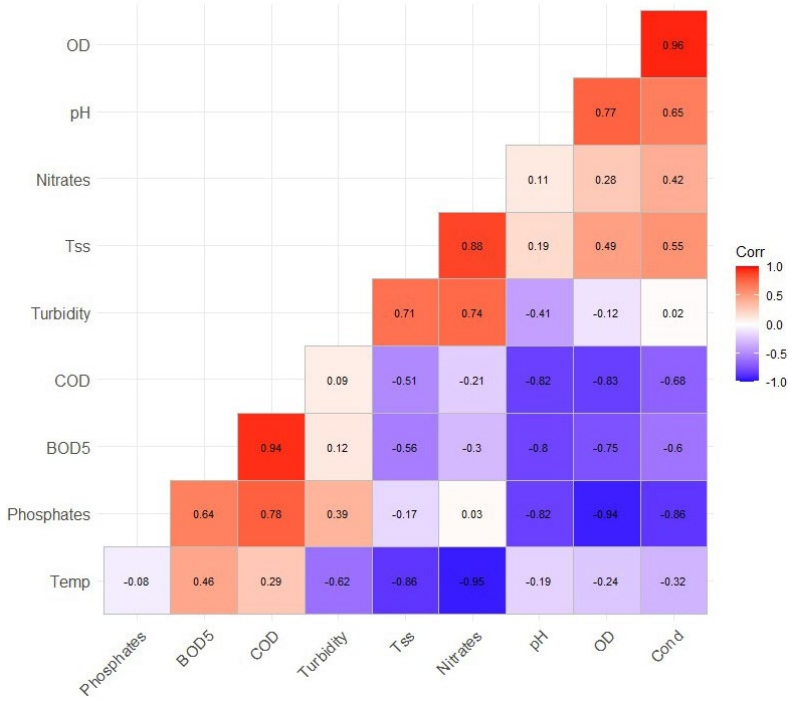

Fig.2. Correlation analysis based on chemical and physical parameters.

Table 2. Functional feeding group ratios as indicators of stream ecosystem attributes.

Attribute	Symbols	Equation	General criteria for ratio levels
Autotrophy to heterotrophy ratio	P/R	$P/R = \frac{Sc}{Sh + CG + CGHb}$	Autotrophic > 0.75
Predator to prey ratio	P/P	$P/P = \frac{Pr}{Total - Pr}$	< 0.15 indicates normal predator/prey ratio
Ratio of coarse particulate organic matter (CPOM) to fine particulate organic matter (FPOM)	Intellectual Property Management Committee (CPOM) and the Intellectual Property Management Committee (FPOM)	$CPOM/FROM = \frac{Sh}{CG + CGFt + CGHb}$	Normal association of shredders linked to riparian zone functioning > 0.25
FPOM in transport (suspended) to FPOM storage in sediment	TFPOM/BFPOM	$TFPOM/BFPOM = \frac{CGFt}{CG}$	Transport of FPOM (in suspension) enriched with (unusual) particle load > 0.50
Substrate stability	Substrate stability	$Est = \frac{Sc + CGFt}{Sh + CG}$	Abundant stable substrates > 0.50

Table 3. Chemical and physical parameters.

Station	Period	Turb	OD	T °C	pH	Cond	BOD_5	COD	TSS	Nitrates	Phosphates
E1	Wet	10.5	5.32	29	7.2	249	2.00	32	22	31.24	0.53
	Dry	9.26	3.3	29.9	7.11	126.3	2.00	37	14	21.12	0.9
E2	Wet	6.94	6.07	29.4	7.6	252	1.00	10	17	23.76	0.26
	Dry	6.00	4.95	33.5	7.24	201	2.00	31	6.0	8.36	0.42
E3	Wet	9.67	6.08	29.6	7.35	248.5	1.00	6	25	24.2	0.35
	Dry	9.04	4.75	32	7.3	195.3	2.00	24	12	17.16	0.48

Water temperature varied from 29 to 33.5 °C (mean = 30.56 ± 1.78 °C), with higher readings at E2M2 and E3M2. E1M1 recorded the lowest temperature. The coefficient of variation was low (5.82%), indicating relative temperature stability. A moderate negative correlation (-0.62) was found between temperature and turbidity, possibly due to temperature-dependent changes in solubility and particle dispersion. However, this relationship is not very strong, so other factors could influence turbidity.

The pH levels ranged from 7.11 to 7.6 (mean = 7.3 ± 0.17). Slight variation was observed, with a low coefficient of variation (2.31%). Electrical conductivity varied significantly, ranging from 126.3 to 252 μS/cm (mean = 212 ± 49.08 μS/cm), with the lowest value at E1M1 and the highest at E2M1. A moderate positive correlation between pH and conductivity suggests a link to dissolved ion concentrations, such as bicarbonates.

Chemical oxygen demand (COD) ranged from 6 to 37 mg/L (mean = 23.33 ± 12.64), with the lowest and highest values at E3M1 and E1M2, respectively. BOD_5 averaged 1.66 ± 0.52 mg/L, indicating relatively low biodegradable organic matter. COD and BOD_5 showed high variability (CV = 54.18% and 30.98%, respectively) and a strong positive correlation (0.93), reflecting a shared response to organic content in water. Both COD and BOD_5 were negatively correlated with pH (-0.82 and -0.80, respectively), indicating that acidic conditions may elevate oxygen demand due to decreased microbial activity and enhanced resistance of organic matter to degradation.

TSS ranged from 6 to 25 mg/L with an average of 16 ± 6.89 mg/L, a relatively low value, suggesting a potentially low amount of suspended particles in the water. Moreover, the coefficient of variation was 43.12%, indicating significant variability in the number of suspended solids. Similarly, nitrates ranged from 8.36 to 31.24 mg/L, with an average of 20.97 mg/L, implying a moderate concentration of nitrates; the minimum value was at E2M2, while E1M1 showed the maximum value. Additionally, the standard deviation was ±7.710 mg/L, reflecting considerable variability in nitrate levels.

A strong positive correlation (0.74) was observed between TSS and nitrates, suggesting that the presence of organic or inorganic material retains or transports nitrates in the water, which may be indicative of contamination from agricultural or industrial sources. This parameter also showed a strong negative correlation with temperature (-0.95), indicating that temperature affects certain biochemical processes that facilitate the conversion of nitrates to more complex forms or their uptake by aquatic organisms. However, the inverse relationship may also reflect seasonality or variation in the source of nitrate. Finally, phosphates averaged 0.49 mg/L, a relatively low value, indicating a low concentration of phosphates in the water. However, the CV was 45.37%, demonstrating significant variability in the number of phosphates in the water. The ratio of phosphate to nitrate was low (0.03), implying that these two compounds are not strongly correlated in the water samples. Although both are important nutrients that contribute to the eutrophication of water bodies, their behaviour in water may depend on other factors, such as pollution sources and biological interactions in the aquatic ecosystem.

The cluster analysis showed three groups, consisting of stations E1M1 and E1M2, E2M2 and E3M2, as well as E2M1 and E3M1 (Fig. 3A). In the principal component analysis, the first two principal components explain more than 87% of the variability in the data (Fig. 3B), meaning that these components capture most of the underlying structure of the system. Additionally, BOD_5 (0.3756), COD (0.3830), and phosphates (0.3372) have high positive loadings, indicating that they have a major influence on PC1 being relevant in defining the variability in this component; while DO (-0.3952) and Conductivity (-0.3763) have high negative loadings. PERMANOVA revealed no statistically significant differences between stations ($F = 1.0655$; $p = 0.4667$) or between seasons ($F = 3.6966$; $p = 0.1$).

3.2. Biological variables

According to the climatic periods (Fig. 4), the most abundant taxa during the rainy season were *Drepanotrema* sp. (55 individuals) and *Physa* sp. (33 individuals), as they are associated with environments more degraded towards eutrophic conditions. In contrast, during the dry season the most abundant taxa were *Caenis* sp. (261 individuals) and *Tanypodinae* sp. (70 individuals), as they are found in conditions with less sedimentation or greater availability of food in the form of detritus.

The presence of taxa indicative of environmental stress, such as *Tubifex sp.*, *Physa* sp. and *Pomacea* sp., indicates alterations derived from excessive sedimentation or zones rich in organic matter, suggesting eutrophication. Similarly, species such as *Acanthagrion* sp. and *Neurocordulia* sp., commonly associated with

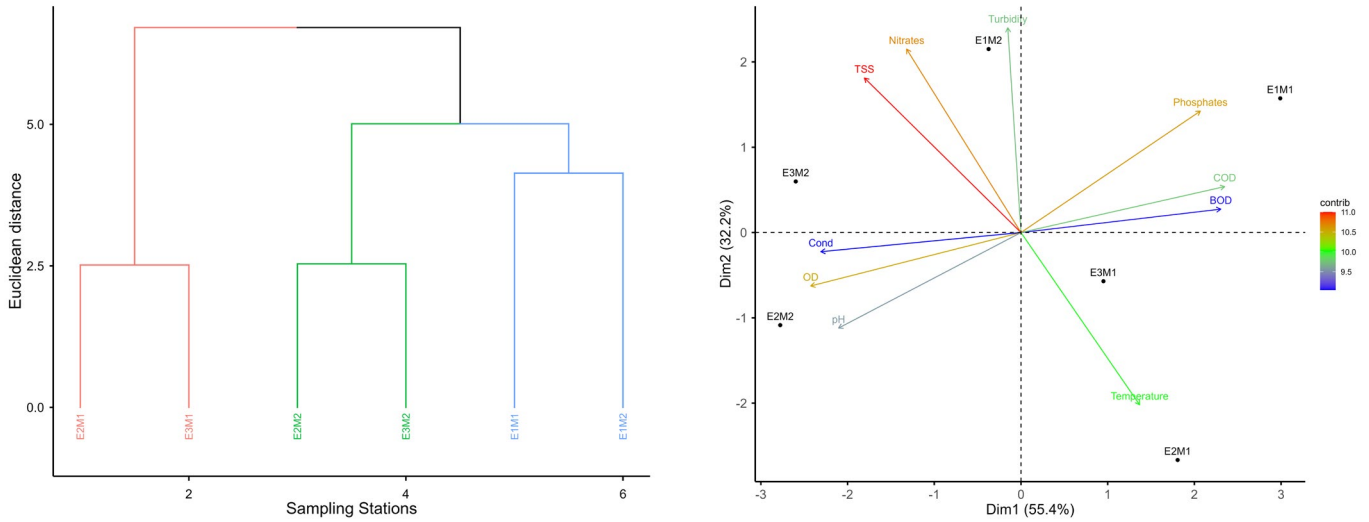

Fig.3. (A) Cluster dendrogram based on chemical and physical parameters of the sampling stations. (B) Principal component analysis (PCA) of the chemical and physical parameters of the sampling sites.

well-oxygenated environments, present a higher biomass in the blue zone, which could suggest better conditions in terms of environmental quality. In contrast, taxa such as *Chironomus* sp. or *Tubifex* sp., which usually thrive in eutrophic or low water quality conditions, have higher biomass in the red zone, which could be an indicator of degradation in certain microhabitats.

In general, among the orders recorded (Fig. 5), Ephemeroptera (297 individuals) was the most abundant, followed by Basommatophora (216 individuals) and Diptera (107 individuals), while Araneae, Trombidiformes and Lepidoptera were the least abundant. However, for the rainy season, the order Basommatophora had the highest abundance with 115 individuals, followed by Odonata with 25 individuals, while for the dry season, the order Ephemeroptera was the most abundant (283 individuals), followed by Basommatophora (101 individuals). Likewise, the orders recorded showed no significant differences in species abundance between sampling stations, except E1M1 and E1M2 ($p = 0.0001$).

The estimated composition and richness of the macroinvertebrate community in the stream section are shown in Table 4. The highest richness occurred in the dry season (46 taxa) compared to the rainy season (28 taxa). This pattern is due to several ecological factors, such as changes in habitat availability, alterations in water conditions, or variations in food resources. Aquatic insects obtained from the three stations were classified as Collector-collectors (n = 18), Collector-filtrators (n = 13), Collector-detritivores (n = 128), Predators (n = 124), Thrashers (n = 32), Scrapers, and Detritivores (n = 228). The difference in taxonomic composition between the epochs suggests that aquatic insect communities are subject to strong seasonal control, where species adapt to or tolerate changing water conditions. Moreover, a value of 0.35 is relatively low, implying that the communities of taxa in rain and drought are quite different, i.e., there is a low overlap between species from both epochs. This pattern may reflect a high specialization of species according to the seasonal conditions of the environment.

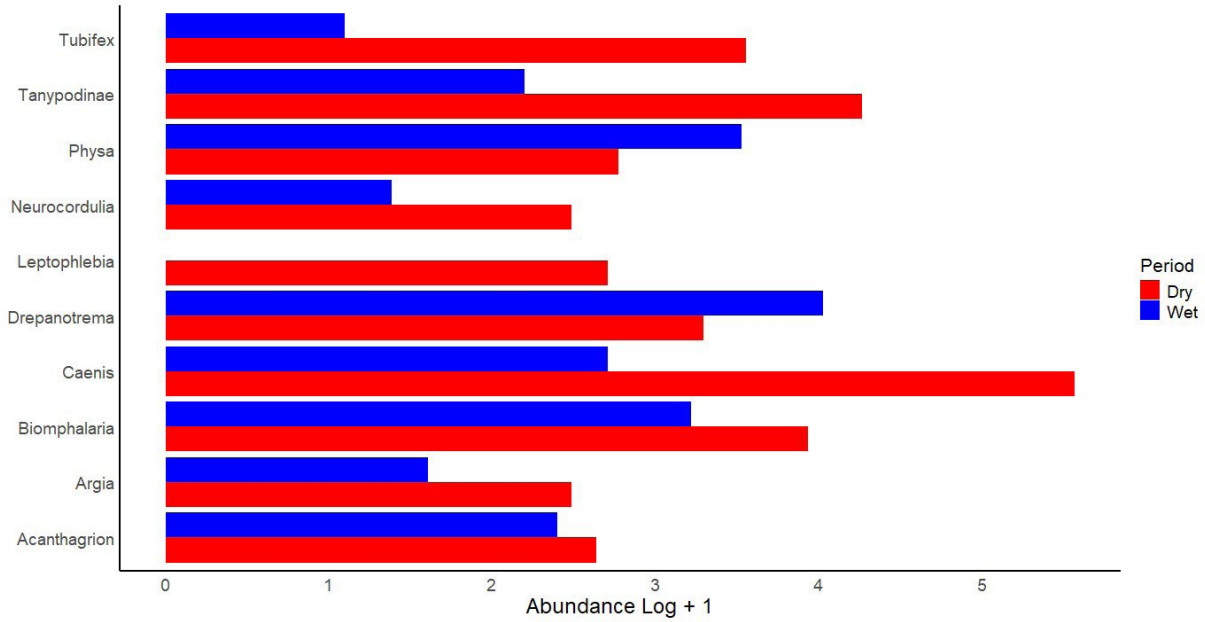

Fig.4. Abundance of taxa (Log + 1) detected in the Gallinazo stream section.

Table 4. Macroinvertebrate community structure with its functional feeding group of the Gallinazo stream section.

Family	Subfamily or genus	Functional Groups		Period	
				Wet	Dry
Dytiscidae	*Dytiscius* sp.	*Predator*	*Pr*	*0*	*1*
	Hydrovatus sp.	*Predator*	*Pr*	*1*	*1*
Scraptiidae	*Tolmetes* sp.	*Predator*	*Pr*	*1*	*0*
Elmidae	*Neocylloepus* sp.	*Collector-collectors*	*CG*	*0*	*1*
	Heterelmis sp.	*Collector-collectors*	*CG*	*1*	*1*
	Noelmis sp.	*Collector-collectors*	*CG*	*1*	*0*
Hydraenidae	*Hydraena* sp.	*Predator*	*Pr*	*0*	*1*
Chrysomelidae	*Bruchia* sp.	*Shredder-Herbivore*	*Sh-Hb*	*0*	*1*
Staphylinidae	*Scaphium* sp.	*Predator*	*Pr*	*0*	*1*
Hydrophylidae	*Derallus* sp.	*Predator*	*Pr*	*1*	*0*
	Hydrochus sp.	*Predator*	*Pr*	*1*	*0*
Staphylinidae	*Codonfomyia* sp.	*Collector-collectors*	*CG*	*0*	*1*
Ephydridae	*Ephydridae* sp.	*Collector-collectors*	*CG*	*0*	*1*
Culicidae	*Aedes* sp.	*Collector-filters*	*CF*	*0*	*1*
Chironomidae	*Tanypodinae* sp.	*Detritivore-collectors*	*CG-Hb*	*1*	*1*
	Chironomus sp.	*Detritivore-collectors*	*CG-Hb*	*0*	*1*
	Ablabesmyia sp.	*Detritivore-collectors*	*CG-Hb*	*0*	*1*
Ceratopogonidae	*Bezzia* sp.	*Predator*	*Pr*	*1*	*1*
Belostomatidae	*Belostoma sp*	*Predator*	*Pr*	*1*	*1*
Pleidae	*Neoplea* sp.	*Predator*	*Pr*	*1*	*1*
Nepidae	*Ranatra* sp.	*Predator*	*Pr*	*1*	*0*
Veliidae	*Rhagovelia* sp.	*Predator* (Perforator-carnivore)	*Pr*	*1*	*0*
Pyralidae	*Pyralidae* sp.	*Shredder-Herbivore*	*Sh-Hb*	*1*	*0*
Leptoceridae	*Nectophyche* sp.	*Shredders*	*Sh*	*0*	*1*
	Oecetis sp.	*Shredders*	*Sh*	*0*	*1*
Leptophlebiidae	*Leptophlebia* sp.	*Collector-collectors*	*CG*	*0*	*1*
Leptohyphidae	*Leptohyphes* sp.	*Collector-collectors*	*CG*	*0*	*1*
	Vacupernius sp.	*Collector-collectors*	*CG*	*0*	*1*
Caenidae	*Caenis* sp.	*Collector-collectors*	*CG*	*1*	*1*
Ephemerelloidae	*Ephemerella* sp.	*Collector-collectors*	*CG*	*0*	*1*
Gomphidae	*Phanogomphus* sp.	*Predator*	*Pr*	*0*	*1*
	Gomphidae sp.	*Predator*	*Pr*	*1*	*0*
Coenagrionidae	*Acanthagrion* sp.	*Predator*	*Pr*	*1*	*1*
	Argia sp.	*Predator*	*Pr*	*1*	*1*
Libellulidae	*Planiplax* sp.	*Predator*	*Pr*	*0*	*1*
	Celithemis sp.	*Predator*	*Pr*	*1*	*0*
	Brachymesia sp.	*Predator*	*Pr*	*0*	*1*
	Brechmorhoga sp.	*Predator*	*Pr*	*1*	*1*
Cordullidae	*Neurocordulia* sp.	*Predator*	*Pr*	*1*	*1*
Macromidae	*Macromia* sp.	*Predator*	*Pr*	*0*	*1*

Family	Subfamily or genus	Functional Groups		Period	
				Wet	Dry
Caloterygidae	*Hetaerina* sp.	*Predator*	*Pr*	*0*	*1*
Trichodactylidae	*Valdivia* sp.	*Shredders*	*Sh*	*0*	*1*
	Trichodactylus sp.	*Shredders*	*Sh*	*1*	*1*
Hydrachnidae	*Hydrachna* sp.	*Predator*	*Pr*	*0*	*1*
	Arrenuridae sp.	*Predator* (Perforator-carnivore)	*Pr*	*0*	*1*
Ancylidae	*Ferrissia* sp.	*Scrapers*	*Sc*	*0*	*1*
Physidae	*Physa* sp.	*Scrapers*	*Sc*	*1*	*1*
Planorbidae	*Biomphalaria* sp.	*Scrapers*	*Sc*	*1*	*1*
	Drepanotrema sp.	*Scrapers*	*Sc*	*1*	*1*
	Helisoma sp.	*Scrapers*	*Sc*	*1*	*1*
Ampullariidae	*Pomacea* sp.	*Scrapers*	*Sc*	*1*	*1*
Sphaeriidae	*Eupera* sp.	*Collector-filters*	*CF*	*0*	*1*
Lumbriculidae	*Lumbriculus* sp.	*Collector-detritivores*	*CG-Hb*	*0*	*1*
Tubificidae	*Tubifex* sp.	*Collector-detritivores*	*CG-Hb*	*1*	*1*
Naididae	*Stylaria* sp.	*Collector-collectors*	*CG*	*0*	*1*
	Naididae sp.	*Collector-collectors*	*CG*	*1*	*0*
Total				*28*	*46*
Jaccard Similarity Index (similarity coefficient)				*0.35*	

As shown in Table 5, ecosystem attribute indicators revealed consistent heterotrophic conditions ($P/R < 0.75$) across all stations, except E2M1 ($P/R = 0.95$). The predator/prey ratio reflected values above 0.15 (high trophic pressure), excluding E2 and E1 during M2 (indicating a normal predator/prey ratio). Similarly, low CPOM/FPOM values (< 0.25 at all stations) reflect a weak contribution of shredders, due to the loss or degradation of riparian vegetation. This coincides with visual observations that showed a reduced strip of riparian vegetation (trees, shrubs, and leaf litter), with eroded banks, sparse herbaceous cover, and no accumulated leaf litter on the shore, which reduces the available substrate. Furthermore, TFPOM/BFPOM is very low at all stations, indicating a predominance of benthic storage rather than transport of organic particles. Finally, E2M1 is the only station that showed channel stability ($0.90 > 0.50$).

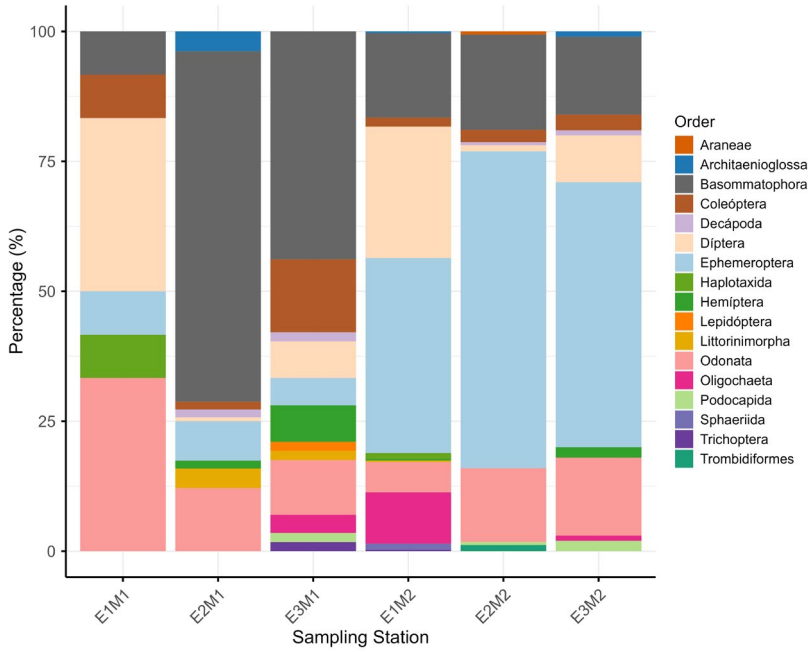

Fig.5. Percentage of aquatic macroinvertebrate orders by sampling stations in the Gallinazo stream section.

Table 5. Aquatic ecosystem attributes of the Gallinazo stream section.

Attribute	E1M1	E2M1	E3M1	E1M2	E2M2	E3M2
P/R	0.10	0.95	0.30	0.25	0.19	0.24
P/P	0.42	0.15	0.33	0.12	0.22	0.25
CPOM/FPOM	0.00	0.00	0.11	0.01	0.04	0.08
TFPOM/BFPOM	0.00	0.00	0.00	0.03	0.00	0.00
Substrate stability	0.17	0.90	0.42	0.44	0.00	0.31

4. Discussion

While some physical and chemical parameters such as turbidity, BOD_5, and COD are in acceptable ranges (Ortiz et al., 2024; Saldaña-Escorcia et al., 2024), low DO concentration may be a consequence of organic decomposition processes in combination with relatively low temperature conditions, which favour oxygen solubility but, in turn, demonstrate excessive organic input or accelerated consumption by microfauna in the water (Nuñez and Fragoso-Castilla, 2019; Kengne Fotsing et al., 2022). Likewise, high nutrient levels represent critical challenges, as these high levels can promote eutrophication processes, which in the long term could lead to algal blooms and episodes of hypoxia (Li and Dudgeon, 2008; Aguiar et al., 2017; Cortés-Guzmán et al., 2021), negatively affecting the biodiversity of the aquatic ecosystem.

Quantitative analyses of richness and abundance revealed greater diversity, more equal distribution, and less dominance in E1 and E3, while E2 showed lower relative richness. Likewise, the presence of taxonomic groups such as Basommatophora, Architaenioglossa, Coleoptera, and Diptera, which showed moderate to high diversity and an even distribution of individuals, demonstrated an ecosystem with good resilience and functionality (Zhao et al., 2017; Addo-Bediako, 2021; Cortés-Guzmán et al., 2021; Doong et al., 2021). In contrast, other taxonomic groups such as Trombidiformes, Araneae, Sphaeriida and Hemiptera showed low diversity and high dominance, reflecting stressful conditions or alterations in water quality in this section of the stream (Rizo-Patrón et al., 2013; Pallottini et al., 2017; Zaghloul et al., 2020; Cortés-Guzmán et al., 2021; Coccia et al., 2022; Fentaw et al., 2024).

The analysis of the functional groups of macroinvertebrates highlights a clear structural and functional response of the ecosystem to the change in hydrological conditions, since for the dry season characterized by a reduction in the flow velocity of the riverbed and accumulation of organic matter, there is a partial functional simplification, with a significant predominance of the Collector-detritivores (CG-Hb) and Collector-filters (CG-Ft) (Abdul and Rawi, 2019). In the rain, on the contrary, there are better oxygenation and water renewal conditions, as well as greater habitat heterogeneity; which promotes greater functional diversity, especially of sensitive groups such as scrapers, shredders and certain collectors (Motta Díaz et al., 2016).

The balance between autotrophy and heterotrophy is one of the most important attributes in the analysis of aquatic ecosystems (Cummins et al., 2005; Addo-Bediako, 2021). The P/R ratio reflected a preference for heterotrophy, excluding E2 during M1 ($P/R = 0.95$), and indicated optimal DO levels for aquatic life (6.07 mg/L) (Yaagoubi et al., 2023). Moreover, the high P/P ratio (< 0.15) found in this study showed signs of high trophic pressure in the stream section, excluding E2 and E1 during M2 with a normal predator/prey ratio. The low abundance of shredders relative to foragers observed in most research suggests a weak CPOM contribution to the system (Cummins et al., 2005; Yaagoubi et al., 2023). Because of the scarcity of leaf litter (Eyes et al., 2012; Aguiar et al., 2017), they are already non-functional riparian zones.

5. Conclusions

The present study allowed us to explore the structural and functional characteristics of aquatic macroinvertebrates and to indicate the relevance of the functional food group method for assessing the general conditions of the Gallinazo stream section at any climatic time. The data obtained and its analysis allowed us to identify signs of both physicochemical and ecological pressure, with significant spatial and temporal variability. This research, in turn, contributes key information for ecological monitoring processes, water quality assessment, and the design of conservation strategies, especially in areas where climate variability, water scarcity, lack of information, and public policies significantly affect the integrity of aquatic ecosystems.

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Conflict of Interest

The authors declare no conflicts of interest

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Bioactivities of freshwater sponges and their associated bacteria: a review

Original Article

LIMNOLOGY FRESHWATER BIOLOGY

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ABSTRACT. This review aims to provide an overview of the biological activities of freshwater sponges and their microbes associated with them. Freshwater sponges, despite their ecological significance and potential as reservoirs of bioactive compounds, remain underexplored compared to their marine counterparts. This review highlights the bioactivities of eight freshwater sponge extracts, among them being *Ochridaspongia rotunda* (Arndt,1937), *Ephydatia fluviatilis* (Linnaeus,1759), *Oncosclera asiatica* (Manconi & Ruengsawan,2012), *Metania reticulata* (Bowerbank,1863), *Drulia browni* (Bowerbank,1863), *Drulia cristata* (Weltner,1895), *Drulia uruguayensis* (Bonetto & Ezcurra de Drago,1969), and *Eunapius carteri* (Bowerbank,1863).Various extracts, including those obtained using methanol, acetone, aqueous, ethyl acetate, and methylene chloride, were employed in previous studies to evaluate a range of bioactivities. Observed bioactivities include antibacterial, antifungal, anti-quorum sensing, antiplasmodial, acetylcholinesterase inhibition, and anticancer properties, which are determined either from the crude extracts of the sponges themselves or by their associated microbes.

Keywords: freshwater sponges, biological activities, bioprospecting, microbial symbionts, anticancer activity, antiplasmodial activity

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1. Introduction

The urgent need to find new drugs to combat existing resistant strains is driven by the increasing prevalence of antibiotic-resistant bacteria, which significantly limits the effectiveness of current treatments. This resistance leads to higher medical costs, prolonged hospital stays, and increased mortality rates, emphasizing the critical necessity for novel antibiotics and alternative therapies to address this global health threat. The development of anti-cancer drugs is crucial due to the limitations and drawbacks of current treatments, such as severe side effects, resistance to chemotherapy, and the inability to effectively target all cancer types.

Extensive research has been conducted to identify effective bioactive molecules from natural sources. Marine sponges have particularly emerged as a prolific source of such compounds (Anbarasu, 2024). However, studies of freshwater sponges remain limited due to various challenges and constraints. Freshwater sponges, belonging to the phylum Porifera, are classified into 45 genera across six families, encompassing a total of 219 species that represent a largely untapped resource of bioactive compounds with significant potential in pharmaceutical and biotechnological applications (Manconi and Pronzato, 2008). They are less diverse compared to their marine counterparts, reducing the likelihood of discovering unique bioactive compounds. These searches for new drugs from freshwater sponges are more challenging.

Sponges are abundant in bioactive molecules due to their high exposure to biotic and abiotic stress. This environmental pressure forces them to develop a wide array of chemical defenses to survive. The need to deter predators has driven sponges to produce various bioactive compounds, ensuring their survival by making

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them less palatable or even toxic to potential threats (Thoms et al., 2007). The symbiotic lifestyle of sponges with numerous microorganisms leads to the production of unique bioactive molecules, resulting from complex interactions and mutualistic relationships with their microbial partners (Taylor et al., 2007). Their filter-feeding behavior increases sponge exposure to hazardous particles, prompting the evolution of a diverse arsenal of bioactive compounds to neutralize potential threats and maintain their health (Webster and Thomas, 2016).

2. Reported bioactivities of freshwater sponges

Freshwater sponges were identified as potential sources of bioactive compounds, exhibiting a range of pharmacological and ecological activities. These bioactivities include antimicrobial, antiquorum sensing, cytotoxic, mutagenic, antiplasmodial, and ecological defense mechanisms such as immobility induction and antipredation effects. Table 1 summarizes these bioactivities from different freshwater sponges. The following subsections provide an overview of these activities, highlighting their significance and potential applications.

2.1. Antimicrobial activity

1. The freshwater sponge, *Ephydatia fluviatilis*, from Vinkeveense Plassen lake in the Netherlands was found to harbor Pseudomonas species. In the study, 90 isolates of Pseudomonas were screened for biofilm production, antimicrobial activity, and protozoan grazing resistance. The *E. fluviatilis* sponge revealed 15 fingerprint clusters (BOX I–XV), containing 2 to 17 isolates after BOX-PCR genotyping. Each cluster was checked against *R. solani*, *F. moniliforme*, *P. ultimum*, or *B. subtilis*. Box 1 represents the *P. protegens*-like isolates, while

Table 1. Summary of reported bioactivities of freshwater sponges, their associated species, mechanisms, and references

Bioactivity	Freshwater sponge	Properties/mechanism	Microbes associated with sponge activity	References
Antimicrobial	****Ephydatia fluviatilis*** (Linnaeus,1759)	1. The sponges harbor *Pseudomonas* species that exhibit antibacterial, antifungal, and anti-oomycetal activities. 2. In-vitro antagonistic activity was observed against *Rhizoctonia solani, Fusarium moniliforme*, *Pythium ultimum*, and *Bacillus subtilis* in 68 out of 90 Pseudomonas isolates. 3. A positive relationship exists between biofilm production capacity and the detection of antagonistic activities in these species.	*Pseudomonas* species 1. *P. protegens* as isolates 2. *P. jessenii* as isolates 3. *P. oryzihabitans* as isolates	(Keller-Costa et al., 2014)
	Ochridaspongia rotunda (Arndt,1937)	1. Five crude extracts showed antimicrobial activity against eight bacterial and eight fungal strains. 2. The methanolic extract exhibited the strongest antibacterial activity with MIC values of 7.5–15 μg/mL and MBC values of 15–30 μg/mL. 3. The acetone extract exhibited maximum antifungal activity with MIC of 7.5–45 μg/mL and MFC of 15–60 μg/mL.		(Pejin et al., 2014b)
	****Oncosclera asiatica*** (Manconi & Ruengsawan,2012)	1. The sponge symbiont bacterium, *Pseudomonas moraviensis*, exhibited antibacterial activity. 2. It was effective against *Escherichia coli* (*E. Coli*) and *Staphylococcus aureus* (*S. Aureus*).	*Pseudomonas moraviensis*	(Wulandari et al., 2023)
	****Metania reticulata*** (Bowerbank,1863)	1. Two bacterial strains, MERETb.761 and MERETb.762, and one fungal strain, MERETf.010, were associated with sponge antimicrobial activity. 2. Both bacterial strains inhibited *Aspergillus* sp. 3. Fractions from fungus MERETb.762 and MERETf.010 inhibited *S. Aureus* 4. HPLC fractions of MERETb.762 inhibited the release of beta-hexosaminidase in the RBL-2H3 degranulation assay.	MERETb.761 (Genbank accession number KF305316) MERETb.762 (Genbank accession number KF305317) MERETf.010 (Genbank accession number KF305318)	(Rozas et al., 2016)

Bioactivity	Freshwater sponge	Properties/mechanism	Microbes associated with sponge activity	References
Immobility and anti-predation (anti protozoan)	****Ephydatia fluviatilis*** (Linnaeus,1759)	The sponge harbors Pseudomonas spp., which shows the immotility effect on the *C. steinii* ciliate. 1. Between 5 and 15 minutes, cells became immotile. After 2 hours, cell lysis of *C. steinii* was initiated. After 24 hours, nearly all *C. steinii* were a broken structure. 2. 35% of isolates resisted to the *C. steinii* ciliate predation.	*Pseudomonas* species 1. *P. oryzihabitans* as isolates 2. *P. protegens* as isolates	(Keller-Costa et al., 2014)
Anti-quorum sensing activity	***Ochridaspongia rotunda*** (Arndt,1937)	1. The methanolic extract of the sponge showed more pyocyanin inhibitory activity than its acetone extract. 2. The acetone extract reduced more biofilm production than the methanolic extract towards *P. aeruginosa*. 3. Both methanolic and acetone extracts reduced the flagellar motility and twitching ability of *P. aeruginosa*.		(Pejin et al., 2014a)
Acetylcholinesterase inhibitory activity	***Ochridaspongia rotunda*** (Arndt,1937)	The acetone extract of sponge inhibited acetylcholinesterase under both liquid and solid conditions.		(Talevska et al., 2017)
	Drulia browni (Bowerbank,1863)	The methanolic extract inhibited acetylcholinesterase.		(Manço da Costa Bolson et al., 2019)
	Drulia cristata (Weltner,1895)	The methanolic extract inhibited acetylcholinesterase.		(Manço da Costa Bolson et al., 2019)
	Drulia uruguayensis (Bonetto & Ezcurra de Drago,1969)	The methanolic extract strongly inhibited acetylcholinesterase.		(Manço da Costa Bolson et al., 2019)
	Metania reticulata (Bowerbank,1863)	The methanolic extract inhibited acetylcholinesterase.		(Manço da Costa Bolson et al., 2019)
Anti plasmodial activity	***Metania reticulata*** (Bowerbank,1863)	The methanolic extract of the sponge inhibited the *P. falciparum* (FCR3) parasite with IC50 of 2.7 μg/mL.		(Manço da Costa Bolson et al., 2019)
	Drulia browni (Bowerbank,1863)	The methanolic extract of the sponge inhibited the *P. falciparum* (FCR3) parasite with IC50 of 9.9 μg/mL.		(Manço da Costa Bolson et al., 2019)
Cytotoxicity	***Ochridaspongia rotunda*** (Arndt,1937)	1. The methanolic extract exhibited cytotoxicity towards the malignant brain U-251 MG tumor cell line with IC 50 of 1.87 ± 0.09 μg/mL at 96h. 2. The micronucleus test revealed that the genotoxicity of the methanolic extract at the chromosomal level was over 4.5 times lower than that of doxorubicin.		(Pejin et al., 2021)
	****Drulia cristata*** (Weltner,1895)	1. Crude extract showed no cytotoxic activity against the colorectal cell line (HCT-116). 2. Ethyl acetate extracts of bacterial strains DTR1 and DTR2 isolated from sponges exhibited the MTT assay with >50% inhibition.	DTR1 and DTR2 bacterial strains	(da Costa et al., 2019)

Bioactivity	Freshwater sponge	Properties/mechanism	Microbes associated with sponge activity	References
Free radical generation	**Eunapius carteri* (Bowerbank,1863)	1. Some fractions (F4) of the sponge isolated by density gradient centrifugation and flow cytometry generated a significant amount of superoxide anions than other fractions (F1, F2, and F3). 2. Fractions P2 and P3 generated nitric oxide ions.		(Mukherjee et al., 2015)

Note: * Indicates crude extract of the sponge used.

** Indicates extracts of bacterial/fungal isolates from the sponge used.

Box 6 represents the *P. jessenii*-like isolate species of Pseudomonas. Of the seven isolates antagonistic to *F. moniliforme*, six belonged to BOX PCR cluster VI (P. jessenii), and all *P. protegens*-like isolates showed strong inhibition towards *B. subtilis*. *P. umsongensis* type isolates, biofilm formers, showed moderate antagonism and resistance against *B. subtilis*. *P. oryzihabitans* showed antagonistic activity towards *B. subtilis*. Biofilm production was observed in 58% of the isolates, dominated by mucilaginous biofilms. Antagonism against phytopathogenic fungi (*Rhizoctonia solani*, *Fusarium moniliforme*, and *Pythium ultimum*) and the *Bacillus subtilis* bacterium was observed in 75% of isolates, with maximum inhibition against *P. ultimum* and *B. subtilis*. Additionally, 35% of the isolates were also resistant to predation by the *Colpoda steinii* protozoan. A principal component analysis (PCA) revealed a co-variation between biofilm production and antagonism, with *Pseudomonas* protegens-like isolates demonstrating high antagonism, particularly against *B. subtilis* and *C. steinii*. (Keller-Costa et al., 2014).

2. Five *O. rotunda* crude extracts were screened for their antibacterial and antifungal activities against eight bacterial strains and eight fungal species by the microdilution method. The methanolic extract of *O. rotunda* displayed the strongest antibacterial activity, with a MIC (Minimum Inhibitory Concentration) of 7.5 to 15 μg/mL and an MBC (Minimum Bactericidal Concentration) of 15 to 30 μg/mL, which was greater than that of the positive control antibiotics streptomycin and ampicillin. *Bacillus cereus* was the most susceptible organism, and *Listeria monocytogenes* was the most resistant to the antibacterial activity of the extracts. The acetone extract of *O. rotunda* was the most active antifungal, with MIC of 7.5 to 45 μg/mL and MFC of 15 to 60 μg/ml. *Trichoderma viride* was the most sensitive fungus, whereas *Candida albicans* was the most resistant to the antifungal action of the extracts (Pejin et al., 2014b).

3. Seventeen bacterial isolates were recovered from *Oncosclera asiatica* from Kali Porong, East Java. Paper disc inhibition assay exhibited isolate 2 with effective antibacterial against *E. coli* and isolate 14–against *S. aureus*. Sequence relationships revealed that isolate 2 had the highest relationship with *Pseudomonas moraviensis* strain 3N. Isolate 2 also had the NRPS gene that encodes antibacterial compounds (Wulandari et al., 2023).

4. Sometimes, symbionts together with sponges produce immunomodulators to interact with the host immune system. Such studies were performed on bacterial and fungal strains that were cultured from the freshwater sponge, *Metania reticulata*, from the Negro River of the Amazon central basin region. Two bacillus strains, MERETb.761(Genbank accession number KF305316) and MERETb.762 (Genbank accession number KF305317), and one fungal strain MERETf.010 (Genbank accession number KF305318) were selected of these MERETb.762 and MERETf.010 exhibited potent antibacterial activity against Staphylococcus aureus, while both bacillus strains inhibited *Aspergillus sp*. Two of its extracts from MERETb.762, containing nitroaromatic compounds, exhibited inhibition to degranulation of RBL-2H3 cells (Rozas et al., 2016).

2.2. Anticancer activity

1. The methanolic extract of *O. rotunda* sponge collected from Lake Ohrid was evaluated for its anticancer activity against brain tumor cell lines (Neuro-2A, U- 251MG, and U-87 MG). MTT assay revealed that the extract was most effective against U-251 MG (IC50 1.87 ± 0.09 μg/mL) and was five times more selective to the U-251 MG than the U-87 MG cell line. At the same time, it was less cytotoxic to normal cells when doxorubicin was used as a control. For assessing its genotoxicity, the micronucleus test was performed, which indicated that the methanolic extract lacks genotoxicity at the chromosomal level (Pejin et al., 2021).

2. The freshwater sponge, *Drulia cristata*, collected from Maracana beach, on the banks of the Tapajos River, the State of Para, was evaluated for its cytotoxic activity. Bacterial strains associated with the sponge were cultured and named DTR-1 and DTR-2. The ethyl acetate extracts of both strains were assessed with the MTT assay against the HCT-116 cell line. Crude extract of *Drulia* did not exhibit any cytotoxic activity, while extracts of bacterial strains showed moderate cytotoxic activity. DTR2 strains showed more potent cytotoxic activity than DTR1 (da Costa et al., 2019).

2.3. Acetylcholinesterase inhibitor activity

1. The Ellman method showed that *O. rotunda* acetone extract in liquid exhibited potent acetylcholinesterase activity with an IC_{50} of 23.07 μg/mL. The Marston method revealed stronger activity in solid form with an IC_{50} of 1.5 μg/mL. (Talevska et al., 2017)
2. *Metania reticulata*, *Drulia uruguayensis*, *Drulia browni*, and *Drulia cristata* collected from the Amazonian water were assessed for their acetylcholinesterase inhibitor activity by the Ellman method. Methanolic extracts of all sponges inhibited the acetylcholinesterase enzyme (AChE from electric eel), while none of them inhibited the butyrylcholinesterase enzyme (BChE from Equine serum). *Drulia uruguavensis* showed the most potent acetylcholinesterase inhibitory activity with 52 percent of enzyme inhibition. The percent inhibition was found as follows in increasing order: *Drulia cristata* < *Drulia browni* < *Metania reticulata* < *Drulia uruguayensis*. IC_{50} of *Drulia uruguayensis* was 1.04 ± 0.01 mg/mL (Manço da Costa Bolson et al., 2019).

2.4. Anti-quorum sensing activity

The anti-quorum sensing activities of the freshwater sponge, *Ochridospongia rotunda*, were assessed towards *Pseudomonas aeruginosa* (PA01). Since twitching and flagella motility are critical for the formation of biofilms, and the ability to form a biofilm is a major Quorum Sensing-controlled virulence trait, the ability of an extract to effectively reduce these motilities is considered a mechanism of anti-QS activity. Methanolic and acetone extracts were used to study pyocynin production, biofilm production, and twitching and motility of the bacterium. Pyocynin is essential for up-regulation of QS genes in stationary phase growth of *Pseudomonas*. Both extracts inhibited pyocyanin production by 49.9% and 42.44%, respectively. Methanolic and acetone extracts exhibited anti-biofilm activity by 48.29 and 53.99, respectively, compared to that of ampicillin (30.84). *Pseudomonas* colonies that were grown in the control group had larger diameters than those grown in methanolic and acetone extracts, suggesting that flagellar motility was reduced, and the twitching ability of *Pseudomonas* was compromised (Pejin et al., 2014a).

2.5. Anti-protozoan activity

Pseudomonas sp. were isolated from *Ephydatia fluviatilis* that showed predation resistance and toxicity towards *Colpoda steinii* strain Sp1, which was assessed by microtiter plate assay adapted from predation resistance strains in Jousset et al. They were defined as bacteria with <40 reductions of initial optical density after 22h of exposure to protozoa. These strains were assessed further for toxicity. Dried bacterial extracts were introduced to the *C. steinii* strain. Extracts of *Pseudomonas proteogens* as isolates, *P. jessenii* as isolates SF-2-02 and SF-3-03 (BOX VI), and isolates SG-1-02, SH-1-17, and SH-2-01 demonstrated toxicity towards protozoa. Within 5-15 minutes, all protozoan motility stopped, and after two hours of exposure, protozoans started to lyse. After 24 hours, all protozoan cells became broken (deformed with broken structures) (Keller-Costa et al., 2014).

2.6. Antiplasmodial activity

FCR3, a chloroquine-resistant strain of *P. falciparum*, was used to assess anti-plasmodial activity by extracts of *M. reticulata* and *Drulia browni*. The evaluation of antiplasmodial activity was assessed according to Rabelo et al. Among all extracts, the methanolic extract of *Metania reticulata* showed IC50 of 2.7μg/mL, while *Drulia browni* showed IC50 of 9.9μg/mL (Manço da Costa Bolson et al., 2019).

2.7. Free radical generation

E. carteri cells were separated into four fractions (F1–F4, top to bottom) using a discontinuous Ficoll gradient (5–25%) as outlined by De Sutter and Van de Vyver (1977) and Zhang et al. (2003) for subsequent analyses. Freshly dissociated sponge cells were filtered through a 35 μm nylon mesh and sorted with a BD FACS Aria III flow cytometer. Cell morphotypes were identified based on size (forward scatter, FSC) and granularity (side scatter, SSC) at 140V and 406V, respectively. Dot plots captured 50,000 events per sample, with three fractions (P1, P2, and P3) gated to exclude debris and cell doublets. Intracellular superoxide anion production in fractions from density gradient centrifugation (F1–F4) and flow cytometry (P1–P3) was quantified spectrophotometrically via the Nitroblue tetrazolium (NBT) reduction method. Fraction F4 produced significantly more superoxide anions than fractions F1, F2, and F3. Nitric oxide production was measured as nitrite release using Griess reagent (Green et al., 1982; Aktas et al., 2013), with fractions P2 and P3 showing nitric oxide generation (Mukherjee et al., 2015).

3. Conclusion

This review emphasizes the diverse bioactivities of freshwater sponges and their associated bacteria, underlining their potential as sources of novel therapeutic agents. The antimicrobial, anticancer, and anti-quorum sensing, as well as anti-plasmodial and acetylcholinesterase inhibitory activities, highlight the medicinal value of these organisms.

Notably, *Ephydatia fluviatilis* and *Ochridaspongia rotunda* with *Pseudomonas* species have very good antimicrobial and anti-quorum sensing activities, while others, such as *Metania reticulata* and *Drulia browni*, have good anti-plasmodial and acetylcholinesterase inhibitory activities. *Ochridaspongia rotunda* and *Eunapius carteri* also exhibit good cytotoxic and nitric oxide production activities, which can be used for cancer and inflammatory disease research. Furthermore, mutagenicity and cytotoxicity assays indicate that

some extracts, such as those of *Ochridaspongia rotunda*, have low genotoxic activity, making them promising candidates for drug development research.

Further exploration of the chemical and microbial diversity within freshwater sponges could uncover new bioactive compounds to combat antimicrobial resistance and neurodegenerative diseases, underscoring the importance of conserving freshwater ecosystems for future bioprospecting efforts.

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Conflict of interest

All authors declare no conflict of interests.

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