ISSN 2658-3518

LIMNOLOGY & FRESHWATER BIOLOGY

2025, № 3

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

Availability and Marketing System of Fish and Crustaceans in an Urban Fish Market: A Study on Retail Practices

Original Article

LIMNOLOGY FRESHWATER BIOLOGY

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ABSTRACT. The marketing system and structure significantly impact the socio-economic conditions and production systems of local communities. Hence, this study aimed to explore the diversity of fish and crustacean species and the marketing system in the urban fish market known as Alexander Fish Market in Lakshmipur. During the study, 32 fish species and 3 crustaceans were identified, representing 35 genera, 21 families, and 11 orders. The majority of species belonged to the orders Cypriniformes (family Cyprinidae) and Siluriformes (families Bagridae and Ailiidae). Among the recorded species, a total of 3 species have been reported as Endangered (EN), 3 as Vulnerable (VU), 4 as Not Listed (NL), 6 as Not Threatened (NT), and 19 as Least Concern (LC). The fish distribution chain involved intermediaries such as beparies, aratders, wholesalers, and retailers. Three types of marketing channels were identified in the study. The average marketing cost, income, and profit for retailers were BDT 97.14/day, BDT 792.71/day, and BDT 695.57/day, respectively. Fish pricing depended mainly on market structure, species quality, size, weight, and season. The average prices per kg for different fish species were recorded as follows: Ilish (BDT 1200 ± 17.89), Ayre (BDT 915 ± 8.64), Bacha (BDT 847 ± 8.24), Ghagra (BDT 816 ± 7.94), Khorsula (BDT 830 ± 5.63), Golda Chingri (BDT 855 ± 8.45), Bagdha Chingri (BDT 712 ± 7.11), Chiring (BDT 715 ± 6.15), and Gang Tengra (BDT 725 ± 8.25). Major challenges in fish marketing included the use of unhygienic ice, lack of financial support from the government and NGOs, and poor knowledge of fish handling and transportation. Organizational and government support, along with extension services on fish preservation, handling, icing, and curing, are crucial for enhancing fish marketing and improving fish quality.

Keywords: Fish Market, Marketing channels, Fish diversity, Income, Profit Margin

For citation: Chakraborty S., Dipty A.K., Rahman M.M., Sarker B.S., Paul S.K., Banik P., Diba S.A., Wasim A. Availability and Marketing System of Fish and Crustaceans in an Urban Fish Market: A Study on Retail Practices // Limnology and Freshwater Biology. 2025. - № 3. - P. 259-267. DOI: 10.31951/2658-3518-2025-A-3-259

1. Introduction

Fish and fisheries resources play a vital role in the socio-economic development of Bangladesh. The fisheries sector contributes 3.00% of the total export earnings, 3.74% to GDP, and 22.23% to the agricultural sector (DoF, 2011). The country is rich in aquatic biodiversity, with 260 species of freshwater finfish, 475 species of marine fish, 63 species of palaemonid and penaeid prawns, 36 species of shrimp, 50 species of reptiles, 24 species of aquatic mammals, 19 species of amphibians, 25 species of tortoises and turtles, and 17 species of crabs, freshwater mussels, and snails (Rahman, 2005; Hossain et al., 2008). However, due to overexploitation and high consumption, fish stocks, particularly in inland open water areas, have progressively declined. The IUCN Red List (IUCN, 2000) identified 54 threatened freshwater species in Bangladesh, of which 12 are critically endangered, 28 are endangered, and 14 are vulnerable.

The marketing system and structure significantly impact the socio-economic conditions of local communities and the production systems of any area. It involves a complex chain of systems linking the production sector to the consumer sector. Since fish and fishery products are highly traded commodities, fish production is an essential part of the marketing process (Alam et al., 2010). In Bangladesh, fish marketing is predominantly managed by the private sector. The distribution channel for small indigenous fish species includes three

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Received: June 06, 2024; ***Accepted:*** May 05, 2025;
Available online: June 11, 2025

market levels: primary, secondary/higher secondary, and final consuming markets. Fish collectors, known as mahajans or aratdars, procure fish from catchers with the help of local brokers who receive a profit margin or commission. However, remote communities face significant marketing challenges due to inadequate transport, lack of ice, poor road facilities, and a weak bargaining position of farmers relative to intermediaries (Rahman, 1997). In addition, middlemen have established a new marketing chain that exploits fish farming communities by enforcing an artificial pricing policy through multiple intermediaries. This results in high marketing margins and prices, causing dissatisfaction among consumers, farmers, fishermen, and small traders (Rahman et al., 2009).

Lakshmipur is recognized as one of the most important fisheries zones in the country and lays a crucial role in the growth of fish production and culture. Various types of fish species (freshwater, brackish, and marine) are available in the Alexander Fish Market. However, the availability of fish species and the marketing channels for this market have received little attention until now. Detailed long-term studies specifically focusing on fish species availability and marketing channels in this market have not yet been published. Therefore, the current study aims to identify the existing marketing system and the availability of fish species in the Alexander Fish Market, Lakshmipur.

2. Materials and methods

Study area and study period. The study was conducted over a period of 7 months, from June 2022 to December 2022, at the Alexander Fish Market in Lakshmipur (Fig. 1). This market, located in Ramgati Upazila, Lakshmipur, Bangladesh, is geographically positioned at 22.6547036°N latitude and 90.908538°E longitude. The site was selected due to its significance as the primary landing center in Lakshmipur. Various activities were carried out using different survey tools and specific methodologies to assess the biodiversity status of the fish market.

Data collection. The study was conducted using a survey method, with data collected directly from wholesalers (aratders) and retailers through on-the-spot interviews. For the questionnaire interviews, 20-25 fish retailers and 10 aratders were randomly selected from the study area using a simple random sampling technique. Data collection focused on the species diversity of fish, including information on species availability, abundance, distribution, and their IUCN status. The activities aimed to understand biodiversity and the marketing channels involved in the entire process.

Primary data were obtained from local people through structured questionnaires designed to meet the study's objectives. Information such as local names, distribution, and species availability was collected at the study sites. Additionally, relevant published and unpublished documents were gathered from various sources for secondary data collection. Research papers on the fish fauna of Bangladesh were also reviewed to compile historical data on species abundance and availability for biodiversity assessment.

After data collection, the information was cross-checked with key informants, including the Upazila Fisheries Officer (UFO), District Fisheries Officers (DFO), and NGO workers. The data were then entered into a database system using Microsoft Excel. All collected information was analyzed using MS Excel and presented in textual, tabular, and graphical forms.

3. Results

3.1. Fish and Crustaceans Diversity

During the study period, a total of 32 fish species and 3 crustaceans were recorded, representing 35 genera, 21 families, and 11 orders. Detailed information on the recorded fish and crustacean species, including specimen collection dates, along with their total length, availability, economic importance, distribution, and IUCN conservation status, is provided in Table 1 and 2. Morphometric and meristic analyses revealed that the order Siluriformes and Cypriniformes contributed the most (11 species of each order), followed by Perciformes and Decapoda (3), Gobiiformes (1), Mugiliformes (1), Aulopiformes (1), Scombriformes (1), Pleuronectiformes (1), Cichliformes (1), and Anabantiformes (1) (Table 1).

Fig.1. Geographical location of the study area (google map).

Table 1. Fish and Crustaceans diversity in the Alexander Fish Market, Ramgati, Lakshmipur.

Order	Family	Local Name	Scientific Name	Collection date (2022)
Cypriniformes	Cyprinidae	Rohu	*Labeo rohita* (Hamilton, 1822)	6th June
		Catla	*Labeo catla* (Hamilton, 1822)	6th June
		Mrigal	*Cirrhinus cirrhosus* (Bloch. 1795)	10th June
		Bata	*Labeo bata* (Hamilton, 1822)	10th June
		Puti	*Puntius sophore* (Hamilton, 1822)	6th July
		Sarputi	*Systomus sarana* (Hamilton, 1822)	6th July
		Gonia	*Labeo boggut* (Sykes, 1839)	12th July
	Xenocyprididae	Silver carp	*Hypophthalmichthys molitrix* (Valenciennes, 1844)	12th July
		Chapila	*Gudusia chapra* (Hamilton, 1822)	23rd July
		Ilish	*Tenualosa ilisha* (Hamilton, 1822)	23rd July
	Engrauliidae	Olua	*Coilia dussumieri* (Valenciennes, 1844)	2nd August
Siluriformes	Bagridae	Tengra	*Batasio batasio* (Hamilton, 1822)	10th August
		Gang Tengra	*Hemibagrus menoda* (Hamilton, 1822)	10th August
		Nuna Tengra	*Mystus gulio* (Hamilton, 1822)	3rd September
		Ayre	*Sperata aor* (Hamilton, 1822)	3rd September
		Rita	*Rita rita* (Hamilton, 1822)	10thSeptember
	Clariidae	Magur	*Clarias batrachus* (Linnaeus, 1758)	10th September
	Ailiidae	Gagra	*Clupisoma garua* (Hamilton, 1822)	14thSeptember
		Baspata	*Ailia coila* (Hamilton, 1822)	14th September
	Schilbeidae	Bacha	*Eutropiichthys vacha* (Hamilton, 1822)	20thSeptember
	Pangasiidae	Pangas	*Pangasius pangasius* (Hamilton, 1822)	20thSeptember
	Ariidae	Ghagra	*Arius gagora* (Hamilton, 1822)	8th October
Gobiiformes	Oxudercidae	Chiring	*Apocryptes bato* (Hamilton, 1822)	8th October
Mugiliformes	Mugilidae	Khorsula	*Rhinomugil corsula* (Hamilton, 1822)	15thOctober
Perciformes	Sillaginidae	Tulardandi	*Sillaginopsispanijus* (Hamilton, 1822)	15thOctober
	Polynemidae	Taposi	*Polynemus paradiseus* (Linnaeus, 1758)	22th October
	Sciaenidae	Poa	*Otolithoides pama* (Hamilton, 1822)	22th October

Order	Family	Local Name	Scientific Name	Collection date (2022)
Scombriformes	Trichiuridae	Chhuri	*Trichiurus lepturus* Linnaeus, 1758	22th October
Aulopiformes	Synodontidae	Loittya	*Harpodon nehereus* (Hamilton, 1822)	27th November
Cichliformes	Cichlidae	Tilapia	*Oreochromis mossambicus* (Peters, 1852)	27th November
Anabantiformes	Anabantidae	Koi	*Anabas testudineus* (Bloch, 1792)	27th November
Pleuronectiformes	Cynoglossidae	Kukur Jeeb	*Cynoglossus cynoglossus* (Hamilton, 1822)	6th December
Decapoda	Palaemonida	Golda Chingri	*Macrobrachium rosenbergii* (De Man, 1879)	6th December
		Goda Chingri	*Macrobrachium dolichodactylus* (Hilgendorf, 1879)	26th December
	Penaeidae	Bagda Chingri	*Penaeus monodon* (Fabricius, 1798)	26th December

All the species collected from the study area were primarily used as food fish, with the exception of *Clupisoma garua* and *Europlichthys vacha*, which were also caught as sport fish. Among these species, 10 were found rarely, 3 species (*Gudusia chapra*, *Mystus gulio*, and *Rhinomugil corsula*) were found moderately, and the remaining species were recorded most commonly (Table 2). The national biodiversity status (Red Book, IUCN, 2000 and 2015) classified the species into various categories: Critically Endangered (CR), Endangered (EN), Vulnerable (VU), Not Threatened (NT), Data Deficient (DD), Least Concern (LC) and Not Listed (NL).

Merging with the IUCN (2000) report for the collected fishes, 5 species (*Systomus sarana*, *Rita rita*, *C. garua*, *E. vacha*, and *Pangasius pangasius*) were recorded as CR, 5 species (*Mystus gulio*, *Labeo boggut, Macrobrachium rosenbergii, Macrobrachium dolichodactylus, Penaeus monodon*) as DD, 1 species (*Sperata aor*) as VU, 1 species (*Labeo bata*) as EN, and the remaining 23 species as NT (Table 2). On the other hand, according to Red Book, IUCN (2015), there were few variations reported over time as 19 species LC, 6 species NT, 3 species VU, 4 species NL and 3 species EN.

In this investigation, the dominant order were Cypriniformes and Siluriformes, comprising 62% of all recorded fish species. The next most dominant orders were Perciformes and Decapoda (18%), and the rest of the orders contributed 3% of each (Fig. 2).

When fish species were grouped into families, Cyprinidae constituted the largest share at 20%, followed by Bagridae 14%, Xenocyprididae 9%, Ailiidae 6% and the rest of the families contributed 3% of each (Fig. 3).

Fig.2. Diagram representing the number of Fish and Crustaceans species contribution of each order in the study area.

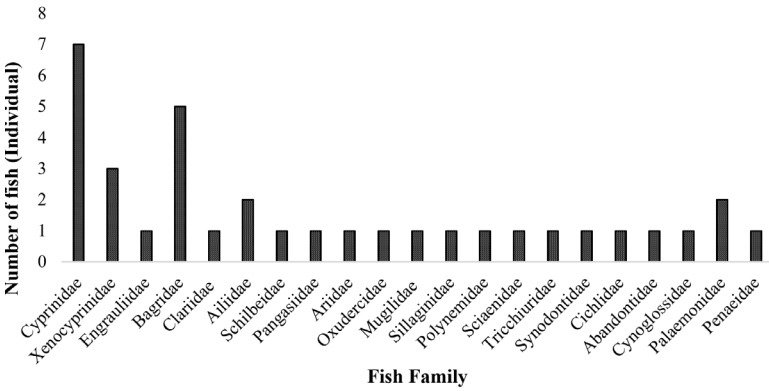

Fig.3. Diagram representing the percentage contribution of each family in the study area.

Table 2. Fish and Crustaceans diversity in the Alexander Fish Market, Ramgati, Lakshmipur.

Scientific Name	Total Length (Average), cm	Economic Importance	Availability in the study area	Distribution	IUCN Status (2000)	IUCN Status (2015)
Labeo rohita	68	Food	Common	Beels, ponds and streams	NT	LC
Labeo catla	70	Food	Common	Rivers, lakes and ponds	NT	LC
Cirrhinus cirrhosus	39	Food	Common	Rivers, lakes and ponds	NT	NT
Labeo bata	61	Food	Common	Ponds, rivers and estuaries	EN	LC
Puntius sophore	08	Food	Common	Rivers, beels and ponds	NT	LC
Systomus sarana	42	Food	Rare	Rivers, lakes and beels	CR	NT
Labeo boggut	29	Food	Common	Rivers	DD	VU
Hypophthalmichthys molitrix	90	Food	Common	Rivers	NT	NL
Gudusia chapra	14	Food	Moderate	Rivers and estuaries	NT	VU
Tenualosa ilisha	23	Food	Common	Marine water	NT	LC
Coilia dussumieri	12	Food	Rare	Estuaries and Bay of Bengal	NT	LC
Batasio batasio	10	Food	Rare	Rivers and canals	NT	NT
Hemibagrus menoda	15	Food	Common	Rivers, tributaries and ponds	NT	NT
Mystus gulio	10	Food	Moderate	Freshwater bodies	DD	NT
Clarias batrachus	51	Food	Rare	Ponds, canals and swamps	NT	LC
Rita rita	60	Food	Rare	Fresh and brackish water	CR	EN
Sperata aor	70	Food	Rare	Canals, lakesand ponds	VU	VU
Clupisoma garua	20	Food, Game	Common	Large freshwater bodies and tidal rivers	CR	EN
Eutropiichthys vacha	20	Food, Game	Common	Tidal rivers and lakes	CR	LC
Pangasius pangasius	15	Food	Common	Rivers and estuaries	CR	EN
Arius gagora	27	Food	Common	Estuaries and tidal rivers	NT	NL
Ailia coila	16	Food	Rare	Rivers	NT	LC
Sillaginopsis panijus	27	Food	Common	River and estuaries	NT	LC
Apocryptes bato	16	Food	Rare	Streams, estuaries and lagoons	NT	LC
Rhinomugil corsula	22	Food	Moderate	Rivers and estuaries	NT	LC
Otolithoides pama	30	Food	Common	Rivers and estuaries	NT	LC
Polynemus paradiseus	23	Food	Common	Sea and rivers	NT	LC
Trichiurus lepturus	198	Food	Rare	Bay of Bengal	NT	NL
Oreochromis mossambicus	32	Food	Common	Lakes, pools and estuaries	NT	NL
Anabas testudineus	10	Food	Rare	Lakes, canals and swamps	NT	LC
Harpodon nehereus	25	Food	Common	Estuaries and coastal water	NT	NT
Cynoglossus Cynoglossus	8.5	Food	Common	Estuaries and tidal rivers	NT	LC
Macrobrachium rosenbergii	30.3	Food	Common	Rivers, estuaries and canals	DD	LC
Macrobrachium dolichodactylus	6.6	Food	Common	Rivers, estuaries and canals	DD	LC
Penaeus monodon	21.9	Food	Common	Estuaries and marine water	DD	LC

Note: EN. Endangered; VU. Vulnerable; CR. Critically endangered; NT: Not threatened, and DD: Data deficient, LC. Least Concern, NL. Not Listed.

3.2. Marketing Chain of fish

The fish marketing chain typically begins with the fish farmer and involves several intermediaries before reaching the end consumer. Three distinct marketing channels were observed in the study area. The first type involves a sequence from fisherman to bepary, then aratdar, wholesalers, retailer, and finally the consumer. The second type includes a route from fisherman to local fish traders, then wholesalers, retailer, and consumer. The third type comprises a direct path from fisherman to retailer, and then to the consumer (Fig. 4). At each stage of the marketing process, the value of fish increases by 5-7%.

3.3. Personnel involved in fish marketing in the landing centre

Intermediaries are fish traders who do not sell fish directly to consumers. Instead, they purchase fish to sell to other traders or intermediaries. There are different intermediaries in the fish marketing system.

Fish harvester group. These groups consist of individuals who harvest fish from ponds, canals, ditches, floodplains, haors, baors, rivers, and other water bodies. Generally, the harvester group earns a 3-5% commission from the market price of fish.

Beparies are professional traders who buy fish from farmers or fishermen and sell them in the wholesale market. They earn profit through the buying and selling process.

Aratders act as agents in the wholesale market. They receive a commission from the fish purchased by retailers. This commission is sometimes paid in cash or after the retailers sell the fish to consumers. Approximately 10 aratders were recorded in the Alexander Fish Market.

Auctioneers are responsible for auctioning fish in the wholesale market. When intermediaries bring fish into the wholesale markets from various sources, auctioneers initiate the auction process. They start with a minimum bid and increase the bid until the highest bidder wins the fish. Auctioneers typically receive a fixed amount or 1-2% of the sold price.

Fish retailers. Retailers are those who buy fishes from intermediaries and sell them to ultimate consumers. Their role involves procuring supplies and displaying them in ways that are convenient for consumers. Retailers often buy fish through open auctions and categorize the fish by species or size before selling them.

Local fish traders. Farmers sell their fish to wholesalers through local traders. Local traders are typically based in local markets near the fish farming communities. These traders may have informal agreements with wholesalers to supply specific quantities despite lower profit margins. The fish then reach consumers through retailers. During the study period, about 20-25 retailers were recorded at the Alexander Fish Market, Lakshmipur. Some individuals also worked as day laborers for retailers. No women's participation was recorded in this marketing channel.

3.4. Marketing cost of retailers in fish market

Icing, watering, packaging, and transportation were all included in the marketing costs. The highest marketing cost was recorded in June at BDT 107/day, while the lowest was in December at BDT 89/day. For the other months, the costs were BDT 103/day in July, BDT 98/day in August, BDT 95/day in September, BDT 96/day in October, and BDT 92/day in November. On average, the marketing cost was estimated to be BDT 97.14/day (Fig. 5).

3.5. Income and profit of fish retailers

The income and profit of fish retailers depended on daily fish supply, marketing costs, weather, and various occasions. The average daily income of fish retailers was recorded as follows: BDT 786/day in June, BDT 787/day in July, BDT 805/day in August, BDT 811/day in September, BDT 795/day in October, BDT 783/day in November, and BDT 782/day in December. Notably, the highest average income and profit were observed in September (BDT 811/day and BDT 697/day, respectively), while the lowest were in December (BDT 782/day and BDT 675/day, respectively). Consequently, the overall average daily income of the retailers was calculated to be BDT 792.71/day, with an average daily profit of BDT 695.57/day in the study area (Fig. 6).

3.6. Amount and prices of available fish species

According to the current survey, a fish retailer sold an average of 45 kg of fish daily. The total daily fish supply was approximately 1.8-2.0 metric tons (average 40 retailers × 45 kg). Fish prices were largely influenced by supply and demand. It was observed that the prices of the same fish remained relatively consistent throughout the study period. The average price per kilogram (with ±SD) of all available fish species in the Alexander fish market is listed in Table 3. The average prices per kilogram for Ilish (1200±17.89), Ayre

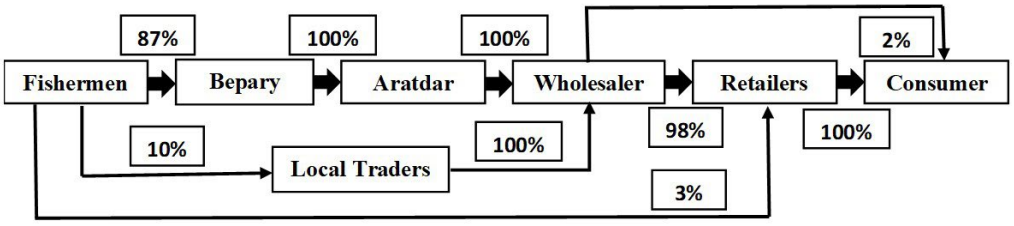

Fig.4. Diagram representing the fish distribution chain from fishermen to consumer.

(915 ± 8.64), Bacha (847 ± 8.24), Ghagra (816 ± 7.94), Khorsula (830 ± 5.63), Golda Chingri (855 ± 8.45), Bagdha Chingri (712 ± 7.11), Chiring (715 ± 6.15), and Gang Tengra (725 ± 8.25) were recorded as higher compared to other fish species (Table 3). However, the supply of carp species was found to be higher than that of other species.

3.7. Constraints of Fish Marketing

There is minimal effort to enhance the quality of fish sold in the market due to the persistent high demand surpassing supply. Consequently, fish of any quality are readily sold, despite traders encountering significant issues such as insufficient capital, high transportation costs, prolonged exposure to high temperatures, improper use of ice, rough and unhygienic handling methods, contamination, and a lack of knowledge about quality standards among the involved parties.

4. Discussion

The total number of fish and crustacean species observed at the Alexander Fish Market in Lakshmipur was 32 species and 3 species respectively. Aktar et al. (2013) reported a higher number of fish species compared to our findings, potentially due to their consideration of seven markets. However, Afroz (2007) and Chakraborty (2023) reported a similar number of fish species to our study. Cypriniformes and Siluriformes emerged as the order with the highest number of fish species, followed by Perciformes, Decapoda, Gobiiformes, Mugiliformes, Scombriformes, Cichliformes, Aulopiformes, Anabantiformes, and Pleuronectiformes.

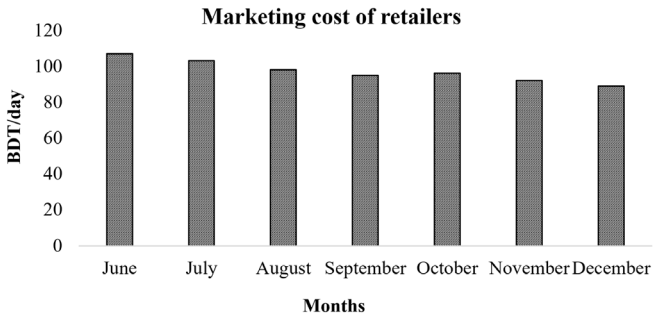

Fig.5. Marketing cost of retailers in the Alexander fish market.

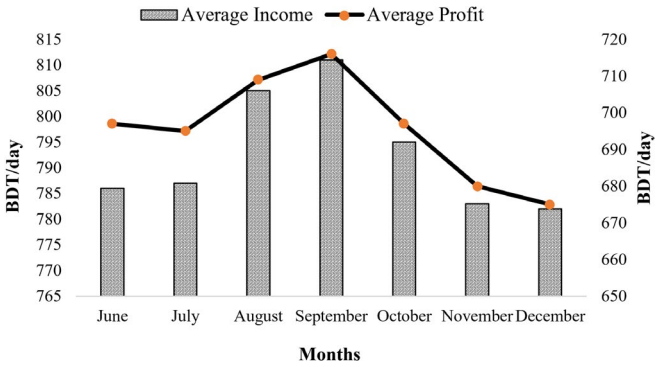

Fig.6. Average daily income (BDT/day) and average daily profit (BDT/day) of retailers across different months at Alexander fish market, Lakshmipur.

Table 3. Prices of available fish and crustacean species in Alexander Fish Market, Ramgati, Lakshmipur.

Sl. No.	Species (Local Name)	Price (Mean ± SD)	Sl. No.	Species (Local Name)	Price (Mean ± SD)
1.	Rohu	335 ± 5.16	19.	Bacha	847 ± 8.24
2.	Catla	300 ± 4.14	20.	Pangas	180 ± 2.90
3.	Mrigal	237 ± 4.23	21.	Ghagra	816 ± 7.94
4.	Bata	251 ± 8.94	22.	Baspata	627 ± 5.24
5.	Puti	200 ± 3.23	23.	Tulardandi	610 ± 5.63
6.	Sarputi	220 ± 2.89	24.	Chiring	715 ± 6.15
7.	Gonia	358 ± 3.23	25.	Khorsula	830 ± 5.63
8.	Silver carp	204 ± 2.41	26.	Poa	155 ± 2.88
9.	Chapila	180 ± 2.80	27.	Taposi	548 ± 4.39
10.	Ilish	1200 ± 17.89	28.	Chhuri	800 ± 6.45
11.	Olua	110 ± 4.21	29.	Tilapia	180 ± 2.89
12.	Tengra	515 ± 5.63	30.	Koi	619 ± 2.89
13.	Gang Tengra	725 ± 8.25	31.	Loittya	150 ± 3.43
14.	Nuna Tengra	415 ± 4.76	32.	Kukur Jeeb	425 ± 5.63
15.	Magur	522 ± 3.31	33.	Golda Chingri	855 ± 8.45
16.	Rita	790 ± 7.56	34.	Goda Chingri	813 ± 6.55
17.	Ayre	915 ± 8.64	35.	Bagda Chingri	712 ± 7.11
18.	Gagra	758 ± 7.16			

Notably, the Cyprinidae family exhibited the highest species count (7 species) and individual abundance, followed by the Bagridae family exhibited the 2nd highest (5 species), and the Xenocyprinidae family exhibited the 3rd highest species count (3 species) and individual abundance among the observed fish. Out of the recorded species, 5 were classified as Critically Endangered, 5 as Data deficient, 1 as Vulnerable, 1 as Endangered, and the remaining 23 were categorized as Not Threatened, based on IUCN classification (IUCN, 2000). While in the updated list on 2015, a total 19 species were reported as Least Concern, 6 as Not Threatened, 3 as Vulnerable, 4 as Not Listed and 3 as Endangered (IUCN, 2015).

4.1. Fish Marketing Systems

In the study area, three distinct marketing channels were identified. Similar observations were made by Mia (1996) in the Mymensingh district, while Khan (2004) reported four types of marketing channels in the Jessore district. At the Alexander Fish Market, wholesalers typically procure fish from araders or beparies, who directly obtain fish from fish farmers or fishermen. These fish are then sold to wholesalers with the assistance of commission agents. Wholesalers, in the next stage, auction off their fish to retailers, who, in turn, sell them to local consumers. Occasionally, local retailers directly purchase fish from fishermen and sell them to consumers. Suppliers typically employ vans, boats, buses, trucks, tempos, pickups, or even rickshaws to transport fish to wholesalers.

4.2. Average marketing cost, income and profit of retailers

In the current study, the average daily marketing cost for retailers was estimated at BDT 97.14, with the highest value recorded in July (BDT 107) and the lowest in December (BDT 89). Aktar et al. (2013) reported a higher average cost (BDT 141.2) in the Noakhali district, possibly due to their inclusion of seven markets, which might have influenced the higher value. However, the average daily income and profit for fish retailers were higher in the present study, recorded at BDT 792.71 and BDT 695.57 respectively, compared to Aktar et al. (2013) in Noakhali and Rashid (2006) in Mymensingh. This discrepancy could be attributed to variations in consumer purchasing power and population density across different regions.

4.3. Amount and prices of available fish and crustacean species

Based on the findings of the present study, the daily fish supply was approximately 1.8-2.0 metric tons, calculated from an average of 40 retailers each selling 45 kg of fish. Certain fish species, including Ilish, Ayre, Bacha, Ghagra, Khorsula, Golda Chingri, Bagdha Chingri, Chiring, and Gang Tengra, commanded higher prices compared to others. Rashid (2006) reported higher daily fish supplies in municipal markets and Maijdee bazar, estimated at 3-3.2 metric tons and 1-1.1 metric tons, respectively. These variations in supply might be attributed to differences in location.

4.4. Constraints of Fish Marketing

During the survey, fish retailers highlighted several constraints in fish marketing. These include insufficient capital, elevated transportation costs, prolonged exposure to high temperatures, improper ice usage, rough handling practices leading to contamination, and inadequate knowledge regarding quality standards among stakeholders. Similar challenges in fish marketing were also observed by Mia (1996) and Rokeya et al. (1997). The involvement of numerous intermediaries in the marketing chain often leads to higher prices for consumers, while fishers themselves may not receive fair prices for their products, with the majority of profits going to intermediaries (Alam et al., 2010).

5. Conclusions

Throughout the study period, a total of 32 fish and 3 crustacean species were recorded, representing 35 genera, 21 families and 11 orders. Present study found that the order Cypriniformes (11 species) and Siluriformes (11) and the family Cyprinidae had the highest contributions, followed by the orders, Perciformes (3) and several others. A total of 3 species were recorded as EN, 3 as VU, 4 as NL, 6 as NT, and 16 as LC (IUCN, 2015). The dominant order, Cypriniformes and Siluriformes, comprised the highest percentage of fish species, followed by the orders, Perciformes, and others. These fish typically inhabit estuaries, rivers, canals, and the Bay of Bengal. Fish availability varies seasonally. The primary causes of the decline in fish populations include overfishing, river siltation, the reckless use of agrochemicals, and the introduction of foreign species, all of which negatively impact fish populations by increasing mortality, spreading diseases, and reducing fertility. In the current study area, three categories of marketing channels were identified. The basic pattern involves intermediaries, known locally as aratdars, who purchase fish from farmers and fishermen, bring it to market, and sell it to wholesalers, known as beparies. Retailers bid at auction to purchase fish from wholesalers, then transport the fish to specific markets where they sell it to consumers. The main issues identified in the Alexander Fish Market were inadequate funding, unsanitary handling practices, contamination, and poor icing facilities. Therefore, further research and understanding of fish marketing, along with government support, institutional and organizational support, and extension services, are necessary.

Acknowledgements

We are grateful to the Department of Fisheries and Marine Science, Noakhali Science and Technology University, for their support and for providing access to laboratory facilities and equipment.

Conflict of interest

The authors declare no conflicts of interest.

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Eukaryotic communities of freshwater bryozoans (Phylactolaemata: Plumatellidae) of the Baikal region

Original Article
LIMNOLOGY FRESHWATER BIOLOGY

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ABSTRACT. The diversity of eukaryotic communities associated with bryozoans was investigated using DNA metabarcoding based on the 18S rRNA gene. The results obtained indicate that each bryozoan species, coexisting in close proximity to one another, harbors a distinctive community of associated eukaryotes, the composition of which likely depends on the form of the colonies. The community associated with the "bushy" colony of *Plumatella* sp. was found to be more diverse and differed in species composition from the community formed on the "creeping" colony of *P. repens*. In the "bushy" bryozoans, diatoms (60%) and ciliates (22%) predominated, along with golden algae (4%), hydras (3%), chytridiomycetes (1.6%), and rotifers (1%). In contrast, unicellular algae (32%), dinoflagellates (27%), apicomplexans (10.6%), and other groups of protists (amoebas, euglenoids, and others) (4.6%) were more frequently associated with the "creeping" bryozoan. Among invertebrates, annelid worms (12.5%), tapeworms (4%), and mollusks (3%) predominated. Notably, the study revealed the presence of protostome animals belonging to the phylum Entoprocta, marking the first documentation of this taxon in the water bodies of the Baikal region.

Keywords: DNA metabarcoding, 18S pPHK, ITS1–5.8S–ITS2, COI, 16S rRNA

For citation: Peretolchina T.E., Shcherbakova A.A., Sitnikova T.Ya., Adelshin R.V., Bukin Yu.S. Eukaryotic communities of freshwater bryozoans (Phylactolaemata: Plumatellidae) of the Baikal region // Limnology and Freshwater Biology. 2025. - № 3. - P. 268-283. DOI: 10.31951/2658-3518-2025-A-3-268

1. Introduction

Bryozoans (phylum Bryozoa) are colonial animals that exhibit a sessile lifestyle, settling on any solid substrate including submerged wood, macrophytes, stones, plastic, polyethylene, and others. Some species are known to encrust the hulls of ships (Gontar, 2010).

The phylum Bryozoa comprises three classes: Phylactolaemata (freshwater bryozoans), Stenolaemata (exclusively marine bryozoans) и Gymnolaemata (mostly marine bryozoans). Phylactolaemata includes one order, seven families, and about 70 species that inhabit exclusively freshwater environments (Ryland, 2005). Members of this class are globally distributed, except in polar regions, and occur in both lentic and lotic ecosystems. Some species are considered cosmopolitan (Wood, 2002). Colonies of phylactolaemates consist of zooids divided into two parts: the soft polypide with its crown of ciliated tentacles, and the gelatinous or chitinous cystid, which functions as an exoskeleton into which the polypide can retract. The mouth, covered by an epistome, is situated within the tentacle crown.

Within the Phylactolaemata, the most diverse family is Plumatellidae Allman, 1856, comprising four genera and more than 20 valid species, most of which belong to the genus *Plumatella* Lamark, 1816. In Russian freshwater ecosystems, seven species of this genus have been recorded (Gontar, 2010), with three species occurring in the Baikal region (Vinogradov, 2008).

Phylactolaemates often dominate among aquatic organisms. As active water filterers, they play a significant role in the self-purification of water bodies, particularly during periods of abundant growth (Protasov, 1994). The size of their colonies varies from a few millimeters to several tens of centimeters, and their rapid growth can result in substantial benthic biomass. Consequently, bryozoans also contribute to nutrient cycling (Sørensen et al., 1986). Phylactolaemates can form associations with other bryozoans, sponges,

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Received: December 17, 2024; ***Accepted:*** April 22, 2025;
Available online: June 11, 2025

hydroids, and caddisfly larvae (Ricciardi and Reiswig, 1994). Their colonies provide habitat for various small organisms, including rotifers, planarians, nematodes, annelids, mollusks, gastrotrichs, copepods, tardigrades, insect larvae, mites, and others (Raddum and Johnsen, 1983; Ricciardi and Reiswig, 1994).

Species of *Plumatella* differ in the morphology and size of their colonies, suggesting that their associated communities may vary depending on the structure of the colonies. To test this hypothesis, we employed the DNA metabarcoding, a method that enables identification of the community taxonomic composition through amplification and high-throughput sequencing of marker gene sequences. The metabarcoding of organism-associated communities also facilitates the identification of interaction mechanisms between different organismal groups and their potential ecological roles within the community.

DNA metabarcoding using the COI gene fragment has been successfully implemented to studies invertebrate communities in Bolshie Koty Bay and Listvennichny Bay of Lake Baikal (Kravtsova et al., 2021; Kravtsova et al., 2023). Furthermore, this method has proven to be effective for investigating the diversity of algae-associated organisms using the 18S rRNA gene fragment (Bukin et al., 2022) and for researching the microeukaryotic planktonic communities in Lake Baikal (Bukin et al., 2023).

This study aimed to investigate and compare the composition of eukaryotic communities associated with two sympatric bryozoan species exhibiting different colony morphologies.

2. Materials and Methods

Bryozoan colonies were collected from submerged wood at the mouth of the Tompuda River (northern Lake Baikal, 55.12122° N, 109.753418° E) in 2022 (Fig. 1). The river mouth valley is characterized by a swampy plain intersected by numerous macrophyte-dominated channels with abundant submerged logs. Bryozoans were observed on the majority of logs. Colonies were collected from a single submerged log to eliminate habitat-associated influences on the taxonomic composition of the bryozoan associated organisms.

Living colony fragments were photographed using an MSP-1 stereomicroscope ("LOMO" JSC) equipped with a Levenhuk C800 digital camera (Fig. 1).

DNA was performed following the protocol described by Doyle and Dickson (Doyle and Dickson, 1987). For Sanger sequencing, DNA samples were obtained from small colony fragments that were carefully cleaned to prevent contamination from bryozoan-associated organisms. For metabarcoding analysis, DNA was extracted from larger colony fragments (five samples per species).

All DNA samples were amplified. Amplification was carried out using the BioMaster HS-*Taq* PCR Kit (Biolabmix, Russia) according to the manufacturer's recommendations. Amplification conditions and primer sequences are detailed in Table 1. For metabarcoding, PCR products from each species were pooled into a single tube.

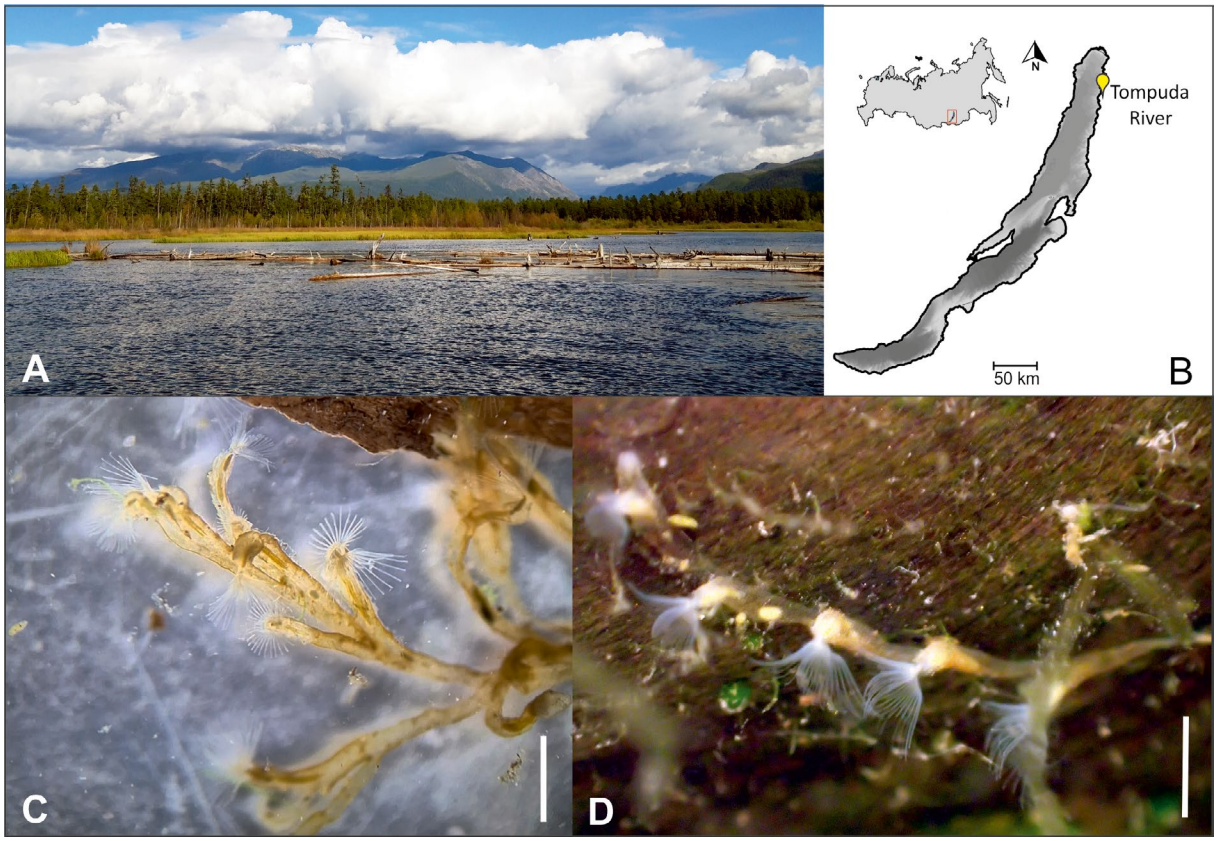

Fig.1. A – photo of Tompuda River valley; B – map of Lake Baikal with collection point indicated; C, D – photos of living colonies of bryozoans. C – *Plumatella* sp.; D – *P. repens*. Scale 1 mm.

Table 1. Amplification conditions and primers used in this study.

Gene	Amplification conditions, 30 cycles	Primers	References
COI	DNA denaturation at 95°C – 40 sec (5 minutes on the first cycle), primer annealing at 50°C – 60 sec, nucleotide chain elongation at 72°C – 60 sec (10 minutes on the last cycle)	LCO1490 (f) 5'-GGT CAA CAA ATC ATA AAG ATA TTG G -3' HCO2198 (r) 5'-TAA ACT TCA GGG TGA CCA AAA AAT CA-3'	Folmer et al., 1994
ITS1–5,8S–ITS2		ITS1 (f) 5'-TCC GTA GGT GAA CCT GCG G-3' ITS4 (r): 5'-TCC TCC GCT TAT TGA TAT GC-3'	White et al., 1990
18S	DNA denaturation at 95°C – 60 sec (5 minutes on the first cycle), primer annealing at 55°C – 60 sec, nucleotide chain elongation at 72°C – 60 sec (10 minutes on the last cycle)	EukA (f) 5'-ACC TGG TTG ATC CTG CCA GT-3' EukB (r) 5'-TGA TCC TTC TGC AGG TTC ACC TAC-3'	Medlin et al., 1988
16S		ZX-1-F 5'-ACC CGC TGA ATT TAA GCA TAT-3' LSUD-R 5'-ACG GAA TGA ACT CAA ATC ATG TAA G-3'	Van der Auwera et al., 1994 Littlewood et al., 2000

PCR products were visualized through electrophoresis on 1% agarose gel. Enzymatic purification of the products was performed using the ExoSAP-IT express kit (Thermo Fisher Scientific, USA) according to the manufacturer's protocol.

The COI, 16S and the ITS1–5.8S–ITS2 markers were sequenced using a NANOFOR 05 genetic analyzer with the Brilliant Dye Terminator (v.3.1) Sequencing kit (NimaGene, Holland). The nucleotide sequences were edited and aligned using the BioEdit software (Hall, 2011).

The taxonomic identification of bryozoans was accomplished through DNA barcoding using COI, 16S mtDNA, and ITS1–5.8S–ITS2 rDNA markers. Taxa delimatation was performed using the web version of the ASAP program (available at: https://bioinfo.mnhn.fr/abi/public/asap/), employing pairwise genetic distances (*p*-distances) as the genetic distance metric. Additionally, nucleotide sequences of various Plumatellidae species for COI, 16S and ITS1–5.8S–ITS2 markers were retrieved from GenBank (Table 2).

18S rRNA metabarcoding of bryozoan-associated eukaryotes was performed using portable nanopore DNA sequencer MinION (Oxford Nanopore Technologies). The quality of reads was assessed using FastQC; reads were filtered by quality using Trimmomatic-0.32 (Bolger et al., 2014). Sequences with an average quality score exceeding 12 were selected for further analysis. Sequence identification was conducted using the SILVA database version 138.2 (Quast et al., 2012), containing full-length 18S rRNA fragments. BLASTn (Altschul et al., 1990) was employed with parameters "word_size = 25, gapopen = 2, gapextend = 1, reward = 1, penalty = -1" to compare sequenced amplicon library fragments with the SILVA database. These parameters allowed for the identification of amplicon sequences with low similarity to the reference database. An 18S rRNA amplicon sequence was considered identified if alignment parameters were E-value $\leq$ 0.00001 and bit score $\geq$ 50, indicating non-random matches. All 18S rRNA amplicon sequences mapping to the same SILVA database sequence in BLASTn alignment results were grouped into operational taxonomic units (OTUs). OTU abundance was quantified by the number of 18S rRNA amplicon reads assigned to each unit.

The results were visualized as histogram where OTUs were grouped into high-rank taxa.

The statistical convergence of the results of taxonomic diversity assessment was characterized using rarefaction curves and the Chao1 index (the expected number of OTUs in samples). Community diversity comparisons were conducted using the Shannon index, as well as species abundance curves, where slower convergence to zero indicates higher community diversity. All statistical analyses and graphical visualizations were performed using the "Vegan" package (Dixon, 2003) and "ggplot2" (Wickham, 2011) in the R programming environment.

3. Results

The bryozoans studied were classified into two species of the genus *Plumatella*, based on the external morphology of zooids and colonies (Fig. 1). For each species, two nucleotide sequences for the COI (629 bp), 16S (439–440 bp), and ITS1–5.8S–ITS2 (738–742 bp) were obtained and submitted in the GenBank database under accession numbers: PQ766342–PQ766343, PQ771669–PQ771670 (COI); PQ770921–PQ770922, PQ772041–PQ772042 (ITS1–5.8S–ITS2); PQ774235–PQ774238 (16S).

Species delimitation analysis using three molecular genetic markers identified the bryozoan with a "creeping" colony type as *Plumatella repens* (Linnaeus, 1758), while the bryozoan with a "bushy" colony type (*Plumatella* sp.) could not be definitively identified to species level due to limited nucleotide sequences available in GenBank. 16S mtDNA fragment analysis revealed that the sister species for this bryozoan is *P. emarginata* Allman, 1844 (with a genetic distance of 2% nucleotide substitutions), while ITS1–5.8S–ITS2 analysis indicated close relation to *P. vaihiriae* (Hastings, 1929) (with a genetic distance of 0.4% substitutions). Species delimitation based on the COI gene fragment could not be performed due to the lack of nucleotide sequences for *P. emarginata* and *P. vaihiriae* in GenBank.

Table 2. List of taxa used for species delimitation analysis with GenBank accession numbers and references.

Species name	COI GB#	References	ITS1–5,8S–ITS2 GB#	References	16S GB#	References
Plumatella fungosa (Pallas, 1768)	KF805632	Dash and Vasemägi 2014, unpublished	GU733426	Rubini et al., 2011	AB365624	Hirose et al., 2011, unpublished
	MH286272	Klass et al., 2018	-	-	-	-
Plumatella repens (Linnaeus, 1758)	FJ196105	Fuchs et al., 2009	GU733417	Rubini et al., 2011	AB365622	Hirose et al., 2011, unpublished
	-	-	EU377576	Taticchi et al., 2010, unpublished	-	-
Plumatella vaihiriae (Hastings, 1929)	-	-	EU377577	Taticchi et al., 2010, unpublished	AB365625	Hirose et al., 2011, unpublished
Plumatella casmiana Oka, 1907	KJ024813	Koletic, 2014	EU377579	Taticchi et al., 2010, unpublished	GQ343297	Briski et al., 2011
	-	-	-	-	AB365629	Hirose et al., 2011, unpublished
Plumatella rugosa Wood, Wood, Geimer & Massard, 1998	-	-	GU733418	Rubini et al., 2011	AB365623	Hirose et al., 2011, unpublished
Plumatella viganoi Taticchi 2010	-	-	GU733422, GU733421, GU733420	Rubini et al., 2011	-	-
Plumatella geimermassardi Wood and Okamura, 2004	-	-	GU733423	Rubini et al., 2011	-	-
	-	-	EU377578	Taticchi et al., 2010, unpublished	-	-
Plumatella emarginata Allman, 1844	-	-	GU733424	Rubini et al., 2011	JN681057	Waeschenbach et al., 2012
	-	-	-	-	AB365623	Hirose et al., 2011, unpublished
	-	-	-	-	GQ343296, GQ343300, GQ343301	Briski et al., 2011
Plumatella reticulata Wood, 1988	-	-	GU733425	Rubini et al., 2011	-	-
Plumatella vorstmani Toriumi, 1952	-	-	-	-	AB365634	Hirose et al., 2011, unpublished
Plumatellidae sp.	KU720129	Jiang et al., 2017	-	-	-	-
	KX620051	Dong et al., 2020	-	-	-	-
Hyalinella punctata (Hancock, 1850)	KJ024814	Koletic, 2014	EU377580	Taticchi et al., 2010, unpublished	AB365631	Hirose et al., 2011, unpublished
	-	-	GU733427, GU733428	Rubini et al., 2011	DQ305342	Okuyama et al., 2006

The initial datasets after nanopore sequencing of the 18S rRNA gene included 44,771 sequences for *Plumatella* sp. and 51,057 for *P. repens*; after quality filtering, the datasets contained 19,027 and 21,762 sequences, respectively, from which OTUs (Operational Taxonomic Units) were formed. Single OTUs (representing fewer than 4 copies per sample) were excluded from analyses. The final analysis identified 299 OTUs for *Plumatella* sp., and 33 OTUs for *P. repens*.

Rarefaction curves and species abundance curves are presented in Fig. 2. The saturation curves for both species reached plateaus, confirming statistical significance of the results. The expected number of taxa, calculated using the Chao1 index, corresponded with the actual number of identified OTUs.

The Shannon diversity index for the community associated with the “bushy” bryozoan *Plumatella* sp was 4.1, while for the “creeping” *P. repens* it was 2.8.

The taxonomic composition (in percentage) of both communities is illustrated in the histogram (Fig. 3). A total of 21 high-rank taxa were identified within the communities. The community associated with the “bushy” bryozoan was dominated by diatoms (60%) and ciliates (22%), with notable presence of

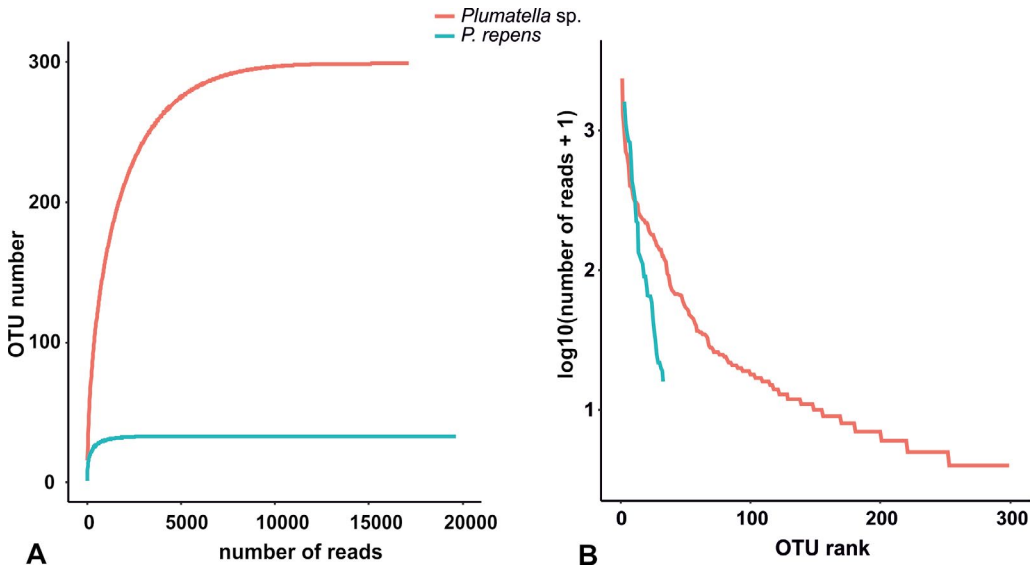

Fig.2. A – rarefaction curves; B – species abundance curves.

golden algae (4%) and hydras (3%), as well as chytridiomycetes (1.6%), which are parasites of algae and invertebrates. Rotifers were also observed (1%), likely attracted by an abundance of ciliates, unicellular algae, and bacteria.

The "creeping" bryozoan-associated community exhibited lower diversity, dominated by unicellular algae (32%) and dinoflagellates (27%). Significant proportions of apicomplexans (10.6%), which are invertebrate parasites, and other protozoan classes (including amoebas, euglena, etc.) (4.6%) were also observed. Among the invertebrates, annelid worms (12.5%), tapeworms (4%), and mollusks (3%) predominated. Additionally, nucleotide sequences of protostome animals belonging to the phylum Entoprocta were identified.

4. Discussion

At the mouth of the River Tompuda, which flows into Baikal, two genetically distant species of the genus *Plumatella* (16% substitutions based on COI, 9% based on ITS1–5.8S–ITS2, and 4% based on 16S) were found living sympatrically. One of them, *P. repens,* is widely distributed in temperate water bodies. The other species, *Plumatella* sp. showed genetic similarity to *Plumatella vaihiriae* (Hastings, 1929) based on the ITS1–5.8S–ITS2 marker (0.4% divergence). *P. vaihiriae* was initially described from a lake on Tahiti and has been recorded in lakes in Hawaii, Argentina, Thailand, and the USA (Taticchi et al., 2008). According to the 16S marker (2% divergence) *Plumatella* sp. is closely related to *P. emarginata*, a species distributed in Eurasia,

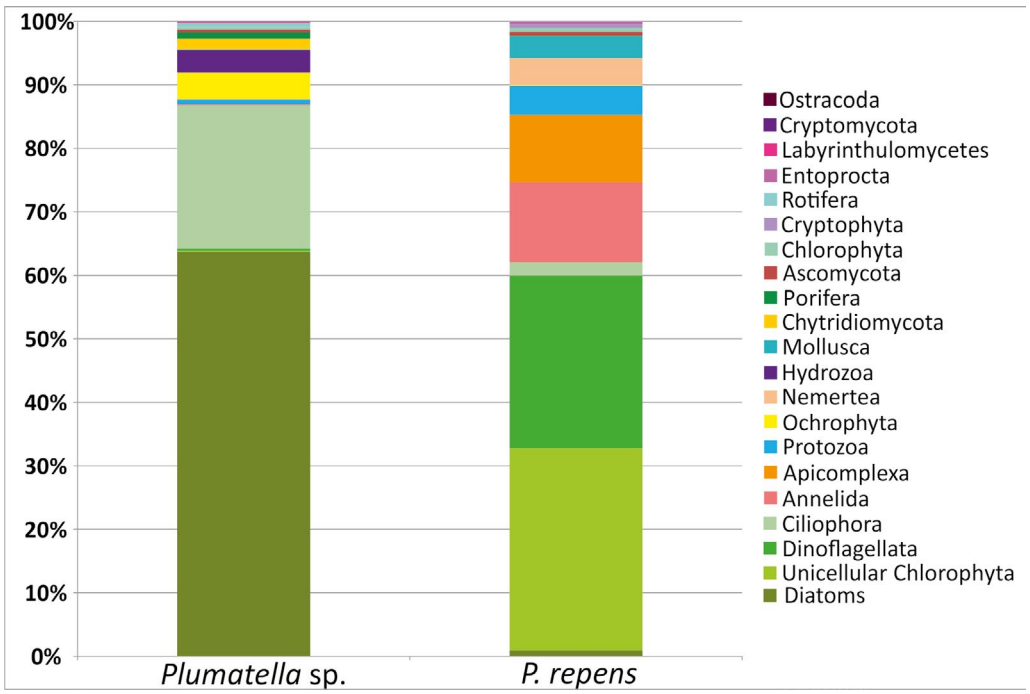

Fig.3. Histogram of OTU distribution across high-rank taxa for *Plumatella* sp. and *P. repens*.

North America, and New Zealand. It should be noted that the presence of *P. emarginata* in the Baikal region has been previously reported (Vinogradov, 2008), but the examined specimens likely represent a distinct species (potentially new to science) based on their level of genetic divergence. However, definitive species identification requires morphological analysis of statoblasts.

The sympatric occurrence of two bryozoan species on the same substrate is not unique to the Baikal region. For example, Taticchi et al. (2008) reported the cohabitation of *P. vaihiriae* with *P. fungosa* (Pallas, 1768), and representatives of Victorellidae Hincks, 1880 in Italian lakes.

The studied bryozoans exhibit different colony morphologies: *P. repens* forms creeping or sprawling branching tubes that spread flat across the substrate, while *Plumatella* sp. develops tubular branched zooids that extend vertically from the substrate in a bush-like formation. We observed that each coexisting bryozoan species harbored a unique eukaryotic community, the composition of which depends on the colony morphology. The community associated with the "bushy" *Plumatella* sp., was much more diverse (Shannon index = 4.1) compared to that of the "creeping" *P. repens* (Shannon index = 2.8), considering both high-rank taxon composition and the number of dominant OTUs. This may be related to the structure of the "bushy" colony, which creates microhabitats within the spaces between zooids that are favorable for diverse epibionts. Various diatoms easily attach to the bryozoan zooids, as evidenced by the abundance of reads corresponding to these organisms. Conversely, "creeping" colony exhibited substantially lower biodiversity, hosting larger invertebrates that potentially feed on the bryozoan itself, along with protozoan Apicomplexa, which are parasites of invertebrates.

An interesting finding was the discovery of the phylum Entoprocta Nitsche, 1870 or Kamptozoa Cori, 1929, which have not previously been reported in the water bodies of the Baikal region. These animals, resembling hydroids and bryozoans, exhibit sessile lifestyles either solitarily or colonially, with individual organisms ranging from 1 to 5 mm in size. Entoprocts are often commensals of invertebrates, including sessile annelids, mollusk shells, and bryozoans (Brusca and Brusca, 2003; Emschermann, 1993; Kristensen, 1970; Wood, 2005). It is possible that the "creeping" colony of *P. repens* allows for the Entoprocta attachment, and the water flow generated by the bryozoan's lophophores potentially facilitates their feeding. Due to limited studies of this phylum, nucleotide sequence data for them are nearly absent in databases, making it possible to identify them only to a high taxonomic level (Entoprocta: Barentsiidae Emschermann, 1972). Currently, approximately 200 species of Entoprocts are known, including sessile solitary species (Loxosomatidae) and colonial species (Loxokalypodidae, Pedicellinidae, and Barentsiidae), most of which are marine. Only two species inhabit freshwater: *Loxosomatoides sirindhorne* Wood, 2005 (fam. Loxosomatidae) and *Urnatella gracilis* Leidy, 1851 (fam. Barentsiidae). It should be noted that area of *L. sirindhorne* is limited to Thailand (Wood, 2005; Schwaha et al., 2010), while *U. gracilis*, originally described as a North American species, is distributed on all continents except Antarctica (Brusca and Brusca, 2003), and has been found in the Don River (Sklyarova, 1969) and the Volga River (Vinogradov, 1997). It is possible that *U. gracilis* occurs in the water bodies of the Baikal region, although this assumption requires detailed morphological analysis.

DNA metabarcoding using the 18S rRNA gene proved to be an effective method for investigating the diversity of eukaryotic communities associated with bryozoans, as indicated by the wide range of identified taxa. Saturation and abundance graphs, as well as the expected species number indices (Chao1), confirm the statistical significance of the results. Nevertheless, it should be noted that nanopore sequencing may introduce errors in the form of insertions and deletions of nucleotides within the 18S rRNA sequences. Analysis of the results from BLASTn alignments of amplicon sequences with the SILVA database revealed that in some cases, sequence similarity (ranging from 97% to 99%) was only determined by single-nucleotide insertions and deletions rather than substitutions, a phenomenon not typically observed in short-read sequencing platforms, like Illumina. This limitation of the MinION portable DNA sequencer (Oxford Nanopore Technologies) should be considered in future software development for amplicon analysis in metabarcoding studies.

5. Conclusions

The results of DNA metabarcoding of the 18S rRNA gene revealed the specificity of the composition of eukaryotic communities associated with bryozoans. It was shown that the colony morphology plays a crucial role in shaping their species diversity. The community associated with *Plumatella* sp., which has a "bushy" colony form, is much more diverse and differs in species composition from the community formed on the "creeping" colony of *P. repens*.

The application of molecular-genetic methods allowed for the identification of representatives of protostome animals of the phylum Entoprocta among organisms associated with *P. repens*, which is significant for the study of aquatic biodiversity. New findings of Entoprocta in the fauna of water bodies of the Baikal region indicate that their distribution is broader than previously assumed.

Acknowledgements

The research was supported by the state projects 121032300196-8 and 121032300180-7. The authors thank team of research vessel "Titov" (The Center for Collective Use «Research vessels Center of LIN SB RAS on Lake Baikal») for assistance in sampling. Sequencing of the samples was carried out at the Collective Use Instrument Center for Physicochemical Ultramicroanalysis of the LIN SB RAS (Center for Collective Use "Ultramicroanalysis").

Conflict of interest

The authors declare no conflict of interest.

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Эукариотические сообщества пресноводных мшанок (Phylactolaemata: Plumatellidae) Байкальского региона

Оригинальная статья

LIMNOLOGY FRESHWATER BIOLOGY

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АННОТАЦИЯ. Методом ДНК метабаркодинга на основе 18S рРНК исследовано разнообразие сообществ организмов, ассоциированных с мшанками. Полученные результаты свидетельствуют о том, что для каждого вида мшанок, сосуществующих в непосредственной близости друг от друга, свойственно своеобразное сообщество ассоциированных с ними эукариот, состав которого, вероятно, зависит от формы колоний. Сообщество, ассоциированное с «кустистой» колонией *Plumatella* sp. оказалось более разнообразным, и отличалось по видовому составу от сообщества, сформировавшегося на «стелющейся» колонии *P. repens*. На «кустистых» мшанках преобладали диатомовые водоросли (60%) и инфузории (22%), а также золотистые водоросли (4%), гидры (3%), хитридиомицеты (1,6%), коловратки (1%). На «стелющейся» мшанке обнаружены одноклеточные водоросли (32%), динофлагелляты (27%), апикомплексы (10,6%), другие классы простейших (амебы, эвглены и др.) (4,6%), из беспозвоночных животных преобладали кольчатые (12,5%) и ленточные (4%) черви, моллюски (3%); обнаружены представители первичноротых животных типа Entoprocta, которые ранее не были отмечены в водоемах Байкальского региона.

Ключевые слова: ДНК метабаркодинг, 18S рРНК, ITS1–5.8S–ITS2, COI, 16S рРНК

Для цитирования: Перетолчина Т.Е., Щербакова А.А., Ситникова Т.Я., Адельшин Р.В., Букин Ю.С. Эукариотические сообщества пресноводных мшанок (Phylactolaemata: Plumatellidae) Байкальского региона // Limnology and Freshwater Biology. 2025. - № 3. - С. 268-283. DOI: 10.31951/2658-3518-2025-A-3-268

1. Введение

Мшанки (тип Bryozoa) принадлежат колониальным животным, ведущим прикрепленный образ жизни. Селятся на любом твердом субстрате – затонувшей древесине, макрофитах, камнях, пластике, полиэтилене и др. Некоторые виды обрастают днища кораблей (Гонтарь, 2010).

В типе выделяют три класса: Phylactolaemata (Покрыторотые), Stenolaemata (Узкоротые) и Gymnolaemata (Голоротые). Phylactolaemata включает один отряд, семь семейств и около 70 видов, обитающих исключительно в пресных водах (Ryland, 2005). Представители этого класса широко распространены по всему миру, за исключением полярных областей, и населяют лентические и лотические экосистемы, некоторые виды считаются космополитами (Wood, 2002). Колонии филактолемат состоят из зооидов, в которых выделяют два отдела – мягкий полипид с венчиком щупалец и желатинизированный (или хитинизированный) цистид, являющийся экзоскелетом, в него втягивается полипид. Внутри венчика щупалец находится ротовое отверстие, прикрытое эпистомом.

Среди филактолемат наиболее разнообразно сем. Plumatellidae Allman, 1856, насчитывающее 4 рода и более 20 валидных видов, из которых наибольшее число принадлежит роду *Plumatella* Lamark, 1816. Для пресных вод России отмечено 7 видов рода (Гонтарь, 2010), три из них встречены в Байкальском регионе (Виноградов, 2008).

Филактолематы нередко доминируют среди гидробионтов. В ряде случаев, как активные фильтраторы, при обильном развитии играют огромную роль в самоочищении водоема (Протасов, 1994). Размер колоний варьирует от нескольких мил-

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Поступила: 17 декабря 2024; ***Принята:*** 22 апреля 2025; ***Опубликована online:*** 11 июня 2025

лиметров до десятков сантиметров. Их быстрый рост может привести к высокой биомассе бентоса. Таким образом, мшанки также играют важную роль в круговороте питательных веществ (Sørensen et al., 1986). Пресноводные мшанки могут образовывать ассоциации с другими мшанками, губками, гидроидами и личинками ручейников (Ricciardi and Reiswig 1994). Их колонии обеспечивают среду обитания для различных мелких организмов, таких как коловратки, планарии, нематоды, аннелиды, моллюски, гастротрихи, копеподы, тардиграды, личинки насекомых, клещи и др. (Raddum and Johnsen, 1983; Ricciardi and Reiswig, 1994).

Виды *Plumatella* имеют разные по форме и размерам колонии, что позволяет предполагать существование различных по составу сообществ, ассоциированных с телом колоний. Для проверки этой гипотезы мы использовали современный молекулярно-генетический метод – ДНК метабаркодинг, который позволяет идентифицировать таксономический состав сообщества путем амплификации и высокопроизводительного секвенирования последовательностей маркерных генов. Метабаркодинг сообществ, ассоциированных с определенным организмом, позволяет также предполагать механизмы и типы взаимодействий между разными группами организмов и их возможную экологическую роль в сообществе.

ДНК метабаркодинг с использованием фрагмента гена COI успешно применяется при исследовании сообществ беспозвоночных животных в бухте большие Коты и Лиственичном заливе озера Байкал (Кравцова и др., 2021; Кравцова и др., 2023). Кроме того, этот метод показал свою эффективность при исследовании разнообразия организмов, ассоциированных с зелеными водорослями на основе фрагмента гена 18S рРНК (Букин и др., 2022), а также применялся при исследовании микроэукариотических планктонных сообществ Байкала (Bukin et al., 2023).

Целью работы было исследовать и сравнить состав сообществ эукариотических организмов, ассоциированных с двумя видами мшанок, обитающих симпатрически и различающихся морфологией колоний.

2. Материалы и методы.

Колонии мшанок были собраны с затопленной древесины в устье р. Томпуда (север оз. Байкал, 55,12122° N 109,753418° E) в 2022 году (Рис. 1). Вблизи устья долина реки имеет характер заболоченной равнины, изрезанной многочисленными протоками, заросшими макрофитами и с большим количеством затопленных бревен. Почти на каждом из них встречались мшанки. Колонии собраны с одного затопленного бревна, чтобы исключить влияние местообитания на таксономический состав организмов, ассоциированных с ними.

Прижизненные фотографии фрагментов колоний (Рис. 1) сделаны под стереоскопическим микроскопом МСП–1 (ОАО «Ломо»), оснащенным цифровой фотокамерой Levenhuk C800.

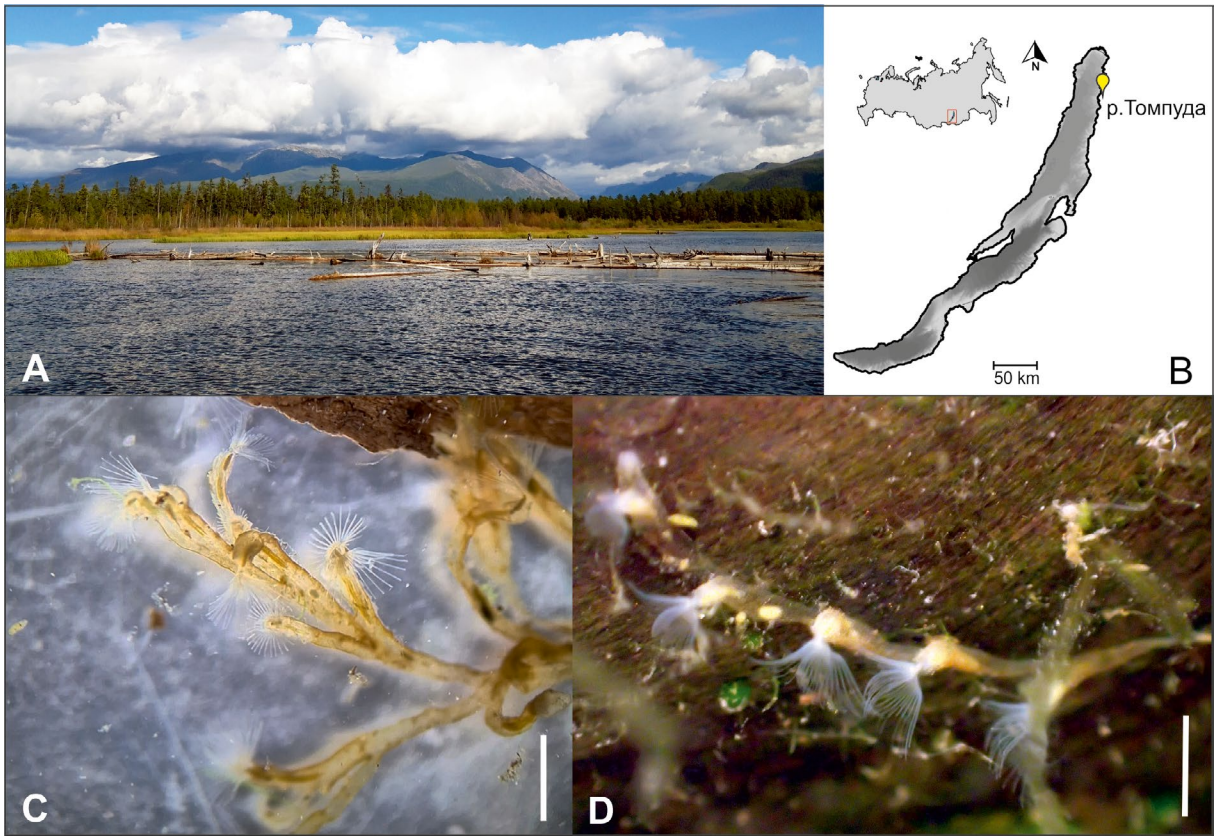

Рис.1. A – фото устья р. Томпуда; Карта оз. Байкал с указанной точкой сбора; C, D – прижизненное фото колоний мшанок. C – *Plumatella* sp.; D – *P. repens*. Масштаб – 1 мм.

ДНК экстрагирована по протоколу, описанному Дойлом и Диксон (Doyle and Dickson, 1987). Препараты ДНК для последующего секвенирования по Сэнгеру получены из небольших фрагментов колоний, которые предварительно тщательно очищали, чтобы избежать контаминации организмами, обитающими на мшанках. ДНК для метабаркодинга экстрагировали из больших фрагментов колоний (по пять для каждого вида).

Все полученные препараты ДНК были амплифицированы. Амплификация проведена с помощью набора реактивов BioMaster HS-*Taq* PCR Kit (Biolabmix, Россия), согласно рекомендациям производителя. Условия амплификации и структура праймеров приведены в таблице 1.

Продукты реакции проанализированы электрофоретически в 1%-ном агарозном геле. В случае метабаркодинга, ПЦР-продукты объединяли в одну пробирку для каждого вида.

Ферментативная очистка продуктов проведена с помощью коммерческого набора ExoSAP-IT express (Thermo Fisher Scientific, США) согласно протоколу, рекомендованному производителем.

Секвенирование маркеров COI и 16S мтДНК и ITS1–5.8S–ITS2 яДНК проведено на генетическом анализаторе «НАНОФОР 05» с помощью набора реагентов Brilliant Dye Terminator (v.3.1) Sequencing kit (NimaGene, Голландия). Нуклеотидные последовательности отредактированы и выровнены в программе BioEdit (Hall, 2011).

Таксономическую принадлежность исследуемых мшанок определяли методом ДНК баркодинга с помощью маркеров COI, 16S мтДНК и ITS1–5.8S–ITS2 яДНК. Делимитация таксонов проведена с помощью веб-версии программы ASAP, доступной по ссылке: https://bioinfo.mnhn.fr/abi/public/asap/. В качестве меры генетических расстояний использовали *p*-дистанции. Дополнительно из базы данных GenBank привлечены нуклеотидные последовательности разных видов сем. Plumatellidae по маркерам COI, 16S и ITS1–5.8S–ITS2 (Таблица 2).

ДНК метабаркодинг ассоциированных с мшанкой эукариот проведен по гену 18S рРНК с помощью нанопорового секвенирования с использованием портативного ДНК-секвенатора MinION (Oxford Nanopore Technologies). Качество полученных прочтений проверено с помощью программы FastQC. Последовательности отсортированы по качеству в Trimmomatic-0.32 (Bolger et al., 2014), для анализа выбраны последовательности со средним качеством прочтения более 12 баллов. Идентификация последовательностей проводилась по базе данных SILVA (Quast et al., 2012). В анализе использована версия базы 138.2, из которой были извлечены полноразмерные фрагменты 18S рРНК. Сравнение расшифрованных фрагментов ампликонной библиотеки с базой SILVA проводилась с помощью алгоритма BLASTn (Altschul et al., 1990) с параметрами word_size = 25, gapopen = 2, gapextend = 1, reward = 1, penalty = -1. Выбранный диапазон параметров позволяет идентифицировать последовательности ампликонов с низкой степенью родства со сравниваемой базой данных. Последовательность 18S рРНК ампликона считалась идентифицированной при параметрах выравниваний E-value $\leq$ 0,00001 и bit score $\geq$ 50, что характеризует неслучайное совпадение. Все последовательности 18S рРНК ампликона, картирующиеся по результатам BLASTn выравниваний на одну и туже последовательность базы SILVA были сгруппированы в OTE (операционная таксономическая единица). Представленность OTE в пробе характеризовалась количеством приходившихся на него прочтений 18S рРНК ампликона.

Результаты визуализированы в виде гистограммы, на которой OTE объединены в таксоны высокого ранга.

Статистическая сходимость результатов оценки таксономического разнообразия охарактеризована с помощью кривых насыщения и индекса Chao1 (индекс ожидаемого числа OTE в пробах). Графическое сравнение разнообразия сообществ проводилось с помощью кривых таксономического

Таблица 1. Условия амплификации и структура праймеров.

Ген	Условия амплификации, 30 циклов	Структура праймеров	Ссылки
COI	денатурация ДНК: 95°C – 40с (5 минут на первом цикле), отжиг праймеров – 50°C - 60с, элонгация нуклеотидной цепи – 72°C – 60с (10 минут на последнем цикле)	LCO1490 (f) 5’-GGT CAA CAA ATC ATA AAG ATA TTG G -3’ HCO2198 (r) 5’-TAA ACT TCA GGG TGA CCA AAA AAT CA-3’	Folmer et al., 1994
ITS1–5,8S–ITS2		ITS1 (f) 5’-TCC GTA GGT GAA CCT GCG G-3’ ITS4 (r): 5’-TCC TCC GCT TAT TGA TAT GC-3’	White et al., 1990
18S	денатурация ДНК: 95°C – 60с (5 минут на первом цикле), отжиг праймеров: 55°C - 60с, элонгация нуклеотидной цепи: 72°C – 60с (10 минут на последнем цикле)	EukA (f) 5’-ACC TGG TTG ATC CTG CCA GT-3’ EukB (r) 5’-TGA TCC TTC TGC AGG TTC ACC TAC-3’	Medlin et al., 1988
16S		ZX-1-F 5’-ACC CGC TGA ATT TAA GCA TAT-3’ LSUD-R 5’-ACG GAA TGA ACT CAA ATC ATG TAA G-3’	Van der Auwera et al., 1994 Littlewood et al., 2000

Таблица 2. Список таксонов, использованных для делимитации таксонов с номерами доступа в GenBank и ссылками.

Название вида	COI GB#	Ссылки	ITS1–5,8S–ITS2 GB#	Ссылки	16S GB#	Ссылки
Plumatella fungosa (Pallas, 1768)	KF805632	Dash and Vasemägi, 2014, неопубликовано	GU733426	Rubini et al., 2011	AB365624	Hirose et al., 2011, неопубликовано
	MH286272	Klass et al., 2018	-	-	-	-
Plumatella repens (Linnaeus, 1758)	FJ196105	Fuchs et al., 2009	GU733417	Rubini et al., 2011	AB365622	Hirose et al., 2011, неопубликовано
	-	-	EU377576	Taticchi et al., 2010, неопубликовано	-	-
Plumatella vaihiriae (Hastings, 1929)	-	-	EU377577	Taticchi et al., 2010, неопубликовано	AB365625	Hirose et al., 2011, неопубликовано
Plumatella casmiana Oka, 1907	KJ024813	Koletic, 2014	EU377579	Taticchi et al., 2010, неопубликовано	GQ343297	Briski et al., 2011
	-	-	-	-	AB365629	Hirose et al., 2011, неопубликовано
Plumatella rugosa Wood, Wood, Geimer & Massard, 1998	-	-	GU733418	Rubini et al., 2011	AB365623	Hirose et al., 2011, неопубликовано
Plumatella viganoi Taticchi 2010	-	-	GU733422, GU733421, GU733420	Rubini et al., 2011	-	-
Plumatella geimermassardi Wood and Okamura, 2004	-	-	GU733423	Rubini et al., 2011	-	-
	-	-	EU377578	Taticchi et al., 2010, неопубликовано	-	-
Plumatella emarginata Allman, 1844	-	-	GU733424	Rubini et al., 2011	JN681057	Waeschenbach et al., 2012
	-	-	-	-	AB365623	Hirose et al., 2011, неопубликовано
	-	-	-	-	GQ343296, GQ343300, GQ343301	Briski et al., 2011
Plumatella reticulata Wood, 1988	-	-	GU733425	Rubini et al., 2011	-	-
Plumatella vorstmani Toriumi, 1952	-	-	-	-	AB365634	Hirose et al., 2011, неопубликовано
Plumatellidae sp.	KU720129	Jiang et al., 2017	-	-	-	-
	KX620051	Dong et al., 2020	-	-	-	-
Hyalinella punctata (Hancock, 1850)	KJ024814	Koletic, 2014	EU377580	Taticchi et al., 2010, неопубликовано	AB365631	Hirose et al., 2011, неопубликовано
	-	-	GU733427, GU733428	Rubini et al., 2011	DQ305342	Okuyama et al., 2006

обилия с учетом того, что чем медленнее кривая стремится к нулю, тем разнообразнее сообщество. Разнообразие исследуемых сообществ оценивали с помощью индекса Шеннона. Все статистические расчеты и графическая визуализация результатов проведены с использованием пакета «Vegan» (Dixon, 2003) и «ggplot2» (Wickham, 2011) для среды программирования R.

3. Результаты

По внешнему строению зооидов и колоний, исследуемые мшанки отнесены к двум видам рода *Plumatella* (Рис. 1). Для каждого вида получено по две нуклеотидные последовательности для маркеров COI (629 п.н.), 16S (439–440 п.н.), ITS1–5.8S–ITS2 (738–742 п.н.) и депонировано в базу данных GenBank с номерами доступа: PQ766342–PQ766343, PQ771669–PQ771670 (COI); PQ770921–PQ770922, PQ772041–PQ772042 (ITS1–5.8S–ITS2); PQ774235–PQ774238 (16S).

По результатам делимитации таксонов с использованием трех молекулярно-генетических маркеров мшанку со «стелющимся» типом колонии отнесли к *Plumatella repens* (Linnaeus, 1758), в то время как мшанку с «кустистым» типом коло-

нии (*Plumatella* sp.) не удалось точно определить до вида, из-за отсутствия достаточного количества нуклеотидных последовательностей в GenBank. По фрагменту 16S мтДНК сестринским видом для этой мшанки является *P. emarginata* Allman, 1844 (генетическая дистанция составила 2% нуклеотидных замен), а по ITS1–5.8S–ITS2 она оказалась близка к *P. vaihiriae* (Hastings, 1929) (генетическая дистанция 0,4% замен). По фрагменту гена COI делимитацию таксонов провести не удалось из-за отсутствия нуклеотидных последовательностей *P. emarginata* и *P. vaihiriae* в GenBank.

Исходные наборы данных после нанопорового секвенирования гена 18S рРНК включали 44771 последовательностей для *Plumatella* sp. и 51057 – для *P. repens*, после фильтрации по качеству наборы включали 19027 и 21762 последовательностей, соответственно, из которых были сформированы OTE. Одиночные OTE (представленность меньше 4 копий на пробу) не принимались в расчет. В итоге, для *Plumatella* sp. идентифицировано 299 OTE, для *P. repens* – 33 OTE.

Графики с кривыми насыщения и таксономического обилия представлены на Рис. 2. Кривые насыщения для обоих видов выходят на плато, что подтверждает результат, как статистически достоверный. Ожидаемое число таксонов, рассчитанное с помощью индекса Chao1, совпадает с фактическим результатом выделенных OTE.

Индекс биоразнообразия Шеннона для сообщества, ассоциированного с «кустистой» мшанкой *Plumatella* sp., составил 4,1, а со «стелющейся» *P. repens* – 2,8.

Таксономический состав (в процентном соотношении) обоих сообществ, представлен на диаграмме (Рис. 3). В составе сообществ отмечен 21 таксон высокого ранга. На «кустистых» мшанках преобладали диатомовые водоросли (60%), инфузории (22%), а также золотистые водоросли (4%) и гидры (3%). Кроме того, в этой пробе обнаружены хитридиомицеты (1,6%), являющиеся паразитами водорослей и беспозвоночных животных. Также среди организмов, ассоциированных с «кустистой» мшанкой, были коловратки (1%), возможно, привлекаемые большим количеством инфузорий, одноклеточных водорослей и бактерий, которыми они питаются.

На «стелющейся» мшанке биоразнообразие меньше. Преобладали одноклеточные водоросли (32%) и динофлагелляты (27%), встречались апикомплексы (10,6%), являющиеся паразитами беспозвоночных животных и другие классы простейших (амебы, эвглены и др.) (4,6%). Среди беспозвоночных животных преобладали кольчатые черви (12,5%), ленточные черви (4%) и моллюски (3%). Обнаружены также нуклеотидные последовательности первичноротых животных типа Entoprocta.

4. Обсуждение

В устье реки Томпуда, впадающей в Байкал, выявлено симпатрическое обитание двух генетически далеких (16% замен по COI, 9% по ITS1–5.8S–ITS2, 4% по 16S) видов рода *Plumatella*. Один из них – *P. repens,* широко распространен в водоемах умеренной зоны. Другой вид – *Plumatella* sp. – по маркеру ITS1–5.8S–ITS2 оказался генетически сходным (0,4% замен) с *Plumatella vaihiriae* (Hastings, 1929), который был описан из озера на о. Таити и обнаружен в озерах Гавайев, Аргентины, Тайланда, США (Taticchi et al., 2008). По маркеру 16S (2% замен) *Plumatella* sp. является сестринским к *P. emarginata*, распространенному в Евразии, Северной Америке и Новой Зеландии. Следует отметить, что присутствие *P. emarginata* отмечалось ранее и в Байкальском регионе (Виноградов, 2008), однако обнаруженные нами мшанки по уровню генетических отличий, скорее всего, представляют собой самостоятельный вид (возможно, новый для науки), для точной таксономической идентификации которого необходим морфологический анализ статобластов.

Совместное обитание 2 видов мшанок на одном субстрате, не является уникальным для Байкальского региона, например Taticchi et al. (2008) показали совместное обитание *P. vaihiriae* с *P. fungosa* (Pallas, 1768), а также представителями Victorellidae Hincks, 1880 в озерах Италии.

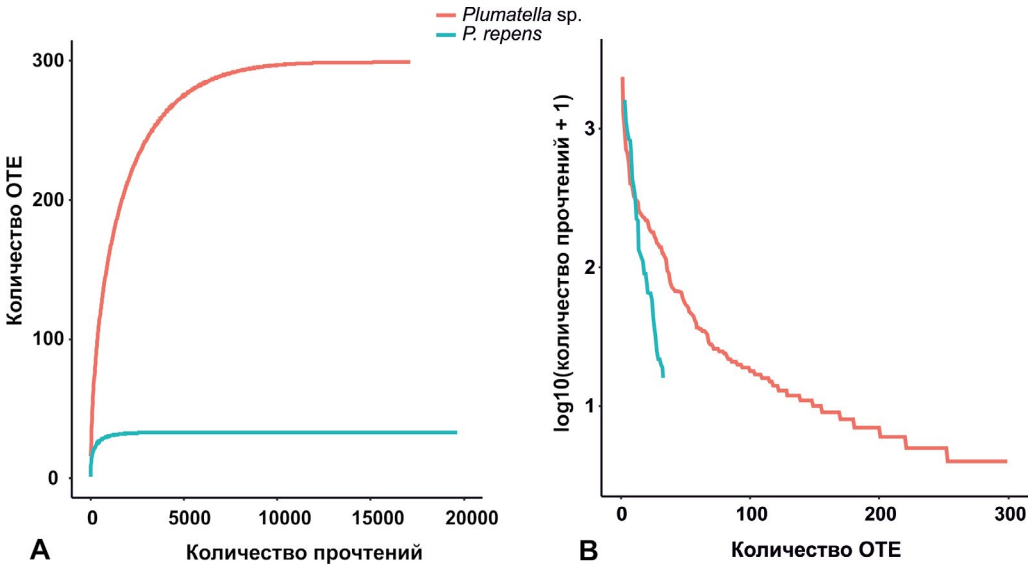

Рис.2. A – кривая насыщения; B – кривая обилия.

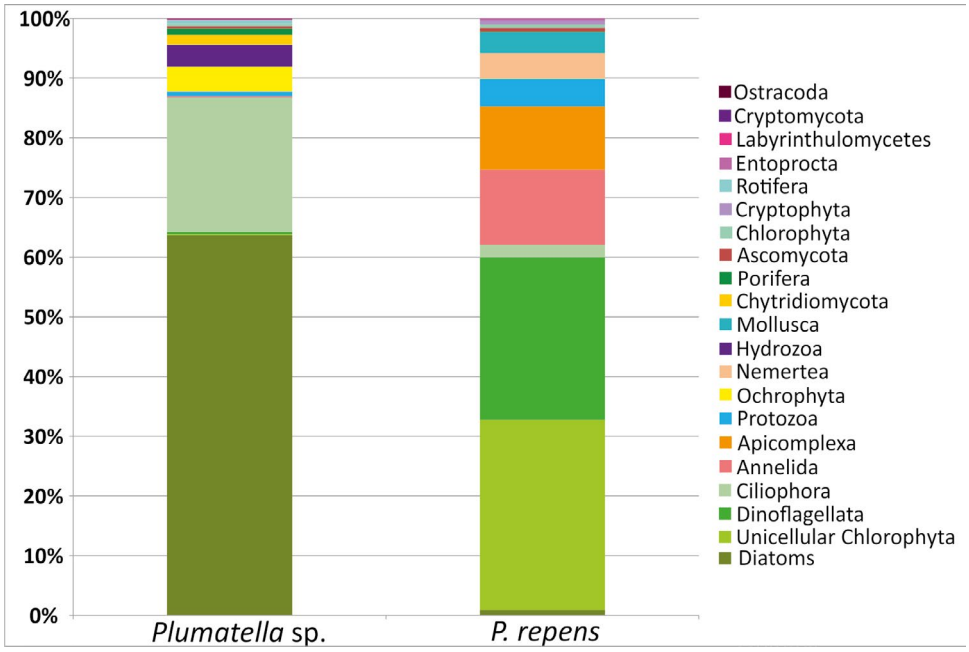

Рис.3. Гистограмма распределения OTE по таксонам высокого ранга для *Plumatella* sp. и *P. repens*.

Исследованные мшанки имеют разные формы колоний: *P. repens*, в соответствии с названием, – ползучая или стелющаяся – образует ветвистые трубки, плоско стелющиеся по субстрату, *Plumatella* sp. имеет трубчатые разветвленные зооиды, высоко поднимающиеся над субстратом (по типу куста). Мы обнаружили, что для каждого вида сосуществующих мшанок свойственно своеобразное сообщество ассоциированных с ними эукариот, состав которого, вероятно, зависит от формы колоний. Сообщество организмов, ассоциированное с «кустистой» *Plumatella* sp., оказалось намного разнообразнее (индекс Шеннона = 4,1) сообщества, ассоциированного с колонией «стелющейся» *P. repens* (индекс Шеннона = 2,8) как по составу таксонов высокого ранга, так и по числу доминирующих OTE. Это может быть связано с тем, что структура «кустистой» колонии формирует пространства между зооидами с микросредой, благоприятной для обитания разнообразных эпибионтов. К зооидам мшанок легко крепятся различные диатомовые водоросли, о чем свидетельствует приходящееся на них большое количество прочтений. На стелющейся колонии, биоразнообразие значительно ниже. Встречаются крупные беспозвоночные, которые, возможно, питаются самой мшанкой, простейшие типа Apicomplexa, являющиеся паразитами беспозвоночных.

Интересной находкой стало обнаружение первичноротых животных типа Entoprocta Nitsche, 1870 или Kamptozoa Cori, 1929 (Внутрипорошицевые или Сгибающиеся), которые ранее не были отмечены в водоемах Байкальского региона. Эти животные внешне напоминают гидроидных и мшанок, ведут прикрепленный образ жизни, живут поодиночке или в колониях, размер одного организма 1–5 мм. Внутрипорошицевые чаще всего являются комменсалами на беспозвоночных, таких как губки, неподвижные кольчатые черви, раковины моллюсков и мшанки (Brusca and Brusca, 2003; Emschermann, 1993; Kristensen, 1970; Wood, 2005). Возможно, «стелющаяся» форма колонии *P. repens* позволяет прикрепляться представителям Entoprocta, а ток воды, создаваемый лофофорами мшанки, облегчает их питание. Поскольку представители данного типа крайне мало изучены, в базах данных практически нет информации об их нуклеотидных последовательностях, поэтому идентификацию удалось провести только на уровне таксона высокого ранга (Entoprocta: Barentsiidae Emschermann, 1972). В настоящее время известно около 200 видов Entoprocta включающих сидячих, одиночных (сем. Loxosomatidae) или колониальных (сем. Loxokalypodidae, Pedicellinidae и Barentsiidae), в основном морских организмов. Известно всего два вида, которые обитают в пресных водах: *Loxosomatoides sirindhorne* Wood, 2005 (сем. Loxosomatidae) и *Urnatella gracilis* Leidy, 1851 (сем. Barentsiidae). Причем ареал *L. sirindhorne* ограничен Тайландом (Wood, 2005; Schwaha et al., 2010), в то время как *U. gracilis*, изначально описанный как Североамериканский вид, распространен на всех континентах, кроме Антарктиды (Brusca and Brusca, 2003), обнаружен в реках Дон (Склярова, 1969) и Волга (Виноградов, 1997). Возможно, именно этот вид Entoprocta встречается в водоемах Байкальского региона, хотя это предположение требует проведения детального морфологического анализа.

ДНК метабаркодинг с использованием гена 18S рРНК оказался эффективным методом для исследования разнообразия ассоциированных с мшанками эукариотических сообществ, о чем свидетельствует широкий спектр выявленных таксонов. Графики насыщения и обилия, а также индекс ожидаемого числа видов в пробах *Chao1* подтверждают статистическую достоверность результата. Тем не менее, следует обратить внимание, что при нанопоровом секвенировании не исключены ошибки в виде вставок и делеций нуклеотидов в последовательностях 18S рРНК. Анализ результатов BLASTn выравниваний последовательностей ампликонов с базой данных SILVA показал, что в некоторых слу-

чаях степень сходства между последовательностями 18S рРНК, варьирующая в пределах от 99% до 97%, определялась только наличием однобуквенных вставок и делеций, а не нуклеотидными заменами, что исключено при секвенировании коротких фрагментов на платформах типа Illumina. Данную особенность портативного ДНК-секвенатора MinION (Oxford Nanopore Technologies) следует учитывать в дальнейшем разработчикам программного обеспечения для анализа ампликонов при метабаркодинговых исследованиях.

5. Выводы

Результаты ДНК метабаркодинга по гену 18S рРНК выявили специфичность состава ассоциированных с мшанками эукариотических сообществ. Показано, что форма колоний имеет критическое значение для формирования их видового богатства. Сообщество, ассоциированное с *Plumatella* sp., имеющего «кустистую» форму колонии, гораздо разнообразнее, и отличается по видовому составу от сообщества, сформировавшегося на «стелющейся» колонии *P. repens*.

Применение молекулярно-генетических методов позволило выявить среди организмов, ассоциированных с *P. repens* представителей первичноротых животных Entoprocta, что представляет интерес с точки зрения изучения биоразнообразия водоемов. Новые находки первичноротых Entoprocta в составе фауны водоемов Байкальского региона свидетельствуют о том, что их ареал шире, чем предполагалось ранее.

Благодарности

Работа выполнена при поддержке бюджетных проектов 121032300196-8 и 121032300180-7. Авторы выражают благодарность команде НИС «Титов» (ЦКП «Центр комплексных судовых исследований Байкала») за помощь в отборе проб. Секвенирование образцов проведено в Приборном центре коллективного пользования физико-химического ультрамикроанализа ЛИН СО РАН (ЦКП «Ультрамикроанализ»).

Конфликт интересов

Авторы заявляют об отсутствии конфликта интересов.

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Original Article

LIMNOLOGY FRESHWATER BIOLOGY

Decoding the Influence of Water Quality and Seasonal Shifts on Phytoplankton Communities in Eastern Indian Freshwater Waterbodies

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ABSTRACT. This study presents a comparative analysis of phytoplankton dynamics and ecological status across two freshwater ponds in Birbhum, West Bengal, India, for the two years (from April 2020 to March 2022). The two study sites included a fish cultivation pond (S1) and an agricultural waste pond used for irrigation (S2). Phytoplankton productivity and environmental parameters, including chlorophyll-a content, temperature, pH, dissolved oxygen (DO), biological oxygen demand (BOD), gross primary productivity (GPP), net primary productivity (NPP), and nutrient levels (nitrate, phosphate, ammonia, silicate, and chloride) were monitored. Both sites were exposed to similar temperature ranges (12˚C to 38˚C), but S2 was more alkaline than S1. Chlorophyll-a content ranged from 1.84 to 5.78 mg/L in S1 and 1.22 to 3.68 mg/L in S2. Nutrient concentrations peaked during post-monsoon period, supporting enhanced phytoplankton growth, and were minimum in summer for both sites. Principal component analysis revealed that nitrate, phosphate, and silicate were primary influencers for S1, while pH, nitrate, phosphate, ammonia, and chloride were influential for S2. GPP and NPP emerged as common factor in both ponds. Correlation analysis indicated that chlorophyll-a in S1 was positively associated with nitrate, phosphate, silicate, and GPP-NPP, whereas, in S2, it correlated positively with pH, nitrate, phosphate, ammonia, and chloride. The post-monsoon season exhibited the highest phytoplankton diversity, dominated by chlorophycean species in S1 and Euglenophyceae in S2, the latter likely due to elevated ammonia levels.

Keywords: Phytoplankton dynamics, physicochemical parameters, nutrient variation, correlation coefficient, PCA

For citation: Garai S., Mondal A., Dey I., Bose R., Pal R. Decoding the Influence of Water Quality and Seasonal Shifts on Phytoplankton Communities in Eastern Indian Freshwater Waterbodies // Limnology and Freshwater Biology. 2025. - № 3. - P. 284-297. DOI: 10.31951/2658-3518-2025-A-3-284

1. Introduction

Phytoplankton growth and productivity controlled by environmental parameters indicated the ecological status of ponds and wetlands used for various purposes (Singha Roy et al., 2018, Dey et al., 2021). They are the chief primary producers and efficient bio-indicators for water quality assessment (Brraich and Kaur, 2015). In an aquatic ecosystem, the base of the food chain is maintained by phytoplankton population (Tas and Gonulal, 2007). Seasonal variation of productivity and diversity of phytoplankton are influenced by different physical, chemical and biological parametersand therefore play a significant role in fish growth and diversity in a particular ecosystem (Angelini and Petrere, 2000; Saifulla et al., 2016). Kaparapu and Gwddada (2015) reported temperature, total phosphorus and nitrate to play major roles in phytoplankton dynamics of reservouirs throughout the year. Bose et al. (2016) investigated phytoplankton diversity from different ecological niches of West Bengal, like freshwater lotic & lentic ponds, oligotrophic and eutrophic water bodies, shallow and deep lakes, and recorded more than 70 microplanktonic taxa belonging to 11 families of Cyanobacteria and 11 families of Chlorophyta. After a thorough study on Santragachi Lake of West Bengal, Barinova et al. (2012) revealed that phytoplankton density became high with increasing temperature and nutrients, where Chlorophycean species dominated over Euglenozoa species during the post-monsoon but minimum during the monsoon period. Bhavya et al. (2016) recorded ammonia as a preferred substrate

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Received: February 12, 2025; ***Accepted:*** April 24, 2025;
Available online: June 11, 2025

for phytoplankton growth both in estuary and coastal waters. Nag and Gupta (2014) analysed physicochemical parameters of some waste ponds in and around Santiniketan of Birbhum district and reported huge variation in physicochemical parameters due to anthropogenic activities. Several authors (Ghosh et al., 2012; Saifulla et al., 2016; Singha Roy et al., 2018) reported positive relation of phytoplankton density with temperature and nutrients. Choudhury and Pal (2010) also reported growth of phytoplankton to be positive with dissolved oxygen, salinity and pH and negative with nitrate, silicate, and BOD in the marine environment.

Seasonal variation of phytoplankton production was reported by several authors. Choudhury and Pal (2011, 2012) concluded that the growth of blue-green and green algal populations were maximum during warmer conditions of summer and monsoon months and diatom population dominated in autumn and winter in estuary water. They found that the total phytoplankton density was highest in winter and lowest during monsoon seasons due to dilution of phytoplankton cells by rainwater. After investigation on a lentic water body in Howrah district Ghosh and Keshri (2011) reported highest phytoplankton diversity and distribution during pre-monsoon and lowest in monsoon. After a thorough study from coastal waters, Vajravelu et al. (2018) reported maximum phytoplankton population density during pre-monsoon and minimum during monsoon. After a thorough study from freshwater ponds of the Hooghly district, Halder et al. (2019) reported that dissolved oxygen, electrical conductivity, pH, light intensity and inorganic phosphorus have important roles in occurrence of microalgal taxa and dominance of chlorophycean members throughout the year.

This research offers a thorough examination of the dynamics of phytoplankton in two divergent freshwater ecosystems, emphasizing the influence of environmental conditions and nutrient availability on the structure and productivity of the community. This research employs a comparative approach, which enables us to distinguish the ecological responses and phytoplankton dynamics of a fish cultivation pond (Site S1) and an agricultural runoff pond (Site S2) that are located in the same region, in contrast to previous studies that have primarily concentrated on individual water bodies only. From this background knowledge, it has been found that a very few studies have done till now about the fresh water phytoplankton diversity in relation to nutrient parameters from Birbhum district with laterite soils of eastern India. Thus, an initiative has been taken to determine the phytoplankton productivity in relation to Chlorophyll-a content with several environmental parameters of two different fresh water ecosystems of Birbhum district in West Bengal- one is used for fish cultivation (S1) and another one for agricultural purposes (S2) surrounded by agricultural field.

2. Material and methods

2.1. Study area

Surface water samples were collected from two physiologically different freshwater ponds located in Birbhum district, West Bengal, India (Fig. 1). Study Site 1 (S1) is the Gangasagar pond in Bolpur, primarily used for freshwater fish cultivation (Latitude: $23^0$39'8" N to $23^0$39'12" N; Longitude: $87^0$41'59"E to $87^0$42'3"E; Total Area: 15,625 m^2). This pond retains a stable water level year-round, with an average depth of 1.5 $\pm$ 0.5 meters, supported by seasonal rainfall and groundwater inputs. The surrounding area is sparsely vegetated, mostly with grasses and aquatic plants, which contribute to habitat structure and nutrient cycling. The pond is subject to occasional organic matter input from fish feed and local vegetation, impacting water chemistry. Study Site 2 (S2) is an agricultural runoff pond (Latitude: 23º40'12" N to 23º40'16" N; Longitude: 87º42'48" E to 87º42'52" E; Total Area: 14,640 m^2), with an average depth of 1.3 $\pm$ 0.5 meters. This pond receives nutrient-rich agricultural runoff from nearby cropland, especially following the monsoon season, which elevates levels of nitrate, phosphate, and other nutrients. Surrounding this pond are fields of rice, mustard, and other seasonal crops that contribute varying levels of sediment and agrochemical residue.

Both sites experience a subtropical monsoon climate, with significant temperature variation (12˚C - 38˚C annually) and distinct wet (June to September) and dry seasons. Rainfall predominantly during the monsoon season affects water quality and nutrient input, influencing phytoplankton dynamics. Additionally, differences in the primary use of these ponds—fish cultivation for S1 and agricultural runoff collection for S2—result in distinct water quality profiles, ecological processes, and seasonal productivity patterns.

2.2. Phytoplankton sampling and identification

Phytoplankton samples were collected from both ponds every 15 days over a two-year period (April 2020 - March 2022), capturing seasonal variations across Summer, Monsoon, Post-monsoon, and Winter. To ensure consistent sampling conditions, all water samples were collected between 8:00 and 10:00 AM, a timeframe chosen to reflect typical diurnal activity levels of phytoplankton and minimize fluctuations due to photosynthetic variation.

Sampling was conducted at the surface layer (0.5 meters depth) to capture the phytoplankton communities that thrive in the photic zone. Using a 20-liter water sampler, 100 litre of water was collected at each site by retrieving five 20-liter subsamples (20 L $\times$ 5 = 100 L total). These subsamples were then pooled and filtered through a 20 μm mesh phytoplankton net to concentrate the phytoplankton biomass, ensuring a representative collection of the community structure at each site. Sampling was performed in triplicate to increase data reliability.

The retained phytoplankton biomass was gently rinsed off the net and combined into a single sample for each site and sampling time. Samples were immediately centrifuged to further concentrate the biomass, then preserved with 4% neutralized formaldehyde to maintain cellular integrity for subsequent analysis.

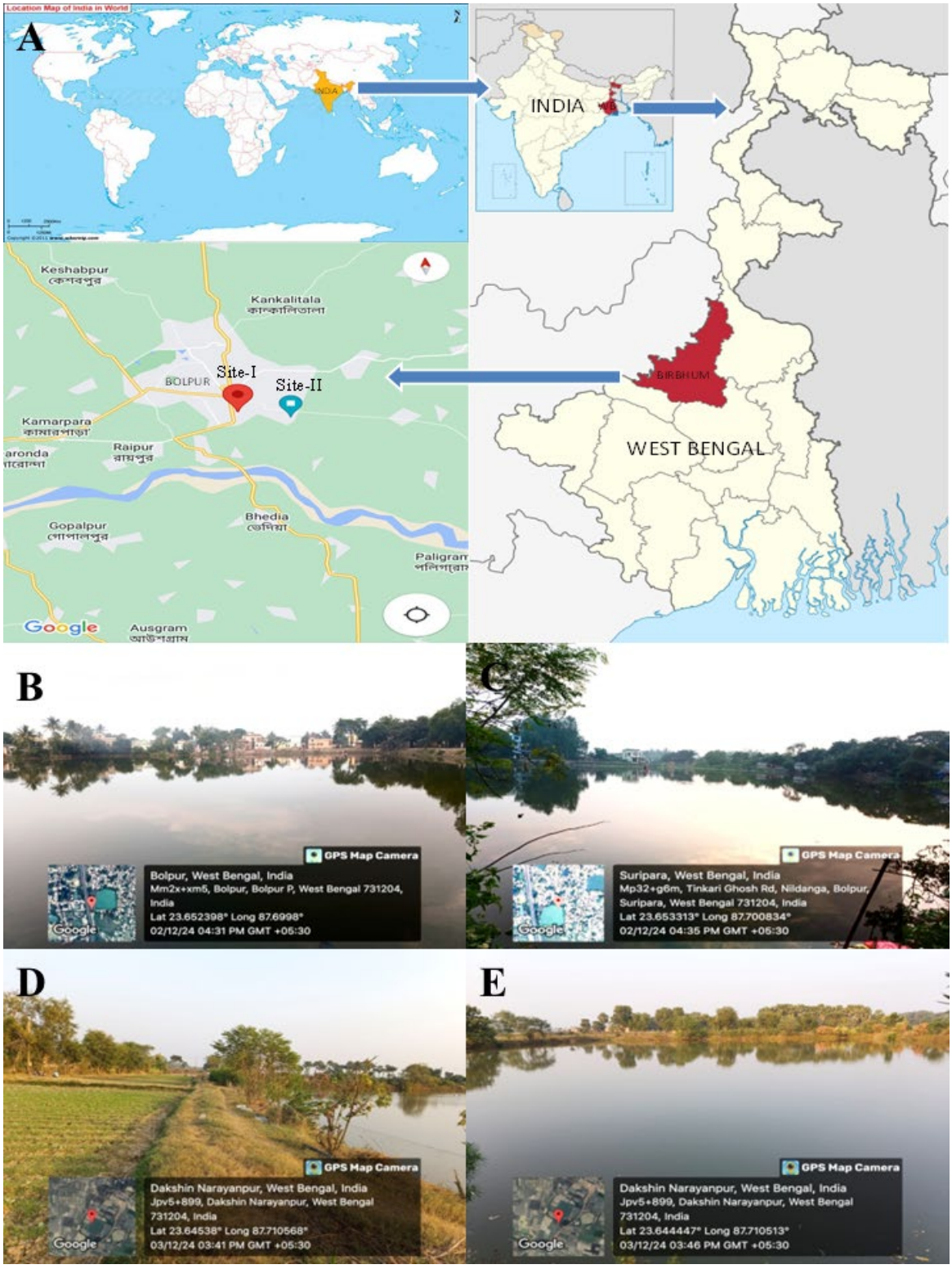

Fig.1. A-Location of study sites B,C Site 1 (S1) and D,E Site 2 (S2).

For microscopic examination, a 200 µl aliquot of the concentrated sample was placed on a glass slide, covered with a cover slip, and examined under a compound microscope (Carl Zeiss Axiostar) at magnifications of 10X, 40X, and 100X. Phytoplankton were identified by morphology and other distinctive features, following standard taxonomic references (Phlipose, 1967; Prescott, 1961; Prescott, 1982; Desikachary, 1989; Komárek and Anagnostidis, 2005; Das and Adhikary, 2014), and cross-verified using Algaebase (Guiry and Guiry, 2002) for confirmation.

2.3. Physico-chemical parameter analysis

Water samples were collected from both sites and immediately filtered through a 20 µm phytoplankton net to remove large particulates and debris. The filtered water samples were then transferred to PVC amber bottles to minimize light exposure and prevent any photochemical changes. Samples were insulated in ice buckets and promptly transported to the laboratory to minimize alterations in water quality parameters.

Upon arrival, several key physicochemical parameters were measured following standard procedures outlined by APHA (2000). Water temperature was recorded in situ using a calibrated glass mercury thermometer (Labworld, -10˚C to 110˚C) at the sampling depth (0.5 meters), while pH was measured on-site with an Ionix digital pH meter, ensuring immediate and accurate readings.

Dissolved Oxygen (DO) was measured using Winkler's iodometric titration method, known for its accuracy in assessing oxygen concentration directly in the field. Biochemical Oxygen Demand (BOD) was determined by incubating the samples at 20˚C for 5 days, following the APHA standard protocol, to assess the organic load in each pond.

In the laboratory, nutrient concentrations (nitrate, nitrite, phosphate, ammonia, silicate, and chloride) were analyzed using spectrophotometric methods. These nutrient levels provided insights into the eutrophic conditions of the ponds and were crucial for understanding phytoplankton growth patterns and seasonal dynamics.

Gross Primary Productivity (GPP) and Net Primary Productivity (NPP) were measured using the light and dark bottle method, which involves incubating samples for 3 hours under natural light conditions to estimate photosynthetic rates. Samples for GPP and NPP were incubated at pond temperature and light levels, simulating natural conditions for accurate productivity measurements.

Chlorophyll-a content, an indicator of phytoplankton biomass and productivity, was determined by the Arnon (1949) method. This involved acetone extraction, followed by spectrophotometric analysis at specified wavelengths to estimate chlorophyll concentration in each sample.

2.4. Correlation coefficient and PCA analysis

To investigate the relationship between phytoplankton community dynamics and environmental parameters, statistical analyses were performed on the collected data. Pearson and Spearman correlation analyses were used to examine associations between various physico-chemical factors (e.g., nitrate, phosphate, ammonia, silicate, chloride, pH, dissolved oxygen) and phytoplankton abundance and diversity metrics, such as chlorophyll-a concentration. Pearson correlation was applied for parameters with normal distributions, while Spearman correlation was used for parameters with non-normal distributions to capture a broader range of relationships. Principal Component Analysis (PCA) was conducted to reduce the dimensionality of environmental variables and identify the key factors contributing to seasonal changes in phytoplankton communities. This analysis highlighted the primary variables influencing productivity and diversity, differentiating key nutrients and other conditions between the two ponds. Additionally, stepwise regression models were applied to determine the influence of environmental variables on gross and net primary productivity (GPP and NPP) and chlorophyll-a content across seasons. These regression models helped quantify the relative impact of each physico-chemical factor on phytoplankton growth patterns and productivity.

Statistical analyses were conducted using GraphPad Prism (version10.10) with significance levels set at $p < 0.05$. This comprehensive approach allowed for a detailed understanding of how seasonal shifts and nutrient availability drive phytoplankton community dynamics in these freshwater ponds.

3. Results

3.1. Phytoplankton Diversity and Composition

A total of 47 phytoplankton species were identified in Site 1 (S1), a fish cultivation pond, whereas Site 2 (S2), an agricultural waste pond, exhibited a lower diversity with 24 species (Table 1). The percentage composition of different phytoplankton groups across the two sites is illustrated in Figures 2a and 2b. In S1, Chlorophyceae emerged as the dominant group, constituting 59% of the phytoplankton population, followed by Bacillariophyceae (23%) and Cyanophyceae (14%). Minor contributions came from Conjugatophyceae (2%) and Euglenophyceae (2%). Contrastingly, S2 exhibited a higher representation of Euglenophyceae (33%), making it the second most dominant group after Chlorophyceae (42%). Cyanophyceae (17%) and Bacillariophyceae (8%) were present in smaller proportions, with Conjugatophyceae being virtually

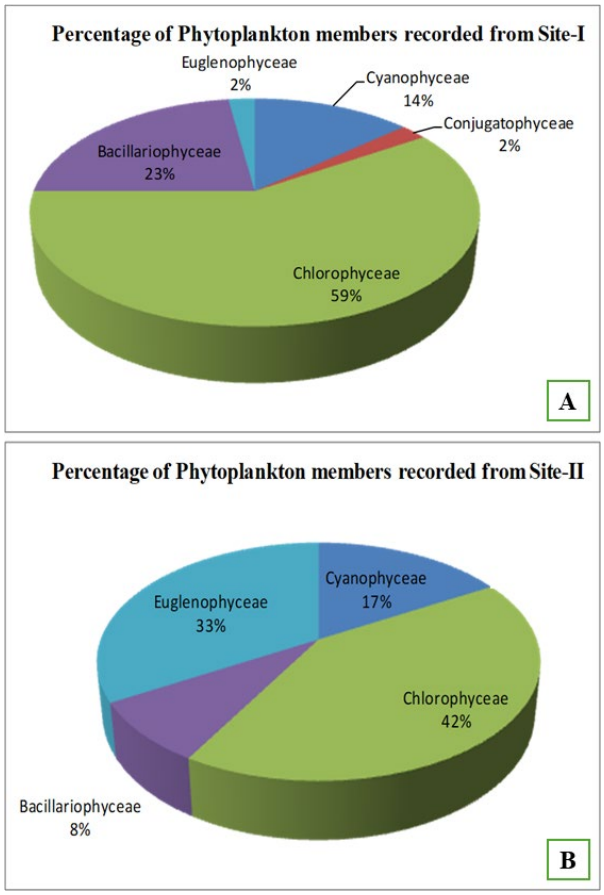

Fig.2. Abundance of different phytoplankton groups in 2a. Site-1, 2b. Site-2.

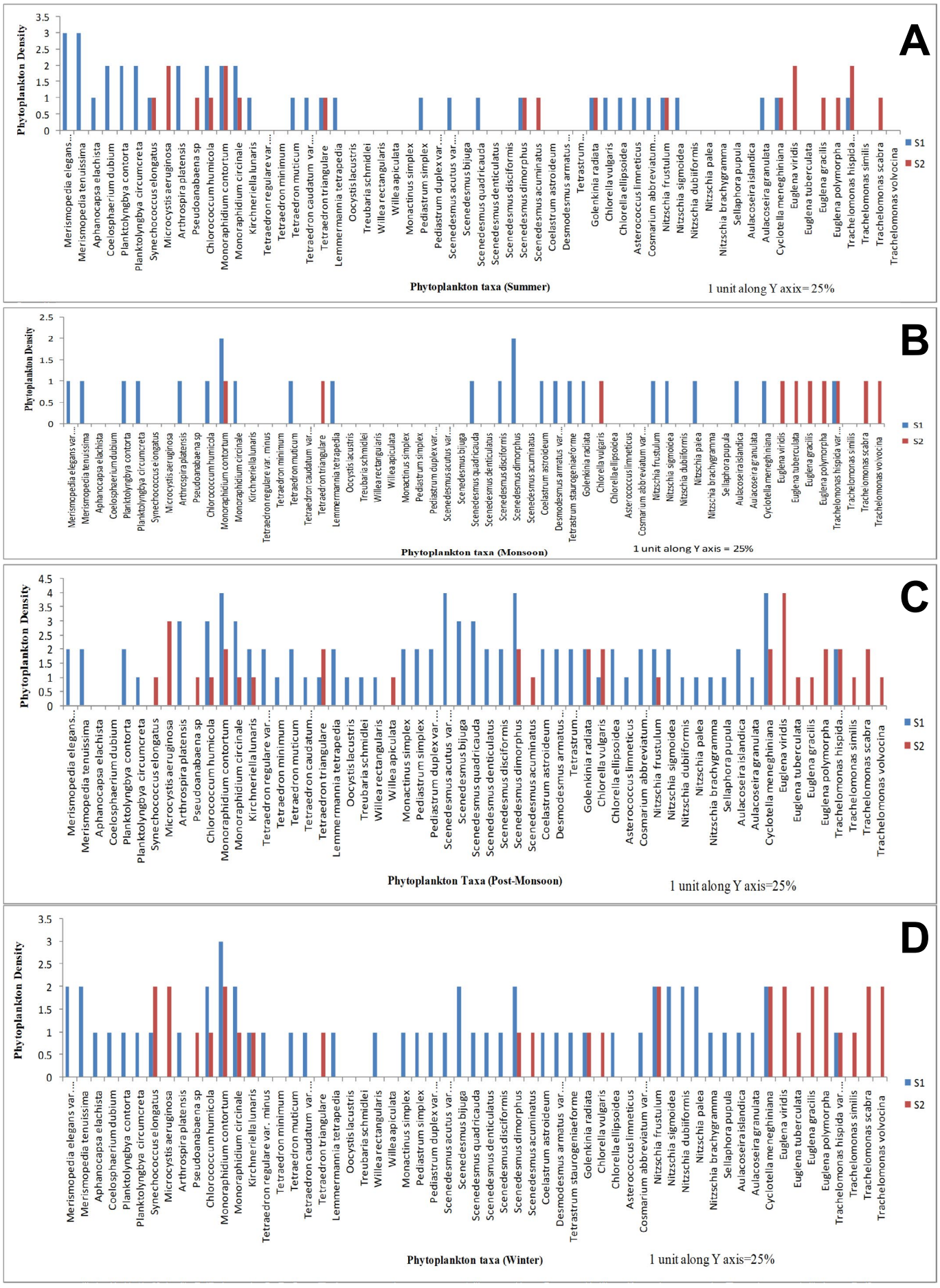

Fig.3. Seasonal variation of phytoplankton taxa in S1 and S2. A. Summer B. Monsoon C. Post-monsoon and Winter.

Table 1. Phytoplankton taxa present in Site 1 and Site 2 in different season.

Systematic Position	Name of the Species	Site-1				Site-2			
		Sum	Mon	Post-Mon	Win	Sum	Mon	Post-Mon	Win
Class-Cyanophyceae									
Order-Synechococcales	*Merismopedia elegans var. major*	3	1	2	2	0	0	0	0
	Merismopedia tenuissima	3	1	2	2	0	0	0	0
	Aphanocapsa elachista	1	0	0	1	0	0	0	0
	Coelosphaerium dubium	2	0	0	1	0	0	0	0
	Planktolyngbya contorta	2	1	2	1	0	0	0	0
	Planktolyngbya circumcreta	2	1	1	1	0	0	0	0
	Synechococcus elongatus	1	0	0	1	1	0	1	2
Order-Chroococcales	*Microcystis aeruginosa*	0	0	0	0	2	0	3	2
Order-Oscillatoriales	*Arthrospira platensis*	2	1	3	1	0	0	0	0
	Pseudoanabaena sp	0	0	0	0	1	0	1	1
Class-Chlorophyceae									
Order-Chlorococcales	*Chlorococcum humicola*	2	1	3	2	1	0	1	1
	Monoraphidium contortum	2	2	4	3	2	1	2	2
	Monoraphidium circinale	2	1	3	2	1	0	1	1
	Kirchneriella lunaris	1	0	2	1	0	0	1	1
	Tetraedron regulare var. minus	0	0	2	1	0	0	0	0
	Tetraedron minimum	0	0	1	0	0	0	0	0
	Tetraedron muticum	1	1	2	1	0	0	0	0
	Tetraedron caudatum var. longispinum	1	0	1	1	0	0	0	0
	Tetraedron triangulare	1	0	1	0	1	1	2	1
	Lemmermannia tetrapedia	1	1	2	1	0	0	0	0
	Oocystis lacustris	0	0	1	0	0	0	0	0
	Treubaria schmidlei	0	0	1	0	0	0	0	0
	Willea rectangularis	0	0	1	1	0	0	0	0
	Willea apiculata	0	0	0	0	0	0	1	0
Order-Sphaeropleales	*Monactinus simplex*	0	0	2	1	0	0	0	0
	Pediastrum simplex	1	0	2	1	0	0	0	0
	Pediastrum duplex var. genuinum	0	0	2	1	0	0	0	0
	Scenedesmus acutus var. globosus	1	0	4	1	0	0	0	0
	Scenedesmus bijuga	0	0	3	2	0	0	0	0
	Scenedesmus quadricauda	1	1	3	1	0	0	0	0
	Scenedesmus denticulatus	0	0	2	1	0	0	0	0
	Scenedesmus disciformis	0	1	2	1	0	0	0	0
	Scenedesmus dimorphus	1	2	4	2	1	0	2	1
	Scenedesmus acuminatus	0	0	0	0	1	0	1	1
	Coelastrum astroideum	0	1	2	1	0	0	0	0
	Desmodesmus armatus var. bicaudatus	0	1	2	1	0	0	0	0
	Tetrastrum staurogeniaeforme	0	1	2	1	0	0	0	0
	Golenkinia radiata	1	1	2	1	1	0	2	1
Order-Chlorellales	*Chlorella vulgaris*	1	0	1	0	0	1	2	1
	Chlorella ellipsoidea	1	0	2	1	0	0	0	0
Order-Chlamydomonadales	*Asterococcus limneticus*	1	0	1	0	0	0	0	0
Order-Desmidiales	*Cosmarium abbreviatum var. planctonicum*	1	0	2	1	0	0	0	0

Systematic Position	Name of the Species	Site-1				Site-2			
		Sum	Mon	Post-Mon	Win	Sum	Mon	Post-Mon	Win
Class-Bacillariophyceae									
Order-Bacillariales	*Nitzschia frustulum*	1	1	2	2	1	0	1	2
	Nitzschia sigmoidea	1	1	2	2	0	0	0	0
	Nitzschia dubiiformis	0	0	1	2	0	0	0	0
	Nitzschia palea	0	1	1	2	0	0	0	0
	Nitzschia brachygramma	0	0	1	1	0	0	0	0
Order-Navicullales	*Sellaphora pupula*	0	0	1	1	0	0	0	0
Order-Aulacoseirales	*Aulacoseira islandica*	0	1	2	1	0	0	0	0
	Aulacoseira granulata	1	0	1	1	0	0	0	0
Order-Thalassiosirales	*Cyclotella meneghiniana*	1	1	4	2	1	0	2	2
Class-Euglenoidea									
Order-Euglenales	*Euglena viridis*	0	0	0	0	2	1	4	2
	Euglena tuberculata	0	0	0	0	0	1	1	1
	Euglena gracilis	0	0	0	0	1	1	1	2
	Euglena polymorpha	0	0	0	0	1	1	2	2
	Trachelomonas hispida var. papillata	1	1	2	1	2	1	2	1
	Trachelomonas similis	0	0	0	0	0	0	1	1
	Trachelomonas scabra	0	0	0	0	1	1	2	2
	Trachelomonas volvocina	0	0	0	0	0	1	1	2

Note: 0→Absent, 1→1-25%, 2→26-50% 3→51-75%, 4→76-100% Occurrence.

absent (Fig. 2, Table 1). The most frequently observed species in S1 included *Merismopedia elegans* var. *major* G.M.Smith, *Monoraphidium contortum* (Thuret) Komárková-Legnerová, *Scenedesmus acutus* var. *globosus* Hortobágyi, *Scenedesmus dimorphus* (Turpin) Kützing, *Desmodesmus armatus* var. *bicaudatus* (Guglielmetti) E.H.Hegewald, *Nitzschia frustulum* (Kützing) Grunow, and *Cyclotella meneghiniana Kützing*. In contrast, S2 was dominated by *Microcystis aeruginosa* (Kützing) Kützing, *Synechococcus elongatus* (Nägeli) Nägeli and *Euglena viridis* (O.F.Müller) Ehrenberg (Table 1).

3.2. Seasonal Variations

Phytoplankton abundance and diversity varied significantly across seasons in both sites. S1 was dominated by *Merismopedia elegans*, *M. tenuissima* and S2 with *Microcystis aeruginosa*, *Monoraphidium contortum*, *Euglena viridis* and *Trachelomonas hispida* during Summer (Fig. 3A). Post-monsoon exhibited the highest species richness and productivity, while summer Monsoon recorded the lowest (Fig. 3B). In S1, the dominant species included *Monoraphidium contortum*, *Scenedesmus acutus* var. *globosus*, *Scenedesmus dimorphus*, *Desmodesmus armatus* var. *bicaudatus*, *Nitzschia frustulum*, and *Cyclotella meneghiniana*. These species thrived in the nutrient-enriched post-monsoon environment, with Chlorophyceae being particularly responsive to increased nitrate and phosphate levels (Table 1). In S2, dominant species included *Microcystis aeruginosa*, *Synechococcus elongatus* and *Euglena viridis*. The dominance of Euglenophyceae in S2, particularly during post-monsoon (Fig. 3C), was likely influenced by elevated ammonia levels from agricultural runoff. This group's resilience to highly alkaline conditions and nutrient enrichment underscores their adaptability to such environments. S1 was influenced by *M. contortum*, many species of *Merismopedia*, *Scenedesmus*, *Nitzschia* and *Cyclotella* and S2 with *S. elongatus*, *M. aeruginosa*, *M. contortum*, *N. frustulum*, *C. meneghiniana* and several species of *Euglena* and *Trachelomonas* during winter (Fig. 3D) due to prolonged nutrient availability.

It was recorded from the results that S2 pond was more alkaline (pH 10.69) than that of S1 (pH 8.16) (Fig. 4A). The seasonal temperature variation was almost similar for both the ponds ranging from 12˚C to 38˚C (Fig. 4B) but dissolved oxygen content was more in S1(17.23 mg/L) than that of S2 (14.66 mg/L (Fig. 4C). The BOD level was 8.25 to 8.73 in S1 and S2 respectively (Fig. 4D). Maximum GPP value recorded as 1.4 mg/L/h in S1 and0.98mg/L/h in S2 during post-monsoon period (Fig. 4E), NPP level was more (0.8 mg/L/h) inS1 than that of S2 again (0.58 mg/L/h) (Fig. 4F). Maximum chlorophyll content was recorded in post monsoon period but more in S1 as expected (5.78 mg/L) compared to S2 (3.68 mg/L) followed by winter season (Fig. 5A). High growth rate of Euglenophyceae is justified with high amount of nitrate (2.52 mg/L) (Fig. 5B), phosphate (4.56 mg/L) (Fig. 5C) and ammonia (0.664 mg/L) (Fig. 5D) contents of S2 pond indicating eutrophication. Chloride content recorded maximum 162.5 mg/during winter

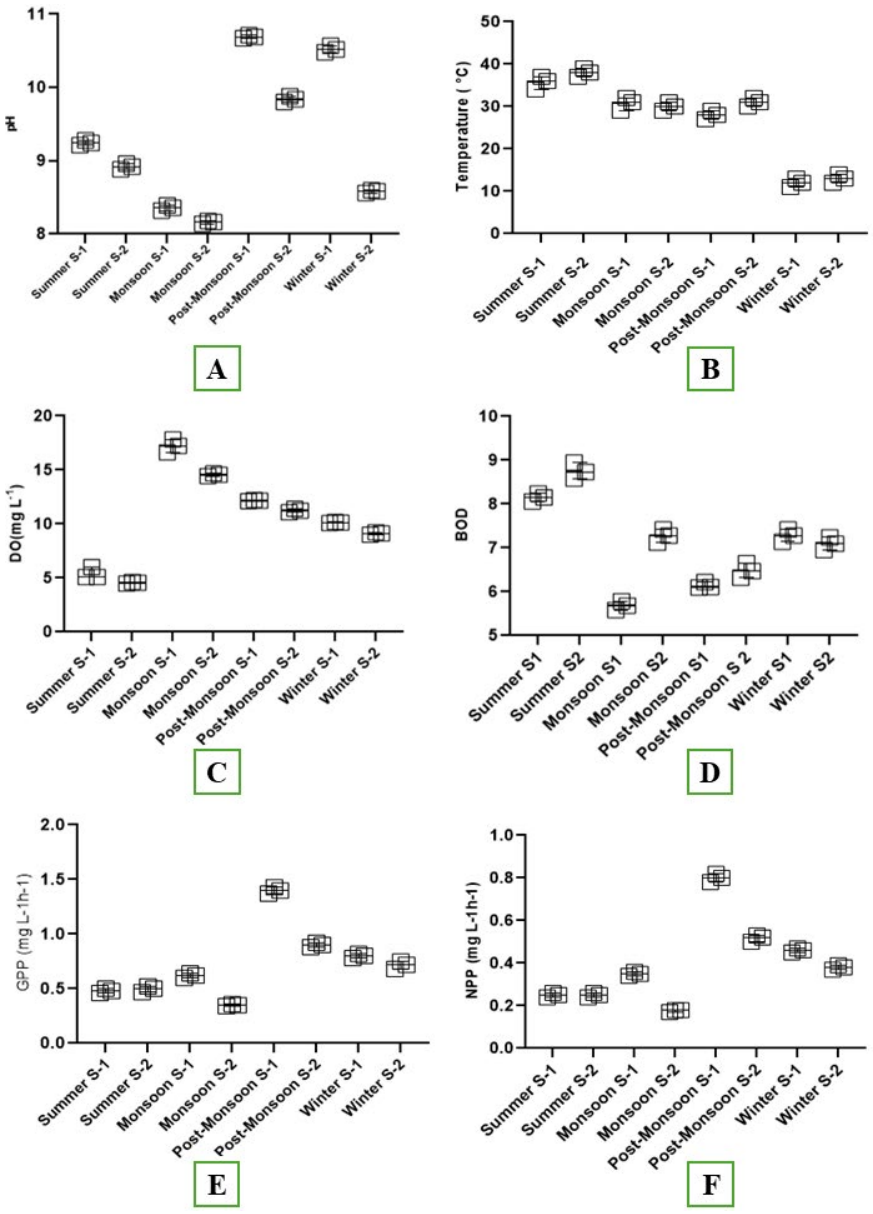

Fig.4. Variations in seasonal mean values with standard error of physicochemical parameters of Site-1(S-1) and Site-2(S-2)- A. pH B. Temperature C. Dissolved oxygen. D. BOD E.GPP, F. NPP.

from S1 and 162.5 mg/L during post-monsoon from S2 (Fig. 5E), and silicate contents were almost high in post-monsoon (6.92 mg/L) and lowest (2.065 mg/L) during summer (Fig. 5F).

3.3. Correlation with Environmental Parameters

The environmental parameters monitored across seasons included temperature, pH, dissolved oxygen (DO), biological oxygen demand (BOD), gross primary productivity (GPP), net primary productivity (NPP), and key nutrients such as nitrate, phosphate, ammonia, silicate, and chloride.

The chlorophyll-a content, an essential indicator of phytoplankton biomass, varied significantly between the two sites (S1 and S2). At Site S1, chlorophyll-a levels ranged from 1.84 to 5.78 mg/L, while at Site S2, values were lower, ranging from 1.22 to 3.68 mg/L. These variations highlight differences in the ecological dynamics and nutrient availability between the sites.

In S1, a strong positive correlation was observed between chlorophyll-a content and nutrients such as nitrate, phosphate, and silicate, indicating that nutrient enrichment plays a critical role in promoting phytoplankton growth. Additionally, chlorophyll-a showed a positive relationship with both gross primary productivity (GPP) and net primary productivity (NPP). This suggests a synergistic effect where higher nutrient concentrations enhance productivity, further stimulating phytoplankton biomass. Notably, during the post-monsoon period, both nutrient levels and chlorophyll-a content peaked, emphasizing the significance of nutrient runoff and seasonal mixing in driving primary productivity.

At Site S2, chlorophyll a demonstrated a positive correlation with pH, nitrate, phosphate, ammonia, and chloride. The strong relationship with pH indicates the influence of alkaline conditions in shaping the phytoplankton community structure. Unlike S1, nutrient levels at S2 were comparatively lower, yet the correlation between chlorophyll-a and ammonia highlights the role of ammonium as a preferred nitrogen source for

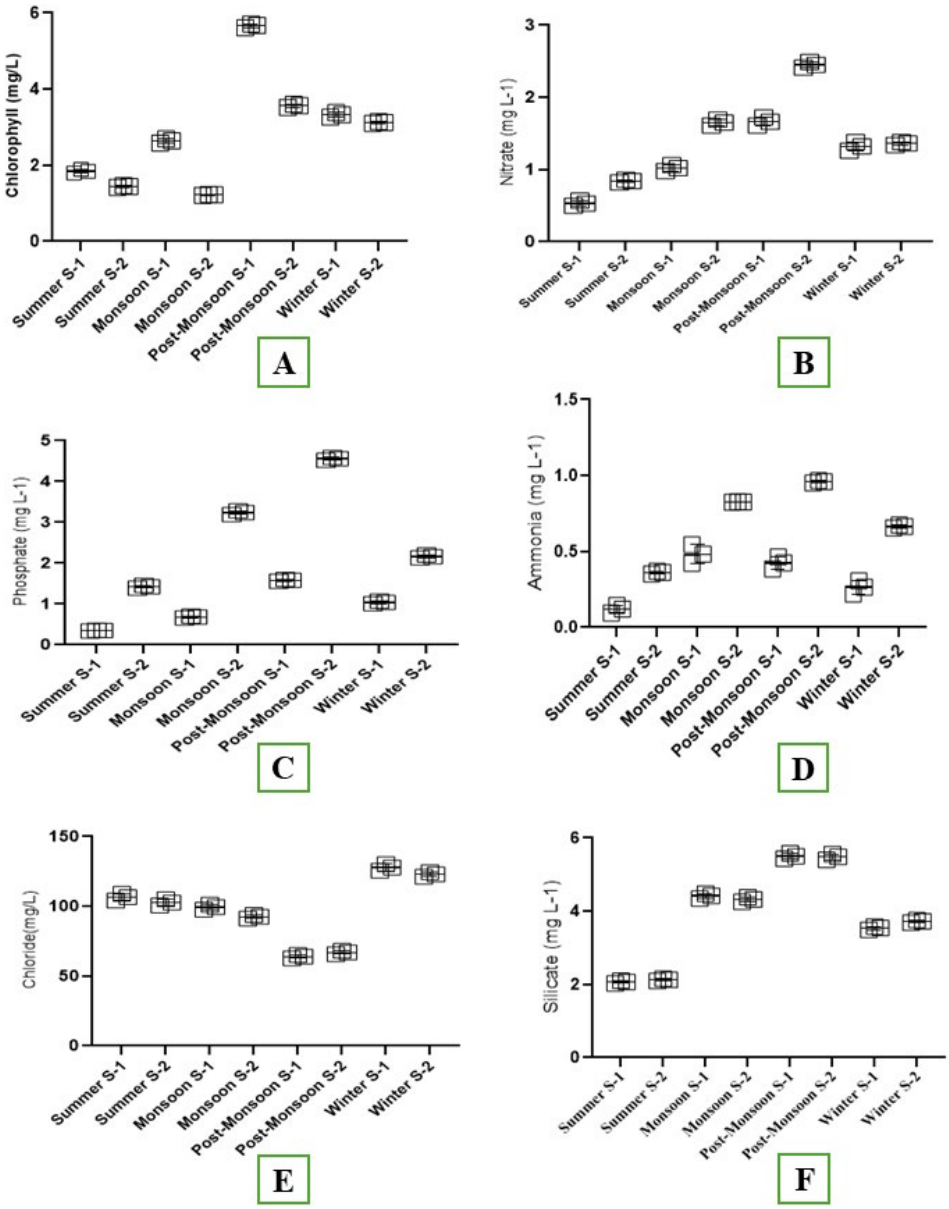

Fig.5. Variations in seasonal mean values with standard error of physico-chemical parameters of Site-1(S-1) and Site-2(S-2)- A. Chlorophyll B. Nitrate C. Phosphate D. Ammonia E. Chloride F. Silicate.

phytoplankton. Seasonal patterns showed a moderate increase in chlorophyll a during the monsoon season, likely driven by nutrient inputs from surrounding areas.

From the Correlation matrix and PCA biplots of S1 and S2 of chlorophyll-a content during different seasons, it was found that PCA of S1 were Nitrate and Silicate (Table 2A, Fig. 6A) and PCA of S2 were pH, phosphate, ammonia and Chloride (Table 2B, Fig. 6B). Among the PCA, GPP and NPP were common for both sites and showed positive relations. Temperature and BOD showed a negative correlation with chlorophyll-a in both the wetlands (Table 2A, 2B and Fig. 6A, 6B).

The graphical representation of environmental parameters across seasons provides a clear insight into the interplay between physical and chemical factors influencing phytoplankton biomass. The pH values (Fig. 4A) exhibited a seasonal trend, with higher alkalinity during the summer and post-monsoon periods, particularly at Site S2, indicating favourable conditions for phytoplankton growth under alkaline environments. Temperature (Fig. 4B) followed a predictable seasonal pattern, peaking during summer and declining in winter. This rise in temperature corresponded with increased metabolic and photosynthetic activity, as reflected by higher gross primary productivity (GPP) and net primary productivity (NPP) during these periods, especially at Site S1.

Dissolved oxygen (DO) levels (Fig. 4C) showed seasonal peaks that aligned with periods of heightened primary productivity. The post-monsoon period, in particular, recorded elevated DO concentrations due to increased photosynthetic activity driven by nutrient enrichment. Conversely, biological oxygen demand (BOD) values (Fig. 4D) were moderately higher at Site S2, indicative of active decomposition processes likely linked to organic matter inputs. These opposing trends in DO and BOD emphasize the dynamic balance between oxygen production and consumption across seasons.

The productivity parameters, GPP and NPP (Figs. 4E and 4F), exhibited strong correlations with chlorophyll-a levels, underscoring the role of pri-

Table 2A. Correlation coefficient of Chlorophyll-a and different physico-chemical parameter of Site-I.

	Chloro-a	Temperature	pH	Nitrate	Phosphate	Ammonia	Chloride	Silicate	DO	BOD	GPP	NPP
Chloro-a	1	-0.265	0.710	0.928	0.974*	0.546	-0.679	0.847	0.237	-0.487	0.993**	0.997**
Temperature	-0.265	1	-0.568	-0.477	-0.455	-0.055	-0.507	-0.203	-0.075	0.030	-0.214	-0.242
pH	0.710	-0.568	1	0.590	0.738	-0.130	-0.108	0.285	-0.385	0.193	0.709	0.726
Nitrate	0.928	-0.477	0.590	1	0.972*	0.717	-0.505	0.930	0.495	-0.666	0.902	0.905
Phosphate	0.974*	-0.455	0.738	0.972*	1	0.563	-0.528	0.853	0.290	-0.500	0.961*	0.962*
Ammonia	0.546	-0.055	-0.130	0.717	0.563	1	-0.574	0.906	0.934	-0.994**	0.518	0.506
Chloride	-0.679	-0.507	-0.108	-0.505	-0.528	-0.574	1	-0.678	-0.312	0.546	-0.709	-0.684
Silicate	0.847	-0.203	0.285	0.930	0.853	0.906	-0.678	1	0.715	-0.871	0.825	0.819
DO	0.237	-0.075	-0.385	0.495	0.290	0.934	-0.312	0.715	1	-0.953*	0.200	0.189
BOD	-0.487	0.030	0.193	-0.666	-0.500	-0.994**	0.546	-0.871	-0.953*	1	-0.454	-0.447
GPP	0.993**	-0.214	0.709	0.902	0.961*	0.518	-0.709	0.825	0.200	-0.454	1	0.990***
NPP	0.997**	-0.242	0.726	0.905	0.962*	0.506	-0.684	0.819	0.189	-0.447	0.990***	1

Note: * Weak correlation, ** Median correlation, *** strongly correlation, **** Very strong correlation. Correlation is significant at the 0.05 level (2-tailed).

Table 2B. Correlation coefficient of Chlorophyll-a and different physicochemical parameter of Site-II.

	Chloro-a	Temperature	pH	Nitrate	Phosphate	Ammonia	Chloride	Silicate	DO	BOD	GPP	NPP
Chloro-a	1	-0.223	0.700*	0.608*	0.497	0.489	0.797**	0.309	0.039	-0.099**	0.732**	0.927****
Temperature	-0.223	1	0.404	-0.114	-0.033	-0.290	0.017	0.054	-0.428	0.300	-0.243	-0.007
pH	0.700*	0.404	1	0.528	0.483	0.250	0.766**	0.401	-0.261	0.070	0.385	0.832***
Nitrate	0.608*	-0.114	0.528	1	0.987****	0.949****	0.931****	0.936****	0.676*	-0.790***	0.236	0.542
Phosphate	0.497	-0.033	0.483	0.987****	1	0.948****	0.896****	0.977****	0.712**	-0.830***	0.150	0.449
Ammonia	0.489	-0.290	0.250	0.949****	0.948****	1	0.804**	0.906****	0.857***	-0.909****	0.186	0.363
Chloride	0.797**	0.017	0.766**	0.931****	0.896****	0.804**	1	0.802**	0.386	-0.523**	0.443	0.772**
Silicate	0.309	0.054	0.401	0.936****	0.977****	0.906****	0.802**	1	0.738**	-0.865**	-0.015	0.284
DO	0.039	-0.428	-0.261	0.676*	0.711**	0.857***	0.386	0.738**	1	-0.971**	-0.131	-0.125
BOD	-0.099**	0.300	0.070	-0.790***	-0.830***	-0.909****	-0.523**	-0.865**	-0.971**	1	0.157	0.028
GPP	0.732**	-0.243	0.385	0.236	0.150	0.186	0.443	-0.015	-0.131	0.157	1	0.643*
NPP	0.927****	-0.007	0.832***	0.542	0.449	0.363	0.772**	0.284	-0.125	0.02	0.643*	1

Note: * Weak correlation, ** Median correlation, *** strongly correlation, **** Very strong correlation. Correlation is significant at the 0.05 level (2-tailed).

mary productivity in driving phytoplankton biomass. Seasonal peaks in GPP and NPP during the post-monsoon period at Site S1 highlighted the combined effect of nutrient runoff and optimal environmental conditions. In contrast, the comparatively lower productivity observed at Site S2 reflected nutrient limitations, with ammonium playing a critical role in sustaining phytoplankton communities during certain seasons.

Collectively, these graphs illustrate the seasonal and site-specific dynamics of environmental factors, reinforcing the pivotal role of nutrient availability, temperature, and pH in regulating primary productivity and phytoplankton growth. Site S1's nutrient-enriched conditions supported higher productivity and chlorophyll-a content, whereas Site S2 displayed a more nuanced response influenced by pH and specific nutrient parameters.

3.4. Ecological Implications and Status

From the study, it was revealed that the post-monsoon season was ideal for maximum nutrient availability as well as peak phytoplankton production. Site S2, with its higher nutrient content, showed some degree of eutrophication, evident from the occurrence of Euglena and Microcystis species. Correlation and PCA analyses identified nitrate, phosphate, and silicate as the principal components influencing phytoplankton dynamics in Site S1, while pH, nitrate, ammonia, and chloride played dominant roles in Site S2. Among the principal components, GPP, NPP, and chlorophyll-a were common drivers across both sites and displayed strong positive correlations. The PCA analysis provided a comprehensive understanding of the environmental variables shaping phytoplankton dynamics. In Site S1, nitrate, phosphate, and silicate emerged as key contributors to phytoplankton growth, as reflected in the PCA biplots (Fig. 6A). These variables formed a tight cluster aligned with post-monsoon conditions, underscoring their importance in enhancing chlorophyll-a concentration and supporting chlorophycean species. The alignment of temperature and dissolved oxygen (DO) with summer and monsoon seasons further highlighted their seasonal influence, while the lower influence of chloride and BOD during winter suggested reduced nutrient input and productivity in this period.

In contrast, the PCA biplot for Site S2 (Fig. 6B) revealed distinct drivers. Here, pH, nitrate, ammonia, and chloride were the most influential parameters, closely associated with the post-monsoon season. Their strong alignment with GPP, NPP, and chlorophyll-a content illustrated their role in promoting eutrophication events and supporting Euglenophyceae and Cyanophyceae abundance. Temperature and DO showed moderate influences during summer and monsoon seasons, while BOD was prominent during winter, indicating seasonal variations in organic load and decomposition rates.

The principal component analysis (PCA) highlights distinct environmental and nutrient-driven factors influencing phytoplankton dynamics in the two ponds. At Site S1, nitrate, phosphate, and silicate were

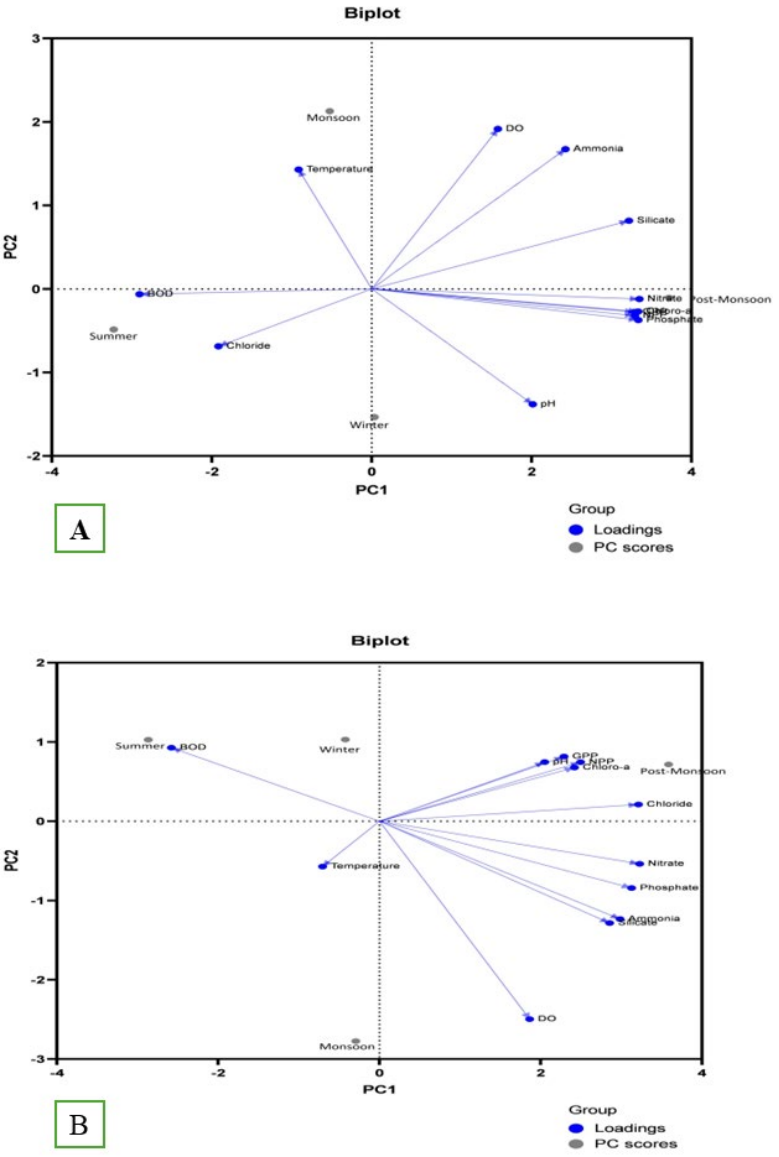

Fig.6. Principal Component Analysis of environmental variables recorded from Site-1 (A) and Site-2 (B).

identified as the primary drivers of phytoplankton diversity and productivity, supporting the dominance of Chlorophyceae and Bacillariophyceae. These groups thrived in the nutrient-enriched conditions typical of a fish cultivation pond, where the interplay between nutrient availability and seasonal variations fostered a robust and responsive phytoplankton community. In contrast, Site S2, heavily influenced by agricultural runoff, demonstrated a more specialized ecological profile. Here, pH, nitrate, phosphate, ammonia, and chloride emerged as critical determinants, reflecting the alkaline and nutrient-specific conditions that favoured the proliferation of Euglenophyceae and Cyanophyceae. The ammonia-rich environment, coupled with elevated chloride levels, provided a competitive edge to these phytoplankton groups, enabling them to adapt and sustain productivity despite lower diversity compared to S1. These findings underscore the ecological dichotomy between the two sites: S1, characterized by a nutrient-responsive and diverse phytoplankton community, and S2, exhibiting a nutrient-adaptive yet less diverse assemblage shaped by its unique environmental stressors. Seasonal nutrient fluctuations further ampli-

fied these site-specific dynamics, reinforcing the pivotal role of both natural and anthropogenic influences in governing phytoplankton diversity and ecological health.These findings have significant ecological and practical implications. The study enhances understanding of phytoplankton dynamics in nutrient-rich freshwater ecosystems, offering insights into the ecological models of fish cultivation ponds and agricultural runoff ponds. For instance, the dominance of chlorophycean species in Site S1 highlights the potential for optimizing fish pond management through nutrient modulation. Similarly, the eutrophication observed in Site S2 underscores the need for strategies to manage agricultural runoff to maintain water quality. Moreover, this research suggests the potential for predictive modelling of phytoplankton dynamics based on environmental parameters. Such models could aid in sustainable water resource management, ensuring balanced ecosystem productivity while mitigating adverse effects like eutrophication.

4. Discussion

The phytoplankton population of the particular ecosystem is controlled by various physical, chemical, and biological factors, which ultimately regulate the phytoplankton dynamics of particular ecosystems (Cole and Cloern, 1984). Results indicated a diverse phytoplankton population, primarily composed of members of Cyanobacteria, Chlorophyceae, Euglenophyceae, Bacillariophyceae, and Conjugatophyceae. Members of Chlorophyceae made up the bulk of the population for both the ponds, followed by Bacillariophyceae, Cyanobacteria, Euglenophyceae and Ochlorophyceae for fish pond and Euglenophyta, Cyanobacteria and Bacillariophyta for the Agricultural pond (Table 1 and Fig. 2a, 2b). Similar results of dominance of Euglenophyceae, Chlorophyceae, followed by cyanobacteria, were recorded from East Kolkata Wetland- a Ramsar site by other authors (Singha Roy et al., 2018), wetlands of tropical region (Bose et al., 2016). The dominant genera recorded from the study area included *Merismopedia* from Cyanobacteria, *Scenedesmus* from Chlorophyta, *Nitzschia* from Bacillariophyta, and *Euglena* from Euglenophyta as already published (Garai et al., 2022). In a similar study at different discharge points of the Tannery industry, Dey et al. (2021) reported the role of chemical parameters in phytoplankton productivity and recorded *Phormidium*, *Leptolyngbya, Pseudoanabaena, Amphora* and *Nitzschia* as major taxa.

No significant interseasonal variation was noted among different groups of phytoplankton population. In case of both the sites, almost same groups appeared, except dominance of Euglenophyceae members in post-monsoon and winter in Site-2.

Wassie et al. (2017) reported that *Melosira* and *Microcystis* were dominated in polluted water with high Nitrate and phosphate content polluted water. We also noticed that S2 contained high amount of nitrate, phosphate and dominance of cyanobacteria and *Euglena* signifying eutrophication of the water body. The nutrient concentration and pH play an important role in phytoplankton productivity as evident from correlation matrix and PCA studies. Among different factors, pH, nitrate, phosphate, ammonia, silicate, GPP, and NPP played the most significant role as they are highly positively correlated with chlorophyll productivity. On the other hand from the PCA plot also Nitrate and Silicate in S1 and pH, phosphate, ammonia and chloride in S2 appeared as principal components for phytoplankton dynamics.

Karak et al. (2013) estimated total nitrogen (16.9 g/kg), total carbon (321.4 g/kg) from agricultural fish pond sediment, which was better than the Indian compost standard. Our results showed that the post-monsoon period was most productive for both the ponds, showing high chlorophyll content with maximum level of nutrients like nitrate, phosphate, silicate etc, along with high GPP value as expected. Therefore, the fishpond sediment would be very good compost as well.

Highly significant correlations between chlorophyll content and GPP clearly established that GPP was the estimation of total fixed carbon by phytoplankton population (Choudhury and Pal, 2012). Thus, the overall productivity is totally dependent on phytoplankton photosynthetic activity for both ponds.

This study provides a comprehensive analysis of phytoplankton dynamics in two distinct freshwater ecosystems, highlighting how nutrient availability and environmental conditions shape community structure and productivity. Unlike previous studies that have primarily focused on individual water bodies, our research adopts a comparative approach, allowing us to differentiate the ecological responses and phytoplankton dynamics of a fish cultivation pond (Site S1) and an agricultural runoff pond (Site S2) belonging from the same area.

A key novelty of this study lies in its demonstration of site-specific phytoplankton adaptation to varying nutrient inputs. At Site S1, the dominance of Chlorophyceae and Bacillariophyceae under nutrient-rich post-monsoon conditions aligns with findings from managed aquaculture systems in the region. However, this study reveals a unique interplay between nitrate, phosphate, and silicate, which together foster a more diverse and resilient phytoplankton community. The seasonal influence of temperature and dissolved oxygen further underscores the dynamic nature of fish ponds, distinguishing them from static water bodies like reservoirs or lakes, where nutrient turnover rates may be slower. Conversely, Site S2 exhibited a phytoplankton community composition strongly influenced by agricultural runoff, with Euglenophyceae and Cyanophyceae emerging as dominant groups. This pattern aligns with eutrophic conditions observed in other anthropogenically impacted freshwater bodies, yet this study provides novel insights into the role of ammonia and chloride in shaping these assemblages. Unlike previous reports where nutrient enrichment primarily favored cyanobacterial blooms, our results suggest a co-dominance of Euglenophyceae, indicating a more complex response to agricultural inputs.

Comparative studies from regional lakes and reservoirs have reported different phytoplankton compositions, often dominated by diatoms (Bacillariophyceae) in winter and Cyanophyceae in summer. In contrast, our findings highlight how specific nutrient regimes in managed ponds can sustain diverse communities year-round, emphasizing the importance of localized environmental factors. This distinction underscores the need for tailored water management strategies, as generic models based on large water bodies may not accurately predict phytoplankton dynamics in smaller, human-influenced ecosystems. By integrating PCA and correlation analyses, this study not only identifies key environmental drivers but also provides a framework for predicting phytoplankton shifts in response to nutrient fluctuations. These findings contribute to the broader understanding of freshwater ecology by offering a site-specific perspective on phytoplankton responses, which can be valuable for both aquaculture management and eutrophication mitigation strategies in similar water bodies.

5. Conclusion

From the study, it was revealed that the post-monsoon season was ideal for maximum nutrient availability as well as peak phytoplankton production. Site S2, with its higher nutrient content, showed some degree of eutrophication, evident from the occurrence of *Euglena* and *Microcystis* species. Correlation and PCA analyses identified nitrate, phosphate, and silicate as the principal components influencing phytoplankton dynamics in Site S1, while pH, nitrate, ammonia, and chloride played dominant roles in Site S2. Among the principal components, GPP, NPP, and chlorophyll-a were common drivers across both sites and displayed strong positive correlations. These findings have significant ecological and practical implications. The study enhances understanding of phytoplankton dynamics in nutrient-rich freshwater ecosystems, offering insights into the ecological models of fish cultivation ponds and agricultural runoff ponds. For instance, the dominance of chlorophycean species in Site S1 highlights the potential for optimizing fish pond management through nutrient modulation. Similarly, the eutrophication observed in Site S2 underscores the need for strategies to manage agricultural runoff to maintain water quality. Moreover, this research suggests the potential for predictive modelling of phytoplankton dynamics based on environmental parameters. Such models could aid in sustainable water resource management, ensuring balanced ecosystem productivity while mitigating adverse effects like eutrophication. Future studies should expand to other freshwater ecosystems with varying anthropogenic impacts to validate these findings. Investigating intervention strategies, such as controlled nutrient inputs or bioremediation techniques, could further enhance the applicability of this research in ecological conservation and resource management.

Acknowledgements

The infrastructural facility was supported by CAS Phase VII and DST-FIST Level II, Department of Botany, University of Calcutta.

Authors contribution

Sumanta Garai: Writing – original draft, Writing – review & editing, Visualization, Methodology, Investigation, Formal analysis. Anwesha Mondal: Writing – review & editing, Validation, Methodology, Formal analysis, Data curation. Iman Dey: Methodology, Formal analysis, Rahul Bose: Visualization, Methodology, Ruma Pal: Writing – review & editing, Investigation, Conceptualization, Supervision.

Funding

Author (s) received no financial support for the research, authorship and publication of the article.

Availability of Data and materials

All the data are enclosed within the manuscript. There is no associated data.

Conflict of interest

The authors declared no potential conflicts of interest with respect to the research, authorship and publication of the article.

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Short communication

LIMNOLOGY FRESHWATER BIOLOGY

New locality records of invasive freshwater jellyfish *Craspedacusta sowerbii* (Lankester, 1880) in Türkiye

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ABSTRACT. Invasive jellyfish *Craspedacusta sowerbii* (Lankester, 1880) is a hydrozoan (Phylum Cnidaria, Class Hydrozoa), most easily identified in its hydromedusa form as a small, bell-shaped jellyfish. Indigenous to the Yangtze River valley in China, this species has spread across temperate climates, including Türkiye, for over a century. In this study, three new locality records were discovered in Türkiye: Umurbey (Çanakkale), Geyik (Muğla), and Seyhan (Adana) dams. Some physicochemical parameters (pH, dissolved oxygen, temperature and electrical conductivity) of the sampling points were measured. Although there is no definite information on the origin of this species in the dams, it is thought that it is hypothesized that it may have been introduced by fish stocking, aquatic birds and human activities.

Keywords: Hydrozoan, Muğla, Çanakkale, Adana, Invasiveness

For citation: Seçer B., Sungur S., Çiçek E. New locality records of invasive freshwater jellyfish *Craspedacusta sowerbii* (Lankester, 1880) in Türkiye // Limnology and Freshwater Biology. 2025. - № 3. - P. 298-301. DOI: 10.31951/2658-3518-2025-A-3-298

1. Introduction

Currently, numerous species are voluntarily or involuntarily relocated outside their natural or potential distribution areas for various reasons, including aquaculture, research, biological control, bio-manipulation, sport fishing, replenishment of declining stocks or ballast water releasing ornamental fishes deliberately or unintentionally (Lockwood et al., 2007; Çiçek et al., 2022). A range of anthropogenic factors, such as global warming, climate change, pollution, unintentional water consumption, eutrophication, and urbanization, present significant challenges to the integrity and biodiversity of aquatic ecosystems. These factors facilitate the spread of non-native species and support the establishment of large populations in new areas (Galil et al., 2014; Mannino et al., 2017).

Globally, over 20 species of freshwater jellyfish exist, with *Craspedacusta sowerbii* (Lankester, 1880) being the most widespread. This small freshwater hydrozoan cnidarian, originally from the Yangtze River region in China, has now spread to many regions worldwide (Kramp, 1950). *Craspedacusta sowerbii* is typically found in natural lakes but is more commonly present in dams and ponds (Augustin et al., 1987). Hydrozoans have two adult forms: polyps and medusae. Polyps can develop a protective membrane called the podocyst, allowing them to withstand prolonged food shortages and extreme conditions. When conditions become favorable, podocysts revert to polyps. This adaptive ability allows the species to colonize various freshwater habitats (Bouillon and Boero, 2000; Lucas et al., 2013).

The first record of *C. sowerbii* in Türkiye was documented by Dumont (1994) from Keban Dam Lake. Subsequent records include Balık et al. (2001) from Topçam Dam Lake, Bozkurt (2004) from Kozan Dam Lake, Bekleyen et al. (2011) from Kıralkızı Dam Lake, Akçaalan et al. (2011) from Sapanca Lake, Gülşahin (2017) from Ula Pond, Kutlu (2020) from Uzunçayır Dam Lake, Özbek and Sömek (2020) from Ürkmez Dam Lake and Killi et al. (2021) from Akdeğirmen and Ataköy Dam Lake.

Recently, several studies have been focused on new localities and mapping the spatial distribution of *C. sowerbii*. In this study, the presence of *C. sowerbii* was discovered for the first time in the Geyik (Muğla), Umurbey (Çanakkale), and Seyhan (Adana) Dam lakes.

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Received: January 07, 2025; ***Accepted:*** April 22, 2025;
Available online: June 11, 2025

2. Material and Method

2.1. Study area

The sampling studies were conducted in 2022 at the following locations: Geyik (37.405760N, 27.87983E), Umurbey (40.243824N, 26.676228E), and Seyhan (37.074054N, 35.272088E) dam lakes.

The Umurbey Dam, constructed in 2003, aims to facilitate irrigation on the Umurbey Stream, located within the boundaries of Çanakkale province in the Marmara basin. This dam covers a surface area of 213 km^2 and is fed by three rivers. It is in 55 meters depth.

Geyik Dam, was constructed in 1998, provides drinking water in the Western Mediterranean basin on Sarıçay in Milas district of Muğla province. The dam has a surface area of 320 km^2 and is fed by three river sources. The depth of the dam lake is 12 meters.

Seyhan Dam, was builted in 1956 in the Seyhan basin, serves the dual purposes of agricultural irrigation and electricity generation. The dam stands at 67 meters above sea level, with the deepest point reaching approximately 45 meters. The lake's surface area of the lake varies seasonally, averaging around 53 km^2 (Fig. 1).

2.2. Physicochemical analysis and sampling

Water samples were collected to measure environmental parameters at three locations within the dam lakes from the bottom, middle, and surface waters. Samples from the surface and bottom of the dam were collected using a Nansen bottle. Light transmittance was determined with a 30-centimeter diameter Seki disk. In situ measurements of temperature (°C), salinity (ppt), dissolved oxygen (mg/L), electrical conductivity (µS/cm), and pH parameters were taken using a Hach Lange HQ 40 D multi-parameter.

To determine the current distribution of *C. sowerbii*, a review of the relevant literature was conducted, and a map was created for each locality (Fig. 1). The taxonomic identification of the medusa was performed using the methodology proposed by Jankowski (2001) with a Leika EZ-4D stereomicroscope. The density of individuals was calculated in situ through instantaneous observations over an area of approximately one square meter.

3. Result and Discussion

This study presents additional records of *C. sowerbii* from freshwaters bodies in Türkiye (Fig. 2). A dense population was found in Geyik Dam Lake in September 2022 with 9 individuals/m^2 in the dam lake with bell diameters ranging from 1.5 to 3.2 cm. In October 2022, a smaller population was found in Umurbey Dam, with 2 individuals/m^2 with bell diameters between 1.4 and 1.9 cm. In Seyhan Dam Lake, 3 individuals/m^2 were observed in September 2022 with bell diameters ranging from 1.3 to 2.1 cm.

Physicochemical parameters of the sampling points are in Table 1. It found that the optimum temperature range for *C. sowerbii* is between 19 and 30 °C (Dunham, 1941; Reisinger, 1957; Lytle, 1959; Acker and Muscat, 1976). However, some studies have observed medusae at lower temperatures (Milne, 1938; Killi et al., 2021). This demonstrates the species' high tolerance to varying environmental parameters. The formation of medusae is influenced by nutrient abundance and temperature (Matthews, 1966).

The density of *Craspedacusta sowerbii* is significantly influenced by factors such as dissolved oxygen, pH, electrical conductivity, and water transparency. In Geyik Dam Lake, low dissolved oxygen (6.7 mg/L), low pH (7.94), and low electrical conductivity (160 µS/cm) may provide a competitive advantage for the species, while the low Secchi depth (1.5 m) suggests high phytoplankton productivity, potentially enhancing food availability. In contrast, Umurbey Dam Lake, characterized by the highest dissolved oxygen (8.2 mg/L), the highest pH (8.46), and the greatest water transparency (5.9 m), presents a more stable and oxygen-rich environment; however, lower planktonic productivity may make it less favorable for the species. Seyhan Dam Lake, with moderate values across parameters, represents a relatively balanced ecosystem, while its highest electrical conductivity (387 µS/cm) indicates a higher concentration of nutrients, poten-

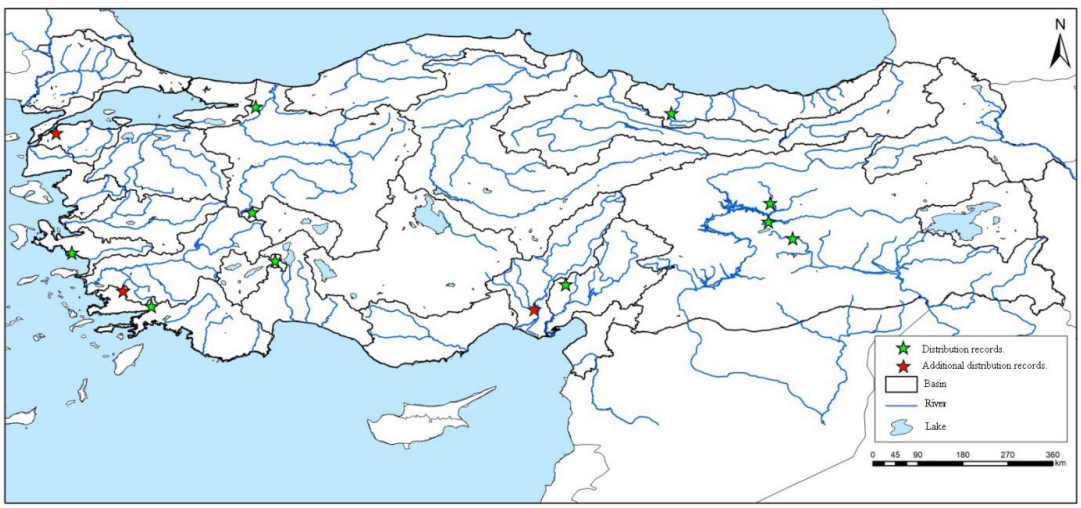

Fig.1. Distribution map of *Craspedacusta sowerbii* in Türkiye.

Table 1. Ecological parameters of sampling stations (DO: Dissolved Oxygen; Temp: Temperature; EC: Electical conductivity)

Station	Parameters					
	DO (mg/L)	Temp (°C)	EC (µS/cm)	pH	Depth (m)	Secchi depth (m)
Umurbey Dam Lake	8.2	18.2	136	8.46	55.0	5.9
Seyhan Dam Lake	8.1	24.4	387	8.23	24.0	5.0
Geyik Dam Lake	6.7	26.6	160	7.94	8.0	1.5

tially supporting greater zooplankton abundance and improving food accessibility for *C. sowerbii*. These findings underscore the critical role of physicochemical water properties in directly shaping the distribution and density of this species.

Anthropogenic impacts on aquatic ecosystems lead ecological degradation, with invasive species exacerbating these disturbances. Increased ecological disturbances facilitate the easier entry of invasive species into aquatic ecosystems (Perdikaris et al., 2010). Especially in fragile areas already inhabited by invasive species, new invasions occur even more rapidly. This is one reason for the high population density observed in Geyik Dam. The region has been invaded by *Lepomis gibbosus* (Linnaeus, 1758), an invasive inland fish; *Dreissena polymorpha* (Pallas, 1771), a highly invasive mussel; and *Elodea canadensis* Michx., an aquarium plant that has become invasive due to its relocation to different regions (personal observation). It is expected that a new invasive species would enter Geyik Dam, which is which is vulnerable to invasions, and establish large populations. In addition to its susceptibility to invasive species, the high air temperature and nutrient availability create a favorable habitat for *C. sowebii*.

The study conducted at Umurbey Dam in October revealed that the water temperature was lower than in other dams, and the nutrients levels in the reservoir were also lower compared to Geyik Dam. However, interviews with local fishermen indicated that *C. sowerbii* was much more abundant in August than it is currently.

In the context of biological invasion, identifying the exact entry-dispersal vectors and pathways is challenging. Nevertheless, primary methods of spread include biological control, research, recreation, and the augmentation of declining aquatic bird populations. Proposed vectors proposed for the dispersal of *C. sowerbii* are aquatic birds, aquatic plants, and human activities (Lockwood et al., 2007; Morpurgo and Alber, 2015). Dumont (1994) highlighted the significance of avian migration routes for *C. sowerbii*, citing the presence of podocysts. The irregular and counterintuitive global distribution of different lineage groups of *C. sowerbii* on underscores the importance of the bird migration route hypothesis. However, further detailed studies are needed to substantiate this hypothesis. Based on current evidence, it can be concluded that Seyhan Dam (Adana-Seyhan) serves as a primary migration route, while Umurbey Dam (Çanakkale-Lapseki) and Geyik Dam (Muğla-Milas) constitute secondary migration routes (Kiziroğlu, 2009). The Tuzla wetland in Muğla/Milas is an important important habitat for migratory birds, and the present distribution of this species aligns with the migratory patterns, substantiating Dumont's hypothesis.

Fig.2. *Craspedacusta sowerbii* sample from Geyik Dam Lake.

The detection of *Craspedacusta sowerbii* in three new localities in Türkiye has increased the total number of recorded sites to 13. This invasive freshwater jellyfish primarily preys on zooplankton, disrupting planktonic equilibrium and intensifying resource competition for larval and juvenile fish, potentially reducing their growth rates. The decline in zooplankton populations, coupled with an increase in phytoplankton biomass, may accelerate eutrophication and degrade water quality. Additionally, the competitive advantage of *C. sowerbii* may threaten endemic or narrowly distributed species, posing a significant risk to biodiversity by altering trophic interactions and ecological stability.

This invasion may also have severe implications for ecosystem services. The decline in fishery productivity could result in economic losses, while deteriorating water quality may limit its use for drinking and irrigation. Furthermore, recreational activities in affected freshwater bodies could be negatively impacted by high jellyfish densities. To mitigate these effects, long-term ecological monitoring, environmental DNA (eDNA) analysis, and the development of strategic management plans to preserve local ecosystems are imperative.

Acknowledgements

Authors wish to thank Ümit LAÇİN for their help and sampling.

Conflict of interest

The authors declare no conflicts of interest.

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