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AQUATIC SCIENCES AND ENGINEERING

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Research Article

Evaluation of Histopathological Alterations in the Liver and Kidney of Olive Barb (*Puntius sarana*, Hamilton 1822) as an Indicator of the Surma River's Pollution

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ABSTRACT

Water pollution poses a global hazard to aquatic biota and ecological sustainability, causing a hazard to aquatic organisms such as fish. The aim of this study was to evaluate the impact of toxic chemical contaminants on histopathological biomarkers in the liver and kidney of olive barb (*Puntius sarana*) collected from the Surma River in comparison to a control group of fish. The histological bio-monitoring assessment involved the sampling of thirty (30) table-sized fish collected from the four-sampling sites (i. Kajir Bazar, ii. Keane Bridge, iii. Shahjalal Bridge and iv. Burhanuddin Majar) of the Surma River on a monthly basis during the period of September 2019 to February 2020. Twenty (20) table-sized fish of the same species were reared in the Fish Disease Diagnosis and Pharmacology Laboratory, which has a controlled water quality system. In the present study, the river temperature was found to range from 19.7±1.57°C to 30.00±1.29°C. Dissolved oxygen of the surface water was observed to range from 4.15±0.31 to 4.82±0.67 ppm. The pH was found to range from 7.42±0.33 to 6.50±0.12. We observed several pathological changes viz., haemorrhage, lipid droplet, hypertrophy and hyperplasia, erythrocyte infiltration into blood sinusoid, pyknosis and hepatocyte degeneration in the liver tissues of *P. sarana*. The histopathological analysis of the kidney showed tubular degeneration of the distal tubule, tubular degeneration of the proximal tubule, melanomacrophage centre, glomerular shrinkage, vacuolisation, necrosis and haemorrhage. The evidence of pathological alterations in the liver and kidney of olive barb *P. sarana* appeared to be a useful biomarker to assess the impact of the toxicity of water pollution in the Surma River.

Keywords: Olive barb, *Puntius sarana*, Surma River, Environmental Pollution, Histopathology, Biomarkers

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INTRODUCTION

Bangladesh is a territory which is bordered by around 230 rivers of different sizes and an abundance of water resources. Being one of the most densely inhabited nations in the world, these resources are constantly becoming contaminated (Hasan et al., 2019). Over the last few decades, freshwater contamination with a variety of contaminants has gained noticeable attention (Vutukuru, 2005). The Surma River's water supply has subsequently been in significant danger from anthropogenic activities and wastewater. Urban discharge in various unauthorized dumping zones, farming techniques, and tannery discharges that have been disposed of without sufficient treatment were reported to have degraded the superiority of the water (Howladar, 2013). Besides this, environmental issues have affected all types of aquatic species due to the unregulated dumping of agricultural waste into waterways (Deng et al., 2017). Industrial leakage is also a significant contributor to water pollution because they contain hazardous metal compounds which directly mix with the waterbody (Patra et al., 2007). Aquatic contamination has been reported to be responsible for the survival disturbance and incapability of regular production processes (Ogamba et al., 2016), so these effects may reduce biodiversity and alter ecosystems' constitution.

One of numerous rivers in Bangladesh, the Surma River is located in the northeast part of Sylhet city. It is considered a large river and is abundant with 51 varieties of fish species. At the same time, a majority of people rely on the Surma River basin for their daily needs (Akter et al., 2019). Currently, community waste and metro pollutants are either directly or indirectly dumped into the Surma River. Wastewater discharge has resulted in water quality deterioration over time in the Surma River (Alam et al., 2007). Pollutants discharged from the water body may be ingested by fish through their food and water and may then accumulate in large concentrations in various tissues (Mohammed, 2009).

As a result of aquatic pollution, fish have become vulnerable to water contamination because their morphology is highly dependent on the quality of the surrounding water (Islam et al., 2020). Fish can absorb or accumulate toxic chemicals either from the surroundings or indirectly via other living things such as tiny fish, aquatic invertebrates and underwater vegetation (Polat et al., 2016). Heavy metal (Chromium) contamination has been reported in the Surma River, but no extensive research has been conducted yet (Islam et al., 2019). Effluents of all kinds have negative impacts on metabolism and behavioural propagation, as well as the potential to cause clinical distortions in the cells and tissues of different parts (Nikalje et al., 2012). Numerous harmful xenobiotics, which induce biochemical and histological changes in fish, are often found in water bodies (Reddy, 2012).

Among the diverse array of fish in Bangladesh, *Puntius sarana* (Hamilton, 1822), commonly known as the 'olive barb' and belonging to the group Cyprinidae, is an indigenous fish which is reported to be critically endangered and found at a stage of vulnerability in neighbouring India (Mikherjee et al., 2002). *P. sarana* is a very well-known small indigenous species (SIS), however, aquatic pollution is increasing day by day and generating a lot of complexities in the cell, tissue and other major organs of this small species (Mahabub et al., 2008). To assess the effect of contamination, histological analysis is a widely supported approach (among a number of identifiable approaches) to distinguish between the healthy and unhealthy status of an animal (Vinodhini & Narayan, 2009).

Fish liver is a major organ because it is crucial for the metabolic and biochemical processes involved in consuming food and nutrition storage (Ostaszewska et al., 2016). Variations in fish liver tissue serve as a reliable indicator of the species' overall health (Nunes et al., 2013). Liver is utilized to assess water quality because it is generally accepted in biomarker research and because its histopathological abnormalities might be linked to water contaminants (Marcon et al., 2016). On the other hand, another primary organ responsible for maintaining the equilibrium of bodily fluids in vertebrate creatures is the kidney, considered another vital organ of the fish body (Ojeda et al., 2003). Various hazardous substances disturb the kidney, which impairs its functioning and temporarily or permanently disturbs homeostasis (Miller et al., 2002). Fish have already been found to experience a variety of histological alterations (cardiovascular, degenerating, advanced changes) with a lot of inflammatory changes due to exposure to numerous environmental stresses (Guardiola et al., 2014; Rodrigues et al., 2017).

Histological alterations determine the environmental stressors that can cause organ-specific response in animals (Madureira et al., 2012; Rašković et al., 2013; Rodrigues et al., 2017). Several species were reported as the grand receiver of river water contamination, which led to histological alterations in various organs like the gill and liver of sneep *(Chondrostoma nasus)* and chub *(Leuciscus cephalus)* (Triebskorn et al., 2008), the ovary of freshwater *Channa punctatus* (Magar and Bias, 2013) and the liver and kidney of *Mystus cavasius* (Karim et al., 2022).

Considering the above aspects, the aim of the present study was to assess Surma River pollution through histopathological alterations that took place in the kidney and liver of *P. sarana,* relative to control fish that were reared in laboratory conditions. Also, we anticipated that fish from polluted river sites would exhibit more abnormalities than control fish.

MATERIALS AND METHODS

Fish collection

The Surma River in the Sylhet region is continuously polluted with a large number of toxic chemicals from a variety of industries and sewage wastes, which rapidly reduces the water quality parameters necessary for aquatic flora and fauna. *P. sarana* were harvested from i. Kajir Bazar, ii. Keane Bridge, iii. Shahjalal Bridge, and iv. Burhanuddin Majar (Figure-1). After collection, fish were then immediately transported in an isothermal box (equipped with a refrigeration system) to the Laboratory of Fish Disease Diagnosis and Pharmacology in the Department of Fish Health Management at Sylhet Agricultural University, Bangladesh. The whole experiment was conducted for 6 months (180 days), from

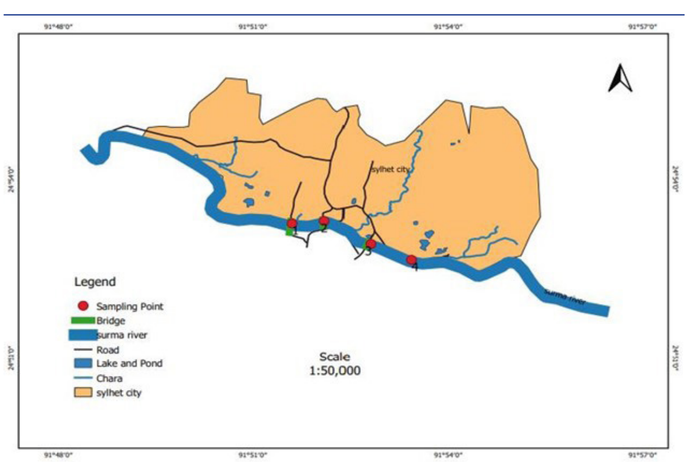

Figure 1. Map showing the sampling sites on the Surma River. (1. Kajir Bazar, 2. Keane Bridge, 3. Shahjalal Bridge and 4. Burhanuddin Majar)..

September 2019 to February 2020. Thirty (30) fish samples were randomly collected on a monthly basis from four sampling sites. For the control study, fish specimens (fingerlings) were collected from a local fish farm (Sreemangal Fish Hatchery) in the Sylhet region. Control fish were fed with commercial food twice per day.

Water sampling

Water samples were collected from the different pre-selected points of the river. The samples were collected at approximately 30 cm depth from the top of the river, and care was taken so that no floating film or organic material could enter the bottle. The samples were analysed in the Fish Disease Diagnosis and Pharmacology Laboratory, under the Department of Fish Health Management. The Digital Multimeter (YSI Professional plus multiparameter water quality meter, USA) recorded various water quality parameters.

Histopathology

The histopathology protocol adopted the following procedure (Sultana et al., 2016). Desired organ liver and kidney tissues were fixed with 10% neutral buffer formalin (40% formaldehyde) and kept for 2-3 days for proper fixation. After, fixation tissue slices were tagged and subjected to a dehydration process in alcohol with a chronological process. After dehydration, tissues were cleared in a Xylene solution. The embedding process was done in paraffin wax overnight at 58°C in the oven. Excess bubbles were removed and kept in refrigerated conditions for solidification. Tissues were sectioned with 5µm size with a microtome. Soon after that, all sections of tissues were placed in an oven at 37°C overnight. Later, tissues were stained with Haematoxylin and Eosin (counter-stains) dye. All prepared sections were mounted with Canada balsam and coverslip used for permanent preservation and kept for one night. The final histological slides were observed and photographed with a microscope (Optika camera; model B9, Italy) at 10X and 40X magnification. All histopathological changes were observed by comparing with the control and updated literature.

RESULTS

Water quality assessment

Several major water quality constraints, such as temperature (˚C), dissolved oxygen and pH, were measured from different sampling sites. All gathered data is calculated and expressed in their mean value ± SD (Table 1). During the experiment, water quality parameters were observed at different times. The average mini-

Table 1. Monthly fluctuation of water temperature, pH and dissolved oxygen of the Surma River during the study period.

Months	Kajir Bazar			Keane Bridge			Shahjalal Bridge			Burhanuddin Majar		
	DO_2 (mg/ml)	pH	Temp. (°C)	DO_2 (mg/ml)	pH	Temp. (°C)	DO_2 (mg/ml)	pH	Temp. (°C)	DO_2 (mg/ml)	pH	Temp. (°C)
Sep	4.65±0.62	7.42±0.33	30.00±1.29	4.58±0.59	7.40±0.31	31.02±1.25	4.66±0.62	7.44±0.25	30.45±1.20	4.50±0.25	7.45±0.35	31.65±1.27
Oct	4.82±0.67	6.92±0.49	29.50±1.25	4.84±0.68	6.90±0.45	30.60±1.27	5.00±0.48	6.76±0.33	29.92±1.12	4.88±0.65	6.85±0.35	30.56±1.25
Nov	4.41±0.56	6.70± 0.28	27.25±1.25	4.39±0.5	6.72± 0.25	28.19±1.23	4.65±0.5	6.86± 0.28	28.85±1.25	4.58±0.34	6.75± 0.25	28.50±1.33
Dec	4.25±0.35	6.65±0.31	21.50±1.29	4.23±0.37	6.69±0.35	21.67±1.26	4.34±0.42	6.7±0.33	20.54±1.28	4.21±0.25	6.63±0.29	21.76±1.35
Jan	4.15±0.31	6.50±0.12	19.70±1.57	4.10±0.34	6.48±0.15	18.85±1.66	4.26±0.30	6.55±0.18	19.55±1.45	4.15±0.35	6.52±0.19	18.53±1.54
Feb	4.15±0.52	7.12±0.22	22.50±1.57	4.18±0.52	7.18±0.19	21.88±1.55	4.21±0.48	7.12±0.26	21.07±1.28	4.18±0.45	7.14±0.26	21.67±1.55

mum water temperature recorded from four different sampling sites was in January (19.70±1.57 ˚C), while the maximum value was found in September (30.00±1.29 ˚C). The minimum value (4.15±0.31 mg/l) of dissolved oxygen was recorded at Kajir Bazar in January, while the maximum value (4.82±0.67 mg/l) was recorded at Keane Bridge in October. The pH value was observed to be higher in September (7.42±0.33) and found to be lowest in January (6.50±0.12).

Histopathology of Kidney

After observing kidney tissues, no alterations were identified in the control fish (Fig. 2a). On the other hand, several alterations were observed in the wild fish tissues, such as tubular degeneration of distal tubule and melanomacrophage centres (Fig. 2b). Vacuolation, necrosis, haemorrhage, tubular degeneration of the proximal tubule (Fig. 2c, 2d), degeneration of the distal tubule and degeneration of the proximal tubule showed severe damage, while vacuolation, glomerular shrinkage, and haemorrhage were found with moderate levels and mild necrosis was found in the tissue of the kidney from collected wild fish (Table 2).

Histopathology of Liver

The liver of *P. sarana* did not show any histological alterations in the control group fish (Fig 3a). At the same time, liver tissue from wild fish was found with severe histological alterations, including nuclear degeneration, cytoplasmic degeneration, cytoplasmic vacuolisation, melanomacrophage centres, erythrocyte infiltration into blood sinusoid, sinusoid dilation and hepatocyte degeneration (Figs. 3c, 3d, 3e). Melanomacrophage centre, haemorrhage, and hepatocyte degeneration indicated higher severity; on the other hand, nuclear alterations and pyknosis were found to be moderate, and minor hyperplasia was observed (Table 3).

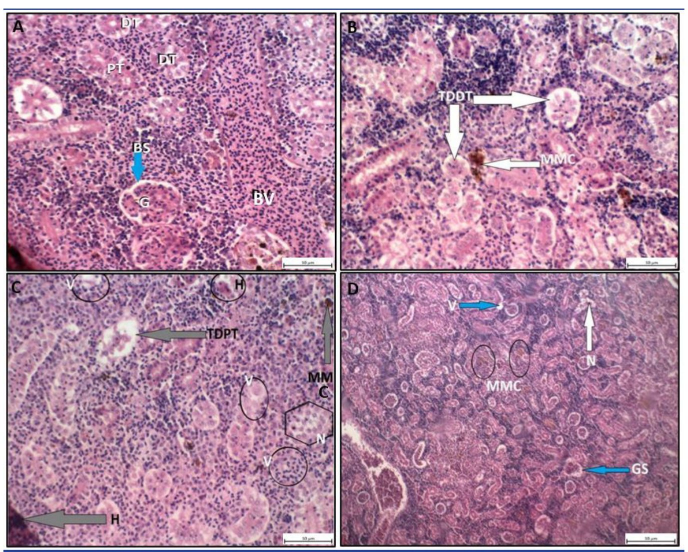

Figure 2. Photomicrograph of the kidney of *P. sarana* from control and experimental site (wild Group). Photomicrograph of kidney from **(A)** Control Group- Distal Tubule (DT), Proximal Tubule (PT), Glomerulus (G), Bowman's Space (BS); (H and E; 40X). and **(B, C, D)** from wild Group- Vacuolation (V), Necrosis (N), Haemorrhage (H), Tubular Degeneration of Distal Tubule (TDDT), Tubular Degeneration of Proximal Tubule (TDPT), Glomerular shrinkage (GS), Melanomacrophage Centres (MMC). (H and E; 10X, 40X).

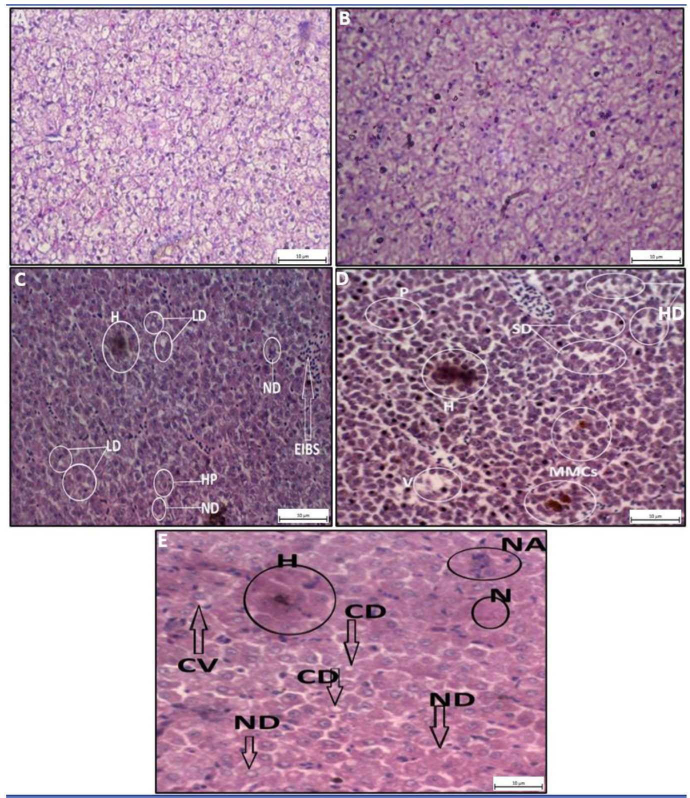

Figure 3. Photomicrograph of the liver of *P. sarana* from control and experimental site (wild Group). Photomicrograph of liver from **(A)** Control Group (H and E; 10X), **(B)** Control Group (H and E, 40X); **(C, D, E)** from wild Group (H and E; 40X). Haemorrhage (H); Lipid Droplet (LD); Necrosis (N); Pyknosis (P); Nuclear Alteration (Na); Hyperplasia (HP) and hypertrophy; Vacuole (V); Nuclear Degeneration (ND); Cytoplasmic Degeneration (CD); Cytoplasmic Vacuolisation (CV); Melanomacrophage Centres (MMC); Erythrocyte Infiltration into Blood Sinusoid (EIBS); Sinusoid Dilation (SD); Hepatocyte Degeneration (HD).

Graphical presentation of histological alteration observed in the kidney and liver of *P. sarana*

In this figure, No. of histological alteration is represented in the Y axis and observed months are represented in the X axis. Several histological alterations of the kidney and liver were observed. On the other hand, the control group of fish species represents low to no histological changes in this study period, both in the kidney and liver (Fig 4a, b).

Table 2. Histopathological alterations observed in the kidney during the experiment.

Abnormalities	Control fish	Wild fish			
		Kajir Bazar	Keane Bridge	Shah-jalal Bridge	Burha-nuddin Majar
Haemorrhage	-	++	+	+++	+
Necrosis	-	+	+++	+	++
Degeneration of Distal Tubule	-	+++	+	++	+
Vacuolation	-	++	++	+	+++
Degeneration of Proximal Tubule	-	++	+++	+	+
Glomerular shrinkage	-	++	+++	++	+

-, None (0%); +, mild (<10%); ++, moderate (10 to 50%); +++, severe (>50%).

Table 3. Histopathological alterations observed in the liver during the experiment.

Abnormalities	Control fish	Wild fish			
		Kajir Bazar	Keane Bridge	Shah-jalal Bridge	Burha-nuddin Majar
Hyperplasia	-	+	++	+++	+
Nuclear alterations	-	++	+	+	+++
Haemorrhage	-	+	+++	++	+
MMCs	-	+++	++	+	++
Hepatocyte degeneration	-	+	+++	+	+++
Pyknosis	-	+	++	++	++

-, None (0%); +, mild (<10%); ++, moderate (10 to 50%); +++, severe (>50%).

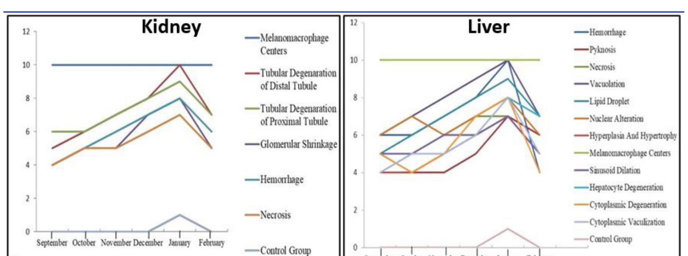

Figure 4. Histological alterations observed at different months. Alterations observed for the kidney (A) and alterations observed for the liver (B).

DISCUSSION

Water quality has been considered an important criterion for successful aquaculture practice, proper growth and good health, which is directly engaged with good water quality. Over the course of time, the Surma River has been disturbed by a huge number of pollutant-discharging industries (Islam et al., 2019). Municipal waste and clinical discharging have a harmful effect on the water quality of a water body (Iqbal et al., 2003). All the sampling sites in this experiment were chosen on the basis of water turbidity and close contact between industries and the river. In our experiment, the minimum temperature was found at the 19.70±1.57°C Kean Bridge site. Water temperature and pH values from different locations in a water body vary at different times (Sazzad et al., 2017). The minimum temperature of a water body plays a crucial role in disease outbreaks (Moniruzzaman, 2000). The lowest value of dissolved oxygen was found at the Keane Bridge and the maximum value was found at the Mendibag station. A DO level of 3mg/L or lower was reported harmful for aquatic organisms; moreover, 5mg/L is considered a preferable limit (Ahmed et al., 2005). In the present study, the pH was found to range from 6.50±0.12 to 7.42±0.33. The minimum pH value was recorded at the municipal sewage disposal site Kajir Bazar and the maximum was observed in Keane Bridge in September. pH value was at its minimum during the time of the monsoon because of heavy rainfall (Sridhar et al., 2008).

Fish liver is the final receiver of all metabolic compounds, and detoxification of all biochemical pollutants takes place in the liver. Exposure to toxic materials and lesion accumulation with other histopathology is very common in this organ (Mohamed, 2009). Wolf et al. (2015) found the liver to be the most sensitive organ damaged due to toxic chemical exposure. The present study demonstrates that the liver of control fish exhibit a normal architecture and there were no pathological abnormalities (Fig 3a, b). In this experiment, liver tissue of *P. sarana* collected from the polluted site (wild group) showed several abnormal changes, including haemorrhage, lipid droplet, necrosis, pyknosis, nuclear alteration, hyperplasia and hypertrophy, vacuole, nuclear degeneration cytoplasmic degeneration, cytoplasmic vacuolisation, melanomacrophage centres and erythrocyte infiltration into blood sinusoid (Fig 3c, d, e). Exposure to contaminated water resulted in increased vacuolisation of the hepatic cell, which is a deteriorating process (Pacheco & Santos, 2002). Reddy & Baghel, (2010, 2012) noticed limited necrosis, vacuolation in cytoplasm and nucleus and other deteriorative signs of parenchymal liver cells in experimental fish exposed to industrial effluent. However, similar histological changes in the liver of *Sarcomento splittail* were reported by (Teh et al., 2005). The *Mugill cephalus*'s liver tissues, which were taken from the contaminated Ennore estuary, exhibited histological changes such as vacuolisation in the hepatocytes, fibroblast growth, vacuole formation, granular degeneration and necrosis (Vasanthi et al., 2013). However, Pinto et al. (2010) reported that histological alterations depend on the length of the individual's exposure to the polluted source. Several histological alterations in the fish liver were also observed by Chavan & Muley, (2014) and Chai et al., (2017). Additionally, diverse complications in the liver tissues of freshwater fish gathered from various contaminated rivers and streams hampered the routine activities of fish (Ahmed et al., 2009; Karim et al., 2022), which fully corroborated our current findings conducted on the Surma River.

Fish kidney is another vital organ that plays an important role in osmoregulation and removes all unnecessary contaminants. Various studies reported that pollutant exposure causes several deformities in kidneys (Velmurugan et al., 2007). So, abnormalities in the kidney can easily predict environmental contamination that can lead to physical stress in any aquatic organisms (Ortiz et al., 2003). Several alterations, including vacuolation, necrosis, haemorrhage, tubular degeneration of distal tubule, tubular degeneration of proximal tubule, glomerular shrinkage and melanomacrophage centres resulted due to environmental contamination. In teleost, cellular hypertrophy was separated from the degeneration of tubular epithelial cells (Happ et al., 2008). Destruction of red blood cells is mainly associated with melanomacrophage centre formation (Agius & Roberts, 2003). Numerous researchers have documented the histological alterations in the kidney at the level of the glomerulus and tubule in fish exposed to pollutants such as insecticides and weedicides (Benli et al., 2008; Yenchum, 2010). Several abnormalities (renal injury, necrosis area, haematopoietic tissue, degradation of renal tubules, shrinkage of glomerulus, increased space within Bowman's capsule, distortion of glomerular capillaries, intracellular droplets, enlarged glomerulus, abnormal nucleus) have been observed in various species by many researches; the occurrence of these abnormalities depends on the sensitivity of the species and the toxic substances that are released into the environment (similar to the liver) (Capkin et al., 2006; Mahmoud et al., 2008; Samanta et al., 2016). Researchers are worried about the severity and consequences of water body contamination on a global scale (Dutta & Dalal, 2008). The cumulative impact of several hazardous contaminants, including heavy metals, industrial effluent, pesticides and fertilizer waste, could have been the cause of all the irregularities seen in the fish's liver and kidney in the proposed investigation. However, an effective study is needed to figure out the precise causes of these histopathological disorders.

CONCLUSION

In the present study, a variety of histopathological changes were caused by the high water pollution of the Surma River. This has a number of harmful consequences on aquatic creatures, particularly fish, and as a result, customers are quite concerned. This could pose a serious danger to the river's biodiversity, causing losses in the near future. The liver and kidney of fish show a number of histopathological diseases that can serve as a biomarker for investigations that are linked. Therefore, the administration and appropriate authorities should take the required steps to safeguard this river's ecosystem against all forms of contamination.

Conflict of interest: We confirm that there are no conflicts of interest among the authors or between the authors and other people, institutions or organisations.

Ethics committee approval: In the present study, fish were sacrificed according to the guidelines as described the Animal Ethics Committee of Sylhet Agricultural University (Memo: SAU/AEC/FOF/FHM-102)

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Research Article

Seasonal Distribution of *Escherichia coli* and Relationship Among Physicochemical Parameters in Lake Water in the Gudiyattam Area, Tamil Nadu, India

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ABSTRACT

The freshwater ecosystem is deteriorating and becoming more polluted due to industrialization, urbanization, and increasing industries. This study is about the bacteriological and physicochemical properties of the lake water in Gudiyattam town and its surroundings. For this research, samples of lake water from different locations were collected monthly during the period from May 2019 to April 2020 and analyzed based on standard procedures. The physicochemical parameters and bacteriological analyses were studied for all four lakes (S1, S2, S3 and S4). The bacterial analysis results showed the presence of coliform bacteria in all lakes. The most dominant value of the MPN index for total coliform number was determined as 80,000 MPN/100mL, at S4 (Valathur) location during monsoon season. It is understood from the fecal coliform count that location S4 was again found to be dominant when compared to the other lakes, with a result of 8,500MPN/100mL in the monsoon season. The possible reason for the high bacteriological values is thought to be the mixing of drainage waters into the lakes. The MPN value was higher in the monsoon season compared to the pre-monsoon season, which indicates that agricultural runoff occurs in the monsoon season. All the lakes were contaminated with bacterial populations, especially at location S4, which needs the most attention among all the lakes. The above-selected lakes are major surveilling factors for humans for various activities.

Keywords: Lake water, bacterial study, *Escherichia coli*, Gudiyattam

INTRODUCTION

Water is the most important natural resource for every lifeform on earth since it is an essential requirement for all living beings. Around 71% of the Earth's surface is covered by water, containing 1,386 million cubic kilometers of water, and only about 2% of the earth's water supply is being used by humans and other activities (Prakash, 2005). Increases in population, pollution, the expansion of industrializing economies, the demand for higher agricultural productivity, water-related disasters, and climate change are all the major contributors to Asia's clean water scarcity. More than 60% of agricultural pollution has been reported to cause high nutrient levels, eutrophication, and algal blooms that greatly disrupt freshwater ecosystems and prevent these ecosystems from providing essential environmental services to humans (Economic and Social Commission for Asia and the Pacific, 2018). Lakes, which give us a variety of commodities and services, play a significant role in the Earth's landscape of valuable ecosystems. They provide priceless habitats for plants and animals, reduce hydrological extreme events (e.g., droughts and floods), alter the microclimate, enlarge the aesthetic value of the environment, and provide enormous recreational opportunities (Ministry of Environment and Forests, Government of India, 2010).

On the other hand, human actions affecting lakes have risen to a great extent in recent decades. Deforestation, agriculture, urban development, and industry are examples of anthropogenic activities that have sped up the aging process by introducing more sediment, nutrients, and harmful compounds into lakes through runoff (CPCB, 2001). The majority of lakes are degrading in various ways, whether it's through eutrophication, chemical contamination, or habitat loss. Catchment-based activities have also been accomplished through lake shoreline encroachment through reclamation of shallow lake margins, sewage disposal, water abstraction, and expansion of in-lake recreational activities. All these particular actions have led to the quick deterioration of lakes. The reduction of water quality is due to organic contamination by the discard of domestic wastewater and other solid garbage into the lakes. This is the most common and major reason for lake deterioration. Nutrient Enrichment happens due to nutrients entering the catchment via water runoff. Apart from positive nutrients and particle matter, water through flood runoff from urban catchments brings several harmful compounds (Rajamanickam & Nagan, 2016). Physio-chemical characteristics and biological diversity help sustain a safe and healthy environment for aquatic living beings (Ekhaise & Omoigberale, 2011). Research involving bacteriological analysis shows that pollution is caused by the overgrowth of microbes, which increases the likelihood of animals and plants becoming infected in the water. Waterborne infections are basically caused by enteric microorganisms (Schlosser & Bitton,1995). The World Health Organization (WHO, 2018) has recorded 1.7 billion cases of diarrhea in children per year, resulting in 525,000 fatalities. It is crucial to monitor the quality of water to prevent water-borne diseases by implementing appropriate water treatment, managing practices, and monitoring (Ekhaise & Omoigberale, 2011). The total coliform count includes the entire members of the coliform group including microorganisms found in vegetation, soil, and water. The total coliform group members originate in the intestinal tract of endotherms and are known as fecal coliforms. Coliforms are essential water-quality indicators in rivers as they can tell us whether consuming this water is safe or not (Seo, Lee, & Kim, 2019). The spread and detection of coliforms as a water quality issue have been studied (Cho & Song,2008; Crabill, Donald, Snelling, Foust, & Southam, 1999; Frena et al., 2019; Kindiki, Kollenberg, Siamba, Sifuna, & Wekesa, 2018; Lee et al.,2016). During the 12 sampling months, 72 surface water samples were collected from six sites in North Florida and 96 surface water samples were collected from eight sites in South Florida. Salmonella, generic Escherichia coli, total coliform, and aerobic bacterial populations were found for each sample (Murphy et al., 2022). In order to maintain the quality of water resources, multivariate statistical approaches like Discriminate Analysis (DA), Factor Analysis (FA), Cluster Analysis (CA), and Principal Component Analysis (PCA) are currently employed to identify the likely source(s) of water pollution. These methods are also employed to group data on water quality and identify similarities between samples or factors (Reghunath, Murthy, & Raghavan, 2002; Simeonova, Simeonov, & Andreev, 2003; Kotti, Vlessidis, Thanasoulias, & Evmiridis, 2005; Singh, Malik, Mohan, & Sinha, 2004). Due to various human activities, agricultural activities, and industrial processes, lake water pollution is a possibility in the Gudiyattam region. The previous findings in the Gudiyattam region showed that the existence of significant industrial and agricultural activity in the major lakes had resulted in pollution of the lakes (Naveen & Nair, 2022). Until now, there have not been any bacteriological analysis studies performed in the Gudiyattam lake region. Therefore, the current study aims to determine the presence of coliform bacteria, assess physicochemical parameters to find the quality of the lakes and perform statistical analysis on the data collected in the Gudiyattam region.

MATERIALS AND METHODS

Area of study

In Tamil Nadu, Gudiyattam is a town situated in the Vellore district. The location is identified by the coordinates Latitude 12.93972ºN and Longitude 78.8644ºE. It has a total size of 30.08 km^2 and is at the basin formed by the Palar stream. In this study, four lakes are identified in the Gudiyattam region namely Thattaparai (S1), Yeripattrai (S2), Thattankuttai (S3), and Valathur (S4). These four lakes are the major utility source providing lake water in above specified location. The monsoonal season for this location starts in the month of October and ends in the middle of January. The location map of the study area is given in Figure 1.

Collection of samples and experimental procedure

In each lake, the water sample was collected monthly from May 2019 to April 2020 and grouped as Pre-Monsoon season and Monsoon season. In each season, a water sample was drawn from every lake at a depth of 0.5 meters and stored in sterilized plastic containers. To preserve the collected lake water sample, 10% of nitric acid was prepared from 69% of nitric acid and used to preserve the collected water samples and the sample was stored at 4^{0}C in a refrigerator for future use. The samples were analyzed for the following parameters: Fluoride(F^-), Sulfate(SO_4), Chloride(Cl-), Magnesium ions (Mg^{2+}), Calcium ions (Ca^{2+}), Total Hardness (TH), Electric conductivity (EC), Turbidity, pH, and elemental analysis: Copper (Cu) and Chromium (Cr). All the param-

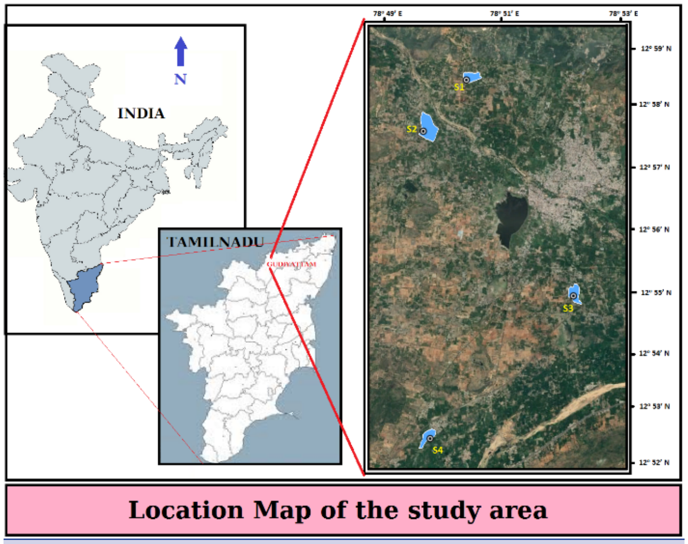

Figure 1. Location Map of the Study area.

eters were determined by the standard procedure recommended by the American Public Health Association (APHA, 2012) and an Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) instrument was used for elemental analysis. The mean value, which was found in each season, was taken into consideration for the physicochemical parameters and bacteriological analysis. All the lakes were dried during the Post-Monsoon season.Thus, only Pre-monsoon and Monsoon seasons were taken into consideration for evaluation.

Bacteriological Analysis (Most Probable Number (MPN)) Test
Determination of total coliform

Multiple tube fermentation technique was performed in a laboratory as outlined by Shariq et al., 2016. Figure 2. depicts the flowchart of the experimental procedure for Presumptive test, confirmed test and completed test.

Principle component analysis (PCA)

Using the water quality dataset from this investigation, all mathematical and statistical calculations were carried out using SPSS Software V.22., in which a large set of highly linked variables were reduced to a more manageable number of independent variables using PCA techniques to infer the variation within them (Regunath et al., 2002).

RESULTS AND DISCUSSION

Physicochemical analysis

Table 1 depicts the interactions between the lake water samples evaluated throughout the study period and the physicochemical features of the distinct seasons. The mean pH value ranged from 5.86 to 8.09. The highest pH value attained at S1 was found to be 8.09 in the Pre-monsoon season and the lowest value attained at S4 was 5.86 in the monsoon season. Especially, at S1 and S4 in the monsoon season, the pH values attained were 6.35 and 5.36, respectively, which is considered to be slightly acidic. The reasons for the acidic nature of the water samples were the waste from households and the municipality. Based on previous studies reported by Bhouyan (1979) and Mahmood et al.,, (1992), pH fluctuation in site due to the industrial and municipal waste dumping. In other sites, the lowest pH value in the monsoon season was due to the dilution of lake water from surface runoff. All the pH values of the all lakes in both seasons lie within the ac-

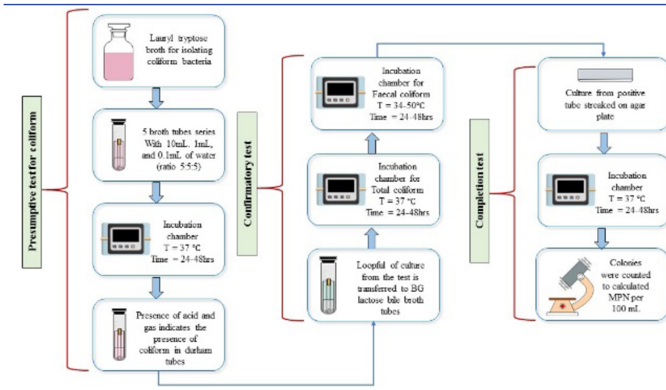

Figure 2. Flowchart for presumptive test, confirmed test, and completed test.

Table 1. Mean and Standard error of Physico-chemical and bacteriological analysis for various seasons.

Parameters	S1		S2		S3		S4	
	Pre-Monsoon	Monsoon	Pre-Monsoon	Monsoon	Pre-Monsoon	Monsoon	Pre-Monsoon	Monsoon
pH	8.09±0.01	6.35 ±0.01	7.83±0	7.21 ±0	7.05 ±0	7.19 ±0.02	7.65 ±0.33	5.86±0.04
Conductivity	264.5 ±1.25	265.2 ±0.33	321.68 ±1.56	619 ±2.56	312.9 ±0.43	315.2 ±0.57	351.1 ±0.33	301.8± 2.13
Turbidity (NTU)	393 ±1.52	73.6 ±0.98	21 ±0.88	2.2 ±0.01	123.5 ±0.03	69.3 ±0	290 ±0.04	2.6±0
Total Hardness (mg/L)	180 ±1.99	250 ±2.6	230 ±1.76	280±0.33	150 ±0.67	120 ±0.01	250 ±0.33	180±0.47
Ca^{2+}(mg/L)	48 ±0.88	20 ±0	50.66 ±0.7	48±0.01	40 ±0.33	16 ±0	52 ±0.01	20.8±0
Mg^{2+}(mg/L)	14.58 ±0.74	31.59 ±0.33	25.11 ±0.85	31.59±0.67	12.15 ±0.47	4.86 ±0	29.16 ±0.01	12.15±0.03
Chlorides (mg/L)	84.97±0.67	149.95 ±0.53	164.9 ±0.03	122.46±0.74	89.97 ±0.01	127.46 ±0.04	134.95 ±0.88	134.95±1.23
Sulfate (mg/L)	14.47±0	0.78 ±0	12.84±0.01	10.26±0.03	11 ±0	10.4 ±0.33	14 ±0.01	4±0
Fluoride (mg/L)	0.54 ±0	0.15 ±0	0.89±0	0.12±0	0.46 ±0.01	0.21 ±0	0.65 ±0	0.14±0
Chromium (mg/L)	-	-	0.0043±0	-	-	-	-	-
Copper (mg/L)	-	-	0.0136±0	-	-	-	-	-
Total Coliform	9600 ±10	15000 ±8.3	1600±0.58	2500±0.3	2240 ±0.88	3500±0.33	51200±12.88	80000±20
Fecal Coliform	3520 ±0.33	5500 ±0.57	960±0.83	1500±1.12	1600 ±2.83	2500 ±3.33	5440±0.33	8500±1.87

ceptable limit, which is mentioned in the standard BIS:(10500-2012). The mean conductivity value ranged from 264.5 (µs/cm) to 619 (µs/cm). The lowest conductivity value attained at S1 was 264.5 (µs/cm) in the pre-monsoon season and the highest value attained at S2 was found to be 619 (µs/cm) in the monsoon season. The variation in conductivity may be due to the agricultural activities happening around lakes. All the conductivity values in each lake were above the acceptable limit, which is outlined in BIS:(10500-2012).

The mean turbidity value for the different seasons ranged from 2.2 NTU to 393 NTU. The highest value of turbidity was obtained at location S1 (393 NTU) in the pre-monsoon season and the lowest value was 2.2 NTU in the monsoon season at S2. The lowest value in the monsoon season was due to the dilution of lake water by surface runoff and rainfall in the monsoonal season. In each lake, the turbidity value was found to be higher than the acceptable limit as per BIS:(10500-2012). The turbidity value was higher than 5 NTU, which has a link to the risk of microorganisms such as coliform bacteria (McCoy & Olson, 1986). The concentration of chloride ranged from 84.97 mg/L to 149.9 mg/L. The highest concentration of chloride was obtained (149.9 mg/L) at S1 in the monsoon season and S1's lowest concentration was in the pre-monsoon season. The chloride concentration of all the locations was below the acceptable limit, which is prescribed in BIS:(10500-2012). Ions of calcium and magnesium are responsible for total hardness. When water has a lot of hardness, scale starts to build inside utensils. The mean lowest concentration of total hardness was obtained at S3 in the monsoon season and the highest concentration was attained at S2 in the monsoon season. The location at S4 in the pre-monsoon season and the location at S1 in the monsoon season were at the border level of the acceptable limit as suggested in BIS:(10500-2012). Location S2 was above the BIS standard. It is thought that the proportions of the minerals calcium and magnesium in water are related (Worcester & Coe, 2010). The mean concentration of calcium ranged from 16 mg/L to 50 mg/L. The concentration was lower in the monsoon season compared to the pre-monsoon season due to surface runoff, and also the dilution of lake water due to the monsoonal effect. The concentration was below the acceptable limit in all the lakes, which is specified in BIS:(10500-2012). The mean lowest concentration of Mg^{2+} was obtained at S3 in the monsoon season (4.86 mg/L) and a higher concentration of Mg^{2+} at S1 and S2 in the monsoon season (31.59 mg/L and 29.16 mg/L). The concentration of Mg^{2+} in each lake was below the acceptable limit, which is prescribed in BIS:(10500-2012). A higher mean concentration of sulfate was attained at S1 (14.47 mg/L) in the pre-monsoon season and a lower mean concentration in the monsoon season at S1 was obtained (0.7 mg/L). The concentration of sulfate in the monsoon season was lower than in the pre-monsoon season due to the dilution of lake water, surface runoff, etc. The mean concentration of sulfate in each lake was below the acceptable limit, which is prescribed in BIS:(10500-2012). Fluoride was high at S4 in the pre-monsoon season (0.65 mg/L) and the lowest concentration obtained in the monsoon season was 0.14 mg/L at S4. The concentration of fluoride in each lake was below the acceptable limit, which is prescribed in BIS:(10500-2012). Only at S2, the heavy metal chromium was found compared with all other lakes. S4 obtained a mean concentration of 0.004 mg/L, which was below the acceptable limit as prescribed in BIS:(10500-2012). S2 attained a mean concentration of 0.01365 mg/L of copper. It was less than the acceptable limit, which is prescribed in BIS:(10500-2012).

Bacteriological analysis

Total coliform is a type of bacterium that is prevalent in water. Fecal coliform is a kind of total coliform that is found in feces. *E. coli* is a subcategory of fecal coliform. Fecal coliform contamination in water serves as a warning sign that infectious microorganisms are present in that water. In this study, multiple tube fermentation was carried out and the results showed that MPN values ranged from 1,600 MPN/100mL to 80,000 MPN/100mL. All the lake water samples were above the limits which are specified in BIS:(10500-2012). At location S2, the pre-monsoon season obtained 1,600 MPN/100mL, which is the lowest range among all the lakes, and location S4 in the Monsoon season obtained 80,000 MPN/100mL, which is recorded as the highest value among all the lakes. It clearly shows that there is huge contamination of the lake at the S4 location both in the monsoon and pre-monsoon seasons. It might be due to the availability of waste from animals and the plantation processes, which pollute the lakes nearer to inhabited regions that take in surface runoff from pasture and agricultural land (Leong et al., 2018). The MPN value in the monsoon season is higher than the pre-monsoon in all the lakes. This is because of the agricultural runoff. During the monsoon season, when it rains a lot, sanitary waste from a vast area is transported into nearby low places or depressions, where dangerous microbes can reach a surrounding shallow aquifer (Escamilla et al.,2013). The minimum and maximum concentration of coliform in each location is tabulated in Table 2.

For the confirmation of fecal coliform, the required incubation temperature is 44.50°C and the results showed that the MPN value ranged from 960 MPN/100mL at S2 in the pre-monsoon sea-

Table 2. Minimum and Maximum value with standard error of Total coliform count and Fecal coliform (MPN/100mL) for all the lakes.

S.No	Place	Total Coliform (MPN/100mL)		Fecal coliform (MPN/100mL)	
		Minimum	Maximum	Minimum	Maximum
S1	Thattaparai	2400 ± 20	16800 ±23.3	2150 ±2.96	1,120±2.30
S2	Eripattrai	800 ± 8.81	3300 ± 5.77	448±0.66	2850±3.33
S3	Thattankuttai	1250 ±7.81	3230 ± 3.33	1,118±0.66	3250±1.99
S4	Valathur	30000 ± 10	72400 ±16	3680±3.33	11,150±8.81

son to 8,500 MPN/100mL at S4 in the monsoon season. In all the lakes, MPN values were greater than the value which is specified in BIS:(10500-2012). The monsoon season dominated the MPN values compared with the pre-monsoon season. It is because the greater load of *E. coli* and consequent high *E. coli* levels in lake water were caused by a wet season. Additionally, if *E. coli* grows at all, increased nutrient loads with runoff may aid in that growth.

Location S4 achieved a higher MPN value in Total coliform and also in fecal coliform, which indicates that there may be an influence of the household drainage and the results were supported by unpredictable changes in the coliform bacteria caused by seasonal fluctuations (Legenre, Baleux, & Troussellier, 1984; Barcina, 1986; Ramanibai, 1996). The fecal coliform value for each site and the minimum and maximum concentrations at each location are shown in Table 2.

The occurrence of *E. coli* was confirmed with EMB (Eosin-Methylene Blue) agar and is shown in Table 3.

PCA (Principal Component Analysis) analysis

Thirteen parameters were analyzed for the lake water samples and the results were subjected to Principal Component Analysis(PCA) and the relationship between the parameters was determined. The identified parameters were regarded as independent variables and for the dependent variable, the sampling site was taken into consideration. The output of PCA analysis is depicted in Table 4 and Table 5 for the pre-monsoon and monsoon seasons.

In the pre-monsoon season, the entire 13 parameters were classified into 3 components in the Principal Component Analysis(PCA). In PC (principal component) 1, chromium, copper, andfluoride had a total variance of 47.2%. This may be a result of human-made contaminants released from the disposal of household garbage and liquid wastes from industries. In PC2, Total coliform, magnesium, Total Hardness, and Conductivity had some similarities and the total variance was 37.1%. In PC3, the parameter pH and sulfate had similar patterns and showed a 17.9% total variance.

In the monsoon season, all the water quality parameters were divided into 3 components in the PCA analysis. In PC1, the parameter pH and sulfate had similar patterns with a total variance of 41.3%. In PC2, Total Hardness and magnesium had unique patterns with a total variance of 36.4%. In PC3, the heavy metal copper and Conductivity had unique trends with a variance of 22.2% and the presence of these pollutants is due to industrial activity and also dumping of waste near the lakes.

Table 3. Completed test for *E. coli* for all the lakes.

S.No	Place	Sample concentration (Pre-monsoon)			Sample concentration (Monsoon)		
		10ml	1ml	0.1ml	10ml	1ml	0.1ml
S1	Thattaparai	+++++	+++	+++	+++++	+++	+++
S2	Eripattrai	+++++	+++++	+++++	+++++	+++++	+++++
S3	Thattankuttai	+++++	++	++	+++++	++	++
S4	Valathur	+++++	+++++	+++++	+++++	+++++	+++++

Table 4. PCA for Pre-Monsoon season.

Parameters	Component		
	PC1	PC2	PC3
pH	.201	-.017	**.980**
Conductivity	.135	**.891**	-.433
Turbidity	-.788	-.050	.613
Total Hardness	.310	**.863**	.399
Calcium	.275	.645	.713
magnesium	.312	**.914**	.258
Chloride	**.759**	.647	.075
Sulfate	-.189	.258	**.948**
Fluoride	**.861**	.422	.285
Chromium	**.995**	.074	.071
Copper	**.995**	.074	.071
Total coliform	-.490	**.847**	.206
Fecal coliform	-.708	.568	.420
Eigen Value	6.143	4.569	2.287
Variability %	47.258	35.147	17.595
Cumulative %	47.258	82.405	100%

Table 5. PCA for Monsoon season.

Parameters	PC1	Component PC2	PC3
pH	**.987**	-.083	.135
Conductivity	.565	.532	**.630**
Turbidity	.269	-.380	-.885
Total Hardness	.103	**.994**	-.021
Calcium	.430	.716	.549
Magnesium	.151	**.962**	-.230
Chloride	-.531	.188	-.826
Sulfate	**.736**	-.301	.606
Fluoride	.255	-.926	-.278
Chromium	.552	-.834	-.010
Copper	-.068	-.062	**.996**
Total Coliform	-.943	-.109	.314
Fecal Coliform	-.994	-.061	-.093
Eigen Value	5.369	4.714	2.889
Variability %	41.301	36.473	22.226
Cumulative %	41.301	77.774	100

The scree plot displays the change in eigenvalue as the number of components is increased while keeping the cutoff eigenvalue at 1. The entire data set was grouped into 3 principal components in the pre-monsoon season and for the monsoon season, the entire data set was grouped into 3 components based on pollution load. The Scree plots for the pre-monsoon and monsoon seasons are depicted in Figure 3(a) and Figure 3(b).

Relationship between physicochemical and bacteriological analysis

Physicochemical and bacterial interaction for the pre-monsoon and monsoon seasons at The Gudiyattam Lake region is shown in Table 6 and Table 7 respectively. During the pre-monsoon season, FC had a significant positive correlation with turbidity and also had a positive correlation with TC. For the monsoon season, FC had a negative correlation with pH and a positive correlation with TC. Pereira et al.,2011) reported that there was an inverse relationship between the mean concentrations of bacterial population and the mean value of pH in the water.

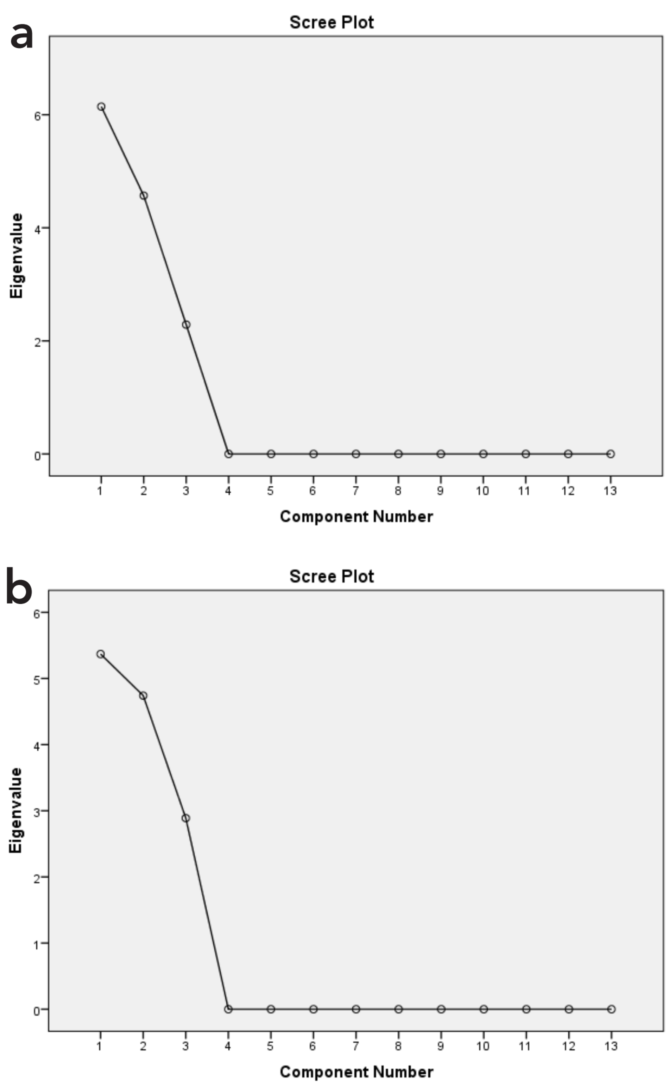

Figure 3. Scree plot for pre-monsoon and post monsoon seasons.

CONCLUSION

The major physio-chemical result lies within the standards except for the following parameters,hich are conductivity, turbidity, and magnesium ion which were specified in BIS:(10500-2012). All the lakes were contaminated with bacteria in the higher range. Especially, location S4 dominated when compared to all the other lakes for total coliform and fecal coliform as well as in the monsoon season. This happens due to the runoff caused by rainfall, which contributes significantly to the influx of fecal debris into the lake, and thus the results revealed there was a correlation between fecal coliform concentrations in the river or lake water and runoff. Also, S4 is exposed to a variety of daily human activities as well as receiving the direct waste from dwellings. The remaining sites also achieved higher bacterial contamination than the standard value, which was specified in BIS10500-2012). This might be due to various human, agricultural, and industrial activities having a serious impact on the microorganisms in the water. Surface runoff from agricultural and grazing land, animal waste, and effluent runoff discharges from industrial sites and plantations will contaminate lakes near populated areas, resulting in bacterial infection and flues via water. The fecal counts were typically high, notably at locations S1, S3, and S4, implying the presence of human or animal waste, as well as harmful bacteria. Nonpoint sources of fecal coliform bacteria in urban and rural regions include bird feces, rats, wild animals, and even domestic animals are also responsible for the microbial load. Organic waste produced from industries, like raw sewage, and other drainages may contain an enormous number of chemicals, disrupting the surface water ecological food chain and is forecasted to be the cause of high MPN indices. Considering the correlation analysis, FC was significantly positive with turbidity and negative correlation with pH, which clearly indicates the lakes had been polluted by various actions.

Overall, this study gives an output that S4 (valathur) needs serious attention compared to all the other lakes concerning bacteriological pollution and the current study recommends continuous monitoring of this region and efficient management to prevent future contamination of this aquatic environment.

Ethics Committee approval: Ethics Committee approval was not required for this study.

Competing Interest: To the best of our knowledge, we haven't ever sent identical work for review or publication to another journal.

Conflict of Interest: There was no conflict of interest in this study.

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Table 6. Correlation Matrix of Physico-chemical and bacterial parameters of the lake water in Gudiyattam region during Pre-monsoon season.

Parame-ters	pH	Con-duct ivity	Turbi dity	TH	Ca^{2+}	Mg^{2+}	Cl	SO_4	Fluor ide	Cr	Cu	TC	FC
pH	1												
Conducti Vity	0.606	1											
Turbidity	0.139	-0.057	1										
TH	0.207	-0.129	-0.256	1									
Ca+	-0.095	-0.441	0	0.54	1								
Mg+	0.3	0.102	-0.299	0.874	0.064	1							
Chloride	0.129	-0.13	-0.542	0.677	-0.105	0.861	1						
Sulfate	0.512	-0.201	-0.219	0.252	-0.03	0.311	0.572	1					
Fluoride	0.744	0.837	-0.2843	0.138	-0.517	0.461	0.378	0.287	1				
Cr	-0.33	-0.748	-0.419	0.472	0.21	0.435	0.737	0.553	-0.296	1			
Cu	0.498	0.357	-0.6	0.346	-0.417	0.647	0.804	0.642	0.795	0.329	1		
TC	-0.005	0.072	0.617	0.379	0.216	0.331	-0.111	-0.512	-0.123	-0.264	0.397	1	
FC	0.076	-0.025	0.939	-0.01	0.052	-0.037	-0.376	-0.35	-0.24	-0.361	-0.55	0.845	1

TH(mg/L):Total Hardness,Ca^{2+}(mg/L):calcium ion, Mg^{2+}(mg/L):magnesium ion, Cl(mg/L):Chloride, SO_4(mg/L):Sulfate, Cr(mg/L): Chromium, Cu(mg/L):Copper, TC:Total coliform, FC: Faecal Coliform

Table 7. Correlation Matrix of Physico-chemical and bacterial parameters of the lake water in Gudiyattam region during Monsoon season.

Parameters	pH	Conduct ivity	Turbi dity	TH	Ca^{2+}	Mg^{2+}	Cl	SO_4	Fluor ide	Cr	Cu	TC	FC
pH	1												
Conductivity	0.608	1											
Turbidity	0.071	-0.466	1										
TH	0.364	0.805	-0.327	1									
Ca+	0.519	0.918	-0.555	0.927	1								
Mg+	0.407	0.816	-0.214	0.988	0.896	1							
Chloride	-0.61	-0.554	0.67	-0.21	-0.55	-0.139	1						
Sulfate	0.583	0.942	-0.385	0.947	0.976	0.946	-0.437	1					
Fluoride	-0.32	-0.794	0.653	-0.91	-0.96	-0.851	0.493	-0.898	1				
Cr	0	0	0	0	0	0	0	0	0	1			
Cu	0	0	0	0	0	0	0	0	0	0	1		
TC	-0.83	-0.186	-0.311	-0.21	-0.26	-0.227	0.383	-0.307	0.16	0	0	1	
FC	-0.97	-0.472	-0.039	-0.3	-0.47	-0.315	0.661	-0.493	0.321	0	0	0.921	1

TH (mg/L): Total Hardness, Ca^{2+} (mg/L): calcium ion, Mg^{2+} (mg/L): magnesium ion, Cl (mg/L): Chloride, SO_4 (mg/L): Sulfate, Cr (mg/L): Chromium, Cu (mg/L): Copper, TC: Total coliform, FC: Fecal Coliform

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Research Article

Effect of Stocking Densities on Growth and Production Performance of Bheda (*Nandus nandus*) in Pond Aquaculture

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ABSTRACT

The current study's goal was to look at how stocking densities affected Bheda (*Nandus nandus*) growth and production over the course of a 120-day period. Three treatments were T_1 (20,000 fish ha^{-1}), T_2 (30,000 fish ha^{-1}), and T_3 (40,000 fish ha^{-1}), each with three replicates. Those fishes were fed daily with commercial sinking feed and a live food mixture at 9-3% fish body weight up to harvesting. Temperature, dissolved oxygen, pH, and other water quality indicators were measured every two weeks, along with the growth performance of Bheda fish. T_2 had the highest yield of Bheda (3439.08±207.31 kg ha^{-1}), followed by T_3 (3422.78±224.42 kg ha^{-1}), and T_1 (3136.62±150.00 kg ha^{-1}). The harvesting weight (g), individual weight gain (g), individual percent (%) weight gain (g), SGR (% per day), and survival rate (%) of fish were significantly higher ($p<0.05$) in T_1, followed by T_2 and T_3, respectively, where combined production of fishes was significantly higher at T_2 followed by T_3 and T_1. Net profit and benefit-cost ratio were significantly higher in T_1 than T_2 and T_3. Based on the results of this experiment, it can be concluded that stocking density of Bheda fingerlings at the rate of 20,000 fish ha^{-1} in T_1 showed the highest production performance for profitable pond aquaculture. However, further research on the standardisation of stocking density with economic profitability of this fish at the on-station or on-farm level in ponds may be required before widespread dissemination of this culture technology to farmers, particularly in Bangladesh and elsewhere.

Keywords: Stocking density, Growth, Production, Bheda

INTRODUCTION

The fishing industry adds substantially to Bangladesh's agro-based economy by supplying food and nutrition, reducing poverty, opening up employment possibilities, and gaining offshore money (Islam *et al.*, 2016). In 2020–2021, there were 4.62 million metric tonnes (MT) of fish produced annually, with aquaculture output accounting for 57.10% of the total (DoF, 2022). In 2020–2021, this sector generated more than 26.50% of the agricultural Gross Domestic Product (GDP) and 3.57% of the national GDP. This industry's average growth rate during the previous ten years was close to 5.43%. The government of Bangladesh is working to maintain this growing performance, which has enabled the fisheries sector to reach this significant milestone (DoF, 2022). Bangladesh presently produces enough fish to meet its own needs, accounting for about 60% of the population's daily need for animal protein. More than 12% of Bangladesh's entire population work in this industry either full-time or part-time as a means of subsistence (DoF, 2022). Through fishing-related activities, more than 17 million people (BFTI, 2016), afford their living where more than 80% are women (DoF, 2015). According to an

FAO report, Bangladesh ranked third in inland open water capture production and in world aquaculture production. Bangladesh also ranked in tilapia production in the world and third in Asia (FAO, 2022; Pandit *et al*., 2021).

The people of Bangladesh have relied on fish and fisheries for their nourishment, economics, culture, and traditions from the onset of time, and they strive to do so now (Arefin *et al*., 2018). The production of fish per hectare in this country is still lower than in some fish-producing countries of the world (FAO, 2022). This is mainly due to a lack of knowledge of the scientific fish culture and management practises used by countries like China and Vietnam. To increase the productivity of water bodies efficiently, an understanding of the various aspects of scientific fish culture and management of fisheries resources is required.

Small indigenous species (SIS) of fish have huge culture potential in Bangladesh. There are about 150 different SIS available in Bangladesh, such as *Anabas testudineus* (Bloch, 1792), *Channa striata* (Bloch, 1793), *C. punctata* (Bloch, 1793), *Nandus nandus* (Hamilton, 1822), *Ompok pabda* (Bloch, 1794), *Salmostoma phulo* (Hamilton, 1822), etc. Some of them have high nutritional value in terms of proteins, vitamins, and minerals which are not commonly available in other foods (Sharmin *et al*., 2016; Mustafi *et al*., 2022; Kawsar *et al*., 2023). Among the available SIS, Bheda (*N. nandus*), locally called Meni or Nondoi, is an indigenous, near threatened, and small food fish in Bangladesh. *N. nandus* exclusively a bottom and column feeder (carnivorous) (Mustafa *et al*., 1980; Saha *et al*., 2000; Kawsar *et al*., 2023) which is widely distributed in India, Pakistan, Nepal, Myanmar, and Thailand (Froese and Pauly, 2019; Gupta, 2018). Muddy streams, rivers, pools, and marshes are its main habitats (Bhuiyan, 1964) and it inhabits both fresh and brackish waters (Hossain and Afrose, 1991) and uses the floodplains as its breeding, nursing, and rearing grounds (Sharmin *et al*., 2016). It is a high-priced fish despite its spiny fins and ugly black bands and blotches all over its body (Talwar and Jhingan, 1991). It has a breeding period from April to September (Hossain and Afrose, 1991).

However, indiscriminate fishing, unplanned construction of bridges and flood protection embankments, destruction of breeding grounds, rapid habitat degradation, siltation, and anthropogenic induced climate change are decreasing its population in rivers and wetlands (Sarkar *et al*., 2020; Kawsar *et al*., 2023). The International Union for Conservation of Nature (IUCN) categorises Bheda as lower risk least concern, though it is a near threatened species in Bangladesh (IUCN, 2015); Kawsar *et al*., 2023). An induced breeding technique that is successful and culture technology development can offer cultivation of these fish in shallow waterways like rice fields, which would play a significant part in Bangladeshi people's total nutrition, especially the poor and lower middle-class inhabitants of rural Bangladesh. By boosting its yield, induced breeding, rearing, or the creation of culture techniques for *N. nandus* can contribute significantly to its conservation and rehabilitation.

There is little research on Bheda artificial breeding for the purpose of producing its fry and raising it in cisterns in Bangladesh (Das *et al*., 2022) in order to save this slightly grey and dark brown striped fish from extinction. As stocking density plays an important role in the growth and production of fish in fish culture (Backiel and Le Cren, 1978; Kunda *et al*., 2021), nevertheless, stocking densities have not yet been standardised for the successful rearing or culture of *N. nandus* in earthen ponds so far. Therefore, this experiment was undertaken for the successful rearing or culture of *N. nandus* to assess the growth performance and economics of this fish at different stocking densities in earthen ponds.

MATERIALS AND METHODS

Location and duration of study

The experiment was carried out by the Bangladesh Fisheries Research Institute of Floodplain Sub-station, Santahar, Bogura (Figure 1) during the period from 01 August to 31 November 2020.

Experimental design and stocking of fishes

A randomised complete block design (RCBD) was followed for this assessment with three different stocking densities viz. T_1 (20,000 fish ha^{-1}), T_2 (30,000 fish ha^{-1}), and T_3 (40,000 fish ha^{-1}), each with three replicates (Table 1). All fish were stocked at the same weight (an average of 3.64 g) for all treatments. Fish seeds from the *N. nandus* species were mostly procured at the Bangladesh Fisheries Research Institute's Flood-plain Sub-station hatchery. The fries were maintained in a nursery pond at a high stocking density after being collected. Fries were relocated and provisioned in nine experimental grow-out ponds after three days in accordance with the research setup. During the discharge of the fry into the ponds, proper procedures and hygienic conditions were upheld.

Pond management

The ponds' water was drawn out and left to dry in the sun for approximately a week, or until the bottom finally cracked or hardened enough to keep a man standing without sinking more than 1 cm. Draining and drying eradicate competitor predatory (*Channa* spp., *Wallago attu*, *Clarias batrachus* and *Notopterus chitala*) and weed fishes (*Puntius* spp., *Colisa* spp. and *Ambassis* spp.). The pond's bottom was then dug up and dried. In addition to eradicating undesired species and burrowing predators like mudfish, ploughing makes underlying nutrients accessible at the surface for the development of fish food in the pond. Then the bottom of the pond was levelled. To keep predators and pests out of the pond system, dikes and all gates were fixed. Additionally, pipelines were examined for cracked slabs and other damage. They were cleansed to get rid of any obstruction-causing material. Aquatic weeds were removed (*Colocasia esculenta*, *Enhydra fluctuans, Marsileaquadri folia, Eichhornia crassipes* and *Ipomaea aquatica*) manually from the ponds. Additionally, the

Table 1. Experimental blueprint (mean±SD).

Variables	Treatments		
	T_1	T_2	T_3
Pond dimension (m³)	720±10	720±15	720±20
Pond profundity (m)	1.2±0.5	1.2±0.6	1.2±0.3
Stocking density (fish ha^{-1})	20,000	30,000	40,000

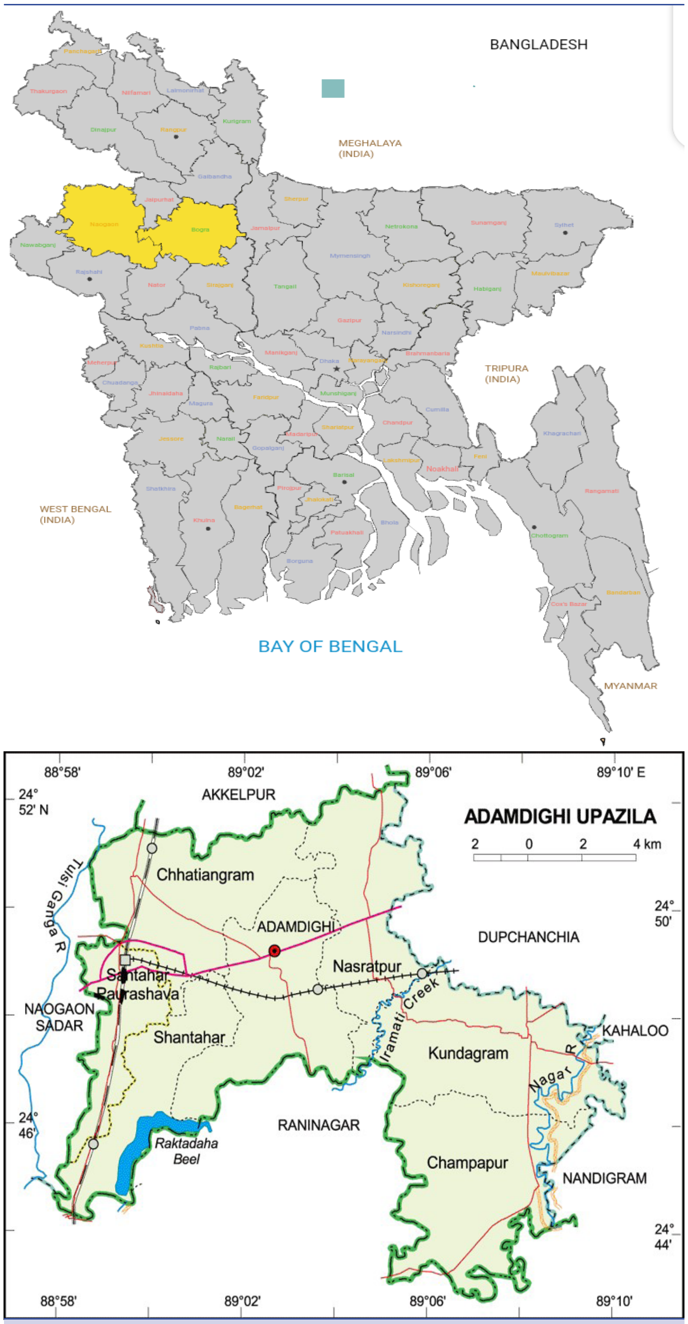

Figure 1. Experimental site.

grasses on the pond dike were carefully pruned down to an extremely tiny size. A deep tubewell was utilised when necessary to maintain the water depth of 1.20 m in all of the ponds utilising ground water. To keep the proper water level, each pond has a well-designed input and outflow system. Lime was applied at a rate of 250 kg ha^{-1} after 3 days. Lime was put in the pond water and embankment after being liquefied in an earthen pot. $CaCO_3$ (agricultural lime) and CaO (quicklime) were utilised for this. The ponds were fertilised with cow dung, urea, and triple superphosphate (TSP) at the rates of 500 kg ha^{-1}, 50 kg ha^{-1}, and 25 kg ha^{-1}, respectively, after 5 days of liming. The water was examined for natural food after a week of fertilising. To improve fish development performance, additional feed continued to be used on a regular basis.

Feeding of Bheda

After stocking, Bheda fish were fed with commercial sinking feed and a live food mixture at 9-3% of the body weight of stocked fish and supplied twice daily. Commercial sinking feed (Quality Feed Co. LTD.) contains 27.94% crude protein, 12.14% moisture, 7.95% crude fat, 8% crude fibre, 15.38% ash, and 36.59% nitrogen free extract. Throughout the period of cultivation, three different types of commercial fish feeds were used, including starter, grower, and finisher feeds. From the time of stocking until three weeks later, starter feed was administered at 6% of the body weight of the fish. Grower feed was then applied until 14 weeks at 4% of the body weight of the fish. Finisher feed was used the last two weeks at 2% of the body weight of the fish. At first, a live food mixture was given at 3% of the body weight of the stocked fish, and the amount was decreased to 1% in the last month according to the different types of commercial feed.

The mixture of live foods contained 75% trash fish fry (*Lepidocephalus guntea*, *Esomus danricus*, *Channa punctata*) and 25% zooplankton (*Notonectidae*, *Cyclops* sp., *Daphnia* sp.).

Growth sampling of *N. nandus*

A seine net and a scoop net were employed during sampling at intervals of 15 days to track Bheda's growth performance and to alter the feeding rate in light of this fish's body weight. To evaluate the health, growth, and even some hazy growth tendencies of the Bheda fish, individual weights of at least 10% of the initially supplied fish were obtained. The weight of the fish in each sampling was determined using a portable balance (OHAUS, model No. CT-1200-S). Throughout the culture phase, pond conditions in general and fish health were regularly checked. Because the sampled fish are sensitive to handling stress, care was taken when handling them.

Harvesting

All fishes were entirely taken at the conclusion of the experiment (after a 120-day culture period). Bheda fish were mostly caught by repetitive netting using a fine-mesh net towed over the pond. After that, a shallow pump was used to entirely drain the water out of each pond individually. The remaining Bheda fish were then taken separately from each pond. In order to determine the survival rate and productivity, each Bheda fish was then numbered, measured, and weighted separately for each pond.

Monitoring of water quality parameters

Throughout the study period, water quality parameters were sampled between the hours of 9:00 and 10:00 a.m. in the morning on each sampling day at intervals of 15 days. A Celsius thermometer was placed 20–30 cm under the water's surface to measure the water's temperature. A black and white veiled Secchi disk was used to measure water transparency (cm). Alkalinity (mg/l) and ammonianitrogen (mg/l) were dogged with a HACH kit (FF2, USA). Dissolved oxygen (mg/l) was listed through a dissolved oxygen metre (DO-5509), pH was recorded with a pH metre (HANNA instruments, HI 98107), and total dissolved solids (TDS) were analysed with a Multimeter (HQ 40 D, HACH, USA).

Fish growth monitoring

Data were gathered every two weeks, with 10% of the supplied fish from each pond being sampled using a seine net on each sampling day. The fish were then weighed in a computerised electronic scale with a 0.01 g precision before being immediately released into the ponds without inflicting any discernible harm to the fish. Following some previous research (Brett and Groves, 1979; Kunda *et al.*, 2021, 2022), the growth, survival, and production abilities of fish were examined as follows:

Initial weight (g) = Fish stock weight

Final weight (g) = Fish harvest weight

$$\text{Weight gain} = \frac{\text{Mean final fish weight (g)} - \text{mean initial fish weight (g)}}{\text{mean initial fish weight (g)}} \times 100$$

Specific growth rate (SGR) (% day^{-1}) = $\frac{\text{Log}_e W_2 - \text{Log}_e W_1}{T_2 - T_1} \times 100$ (Brown, 1957)

Where,

W_1 = The initial body weight (g) at time T_1 (day)

W_2 = The final live body weight (g) at time T_2 (day)

T_2 - T_1 = Duration of fish rearing (days)

$$\text{Survival (\%)} = \frac{\text{No. of fish harvested}}{\text{No. of fish stocked}} \times 100$$

Gross production = No of fish harvested × Final mean weight of fish

Net production = No of fish harvested × Mean weight gain of fish

Economic analysis

On the basis of the expenses (cost of fish fry, energy, lime, fertiliser, labour, and harvest) and the revenue from selling fish, cost-benefit analyses of the treatments were developed. The cost of goods and fish sales are indicated in Bangladeshi Taka (BDT) (1 USD = 85 BDT, November 2020). Due to the varying sizes, the average selling prices of fish at T_1, T_2, and T_3 were 300, 280, and 250 BDT/kg, respectively.

The net return was calculated using the following formula: R = I − (FC + VC + Ii)

where, R = net return, I = fish sale revenue, FC = fixed costs (costs that apply to all treatments equally), VC = variable costs and Ii = interest on inputs.

Benefit-cost ratio (BCR) = Fish selling revenue / Total input costs

Statistical analysis

One-way analysis of variance (ANOVA) was used to examine the relationship between stocking densities and the growth and production performances of Bheda in pond aquaculture. While a mean impact was significant, Duncan New Multiple Range Test (Duncan, 1955) was conducted after the ANOVA at a 5% level of significance (Gomez and Gomez, 1984). After the arcsine transformation, the percentages and ratio data were examined. Prior to analysis, the assumptions of a normal distribution and variance homogeneity were verified. Version 20.0 of SPSS (Statistical Package for Social Science) was used for all analyses (IBM Corporation, Armonk, NY, USA).

RESULTS

Hydrological properties of the pond water

Water quality parameters of the pond water were analysed in this experiment to observe any appreciable variation among the treatments (Table 2). Water quality parameters viz. temperature (°C), dissolved oxygen (mg/l), pH, transparency (cm), total alkalinity (mg/l) and NH_3 (mg/l) did not vary significantly (p>0.05) among the treatments.

Growth and yield parameters of *N. nandus*

Table 3 displays growth and yield metrics of Bheda. Fish stocking density has a noticeable impact on the overall growth presentation of this fish (Table 3 and Figure 2). Individual harvesting weight (g), weight growth (g), weight gain percentage (%), and survival rate (%) of Bheda were all significantly greater in T_1 treatment than in T_2 and T_3 treatments, respectively. As a general rule, a higher specific growth rate (% bw d^{-1}) was also observed in T_1, despite the fish being stocked in that treatment at a much lower rate than those in the other treatments. The fish at T_1 had a considerably (p<0.05) greater survival rate than the others (Table 3).

Table 2. Water quality variables (mean±SD) recorded under different treatments during the study period of August-November, 2020.

Months	Treatments	Temp. (°C)	pH	Dissolved oxygen (mg/l)	Transparency (cm)	Total alkalinity (mg/l)	NH_3 (mg/l)
	T_1	28±1.11	7.88±0.5	5.81±0.50	36.88±3.22	82.10 ± 2.47	0.49±0.10
Aug.	T_2	28.18±1.3	7.93±0.5	5.91±0.39	34.5±2.8	83.11 ± 2.85	0.50±0.13
	T_3	28.21±1.5	7.96±0.5	5.96±0.37	34.8±4.45	85.20 ± 2.95	0.47±0.18
	T_1	31.99±1.2	7.3±0.8	6.2±0.31	36.33±4.22	84.10 ± 1.23	0.48±0.11
Sept.	T_2	32.23±0.7	7.6±0.4	5.4±0.31	35.6±2.5	81.11 ± 2.11	0.49±0.12
	T_3	30.22±1.2	7.2±0.14	5.9±0.55	34.66±5.1	81.25 ± 2.31	0.51±0.11
	T_1	31.88±2.3	7.2±0.41	6.2±0.11	36.33±2.01	82.10 ± 2.94	0.50±0.09
Oct.	T_2	29.88±2.1	6.9±0.42	5.81±014	33.99±4.22	83.11 ± 2.92	0.54±0.12
	T_3	30.44±1.8	7.3±0.32	5.74±0.35	34.88±4.31	82.20 ± 1.99	0.49±0.10
	T_1	27.19±1.1	7.1±0.81	6.64±0.41	36.66±3.33	85.10 ± 3.25	0.50±0.10
Nov.	T_2	29.55±1.0	6.9±0.28	5.64±0.55	36.33±3.18	80.11 ± 2.23	0.48±0.07
	T_3	25.09±1.0	7.3±0.47	5.51±0.19	35.5±5.50	79.20 ± 2.99	0.49±0.08

Feed utilisation

N. nandus stocking at different stocking densities had a significant impact on the FCR (Table 3). FCR was lowest at treatment T_1's lowest stocking density, and subsequent increases in stocking density at treatments T_2 and T_3 resulted in a considerable drop in feed consumption, which was shown by a magnified FCR value.

Economic analysis

Economic illustration of *N. nandus* under various stocking density is given in Table 4. In this experiment, fish fry cost was an important item for being a SIS among the treatments. Only few hatcheries do induced breeding of this species. The greatest feed cost was determined at treatment T_3, despite the fact that feed cost for treatments T_2 and T_1 also fluctuated considerably over the research period due to the large influence of stocking density that was seen in the experimental ponds. Feed costs made up the majority of the study's costs, accounting for 42.07, 50.34, and 55.87% of the total costs for T_1, T_2, and T_3, respectively. In addition to this, a few other expenses had fixed prices, most

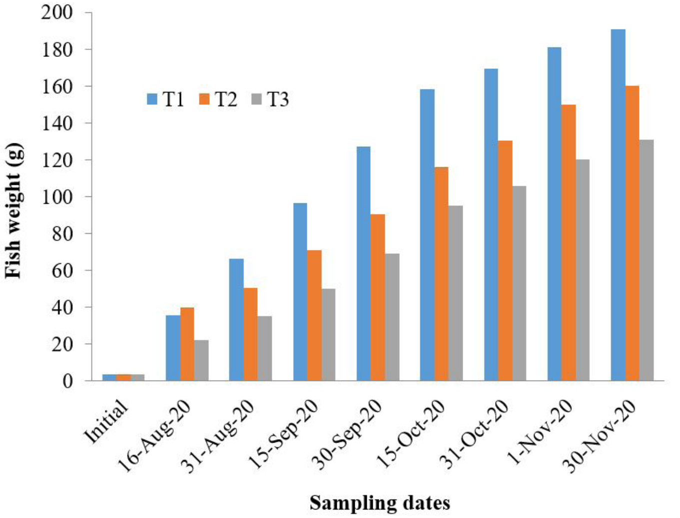

Figure 2. Fish growth in different stocking density.

Table 3. Growth and yield parameters (mean±SD) of Bheda fish in different treatments during study period.

Growth parameters	Treatments		
	T_1	T_2	T_3
Initial weight (g)	3.64±0.31^a	3.64±0.23^a	3.64±0.16^a
Final weight (g)	190.56±0.49^a	160.33±1.26^b	130.84±3.46^c
Weight gain (g)	186.92±1.80^a	156.69±1.30^b	127.2±2.30^c
% weight gain	5135.17±0.58^a	4304.67±0.31^b	3494.51±1.50^c
Specific growth rate (% bwd $^{-1}$)	2.12±0.05^a	2.41±1.70^b	2.81±0.07^c
Survival rate (%)	82.3±0.45^a	71.50±0.65^b	65.40±0.03^c
FCR	1.09±0.51^a	1.29±0.55^b	1.46±0.99^c
Total yield (kg ha^{-1} 120 d^{-1})	3136.62±150^b	3439.08±207.3^a	3422.78±224.4^a
Net yield (kg ha^{-1} 120 d^{-1})	3076.70±200^b	3361.00±250.80^a	3327.55±278.30^a

*Mean values with different superscripts letters in the same row indicate a significant difference (p<0.05).

Table 4. Costs associated with carp fattening at various pond stocking densities.

Items	Treatments (mean ± SD)		
Fixed costs (BDT/ha)	T_1	T_1	T_1
Pond lease	120000	120000	120000
Variable cost (BDT/ha)			
Fish fry	40000	60000	80000
Feed	200000	300000	400000
Electricity bill (Pump)	13000	13000	13000
Labour	60000	60000	60000
Fertilizer	20000	20000	20000
Harvest	13000	13000	13000
Total cost (BDT/ ha)	476000±2000 ^{c}	596000±2250 ^{b}	716000±1500 ^{a}
Total return (BDT/ ha)	940985.28±2000 ^{a}	962941.98±2000 ^{a}	855693.6±2000 ^{a}
Net benefit/profit (BDT/ ha)	464985.28±2000 ^{a}	366941.98±2000 ^{b}	139693.6±2000 ^{c}
BCR	1.97 ^{a}	1.62 ^{b}	1.19 ^{c}

Values in the same row with different superscripts are significantly different (p<0.05).

notably the cost of electricity (for the pump), lime, fertiliser, and harvesting for all treatments. However, the full refund from the fish vending industry claimed a different setup of total costs, and treatment T_1, where the stocking density was lower, filed for a substantially larger total reimbursement. Additionally, treatment T_1 generated a much bigger net benefit; hence, the economic analysis revealed a higher BCR at a lower stocking density for treatment T_1. Additionally, for T_1, T_2, and T_3, the net benefit was 97.68%, 61.56%, and 19.51%, respectively. The growth of Meni/Bheda was confirmed to be considerably more lucrative for the farmer and to be economically beneficial at a lower stocking density, and higher stocking density was always lacking in number of multiplication in comparison with the lower stocking density ponds.

DISCUSSION

The most significant elements influencing fish growth and productivity in aquaculture ponds are fish stocking size and density (Mazlum, 2007; Zhu *et al*., 2011; Garr *et al*., 2011; Kunda *et al*., 2021). Fish growth and survival were negatively impacted by higher stocking density (40,000/ha) at treatment T_3. Additionally, it has been confirmed by Pouey *et al*. (2011), Sorphea *et al*. (2010), Mohanty *et al*. (2004), Rahman and Verdegem (2010), and Mridha *et al*. (2014) that fish at higher stocking density are subjected to a relatively higher struggle for food and space, which causes physiological stress to fish and results in lower growth, while some can tolerate extreme crowding, competition for food, and DO (Stickney, 1994).

The significant impact of stocking density on FCR is shown in Table 3. As shown by the FCR value, treatment T_1's lower stocking density resulted in greater feed consumption. Whereas Buentello *et al*. (2000) reported that low dissolved oxygen resulted in a stress situation that altered the food intake of fish, higher stocking density at treatment T_3 may produce overcrowding and a consequent decrease in oxygen consumption. Several studies have also documented a detrimental negative impact of increasing stocking density on the aquaculture feed efficiency of farmed fish. Haque *et al*. (2018) and Rahman *et al*. (2010) reported that the individual final weight of cultured fish may be reduced with the increase of their stocking density due to intra-specific competition for the same resources. In this experiment, the mean values of individual weight gain of Bheda fish were significantly higher ($p<0.05$) in T_1 than in T_2 and T_3, where stocking density was low, which could be due to high competition for feed and space, which slows down weight gain. This finding, however, was nearly identical to that of Begum *et al*. (2017b), who discovered better individual weight gain in Bheda at a lower stocking density fed with artemia. Bheda's individual percent (%) weight gain was significantly higher ($p<0.05$) in T_1 and T_2 than in T_3. This could be attributed to the T_1 treatment's higher individual harvesting weight and weight gain, followed by the T_2 and T_3 treatments. The result also indicated that the growth rates varied due to different stocking densities, which coincides with the findings of Kunda *et al*. (2021), Begum *et al*. (2017b), Begum (2009), Rubel (2008), and Rashid (2008). The specific growth rate of the fish was significantly higher ($p<0.05$) in the T_1 treatment than in the T_2 and T_3 treatments, respectively. This finding was more or less similar to the findings of Haque *et al*. (2018), Begum *et al*. (2017b), and Ronald *et al*. (2014). However, the highest gross and net yields of Bheda were found in T_2 compared to T_3 and T_1, respectively. Although the individual harvesting weight (g), individual weight gain (g), individual percent (%) weight gain, specific growth rate (% bw d^{-1}) and survival rate (%) were higher in T_1 than T_2 and T_3, respectively, production does not depend only on the average individual weight, individual weight gain, specific growth rate (SGR), and survival rate (%) of fish but also on the number of individuals stocked (Rahman *et al*., 2010). Backiel and le Cren (1978) stated that stocking density has a direct effect on growth and hence on production. They also mentioned that the growth rate progressively increases as the stocking density decreases and vice-versa. However, the findings of gross and net production support the findings of Haque *et al*. (2018), Rahman *et al*. (2016), and Sarker (2016). Considering the overall growth performance, weight gain, percentage weight gain, specific growth rate (% bw d^{-1}), survival rate (%) gross and net production, the best result was obtained in T_1 with lower stocking density.

The appropriateness of water for the growth and existence of fish in fish farming is the standard definition of water quality. In T_1, T_2, and T_3, the mean water temperatures were 28±1.11°C, 28.17±1.3°C, and 28.21±1.58°C, respectively. The fact that there were no clouds in the sky and a strong sun on October 3 may have contributed to the highest temperature of 30.4°C that was recorded in T_1. It is possible that the low intensity of sunshine and some rain on August 19 led to the lowest water temperature (25.7 °C) ever recorded in T_3. Begum *et al*. (2017a) noted a temperature range of 28.50°C to 28.60°C for the water, which is somewhat comparable to the present findings. The dissolved oxygen concentration of the water in treatments T_1, T_2, and T_3 in the current investigation had mean values of 5.81±0.50, 5.91±0.39, and 5.96±0.37 mg/l, respectively. On August 19, T_1 had the greatest dissolved oxygen level (6.23 mg/l), whereas on 3 December, it had the lowest dissolved oxygen content (5 mg/l). Rahman *et al*. (2018) in primary nursing of bata (*Labeo bata*), Rayhan *et al*. (2018) in recirculating aquaponic system of monosex tilapia (*Oreochromis niloticas*), and Yasmin *et al*. (2019) in primary nursing of Mahseer (*Tor putitora*) all found decreased DO with higher stocking density.

Another potential explanation for treatment T_3's decreased DO concentration was the considerable decline in total plankton population brought on by the presence of more fish (Monir and Rahman, 2015). According to Boyd (1998), the ideal range for dissolved oxygen content in water is 5 to 15 mg/l. The DO values below 3.5 mg/l are not acceptable for fish farming, according to Neill and Bryan (1991) and Daniel *et al*. (2005). In order to support fish life in freshwaters, 5.0 mg/l of dissolved oxygen is necessary, according to Chapman and Kimstach (1996). According to Mallasen *et al*. (2012), oxygen concentrations below 4.0 mg/l are necessary for tropical fish growth. As a result, the experiment's DO values support the conclusions of the authors mentioned above. The water in tests T_1, T_2, and T_3 had mean pH values of 7.9, 7.91, and 7.92, respectively. Boyd (1979) asserted that an ideal pH range for enhancing fish output is between 6 and 9. According to Hepher and Pruginin (1981), fish culture is best in a pH range of 6.5 to 9.0. In pond water, Dinesh *et al*. (2017) discovered a pH range of 7.1 to 8.0. The pH readings from this investigation, however, were consistent with the findings of the authors mentioned above. Therefore, it could be argued that the water quality standards were satisfactory for *N. nandus* culture or upbringing.

According to the study, net profit declined as stocking density increased, which was consistent with Dasuki *et al*. (2013) and Hossain *et al*. (2022). The BCR for every treatment exhibits a significant heterogeneity in density. The 20,000 fish ha^{-1} stocking density of Bheda was therefore found to be the best among the three treatments in terms of growth, survival rate, output, and economic return, according to the overall findings of the current study. The BCR calculated for this trial run was between 1.19 and 1.97. The T_1 had the significantly highest BCR compared to the T_2 (1.62) and the T_3 (1.19), respectively (Table 4). Benefit cost ratio demonstrated the substantial *($p<0.05$)* differences across treatments. The BCR values that Kunda *et al*. (2021) and Hossain *et al*. (2022) acquired were significantly lower than our latest findings.

CONCLUSION

In this study, the stocking density of Bheda fingerlings at 20,000 fish ha^{-1} significantly improved *($p<0.05$)* the growth performance of fish in terms of individual harvesting weight, individual weight gain (g), individual percent (%) weight gain, specific growth rate (% bw d^{-1}), survival rate (%), and BCR. Despite T_1 having the lowest overall output, it has a more favourable BCR than other regimens. Therefore, it can be said that stocking fingerlings of the Bheda fish (*N. nandus*) at a density of 20,000 fish ha^{-1} has a significant impact on their growth and production performance in ponds. However, more research on standardising the stocking density of this fish at the on-station and on-farm levels may be necessary before this culture technology is widely distributed to farmers, particularly in Bangladesh and other areas in this region.

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Review Article

Application of the IMTA (Integrated Multi-Trophic Aquaculture) System in Freshwater, Brackish and Marine Aquaculture

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ABSTRACT

Aquaculture activities that have been carried out intensively for several decades have made this sector grow rapidly compared to other food sectors. However, intensive activities have negative impacts, one of which is on the environment. To respond to these problems, aquaculture activities have now focused on environmentally friendly aquaculture by implementing various ecosystem-based cultivation systems and improving aquaculture management based on the principle of sustainable aquaculture. The IMTA (Integrated Multi-trophic Aquaculture) system is a cultivation system that uses species with different trophic levels to reuse wasted nutrients to be used as biomass. Currently, the IMTA system has begun to be developed in various countries in fresh, brackish, and marine water cultivation with multiple approaches according to environmental, social, and economic conditions. This review study discusses different IMTA systems and their applications.

Keywords: Ecosystem Approach Aquaculture; Aquatic Sustainable Aquaculture; Integrated Multi-Trophic Aquaculture (IMTA)

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INTRODUCTION

Aquaculture has been one of the fastest-growing sectors in recent years. In 2015, 53% of total fishery production came from aquaculture. In addition, aquaculture production is predicted to increase by 32% (around 26 million tons) or reach approximately 109 million tons in 2030 (FAO, 2018). To meet the increasing market demand, intensive aquaculture activities are carried out. However, there are significant challenges faced by the aquaculture sector where producing organisms still use too many natural resources and impact the environment (Martinez-Porchas & Martinez-Cordova, 2012). In addition, the impact of intensive aquaculture practices creates several problems for the environment. One of the main problems caused is the nutrient load from metabolic waste products and uneaten feed (Tacon et al., 2010; Troell et al., 2017a). Nutrient loads that are wasted into the environment can cause eutrophication, ecosystem damage, biodiversity loss, and decreased oxygen concentration and water quality (Diana et al., 2013; Edwards, 2015; Martinez-Porchas & Martinez-Cordova, 2012). To deal with these problems, it is necessary to implement an environmentally friendly, ecosystem-based cultivation system and improve aquaculture management based on sustainable aquaculture.

Sustainable aquaculture, according to FAO (1995), is an economical and socially acceptable aquaculture activity based on the conservation of natural resources using technology. Sustainable aquaculture is a solution to dealing with the negative impacts of current aquaculture activities. Sustainable aquaculture must be ecologically efficient, environmentally friendly, diverse in products, and economically and socially profitable (Chopin, 2013a). To realise sustainable aquaculture, several criteria must be carried out: aquaculture activities must 1) promote socio-economic aspects, 2) produce and provide safe and nutritious products, 3) increase fish production in terms of land use, water, feed, and energy used, 4) and minimise water pollution, fish disease, and fish escaping into nature (Waite et al., 2014).

The development of cultivation with the concept of sustainability is currently being widely applied. Methods include using a recirculation system (RAS) and an eco-friendly and ecosystem-based cultivation system that has been implemented, one of which is through the multitrophic concept (IMTA). The RAS system (Recirculating Aquaculture Systems) is one of the technologies developed for intensive aquaculture. The RAS system allows the use of 90-99% of the water that has been recycled using specific components. However, this system has several limitations, including greater energy use, high production costs, waste disposal problems, and a high risk of failure due to disease (Badiola et al., 2012). The RAS system is currently only used in aquaculture activities with high selling value, definite production protocols, and large production models for mass-scale efficiencies, such as salmon and shrimp. So the cultivation of the RAS system with other cultivation systems still needs to be determined (Naylor et al., 2021). Meanwhile, multitrophic cultivation (IMTA) develops from the traditional polyculture cultivation model, as well as fish or shrimp farming which is integrated with vegetable plants, microalgae, shellfish, and seaweed. This system has been implemented for the past few decades, especially in Asia (Neori et al., 2004).

History of IMTA

The IMTA concept has been under development for a long time. It is based on the book Nong Zheng Quan Shu (The Complete Book on Agriculture), written in 1639, which explains many topics in the agricultural sector, including irrigation and rotation of fish and plant production in a field, integrated cultivation between fish and livestock, the use of manure for fish farming, as well as integrated cultivation of mulberry, paddy fields, and fish ponds (Chopin, 2013b). Modern IMTA began to develop in 1970 when John Ryther, through a project called "integrated waste-recycling marine polyculture systems", tried to revive the IMTA concept in modern times by developing intensive methods of processing waste outlets using seaweed and Bivalvia (Troell et al., 2003). Then in the 2000s, this concept was applied to aquaculture through various modifications such as polyculture cultivation, integrated marine cultivation, and ecology-based aquaculture (Kodama, 2019; Chopin, 2013b). Then, in 2004, the term *integrated multitrophic aquaculture* emerged and was initiated by Thierry Chopin and Jack Taylor. The basic concept of the IMTA system is the conversion of nutrients produced from aquaculture activities through species diversification (Chopin, 2013b).

IMTA System definition and principles

The IMTA is a cultivation system that utilises species with different eating habits and trophic levels in the same production system. These species are placed in the same compartments or separately in the waste stream (Figure 1). The goal is to be able to utilise waste that is reusable (Barrington et al., 2009; Chopin et al., 2008; Chopin et al., 2013). The IMTA system adheres to the "circular principle". In this system, scientific integration occurs in the utilisation of uneaten feed, waste, nutrients, and by-products produced by the species being fed for conversion into fertiliser, feed, and energy by other species with lower trophic levels (FAO, 2022). The selection and proportion of the use of correct species will balance the biological and chemical processes in the IMTA system. In addition, species use serves as a biofilter, and the species can be harvested and has commercial value (Chopin, 2006). The IMTA system generally aims to improve the ecosystem's condition in the cultivation environment and produce more excellent cultivation production than the monoculture model (Neori et al., 2004).

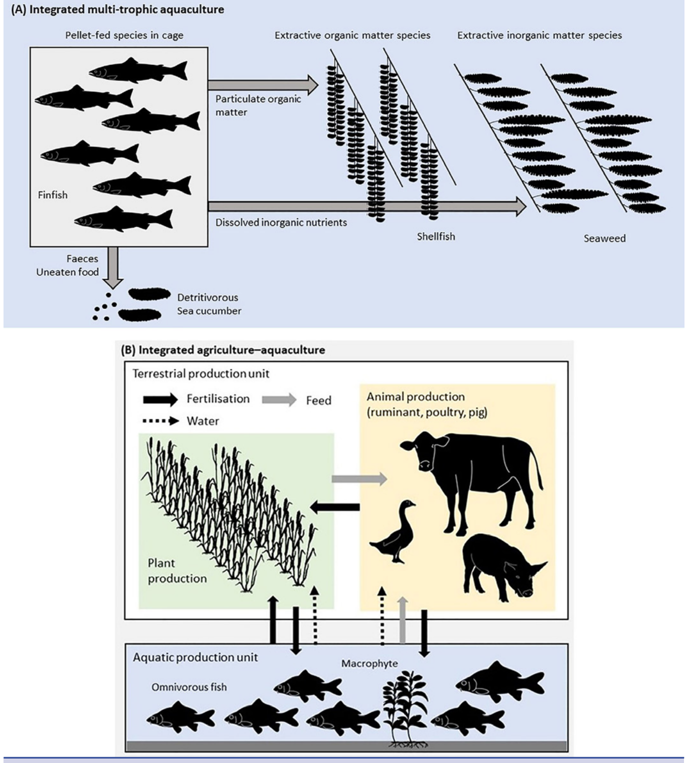

Figure 1. IMTA system concept (Thomas et al., 2020).

Components of the IMTA System

Several essential components in the IMTA system need attention, including species selection, extractive species (*organic and inorganic extractive species*), main species (*fed aquaculture*), and the IMTA design (Sasikumar & Viji, 2015). Species selection will affect cultivation with the IMTA system. The use of the suitable species will affect a stable and sustainable environmental balance, improve the environment, and increase total production (Chopin, 2006; Rosa et al., 2020; Sanz-Lazaro & Sanchez-Jerez, 2020).

Several criteria must be considered in species selection. The selected species must be able to complement each other with other species in a system based on the tropic level so that nutrients can be efficiently produced and able to increase production (Barrington et al., 2009; Khanjani et al., 2022; Sasikumar & Viji, 2015). The selected species must have a good level of adaptation, preferably come from local species, and have a high level of production (Largo et al., 2016). Attention must be paid to cultivation technology and environmental conditions; the selected species have the ability to improve ecological conditions efficiently (bio-mitigation) and sustainably (Sasikumar & Viji, 2015). Finally, species that meet market demand and have commercial value must be used (Carras et al., 2020).

The components of the IMTA system relate to the utilisation of nutrients from each trophic level. The route of nutrient utilisation can be seen in Figure 2. The existence of extractive species (organic and inorganic) has a vital role in the IMTA cultivation system. Extractive species are species that can utilise and reduce waste in the form of organic matter (particulates and suspensions) and dissolved inorganic matter originating from metabolism (ammonium (NH_4^+), nitrate (NO_3^-), phosphate (PO_4^{3-}) and carbon dioxide (CO_2), as well as other by-products derived from main species (fed species) to be used as a source of energy for growth (Barrington et al., 2009; Chopin, 2006; Reid et al., 2018; Rosa et al., 2020). In addition, organic extractive species which function as secondary consumers are also marketable, although this is not an essential factor (Sasikumar & Viji, 2015).

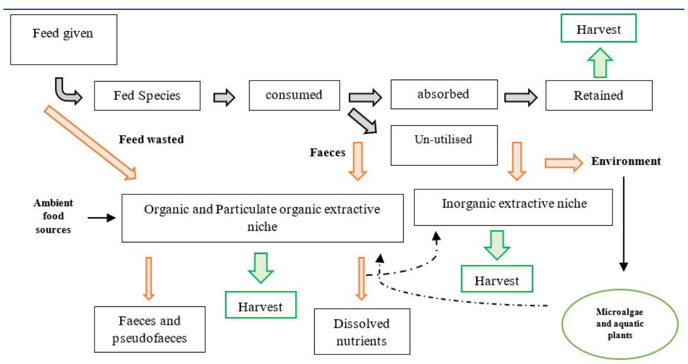

Figure 2. Routes of transfer of nutrients from fed species to extractive species. The black arrows show the nutrient flow of the species being fed. The orange arrows indicate the flow of nutrients extractive species use in addition to those from nearby food sources. The green arrows show the flow of nutrients into biomass during harvesting. Dashed arrows indicate an indirect relationship in nutrient utilisation (Reid et al., 2018).

The use of organic extractive species in IMTA can come from species that act as suspension or deposit feeders. Types of organic extractive species commonly used in the IMTA system include those from the Mussels group. However, several other species have been tried, as seen in the studies by Giangrande et al. (2020), Jerónimo et al. (2020), and Nederlof et al. (2019), who used deposit feeder species from the Polychaetes group and sponges. The results show that using species can increase waste utilisation, efficiency, and profitability (Giangrande et al., 2020). Aquatic plants are inorganic extractive species in the components of the IMTA system that utilise nutrients that are wasted in water (C, N, and P). Through the process of photosynthesis, aquatic plants will form new biomass and cause the formation of a mini ecosystem in the cultivation system (Sasikumar & Viji, 2015). Some inorganic extractive species include seaweed, which is generally used in mariculture cultivation with the IMTA system. Seaweed is used because it can absorb nutrients from cultivation activities or from outside that enter through the exchange of water flows (Yokoyama, 2013).

Fed species is the main component in aquaculture because it is the only component fed to aquaculture with the IMTA system. In this system, fish are the primary source of nutrition for other organisms. Nutrient retention from fed species is influenced by the type and size of fish used, feed composition and management, and environmental factors (Schneider et al., 2005). Fish is the primary source of income in IMTA system cultivation. Therefore, it is necessary to select profitable and marketable fish species. In addition, the selection of fish species and type of feed will also affect the quantity and quality of waste generated in the IMTA system (Sasikumar & Viji, 2015).

The exemplary system design will affect the effectiveness and success of cultivating the IMTA system. The method of the IMTA system must pay attention to the placement, arrangement, and selection of species to be used. System design and species selection must be engineered so that organisms with lower multitrophic levels can utilise the waste from aquaculture activities optimally (Sasikumar & Viji, 2015).

Selection of Extractive Species in the IMTA System

Production of extractive species increased significantly compared to 2000, with a percentage of 43% live weight of total world aquaculture production in 2017 (Naylor et al., 2021). This production increase is generally used for non-food products and ecosystem improvement. Several extractive species are commonly used in the IMTA system, including crustaceans, mussels, sea cucumbers, Polychaeta, sponges, and seaweed (Figure 3) (Granada et al., 2016; Khanjani et al., 2022; Zhang et al., 2019).

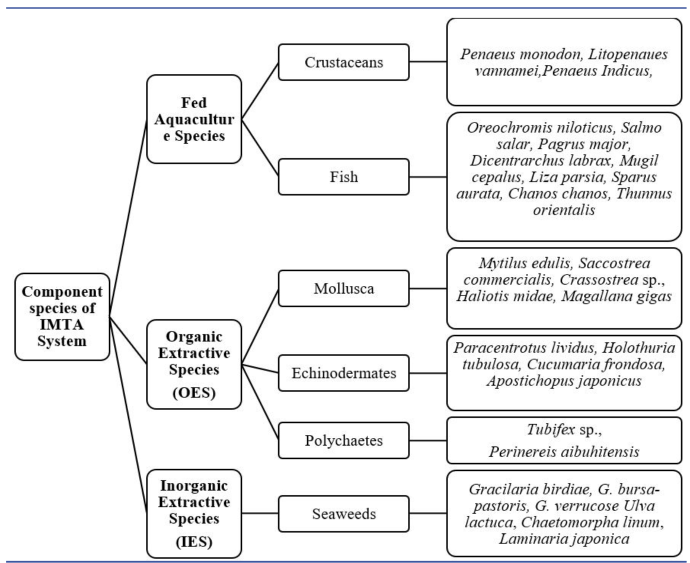

Figure 3. Common types of species used in IMTA cultivation (adapted from Khanjani et al., 2022; Nissar et al., 2023; Zhang et al., 2019).

The selection of suitable extractive species is crucial in supporting success in cultivation with the IMTA system. In general, the use of extractive species in the IMTA system aims not only to improve environmental conditions but also to provide additional benefits such as minimising the risk of failure during the cultivation process, having commercial value, complying with market demand and being accepted by the community (Barrington et al., 2009; Chopin, 2006; Granada et al., 2016). The use of extractive species must also pay attention to growth rates, densities, characteristics, seasonal cycles, and comparison ratios between species, as well as the use of local species that aim to facilitate adaptation and reduce the risk of using introduced species (Ren et al., 2012). Another thing that needs to be considered in selecting extractive species is understanding habitat specifications, because this will affect the growth of the species used (Barrington et al., 2009). With the growing development of IMTA cultivation in the world, the use of potential new species has begun to be tried in several IMTA studies in fresh, brackish, and marine water cultivation (Tables 1 and 2).

Freshwater IMTA

The concept of freshwater IMTA or FIMTA (freshwater IMTA) has long been practised in freshwater aquaculture, especially in Asia countries (Chopin 2013a). Freshwater aquaculture using the IMTA system generally uses tilapia and carp (Bakhsh & Chopin, 2012; Kestemont, 1995). In addition, the application of the IMTA system in freshwater aquaculture is more common in tropical climates than in cold or temperate climates. This is due to differences in water temperature (Bakhsh et al., 2015). Along with the development of increasingly modern aquaculture, FIMTA is also being developed. Aquaponics is one variation of freshwater IMTA cultivation that is currently widely applied (Chopin et al., 2016). Aquaponics is one part of the IMTA system because it utilises at least two species, such as fish and plants. The two species use nutrient sources and have different roles in aquatic ecosystems (Bakhsh & Chopin, 2012; White et al., 2004). Several FIMTA studies have been conducted, as shown in Table 3.

The results from several FIMTA studies show that the IMTA system can improve environmental conditions for cultivation and increase overall biomass production through product diversification. Several things still need to be studied in implementing the FIMTA system, including understanding the flow of nutrients in the system, using local species in the IMTA system, and the proportion of species used.

Marine and Brackish Water IMTA

IMTA is a flexible system and can be developed based on environmental, social, and economic conditions in the area where it is implemented. In addition, the IMTA system can be applied to freshwater, brackish, marine, open-water, and land-based aquaculture activities in temperate to tropical climates (Chopin 2013b). Applying the IMTA system to marine systems is a cultivation model for

Table 1. Several types of species used in Freshwater IMTA.

Fed species	Organic extractive species	Inorganic extractive species
Oreochromis niloticus		*Lactuca sativa*
Anabas testudineus Blk. *Oreochromis niloticus*	*Tubifex* sp.	*Ipomoea reptana* *Brassica juncea*, *Ocimum basiliucum*
Oreochromus niloticus *Clarias gariepinus* *Liza ramada*	*Macrobrachium rosenbergii* *Aspatharia chaiziana* *A. marnoi*	*Brassica oleracea* *Cucumis sativus* *Lactuca sativa* var. *capitata* *Lactuca sativa crispa* *Solanum lycopersicum* *Solanum melongena* *Capsicum annuum*
Salmo salar		*Nasturtium officinale* *Lactuca sativa* *Beta vulgaris cicla* *Allium schoenoprasum* *Cichorium endivia* *Solanum lycopersicum* *Tagetes patula* *Tropaeolum majus* *Achillea millefolium* *Matricaria chamomilla* *Mentha* sp.
Oreochromis niloticus	*Macrobrachium amazonicum*	
Catla catla *Hypophthalmichthys molitrix* *Labeo rohita* *Cirrhinus mrigala* *Heteropneustes fossilis*	*Viviparus bengalensis*	*Ipomoea aquatica*

Table 1. Continue.

Fed species	Organic extractive species	Inorganic extractive species
Cyprinus carpio *Rutilus rutilus* *Tinca tinca*		*Nuphar lutea* *Mentha aquatica* *Typha latifolia* *Glyceria aquatica* *Ceratophyllum demersum* *Phalaris arundinacea*
Colossoma macropomum	*Macrobrachium amazonicum*	
Colossoma macropomum	*Macrobrachium amazonicum* *Prochilodus lineatus*	
Cyprinus carpio *Rutilus rutilus* *Tinca tinca*		*Ceratophyllum demersum* *Glyceria aquatica* *Nuphar lutea* *Nasturtium officinale* *Pondeteria cordata*
Labeo rohita	*Lamellidens marginalis*	*Wolffia globose*
Oncorhynchus mykiss *Perca fluviatilis*		*Lemna minor* *L. minor* Keywaters *L. gibba.*
Oreochromis niloticus	*Liza ramada* *Procambarus clarkia* *Aspatharia chaiziana* *Hypophthalmichthys molitrix*	*Lactuca sativa crispa* *Capsicum annuum* *Cucumis sativus* *Solanum melongena* *Althea Officinalis* *Nasturtium officinale* *Apium graveolens*

References: (Bakhsh & Chopin, 2012; Chopin et al., 2016; David et al., 2017; Flickinger et al., 2019a; Flickinger et al., 2020; Flickinger et al., 2019b; Franchini et al., 2020; Goada et al., 2015; Ibáñez Otazua et al., 2022; Jaeger & Aubin, 2018; Jaeger et al., 2021; Kibria & Haque, 2018; Nath et al., 2021; Paolacci et al., 2021)

Table 2. Several types of species used in marine-brackish water IMTA studies.

Fed species	Organic extractive species	Inorganic extractive species
Crassostrea gigas	*Apostichopus japonicus*	
Litopenaeus vannamei		*Gracilaria tikvahiae*
Sparus aurata	*Mugil cephalus*	
Litopenaeus vannamei		*Gracilaria birdiae*
Pseudosciaena crocea		*Gracilaria lemaneiformis*
Sparus aurata		*Ulva lactuca*
Sparus aurata *Paracentrotus lividus*		*U. lactuca*
Argyrosomus regius *Diplodus sargus*	*Mugil cephalus* *Crassostrea gigas*	Microalgae (Naturally developed in ponds/ *Tetraselmis* spp.) *Ulva* sp.
Dicentrarchus labrax	*Crassostrea gigas*	Microalgae (*Tetraselmis* sp., *Stauroneis* sp. *Phaeodactylum* sp.)
Litopenaeus vannamei	*Mugil liza*	
Litopenaeus vannamei	*Mugil liza*	
Mugil cephalus *Liza parsia*	*Crassostrea cuttackensis*	*Ipomoea aquatica*
	Sarcotragus spinosulus *Sabella spallanzanii* *Mytilus galloprovinciali*	*Chaetomorpha linum* *Gracilaria bursa-pastoris*
Hexagrammos otakii	*Perinereis aibuhitensis*	

Table 2. Continue.

Fed species	Organic extractive species	Inorganic extractive species
Litopenaeus vannamei		*Agarophyton tenuistipitatum*
	Holothuria scabra *Anadara antiquata*	
Litopenaeus vannamei	*Crassostrea* sp.	
Penaeus monodon	*Anadara granosa*	*Gracilaria verrucose*

References: (Amalia et al., 2022; Biswas et al., 2019; Borges et al., 2020; Brito et al., 2016; Cunha et al., 2019; Giangrande et al., 2020; Holanda et al., 2020; Hu et al., 2021; Li et al., 2019; Lima et al., 2021; Magondu et al., 2022; Samocha et al., 2015; Sarkar et al., 2021; Shpigel et al., 2016; Shpigel et al., 2017; Shpigel et al., 2018; Wei et al., 2017; Yokoyama, 2013)

Table 3. Study research with fresh water IMTA system (FIMTA).

Treatments	Parameters	Conclusions and recommendations	References
Use of inorganic and organic nutrients with different concentrations with a maintenance period of 45 days.	-Growth performance: feed conversion ratio (FCR), absolute growth rate (AGR), specific growth rate (SGR), survival rate (SR), weight gain (WG), protein efficiency ratio (PER), and total plant yield (TPY)). -Water quality: water temperature, pH, dissolved oxygen (DO), nitrate (NO_3^-), and phosphate (PO_4^{3-}). -Proximate analyses: crude protein, crude fibre, crude lipid, ash, and moisture of feed and fish.	The use of tilapia and lettuce positively responded to feed enriched with organic-inorganic materials.	(Bakhsh & Chopin, 2012)
The use of different types of inorganic extractive species with a maintenance period of 32 days.	-Water quality: pH, DO, alkalinity, carbon dioxide (CO_2), total ammonia nitrogen (TAN), NO_3^-, and PO_4^{3-}.	The extractive species can adsorb TAN by 86.5% and increase fish biomass to 62.69 kg m^{-3}.	(Sumoharjo & Maidie, 2013)
Use of two different IMTA-integrated aquaponics systems (NFT and FRS systems) with a maintenance period of 2 periods (2 years).	-Water quality: water temperature, pH, DO, ammonia (NH_3^+), nitrite (NO_2^-), nitrate (NO_3^-), alkalinity, and electrical conductivity (EC). -Growth performances: mean final body weight (FBW), SGR, and FCR. -Economic analysis: operating ratio, return on revenue, ratio of income to costs, capital payback period, and return on equity.	The IMTA-FRS (Floating Raft system) is a good model for small-scale businesses with a payback investment period ranging from 2.17-3.34 years.	(Goada et al., 2015)
The utilisation of various types of inorganic extractive species in salmon hatcheries.		The ability of extractive species to absorb nutrients depends on the type of species used, the amount of biomass produced, and the nutritional level of the waste produced.	(Chopin et al., 2016)
The use of different substrates in rearing tilapia, which is integrated with shrimp, with a maintenance period of 140 days.	-Water quality: water temperature, DO, pH, TAN, NO_2^-, NO_3^-, transparency, and total suspended solids (TSS). -Growth performances: SR, individual mean weight, and productivity. -Nitrogen budget.	-Using different substrates does not increase nitrogen retention in fish and shrimp. -Recovery of Nitrogen in ponds depends on periphyton development and substrate cover area. -The application of integrated aquaculture in stagnant ponds can absorb most of the nitrogen from the culture media.	(David et al., 2017)

Table 3. Continue.

Treatments	Parameters	Conclusions and recommendations	References
The use of a combination of different extractive species in carp and stinging catfish cultivation in a cage-cum-pond model maintained for six months.	-Water quality: Temperature, pH, DO, NH_3^+, NO_2^-, and PO_4^{3-}. -Growth performances: percent weight gain, SGR, FCR. -Yield estimation: gross yield of fish and total yield.	Using species diversification can increase productivity and maintain water quality balance.	(Kibria & Haque, 2018)
Use of pond models with different systems with a maintenance period of 7 months.	-Water quality: water temperature, pH, DO, oxygen saturation, EC, redox potential, transparency, and nutrient concentrations (N and P) in water and sediments. -Growth performance: SR, SGR, and FCR. -Biological parameters: chlorophyll-a, total chlorophyll, blue, green, and brown chlorophyll.	-The use of plants in carp, roach, and tench co-culture made the water quality better and more stable (pH and oxygen) in spring and summer compared to other treatments (extensive and semi-intensive ponds). The use of plant ponds can also store P in the sediment properly. -Research on the economical use of macrophytes and exploration studies on biodiversity (zooplankton and macro-invertebrates); the nutrition cycle of the feed of each cultivated species needs to be done.	(Jaeger & Aubin, 2018)
Integrated maintenance between tambaqui-prawn in earthen ponds with different production systems was carried out for 171 days.	-Water quality: water temperature, pH, DO, oxygen saturation, EC, total nitrogen (TN), TAN, NO_2^-, total phosphorus (TP), and transparency. -Biological parameter: chlorophyll-α. -Phosphorus budget	-Using species with different tropic levels can increase productivity and more efficient phosphorus uptake ranging from 24-34%, converted into harvestable biomass products. -There is a need for further studies on using phosphorus as waste from aquaculture activities and adding other detritivores and hylophagous (mud-eating) species.	(Flickinger et al., 2019a)
Integrated rearing between tambaqui-prawn in earthen ponds with different production systems was carried out for 171 days of maintenance.	-Water quality: water temperature, pH, DO, Oxygen Saturation, EC, TN, TAN, NO_2^-, TP, and transparency. -Biological parameter: chlorophyll-α. -Nitrogen budget.	-Fish-prawn cultivation using the IMTA system is more efficient in utilising nitrogen from feed into harvested biomass than monoculture. -Further studies on the flow of nitrogen and sediment in ponds and the bottom soil need to be carried out. It is necessary to study the effect of the bioturbation of cultivated species on the biological processes that occur in the bottom sediments.	(Flickinger, et al., 2019b)
Integrated rearing between tambaqui-prawn in earthen ponds with different production systems was carried out for 171 days of maintenance.	-Water quality: water temperature, pH, DO, oxygen saturation, EC, TN, TAN, NO_2^-, TP, and transparency.-Biological parameter: chlorophyll-α.-Carbon budget.	-The IMTA system can maintain water quality in acceptable conditions and improve the cultivation system with earthen ponds by producing organic matter that settles to be used as fertiliser for other agricultural activities. -The need for further research on the bioavailability of organic carbon accumulated in settled solids, silt, and the water column.	(Flickinger et al., 2020)

Table 3. Continue.

Treatments	Parameters	Conclusions and recommendations	References
The use of a combination of extractive species was carried out for 53 days of the rearing period.	-Growth performances: SR, total length, body mass, body mass gain, FCR total, and total yield.-Water quality: water temperature, DO, EC, pH, TAN, TP, and chlorophyll-a.	-The use of curimbata can increase biomass production and efficiency of feed utilisation. -This study also shows that the integrated cultivation of tambaqui, amazon river prawn, and curimbata is feasible, especially in the nursery phase. -Further studies are focused on stocking density and the proportion of each species based on its growth phase.	(Franchini et al., 2020)
Use of lagoon plants in semi-intensive coupled (fishpond and lagoon) model, semi-intensive with water movement, and extensive fishponds.	Growth performances: Condition Factor (K), SR, SGR, and FCR.	Nutrients from aquaculture ponds can increase primary and invertebrate production, providing additional natural food for cultivated fish species. In addition, it can maintain water quality conditions in optimal conditions.	(Jaeger et al., 2021)
Co-culture maintenance with a combination of different extractive species with a maintenance period of 90 days.	-Water quality: transparency, ph, total alkalinity, hardness, NH_3^+, NO_2, NO_3^-, and PO_4^{3-}. -Growth performances: SR, daily growth index (DGI), daily weight gain (DWG), net fish yield (NFY), apparent feed conversion ratio (AFCR), apparent protein efficiency ratio (APER), and apparent protein retention (APR). -Biological parameters: hemoglobin (Hb), erythrocytes (RBC), and leucocytes (WBC). -Immunological responses: nitroblue tetrazolium (NBT) and total myeloperoxidase. -Antioxidant stress responses: catalase activity, superoxide dismutase (SOD). -Biochemical responses: total protein, albumin, globulin, serum glutamic pyruvic transaminase (SGPT), and serum glutamic oxaloacetic transaminase (SGOT).	- Rohu, bivalve, and floating weed co-cultures can improve water quality, increase fish immunity, and increase productivity and survival rates. -It is necessary to conduct a study on the use of other types of extractive species (plants and shellfish) originating from local areas that are relevant to the cultivation activities; another study is the calculation of nutrient load and sequestration in co-culture cultivation to determine the value of the overall system effectiveness.	(Nath et al., 2021)
The utilisation of three strains from Lemna minor in the bioremediation process in aquaculture waste disposal with a maintenance period of 5 weeks (part 1) and one year (part 2).	-Water quality: water temperature, pH, NO_2^-, NO_3^-, NH_4^+, and PO_4^{3-}. -Bioremediation performances: concentration of chlorophyll, and cyanobacteria. -Monitoring of duckweed surface coverage.	*Lemna minor* can maintain water quality under controlled conditions in the IMTA system.	(Paolacci et al., 2022)
IMTA system fish farming integrated with NTF and FRS system hydroponic cultivation.	-Water quality: NH_3^+, NO_2^-, NO_3^-, PO_4^{3-}, carbonate, (CO_3^{2-}), bi-carbonate, (HCO_3^-), TDS, BOD, COD, major cations (Ca, Mg, K, Na) and major anions (F, SO4) Metals (Al, As, Ba, Cd, Cr, Co, Cu, Fe, Mn, Ni, Pb, Se, Sb, Sn, Zn).-Water use efficiency (WUE).-Nutrient use efficiency (NUE).-Biomass production.	- Integrated application of IMTA with hydroponic cultivation can significantly increase production and water and nutrient use efficiency (WUE and NUE) compared to traditional horticultural models and monoculture aquaculture.	(Ibáñez Otazua et al., 2022)

commercial purposes and overcoming pollution. In the IMTA system, the species used are commercial and environmentally friendly (Chopin, 2006; Troell, 2009). Several studies on marine-brackish water IMTA have been carried out and can be seen in Table 4.

The results of several studies show that the IMTA system's application to marine-brackish water improves water quality in the culture media and increases profitability and balance in the system. Further research needs to be done using a combination of potential local extractive species on a larger scale to understand better system performance, the ratio of species used in the systems, and integration between IMTA and other systems.

IMTA integrated BFT and RAS

The current IMTA system has evolved in its application. One development model combines it with other systems, such as the RAS and BFT (Biofloc Technology) systems. The purpose of the combination of these systems is to find a model that is suitable for sustainable and environmentally friendly aquaculture activities. Cultivation with the BFT system generally produces solids (TSS) and nutrients (especially nitrate and phosphate), which

Table 4. Study research on marine-brackish water IMTA.

Treatments	Parameters	Conclusions and recommendations	References
Use oysters in the sea cucumber cultivation for 216 days of the rearing period.	-Growth performances: initial wet, final wet, specific growth rate (SGR of sea cucumber). -Water quality: water temperature, total nitrogen (TN), total organic carbon (TOC), isotopic analysis, stable carbon, and nitrogen isotope ratios (d13C, d15N).	-Co-culture activities on sea cucumber with Pacific oyster showed good growth and high SR (100%) for 216 days of the rearing period. -The need for research on a larger scale to better understand the capabilities of Pacific oysters and sea cucumber cultivation using the IMTA system.	(Yokoyama, 2013)
The use of algae integrated with shrimp in two IMTA systems with a maintenance period of 7-18 days.	-Growth performances: density, weight, and shrimp biomass. -Water quality: water temperature, salinity, oxygen saturation, dissolved oxygen (DO), pH, NO_2^-, NO_3^-, NH_4^+, light insolation, total dissolved inorganic nitrogen flux (TIDN Flux), water turnover rate. -Macroalgae performance: algae stocking density, specific algal growth rate, tissue nitrogen content in dry weight (N in DW), algal tissue, the content ratio of carbon to nitrogen in dry weight (C:N ratio), and nitrogen assimilation rate into algal tissue per square meter.	*Gracilaria tikvahiae* and *Litopenaeus vannamei* grow quickly, and most of the nitrogenous waste is converted into algal biomass, which can be harvested and marketed.	(Samocha et al., 2015)
Co-culture between sea bream and mullet compared to sea bream and mullet monoculture with a rearing period of 284 days.	-Growth performances: fish weight, yield, SGR, FCR, and SR. -Biochemical composition: protein, carbohydrate, lipids of the fish feed and the sludges. -Water quality: water temperature, pH, oxygen saturation.	Co-culture between sea bream and mullet increases biomass, nitrogen assimilation, and dissolved oxygen emissions, reducing FCR, sediment amount, and nitrogen in sediment.	(Shpigel et al., 2016)
Comparison between monoculture shrimp rearing and shrimp-seaweed integration with the biofloc system with a rearing period of 42 days.	-Growth performances: final weight, survival, yield, biomass gain, weight gain, weekly growth, FCR, SGR of shrimp. -Water quality: temperature, salinity, settleable solids (SS), total suspended solids (TSS), pH, DO, TAN, NO_2^-, NO_3^-, PO_4^{3-}, and Alkalinity. -Vibrio monitoring. -Proximate analysis: crude protein, crude lipid, moisture and ash.	Shrimp-seaweed integration in the biofloc system (BFT) can improve water quality, reduce Vibrio density, and increase growth and protein content in shrimp bodies.	(Brito et al., 2016)

Table 4. Continue.

Treatments	Parameters	Conclusions and recommendations	References
The utilisation of red algae for the bioremediation process in cage cultivation was carried out for 35 days.	-Water quality: water temperature, salinity, pH, DO, chemical oxygen demand (COD), NO_2^-, NO_3^-, NH_4^+, and PO_4^{3-}, dissolved inorganic nitrogen (DIN), dissolved inorganic phosphate (DIP), and eutrophication index (E). -Macroalgae performances: growth rate of red algae. -N and P content in yellow croaker, red algae, and feed.	-Utilisation of the optimal proportion of red algae will balance the nutrients produced from fish farming in the IMTA system. -The need for further studies focusing on bioremediation by cultivating macroalgae throughout the year to improve the aquaculture environment.	(Wei et al., 2017)
Use of *Ulva lactuca* with different percentage ratios as feed on sea bream.	-Growth performances: SGR, yield, FCR, protein productive value (PPV), and SR. -Food biochemical composition: proteins, carbohydrates, lipids of ulva, and fish feed.	The utilisation of *Ulva lactuca* as a protein supplement can save feed production costs by 10%, reduce the concentration of nitrogenous waste in the culture media, and save water treatment costs.	(Shpigel et al., 2017)
The use of sea urchins as algivorous in semi-commercial groundfish-seaweed-sea urchin IMTA systems was carried out for 460 days of the rearing period.	-Water quality: water temperature, DO, and pH. -Growth performances: SGR of fish and sea urchins, FCR, gonad somatic index (GSI) of sea urchins.-Roe colour assessment for the sea urchin. -Biochemical composition: protein, carbohydrate, lipids, ash of fish, sea urchins, and ulva. -Nitrogen and biomass budgets.	Using *U. Lactuca* as a biofilter can improve waste treatment and the system's sustainability.	(Shpigel et al., 2018)
The use of a combination of extractive species in rearing three types of fish with different trophic levels in semi-intensive seawater aquaculture in earthen ponds.	-Water quality: water temperature, DO, pH, salinity, turbidity, transparency, NO_2^-, NO_3^- NH_3^+, PO_4^{3-} and silica (Si). -Phytoplankton analysis: chlorophyll *a*, phaeopigments, and degradation ratio. -Growth performances: biomass, fish density, FCR, total weight (TW), total length (TL), condition factor (K), specific growth rate (SGR), and daily growth index (DGI) of fish); biomass, density, and FCR for all species. -Fish parasite analysis: prevalence and infestation level of ectoparasites in reared fish.	-Integration of fish, oysters, and macroalgae can improve water quality, increase biomass production, reduce energy use by 14% daily, and generate greater profitability.	(Cunha et al., 2019)
The use of extractive species (organic and inorganic) in utilising waste from rearing European sea bass with the RAS system, as well as the use of algae as feed in oyster production, which is reared for 60 days.	-Water quality: RAS-IMTA systems: water temperature, salinity, pH, DO. -Nutrients: Algae: CO_2 concentrations; Oyster: temperature, salinity, and nutrients (NO_2^-, NO_3^-, NH_4^+, and PO_4^{3-}).	-The application of the RAS-IMTA system can remove nutrients like the microalgae-based IMTA system. -The microalgae-based RAS-IMTA system can produce as much as 20.5–33.3 g Algae gN^{-1} and 0.07–0.09 g Chla gN^{-1}. -Oyster growth is prolonged. -The need for research is to increase oysters' growth by optimising access to food and adding a CO_2 source to stabilise the media's pH value and maintain the microalgae stability.	(Li et al., 2019)

Table 4. Continue.

Treatments	Parameters	Conclusions and recommendations	References
Comparison between monoculture shrimp and mullet with integrated shrimp-mullet models with co-culture and sequential/ different tank models.	-Water quality: water temperature, DO, salinity, pH, alkalinity, TSS, TAN, NO_2^-, NO_3^-, and settleable solids (SS). -Growth performance: Shrimp: final mean weight, SR, SGR, FCR, biomass, and yield; Mullet: weight gain (WG), SGR, biomass, and SR. -Hepatosomatic index (HSI) and condition factor (K) of Mullet, total vibrio count (TVC). and total heterotrophic bacteria.	-Adding a mullet to an integrated system can reduce the population of *Vibrio* spp. in an integrated pond. -Integration between mullet and shrimp can be done in the same tank.	(Borges et al., 2020)
Comparison of shrimp monoculture with integrated shrimp-mullet rearing with co-culture and sequential/ separate models carried out for 31 days of the rearing period.	-Water quality: DO, water temperature, pH, salinity, turbidity, TSS, TAN, NO_2^-, NO_3^-, PO_4^{3-}, and alkalinity. -Growth performance: SR, final mean weight, total biomass, weekly growth gain, apparent feed conversion ratio (AFCR), productivity.	-Mullet can utilise solids derived from shrimp production with the BFT system. -There is a need for further research to find out better mullet and shrimp ratios and feeding rates and studies on a mass scale to determine the economic analysis.	(Holanda et al., 2020)
Fish rearing using the polyculture model was compared to IMTA using a combination of different extractive species with a rearing period of 150 days.	-Water quality: water temperature, pH, salinity, DO, NO_2^-, NO_3^-, NH_3^+, and PO_4^{3-}. -Body composition analysis of harvested fish and shrimp. -Economic performance: total income, net income, and benefit-cost ratio (BCR).	Using mullets and tiger shrimp as fed species and oysters and water spinach as extractive species increased net income by 3.35, 3.48, and 1.6 times in the IMTA system with low-salinity brackish water. Furthermore, the water quality with the IMTA system is better than a conventional polyculture using mullets and shrimp.	(Biswas et al., 2020)
Utilisation of micro-invertebrates and seaweed as extractive species placed around seabass or sea bream production areas using the long-line method.	-Growth performance: Sponge: survival (living, dead or damaged), and SGR; Worm: length and weight measurements; Seaweed: SGR. -The health status of the worm. -Analysis of the consistency and colouring of the algae.	-Utilisation of species diversification can help improve efficiency in screening systems and provide commercial advantages. -Need to pay attention to the rearing time and the harvested biomass of each species reared. -Need for investigations regarding market analysis of using sponges and Polychaeta as species that utilise by-products in the IMTA system.	(Giangrande et al., 2020)
The rearing of fat greenling monoculture integrated with polychaetes as an extractive species using different stocking densities was carried out for 30 days.	-Chemical analysis: Carbon and nitrogen in fish, polychaetes, and sediment.-Growth performances: initial body weight, final body weight, SGR, SR, moisture content, feed consumption. -Nitrogen and carbon budget. -Nitrogen and carbon discharge comparison.	The use of Polychaeta can utilise particulate organic waste from intensive fish farming.	(Hu et al., 2021)
The rearing of vannamei shrimp with monoculture, co-culture with BFT, and shrimp monoculture with BFT was carried out for 30 days.	-Water quality: water temperature, Salinity, pH, DO, NH_4^+, NO_2^-, NO_3^-, and PO_4^{3-}. -Growth performance: shrimp: length gain, average percentage weight gain (AWG), SR; shrimp and seaweed: final biomass, SGR.	Using seaweed integrated with the BFT system has the potential for sustainability and good production. The use of seaweed can improve water quality in intensive shrimp farming in brackish water.	(Sarkar et al., 2021)

Table 4. Continue.

Treatments	Parameters	Conclusions and recommendations	References
Shrimp rearing with monoculture and IMTA systems using two types of organic extractive species was carried out for 135 days.	-Growth Performance: ABW, net weight gain (NWG), DWG, SGR, daily growth rate (DGR), AFCR, survival, and yield.-Water quality: Salinity, pH, DO, water temperature, EC, TDS, TAN, NO_2^-, NO_3^- and PO_4^{3-}.	-Integrated system cultivation shows better results than monoculture cultivation. -Utilisation of extractive species can increase profitability and improve the balance of the system in the cultivation environment. -The need for further research on using different and commercial combinations of extractive species and more extended cultivation periods to assess the suitability of the cultivation and application of the IMTA system.	(Magondu et al., 2022)
The use of oysters with different stocking densities in shrimp rearing using the IMTA-Biofloc system compared to monoculture was used for 45 days.	-Water quality: water temperature, salinity, pH, TDS, TSS, SS, DO, alkalinity, TAN, NO_2^-, NO_3^-, and PO_4^{3-}. -Zootechnical performance: biomass gain, mean final weight, SGR, FCR, SR, and yield. -Proximate composition: crude protein, lipid, ash, and fibre in the floc samples. -Vibrio count -Total haemocyte count for shrimp and oysters.	-Using multitrophic biofloc between shrimp and oysters can reduce nitrogen and solids content in the culture media and reduce vibrio bacteria during the nursery phase. -The high stocking density of oysters (300 oysters m^{-2}) can reduce the water quality in the pond and the nutritional content of the flocs due to the high rate of the filtration process.	(Lima et al., 2021)
The use of a combination of different extractive species in the maintenance of tiger shrimp was carried out for ± 30 days.	-Water quality: water temperature, pH, salinity, DO, TOM, TAN, NO_2^-, and NO_3^-. -Growth performances: SGR and SR.	Integrated farming of tiger shrimp with seaweed and blood cockle can improve water quality by reducing the concentration of TOM, TAN, NO_2^-, and NO_3^- in the rearing medium and increasing the SR level of the shrimp.	(Amalia et al., 2022)

tend to increase at the end of the rearing period (Azhar et al., 2020; Gaona et al., 2011; Khanjani & Sharifinia, 2022; Ray et al., 2010). With the application of the BFT system integrated with IMTA, organic and inorganic extractive species are expected to utilise solids and nutrients to be used as harvestable biomass products and keep the rearing medium in optimal conditions for cultivation.

Several studies on the IMTA-integrated BFT system have been carried out. They show that it can improve water quality in the rearing medium by increasing efficiency in nutrient absorption in the form of inorganic nitrogen (NH_4^+, NO_2^-, NO_3^-) and phosphate (PO_4^{3-}), utilisation of dissolved solids (Borges et al., 2020; Brito et al., 2016; Lima et al., 2021), as well as increasing biomass production with species diversification (Poli et al., 2019). However, the use of proportions of extractive species that are not suitable can also have negative impacts, such as decreasing water quality and the nutrient content of flocs due to the use of high oyster stocking densities so that the filtration rate increases (Lima et al., 2021). However, evaluation of the integrated BFT system still needs to be carried out, especially in commercial applications, and economic analysis of the cultivation application needs to be performed for a longer period of time.

RAS (Recirculating Aquaculture Systems) is a system that is currently being widely used in land aquaculture activities. With the application of the RAS system, it is possible to produce various species by minimising the use of natural resources, especially in utilising water sources (Martins et al., 2010; Orellana et al., 2014). In the RAS system, the level of water consumption for aquaculture activities is reduced without any water changes or maximally with water changes of <1% of the system volume per day (Waller et al., 2015). Bio-process technology using biological and mechanical filters plays a vital role in the RAS system because it functions to maintain water quality to remain stable. However, the accumulation of solids and nitrates resulting from the bio-process significantly affects the system's stability. Based on this, it is necessary to utilise nutrients derived from commercial RAS waste (Orellana et al., 2014).

Future Challenges

The IMTA system is currently developing towards using extractive species that have work potential in the system and have commercial value. In addition, it is also necessary to use technology that can utilise natural energy to be more efficient in energy use. The challenge will be faced by focusing on three aspects: 1) economic elements in the form of investment capital, methods of maintenance and harvesting, and system design for mass scale; 2) from the biological aspect, the effects of weather changes on water quality, limitations on the use of suitable species, residues, and contamination from using chemicals in aquaculture; and 3) from the social aspect, concerns from the community about the impact on the environment, competition in the utilisation of natural resources, and conflicts between stakeholders (Buck et al., 2018; Oyinlola et al., 2018; Rosa et al., 2020; Sasikumar & Viji, 2015; Troell et al., 2017b). Selection of appropriate species and managing the flow of nutrients are essential in increasing production and producing optimal models adapted to cultivation conditions (Granada et al., 2016; Rosa et al., 2020; Sasikumar & Viji, 2015). Currently, the cultivation of the IMTA system is still in the development stage in many countries. This has positive and negative impacts on several sides. Increasing land use and food security must also be considered because IMTA is a complex system. Therefore, it is necessary to enact special laws focusing on socio-economic aspects such as area utilisation and biological elements in residues and contaminants resulting from cultivation activities (Rosa et al., 2020; Sasikumar & Viji, 2015).

CONCLUSION

The concept of eco-friendly aquaculture is the key to sustainable aquaculture in the future. The IMTA system is one of the solutions that can overcome the waste generated from intensive aquaculture activities. Approaches from the environmental side have been carried out through various studies and have shown positive results in improving the ecological conditions of cultivation. However, IMTA studies through economic and social approaches still need to be performed. If more IMTA studies using economic and social approaches are carried out, we suspect that the IMTA system will become popular and widely applied to aquaculture activities.

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Review Article

Potential Use of the Miracle Tree (*Moringa oleifera*) Leaves in Aquaculture: A Recent Update

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ABSTRACT

The superfood *Moringa oleifera* leaves are an alternative food source for human nutrition and animal feeds in different countries. This highly nutritious plant has several medicinal uses as well. Abundant vitamins and minerals make this plant a source of curiosity to underdeveloped and developing countries to meet the requirements of nutrients. Higher crude protein levels in moringa leaves have become popular as alternative feed sources for animals. Moreover, the leaf contains a rich amount of macro and micronutrients such as minerals and vitamins. It is also popular in some countries for its aphrodisiac use. Moringa leaves are used in the diet as a supplement to enhance growth and reproductive performance in animals, including fish. These leaves have been used in diets to replace fishmeal, soybean, and other plant-based meal sources. The antibacterial properties of the leaves are functional as a way of lessening the spread of diseases and as an immunity booster in aquaculture. According to the literature surveyed, moringa leaves can be utilized in the diet at 10-30% in omnivore and herbivore fish and 10-20% in carnivore fish without adverse effects. In this review, we discuss the utilization of supplemented moringa leaves and their effect on the growth and reproduction of fish. We also discuss how these leaves affect the hematological and physiological performance of fish.

Keywords: Fish, growth performance, supplement, reproductive performance, alternative feeds

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INTRODUCTION

Aquaculture industries are growing each day. Sustainable aquaculture depends on economically viable quality feed. The feed industries frequently use fishmeal and oil to maintain the high-quality feed. However, the natural fish stock is limited, which poses a big challenge for those working on getting available fishmeal at the desirable level for the industry. Therefore, scientists are working to find alternative feed sources to cope with the challenges. Much research has been done on plant-based alternative diet sources. Plant-based additives are also used as growth promoters and to boost reproductive performance in aquaculture. Researchers have tested plant-based supplements in aquaculture as an alternative to artificial chemicals. Improving the diet of cultured fish and enabling weight gain are two goals of using plant-based additives in aquaculture (Dada, 2015). It has been the usual practice to use plant-based resources in the feed industry for many years.

Moringa oleifera is called a drumstick plant in English and is known as *shajna* in India and Bangladesh. Due to its extremely high nutritional content, *Moringa oleifera* is a widespread vegetable plant in India, Bangladesh, Africa, and other regions. Leaves can be used in fresh, dried, or powdered form. Fish and animals can consume the bark, leaves, pods, seeds, and roots of the moringa plant (Kou et al., 2018). Its broad beans or pods are mainly used to prepare different curries and soups. In addition, its leaves are also nutritious and full of vitamins and minerals. The pods and leaves of *Moringa oleifera* are an abundant source of minerals like magnesium, calcium, manganese, phosphorus, iron, zinc, copper, and others (Aslam et al., 2005). Moringa leaves have a common practice in ethnomedicine in different countries, especially in India and Africa. In addition to being a widespread plant in the Indian subcontinent, it may also be found in other regions. Even in dry conditions, *Moringa oleifera* may grow rapidly, and it serves as an excellent protein supplement for livestock (Oduro et al., 2008). According to Fuglie (1999), *Moringa oleifera* contains more vitamin A than oranges and carrots, more protein than yogurt, more calcium than milk, more potassium than bananas, and more iron than spinach. The National Institute of Health (USA) recognized it as the "Botanical of the Year 2007" (Gupta et al., 2018).

Moringa oleifera increases antioxidant enzyme levels and enhances detoxification (Hasan et al., 2019). Dry moringa leaves contain 25-30 % protein and a good amount of lipids. In moringa, the polyunsaturated fatty acid is higher than saturated fatty acids. More than 16-19 amino acids are found in moringa leaves, of which tens are essential amino acids (Moyo et al., 2011). Many studies utilize plant-based diets to replace fishmeal (Elumalai et al., 2020; Ahmadifar et al., 2019) as they are easily accessible, safe for the environment, affordable, sustainable, and eco-friendly (Hardy, 2010). Fish are influenced in a variety of ways by medicinal herbs and their extracts. When added to fish diets, these substances can influence growth performance and boost immune activities.

The oxidative stress brought on by many stressors during fish culture can be lessened using medicinal plants and their extracts (Ahmadifar et al., 2021). According to Siddhuraju et al. (2003), the natural antioxidants contained in moringa leaves have similar functions to popular synthetic antioxidants like butylated hydroxyanisole and butylated hydroxytoluene. As moringa leaves have some significant medicinal value, they can be utilized to treat several diseases in animals. They may perform a variety of roles, including that of bactericides and immunostimulants (Coppin et al., 2013). Research has also demonstrated that *Staphylococcus aureus, Bacillus subtilis*, and *Vibrio cholera* are susceptible to the crude leaf powder and extracts of *Moringa oleifera* (Jayawardana et al., 2015).

Studies have revealed that both fresh and post-thawed semen benefit from the herb or phytochemical extracts (Azimi et al., 2020; Ahmed et al., 2020). It has been shown in various species that utilizing plant additives with antioxidants in the feed improves in vitro fertilization by decreasing oxidative stress production in the medium (Azimi et al., 2020; Ahmed et al., 2019). However, plant-based compounds are also utilized to stimulate animal reproduction. A saponin called protodioscin is found in *Tribulus terrestris* and is assumed to be the compound responsible for improving testosterone levels (Ganzera, 2001). Moringa leaves also contain different saponins, tannins, flavonoids, terpenoids, and glycosides (Fahey, 2005; Singh et al., 2009), which might help to improve the reproductive performance and increase the testosterone level in fish. Additionally, moringa leaves have a good amount of selenium, zinc, calcium, and vital vitamins (Vit-A, Vit-B, and Vit-C).

In the last couple of years, the multipurpose plant moringa has been used in the diets of ruminates, rabbits, rats, chickens, and fish to evaluate its effect on growth and reproductive performance. Moringa leaves have been used as supplemented diet, aqueous extract, ethanol extract, and other forms of inclusions in the diet of cultured fish. This review aims to summarize the findings of published articles on the use of *Moringa oleifera* leaves in aquaculture species.

The Miracle tree - *Moringa oleifera*

Description of *Moringa oleifera*

Moringa oleifera originated from Southeast Asia, the Middle East, and Africa, however, it is now widely distributed throughout America, the Caribbean countries, several countries in Asia, the Philippines and Indonesia (Morton, 1991; Mughal et al., 1999; Seshadri & Nambiar, 2003). *Moringa oleifera* can survive droughts; thus, it grows well in hot regions. The *Moringa oleifera* tree thrives in sandy-loam soil that drains well and has a pH range of 5 to 9. *Moringa oleifera* can easily grow from seeds and cuttings (Hasan et al., 2019).

Moringa oleifera is a leafy plant of the family Moringaceae, commonly called a drumstick tree. The drumstick tree is typically 8–15 m tall. It is solid and has a soft woody stem (Figure 1a, 1b) and a tuberous root. When grown, its leaves are dark green (Figure 1c). The tree produces 30–50 cm long, broad pods (Figure 1d). Most people eat the fleshy part of the pods as vegetables (Seshadri & Nambiar, 2003). Moringa flowers and seeds are presented in Figures 1e and 1f.

Nutritional values of moringa leaves

Due to being rich in micro and macronutrients, *Moringa oleifera*, a well-known vegetable of the Indian subcontinent, is recognized as a miracle tree. In addition to being an abundant source of β-carotene, moringa also contains significant levels of other minerals, including iron, calcium, phosphorus, and zinc, as well as

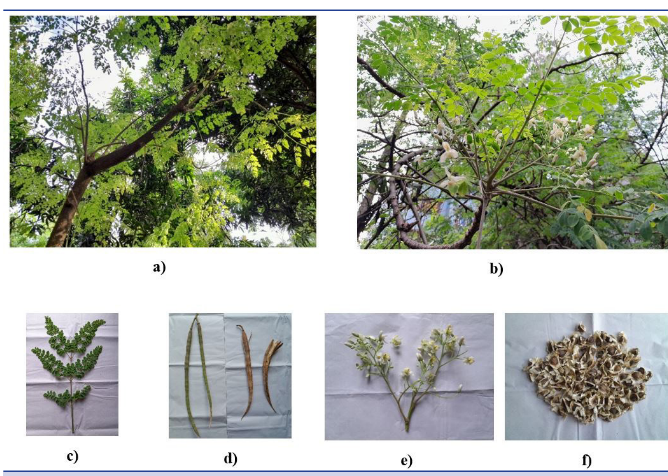

Figure 1. Different parts of *Moringa oleifera*. a) leaves and stem, b) leaves with flower, c) leaves, d) pods or beans (green and dry), e) flowers, f) seeds.

significant amounts of vitamins, including folic acid and ascorbic acid (Seshadri & Nambiar, 2003; Faboya, 1983). Almost every part of the moringa tree, including the leaves, pods, seeds, and flowers, is edible. Moringa leaves are well known for abundant protein, vitamin, and mineral content and also for natural antioxidants, i.e., carotenoids, flavonoids, and phenolic components (Dillard & German, 2000; Siddhuraju & Becker, 2003). The pods and leaves of *Moringa oleifera* are a rich source of minerals like magnesium, calcium, potassium, manganese, phosphorus, sodium, zinc, copper, iron, and others (Aslam et al., 2005). Parts of the moringa plant, such as the leaves, roots, beans, bark, and seeds, are extensively utilized in ethnomedicine in different countries (Stohs & Hartman, 2015). Moringa leaves are well known for having a high nutritional value. In their dried form, they possess 23-30% crude protein, 5.9% crude fiber, 7.6-12% ash, and 7.09% crude lipid (Su & Chen, 2020).

Drumstick leaves have 137.28 mg of vitamin C (ascorbic acid) / 100 g of fresh weight. Compared to spinach, the ascorbic acid concentration of drumstick leaves is 1.5 to 2 times higher (Seshadri & Nambiar, 2003). The highest concentration of β-carotene is found in moringa leaves (19210 µg/100 g fresh weight) (Nambiar & Seshadri, 1998). As a result, animals can consume moringa leaves as a natural supply of vitamin A. Nambiar and Seshadri (2001) worked on the albino rat to find the effect of moringa leaves as a Vit-A source and concluded that both fresh and dehydrated forms of moringa leaves enhance the growth performance more than the synthetic Vit-A.

Ethnomedicinal uses of *Moringa oleifera*

There are many therapeutic uses of *Moringa oleifera,* which have been found in different regions of the world. In addition to having antitumor and antimicrobial properties, *Moringa oleifera* is effective against infection, diabetes, typhoid, HIV, diarrhea, dysentery, ulcer, rheumatism, joint pain, arthritis, etc. (Fahey et al., 2005; Costa-Lotufo et al., 2005; Fuglie, 1999; Caceres et al., 1992; Faizi et al., 1998; Asres, 1995; Bharali et al., 2003).

Phytochemicals in moringa leaves

M. oleifera has high concentrations of several phytochemicals, including glucosinolates, isothiocyanates, kaempferol, caffeoylquinic acid, zeatin, quercetin, kaempferitrin, rhamnose, rhamnetin, and isoquercitrin (Fahey, 2005). Additionally, *M. oleifera* also contains chlorogenic acid, ferulic acid, ellagic acid, vanillin, and gallic acid in their aqueous extracts (Singh et al., 2009).

USE OF MORINGA LEAVES IN AQUACULTURE

Moringa oleifera leaves have been used in the feed of different omnivorous, herbivorous, and carnivorous fish to evaluate the effect on several physiological parameters, such as growth performance, reproductive performance, hematological parameters, enzyme activities, and disease resistant performance.

Effect of moringa leaves on the growth performance of fish

Moringa leaves have been widely utilized in research to assess their impact on the growth performance of various fish species. Dietary fishmeal can be substituted up to 30% with solvent-extracted moringa leaves without adversely affecting growth parameters in *Oreochromis niloticus* (Afuang et al., 2003). In contrast, Richter et al. (2003) demonstrated that more than 10% inclusion of moringa leaves suppressed growth in Nile tilapia. Yuangsoi & Charoenwattanasak (2011) also suggested a 10% inclusion of moringa leaves in Nile tilapia diet. After eight weeks of the feeding trial, El-Kassas et al. (2020) reported that 5% moringa leaves in the feed significantly lowered the lipid profile and enhanced growth performance. The inclusion of moringa leaves up to 20-25% in the diet showed better growth efficiency in *O. niloticus* (Sherif et al., 2014). However, Kasiga et al. (2014) demonstrated that the replacement of soybean meal protein with moringa leaf meals is possible up to 30% without affecting growth in Nile Tilapia.

In *Labeo rohita,* body composition could be unaffected by the substitution of moringa leaves for soybean meal as a cost-effective source of high-quality plant protein (Masood et al., 2020). This statement has been supported by the findings of Arsalan et al. (2016), who demonstrated that fishmeal could be supplemented with moringa leaf meal up to 10% to boost the nutritional value of *L. rohita* fingerlings. Another study opposed the previous findings that fish growth is decreased when *Moringa oleifera* is added to the diet, which may be because some anti-nutrients have a detrimental impact (Mehdi et al., 2016). The contrary might be because the feed composition, size of the fingerlings, and duration of the experiment were different. In *Clarias gariepinus,* fishmeal can be replaced by up to 15% moringa leaf meal without any negative impact on growth parameters (Idowu et al., 2017). In addition, a 15% inclusion of moringa leaves in substitution of fishmeal showed the best performance in mean weight gain, specific growth rate (SGR), and feed conversion ratio (FCR). It is also possible to replace soybean meal at 20% with moringa leaves to get the highest performance of similar parameters in *Clarias gariepinus* (Ncha et al., 2015). According to Dienye and Olumuji (2014), 10% inclusion of moringa leaves showed no negative effect on blood parameters and enzymes level. In Asian seabass (*Lates calcarifer*), replacing 10% of fishmeal with moringa leaves was suggested to improve growth performance (Ganzon-Naret, 2014). The author also recommended that up to 30% of moringa leaves be used instead of fishmeal by adding Methionine to the diet, enhancing protein digestibility. In a 60-day feeding trial of *Pangasius bocourti,* Puycha et al. (2017) demonstrated that the inclusion of moringa leaves at 100 g could be utilized in the feed without negative effect on the growth performance, digestibility, and serum biochemistry.

An overview of the impact of moringa leaves on the growth performance, hematological indices, and other physiological parameters of omnivore, herbivore, and carnivore fish species is provided in Table 1, Table 2, and Table 3.

Effect of moringa leaves on reproductive performance of fish

To date, *Moringa oleifera* leaves have been used in research on several fish species to evaluate their biological and reproductive performance. However, the greater part of the research investigating the value of moringa leaves in fish feed has mainly focused on evaluating the effect on the growth performance, hematological parameters, and immune activities in several fish species, i.e., Tilapia, *Labeo rohita, Puntius altus, Lates calcarifer, Pangasius bocourti*, zebrafish, sea bream, rainbow trout, and

Table 1. Use of *Moringa oleifera* leaves in omnivorous fish culture.

Experimental Fish	Feeding behavior	Doses used	Duration	Remarks	Recommended dose	Ref.
Nile tilapia (*Oreochromis niloticus*)	Omnivore	0, 5, 7, and 9 g/kg diet	60 days	Improvement in growth, maintenance of intestinal health, increase in antioxidant capacity, and boost of immunity in Nile tilapia.	5 g/kg	El-Son et al., 2022
Nile Tilapia (*Oreochromis niloticus*)	Omnivore	Replacement of dietary protein at 0, 10, 20, and 30%	7 weeks	10% showed normal growth, others suppressed growth.	10%	Richter et al., 2003
Nile Tilapia (*Oreochromis niloticus*)	Omnivore	Replacement of 0, and 30% dietary fishmeal	8 weeks	About 30% of the fishmeal could be replaced with solvent extracted moringa leaf meal.	30%	Afuang et al., 2003
Nile Tilapia (*Oreochromis niloticus*)	Omnivore	0, 5, and 10%	8 weeks	Growth performance improved. Significantly lowered lipid profiles.	5%	El-Kassas et al., 2020
Nile Tilapia (*Oreochromis niloticus*)	Omnivore	0, 5, 10, 15, 20 and 25%	6 weeks	Growth parameters were significantly higher in 20 and 25%.	15%	Sherif et al., 2014
Nile Tilapia (*Oreochromis niloticus*)	Omnivore	0, 5, 10, and 15%	60 days	10% moringa leaf had no negative effects on fish health, growth, or protein digestion.	10%	Yuangsoi & Charoenwattanasak, 2011
Nile Tilapia (*Oreochromis niloticus*)	Omnivore	Replacement of soybean meal at 0, 15, and 30%	59 days	No effects on proximate composition, survival rate, FCR, weight gain, lysozyme activity, proteolytic enzyme activity.	30%	Kasiga et al., 2014
Mozambique tilapia (*Oreochromis mossambicus*)	Omnivore	0, 3, 6, 9, and 12%	45 days	Survival rate increased. Without affecting growth capacity, moringa can enhance fish health.	9, and 12%	Mbokane & Moyo, 2018
Fancy carp (*Cyprinus carpio*)	Omnivore	0, 200, & 500 g/kg replacement of soybean meal protein	6 weeks	No negative impact on growth and digestibility at 200 g /kg.	200 g/kg	Yuangsoi & Masumoto, 2012
Fancy carp (*Cyprinus carpio*)	Omnivore	0, 10, 20, 30, 40, and 50% of crude protein replacement	12 weeks	Growth parameters enhanced in 30%.	30%	Adeshina et al., 2018
Zebrafish (*Danio rerio*)	Omnivore	Control, M (only moringa), FM (supplemented moringa without vitamin & mineral), F_{vm} (feed without vitamin, mineral, and moringa)	12 weeks	Normal growth and reproduction in control group, No growth and no eggs in M group, reduced growth and reproductive performance in F_{vm}, recovery of growth and reproductive performance in FM.	Control	Paul et al., 2013

Table 1. Continue.

Experimental Fish	Feeding behavior	Doses used	Duration	Remarks	Recommended dose	Ref.
Bocourti's catfish (*Pangasius bocourti*)	Omnivore	0, 100, 150, and 200 g/kg of fish	60 days	No adverse impact on the growth, digestibility, feed utilization, and serum biochemistry at 100 g/kg.	100 g/kg	Puycha et al., 2017
Red-Tailed Tinfoil (*Puntius altus*)	Omnivore	0, 20, and 60 mg / g of fish feed	28 days	Fish health, growth performance, muscle protein profile improved significantly.	20 mg/g diet	Sirimongkolvorakul et al., 2015

FCR= Feed conversion ratio, FM= Fishmeal

Table 2. Use of *Moringa oleifera* leaves in herbivorous fish culture.

Experimental Fish	Feeding behavior	Doses used	Duration	Remarks	Recommended dose	Ref.
Milkfish *(Chanos chanos)*	Herbivore	Replacement of soybean meal at 0, 10, 25, 50, and 75%	60 days	No problem up to 50% replacement in terms of protein digestibility.	50%	Hamzah et al., 2021
Labeo rohita fingerlings	Herbivore	0, 10, 20, and 30%	2 months	Decrease in growth.	Control	Mehdi et al., 2016
Labeo rohita fingerlings	Herbivore	0, 10, 20, 30, and 40%	70 days	Fish carcass composition and hematological parameters are unaffected by the 10% inclusion of moringa leaves.	10%	Arsalan et al., 2016
Labeo rohita fingerlings	Herbivore	0, 10, 20, and 30%	90 days	Improved weight gain, FCR, SGR.	30%	Masood et al., 2020
Mrigal *(Cirrhinus mrigala)*	Herbivore	0, 1, 2, 3, 4 and 5% of acidified moringa seed meal by citric acid	90 days	Improved weight gain, SGR, and nutrient digestibility.	3%	Hussain et al., 2017
Cirrhinus mrigala	Herbivore	0, 10, 20, 30, 40, and 50% replacement of FM	90 days	10% showed best results, which leads to positive changes in hematological indices.	10%	Tabassum et al., 2021

FCR= Feed conversion ratio, FM= Fish meal, SGR= Specific growth rate.

Table 3. Use of *Moringa oleifera* leaves in carnivorous fish culture.

Experimental Fish	Feeding behavior	Doses used	Duration	Remarks	Recommended dose	Ref.
Rainbow trout (*Oncorhynchus mykiss*)	Carnivore	0, 0.625, 1.25, 2.5, and 5%	8 weeks	5% untreated moringa leaves have no negative impact on fish performance.	5%	Stadtlander, & Tonn, 2015
Rainbow trout (*Oncorhynchus mykiss*)	Carnivore	10, 20, 30, and 40% replacement of fishmeal	90 days	20% showed best growth performance, improvement in immune response and antioxidant activities.	20%	Labh, 2020
Asian sea bass (*Lates calcarifer*)	Carnivore	0, 10, 20, and 30%	75 days	0% showed best result then 10, 20 and 30%.	10%	Ganzon-Naret, 2014

Table 3. Continue.

Experimental Fish	Feeding behavior	Doses used	Duration	Remarks	Recommended dose	Ref.
Gilthead Seabream (*Sparus aurata* L.)	Carnivore	0. 5, 10, and 15% supplementation	4 weeks	Adding Moringa to the diet increases the muscle's antioxidant activity, resulting in improved oxidative stability of the food.	15%	Jiménez-Monreal et al., 2021
Sea bream (*Sparus aurata*)	Carnivore	0, 5, 10, and 15%	4 weeks	5% inclusion showed best immune status, enhanced growth performance and acceptable immune surveillance found in 10%.	10%	Mansour et al., 2018
Sea bream (*Sparus aurata*)	Carnivore	0, 1, 2.5, and 5%	3 weeks	5% boosted the mucosal immune system. No adverse effect in cortisol and glucose level.	5%	Mansour et al., 2020
Heterobranchus longifilis	Carnivore	0, 5, 10, 15, and 20%	5 months	Improve growth parameters in 15% group.	15%	Eyo & Ivon, 2017
Heterobranchus longifilis	Carnivore	0, 5, 10, 15, and 20%	5 months	Final body weight and feed intake improved. No difference in FCR. Higher GSI in 15% group.	15%	Opeh et al., 2018
African catfish (*Clarias gariepenus*)	Carnivore	0, 4.1, 8.2, 12.3 and 16.39 g inclusion of moringa leaves	12 weeks	Moringa enhance growth, and no adverse effect on fish health. FCR, AWG, SGR improved with moringa inclusion.	8.2 g	Ayoola et al., 2013
African catfish (*Clarias gariepenus*)	Carnivore	0, 10, 20, 30, 40, and 50%	8 weeks	Blood serum and enzyme level had no negative effect at 10% inclusion. Mean weight gain, FCR are better in 10%.	10%	Dienye & Olumuji, 2014
African catfish (*Clarias gariepenus*)	Carnivore	0, 20, 40, and 60% of soybean meal replacement	56 days	20% showed best performance in FCR, weight gain, SGR.	20%	Ncha et al., 2015
African catfish (*Clarias gariepenus*)	Carnivore	0, 10, 15, 20, and 25%	8 weeks	Weight gain, FCR, SGR improved in 15%.	15%	Idowu et al., 2017

SGR= Specific growth rate, FCR= Feed conversion ratio, AWG= Average weight gain, GSI= Gonado-somatic index

fancy carp (Yuangsoi & Masumoto, 2012; Mehdi et al., 2016; Paul, 2013; Ganzon-Naret, 2014; Sirimongkolvorakul et al., 2015; Labh, 2020; Puycha et al, 2017; Mansour et al., 2018; El-Kassas et al, 2020). Limited studies were found on the reproductive performance of fish. Opeh et al. (2018) worked on African catfish (*Heterobranchus longifilis*) conducting feeding trials over a period of five months to check the effect of moringa leaves on the gonadosomatic index and fecundity at a rate of 0, 5, 10, 15, and 20%. In this study, 15% supplementation of moringa leaves improved gonadal development and increased fecundity significantly. Twelve weeks of feeding trials on zebrafish were conducted and designed with four groups: control diet, only moringa leaves (M), control diets without vitamins and minerals (F_{VM}), and moringa-supplemented diets without vitamins and minerals (FM). In this research, moringa-supplemented diets (FM) showed a greater increase in egg production than the F_{VM} group. In contrast, zebrafish fed with only moringa leaves without any additional nutrients did not produce eggs (Paul et al., 2013). The findings of this study imply that moringa leaves can be used as a suitable dietary supplement for macro- and micronutrients and may help alleviate the effects of nutritional shortages in zebrafish. Gad et al. (2019) investigated the effect of moringa seeds on the reproductive performance of Nile tilapia. This study used ethanol extract moringa seed (EEM) and petroleum ether extract moringa seed (PEEM) at 0.5 g and 1.0 g. The control group had the lowest ovarian weight, whereas the 0.5 g PEEM group had the highest ovary weight compared to the 1.0 g EEM group. Moreover, in female fish, the gonadosomatic index and estradiol level were higher in moringa-treated groups than in the control group. In male fish, the gonadosomatic index, testosterone level, and cortisol level were higher in moringa-treated groups than in the control group. In a recent study, 14 weeks of feeding experiments were conducted in which moringa leaf was included at 0, 4, 8, and 16% into the diet to assess the impact on the reproductive performance of male rainbow trout (*Oncorhynchus mykiss*) (Momin & Memiş, 2022). The study results suggested that the 8% inclusion of moringa leaf boosts sperm quality parameters and improves reproductive performance. However, in male broodstock rainbow trout, 16% of moringa leaf can be fed without adverse effects (Momin & Memiş, 2022).

Effect of moringa leaves on other physiological parameters of fish

The hematological indicators of *Clarias garipenus*, ssuch as red blood cell count (RBC), packed cell volume (PCV), hemoglobin (Hb), and white blood cell count (WBC), etc., increased significantly when moringa leaves were added to the diets (Ezekiel et al., 2016; Ayoola et al., 2013). According to Sherif et al. (2014), feeding Nile tilapia with *M. oleifera* leaves significantly raised their hematological indices and serum alanine transaminase (ALT) and aspartate transaminase (AST) levels. In addition, Nile tilapia showed a significant drop in blood uric acid, urea, and creatinine when fed 1.5% moringa leaves compared to the control (Salama et al., 2016). Blood parameters such as RBC, WBC, Hb, and PCV increased with a greater inclusion of moringa leaves in the diet of *Cyprinus carpio* (Adeshina et al., 2018). Moringa supplementation at 15% in the diet of *Oreochromis niloticus* showed no significant differences in RBCs, Hb, PCV, mean corpuscular hemoglobin (MCH), and mean corpuscular volume (MCV), but WBC increased (Bbole et al., 2016). Mansour et al. (2020) suggested 5% moringa leaves inclusion in the diet of seabream to enhance skin-mucosal immune activities. In addition, four weeks of feeding trials in gilthead seabream (*Sparus aurata*) revealed that a 15% supplementation of moringa leaves in the diet increased the antioxidant activities of the muscles (Jiménez-Monreal et al., 2021). Arsalan et al. (2016) demonstrated that a 10% replacement of fishmeal with moringa leaves found the highest RBC count and Hb value in *Labeo rohita*. According to research on catfish (*C. gariepinus*), no negative impact was determined in the hematological indices or serum enzymes with 10% supplementation of moringa leaf in the diet (Dienye & Olumuji, 2014).

CONCLUSIONS

Moringa oleifera leaves are a potential alternative feed for fish in aquaculture industries because they contain high levels of nutrients such as proteins, minerals, and vitamins that can boost growth and reproductive performance. Studies have shown that supplementing a fish diet with 10-20% moringa leaves for carnivore fish and 10-30% for herbivore and omnivore fish does not have adverse effects. However, more research is needed to determine the appropriate dose for maximum benefit and to study the effect of moringa leaves on the reproductive performance of fish.

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Short Communication

First finding of live specimens of non-native copepod *Calanus finmarchicus* (Gunnerus, 1770) in the Sea of Marmara

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ABSTRACT

In April of both 2018 and 2019, one specimen each time of live *Calanus finmarchicus* adult females was found during collections of zooplankton samples near Sivri Ada in the Sea of Marmara. This was the first observation of live *C. finmarchicus* (a native species of northern Atlantic waters) in the eastern Mediterranean Sea. This morphological study illustrates the similarity of *C. finmarchicus* specimens found in the Sea of Marmara with existing illustrations and descriptions of this species. It also shows the clear differences between this species and *Calanus helgolandicus* specimens collected during the same or earlier periods from the Marmara Sea. Transportation of *C. finmarchicus* to the Sea of Marmara is possible either via the lower current from the Aegean Sea or through the ballast waters of vessels. In any case, the occurrence of *C. finmarchicus* as a cold-water species in the Sea of Marmara is striking and attracts further investigations.

Keywords: Calanoid copepod, taxonomy, *Calanus*, Marmara Sea, alien

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INTRODUCTION

Calanus finmarchicus (Gunnerus 1770) is widely distributed in the northern hemisphere and is among the most studied copepod species in the northern Atlantic (for example, Marshall & Orr 1972; Heath *et al.* 2000; Melle *et al.* 2014). This boreal species has dominated the North Atlantic Gyre (Fleminger & Hulsemann 1977), and its regional distribution varies with the North Atlantic Oscillation (Greene & Pershing 2000). The Azores and the Iberian coasts are the lower limit of their range in the North Atlantic. Rare records of this species in the western Mediterranean Sea (Rose 1933) may be due to its introduction into these waters via the southern branch of the North Atlantic Current. However, there has been no reliable evidence of this species being found in the central and eastern Mediterranean (Marshall & Orr 1972). Only two species of copepods of the genus *Calanus* have been found in this part of the Mediterranean Sea: *C. helgolandicus* and *C. tenuicornis* (Brodskii 1950; Weikert *et al.* 2001), and only one of them, *C. helgolandicus*, permanently lives in the Sea of Marmara and the Black Sea (Svetlichny *et al.* 2006; 2010). In the Black Sea, the population of this species is also referred to as *Calanus euxinus* (Hulsemann 1991), however, according to the results of a morphological (Isinibilir *et al.* 2009) and genetic analysis (Ünal *et al.* 2006; Yebra *et al.* 2011; Figueroa *et al.* 2019), *C. euxinus* does not have differences in species rank from *C. helgolandicus* living in the Mediterranean basin (e.g. the Black Sea, the Sea of Marmara, the Aegean Sea, the Adriatic Sea) and in the North Atlantic.

The Sea of Marmara is a Turkish inland sea connected to the Black Sea via the Bosporus and to the Aegean/Mediterranean Sea via the Dardanelles straits (Figure 1). It has a surface area of 11,350 km^2 with the greatest depth of 1,370 m. The Sea of Marmara has two distinct vertical layers . In the upper layer, due to the continuous inflow of the less saline (18 PSU) Black Sea, the surface salinity is low averaging about 22 PSU (=Practical Salinity Unit). Mediterranean waters having a salinity of 38.5 PSU dominate the bottom layer 20-25 m, which is the depth of the permanent halocline/pycnocline. Except in the two shallow straits (Jarosz *et al.* 2013), these two layers in the Sea of Marmara do not generally mix, as is the case for the Black Sea. During the last few decades, the Sea of Marmara's ecosystem has faced several anthropogenic problems such as land-based pollution, eutrophication, overfishing, loss of habitats, and introduction of invasive species. The large-scale mucilage events occurring during several months in 2021 received great attention from scientists, the media, and the public. Strong stratification combined with pollution in the Sea of Marmara caused oxygen levels to drop to critical levels for metazoan life in the deeper waters.

The high volume of shipping traffic and particularly the discharge of ballast water by ships are the main reasons for the introduction of new species (Öztürk & Albayrak 2016). By 2014, the number of alien taxa found was 95 belonging to 11 different systematic groups, including 7 copepod species.

This paper presents the first finding of a total of two individual females of *C. finmarchicus* in the northeastern part of the Marmara Sea in the spring of 2018 and 2019.

MATERIALS AND METHODS

Zooplankton samples were collected on 3 April 2018 and 5 April 2019 with a closing Nansen net (opening diameter: 50 cm, mesh size: 200 µm) by vertical hauls from the bottom (150 m) to the surface at a station located (40º 51′ 53″ N – 28º 57′ 44″ E) in the northern Marmara Sea south of Sivri Ada, off Istanbul, Türkiye (Figure 1). The total depth of the station was about 300-500 m since it was in a region steeply deepening. Water temperature and salinity were measured using a Sea-Bird CTD probe.

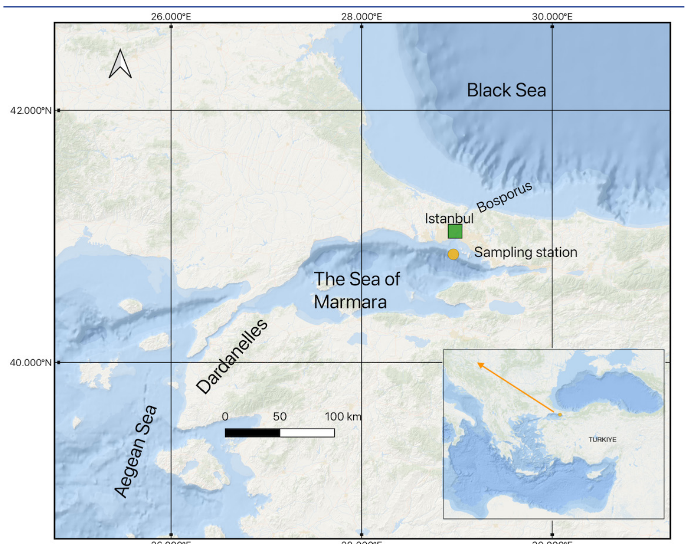

Figure 1. Sampling station (orange circle) in the Sea of Marmara in 2018 and 2019.

Immediately after capture, a sample of the collected species was placed into 5-litre bottles of clean, filtered seawater about 22 PSU and approximately 2 hours after collecting samples, all healthy copepod specimens were sorted individually at the laboratory using a wide pipette and placed in 1-litre volume vessels containing 0.45 µm filtered seawater (temp. 17°C) for further study.

On 3 April 2018 nine females of the genus *Calanus* were caught, of which one individual looked different and was characterized by slow swimming. This specimen soon died in the laboratory. A preliminary morphological examination allowed us to suggest its taxonomic difference from other individuals belonging to the species *Calanus helgolandicus* living in this area. This specimen was photographed (Figure 2a, b) measured and fixed in 96% ethanol for further taxonomic analysis.

On 5 April 2019 fifteen females of the genus *Calanus* were caught, one of which stood out for its larger size, red color, and slow activity. This individual was anaesthetized with MS-222, photographed (Figure 3), measured and fixed in 96% ethanol. For comparison, females of *Calanus helgolandicus* were selected and fixed in the same catches. A detailed taxonomic analysis of selected individuals, including dissection and photography of their swimming legs and abdomen, was performed at the Department of Invertebrate Fauna and Systematics of I.I. Schmalhausen Institute of Zoology NASU, Kyiv (Ukraine).

The identification of the species was based on original descriptions of *Calanus finmarchicus* and their morphological comparison with *Calanus helgolandicus* and other species of the genus *Calanus* by Rose (1933), Brodsky (1950), Marshall & Orr (1972), Williams (1972) and Frost (1974) and Fleminger & Hulsemann (1977).

RESULTS AND DISCUSSION

As is well known, the Marmara Sea is a transit reservoir between the Black Sea and the Aegean Sea. Its upper layers are formed by the brackish water (about 22 PSU) coming from the Black Sea through the Bosporus Strait, while deeper than the sharp halocline lies water with high salinity (38.5 PSU) coming with the lower current through the Dardanelles from the Aegean Sea. Plankton fauna is thus distributed in accordance with these currents and water layers. The lower course brings to the Sea of Marmara species typical of the Mediterranean and the North Atlantic, while the upper course replenishes it with Black Sea species.

C. helgolandicus is the only member of the *Calanus* genus both in the Sea of Marmara and the Black Sea. The Black Sea population of *C. helgolandicus* was allocated a separate species, *C. euxinus,* based on the difference in the distribution of supernumerary pores on the second and third urosome segments of adult females (Fleminger & Hulsemann 1987; Hulsemann 1991). Many authors have also used this naming in publications, however, further morphological (Isinibilir *et al.* 2009) and genetic analysis (Ünal *et al.* 2006; Yebra *et al.* 2011; Figueroa *et al.* 2019) has indicated that the supernumerary pores are not sufficient indicators for species differentiation hence the name *C. helgolandicus* should be used.

The female *Calanus finmarchicus* collected on 3 April 2018 (Figure 2a) had a total body length of L_{tot}=3.60 mm, prosome length l_{pr}=2.84 mm, and prosome width d_{pr}=0.85 mm. Respective measurements for the female collected on 5 April 2019 (Figures 3 and 4a) were even higher with L_{tot}=3.95 mm, l_{pr}=3.14, and d_{pr}=0.98. The length of *Calanus helgolandicus* females from the same area (Figure 2d and Figure 4b) were much lower with values of 2.11±0.12 mm for small forms and 2.66±0.14 mm for the large form.

Differences between the morphologies of the two species were also apparent, as follows:

- The ratio of prosome to abdominal length was 3.74 and 3.87 for the two specimens of *C. finmarchicus* whilst an overall lower value of the ratio of 3.43±0.12 was found in *C. helgolandicus*.

- Compared to *C. helgolandicus,* the frontal part of the female head in *C. finmarchicus* was more evenly rounded and its abdomen was relatively thicker (Figures 2a and d as well as Figures 4a and b).

- There were obvious differences in the shape of their genital segment and spermathecae (Figures 2b and 2e as well as Figures 4a and b).

- The inner edge of the coxa of P5 was less curved and the dentate plate on coxopodite had more pointed and shorter teeth, especially in the area of the distal part of the plate (Figures 2c and 2f as well as Figures 5a and b).

-In *C. finmarchicus* collected on 5 April 2019, the spine on the outer margin of the swimming leg basipodite was smaller and located closer to its center, while the marginal spines of the exopodites were thicker compared to those in *C. helgolandicus* (Figure 5c, d).

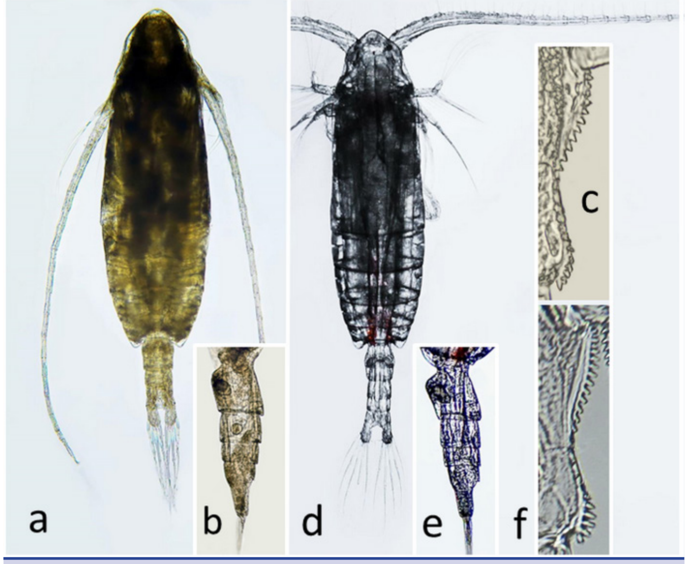

Figure 2. *Calanus finmarchicus*: habitus of female, front view (a); abdomen, side view (b) and coxopodite inner edge of P5 (c). *Calanus helgolandicus*: habitus of female, front view (d); abdomen, side view (e) and coxopodite inner edge of P5 (f). Both specimens were sampled on 3 April 2018.

- There were different proportions of the mandibular gnathobases, as well as different shapes and distances between the opal teeth of the gnathobases (Figure 6).

The first specimen of the female *C. finmarchicus* caught in April 2018 had a prosome length (2.84 mm) as in the largest specimens of *C. helgolandicus* living in the Black Sea, while the prosome length of the female caught in 2019 (3.14 mm) exceeded the known ranges body size of *C. helgolandicus* living in the Black, Mediterranean and North Atlantic seas (Brodskii 1950; Fleminger & Hulsemann 1977, 1987). Besides the total and prosome lengths, ratios of prosome to abdominal length were also much higher in the two specimens found in this study compared to previous studies from the different ratios of prosome to abdominal length (Table 1).

Figure 3. *Calanus finmarchicus*, female collected on 5 April 2019 (front view).

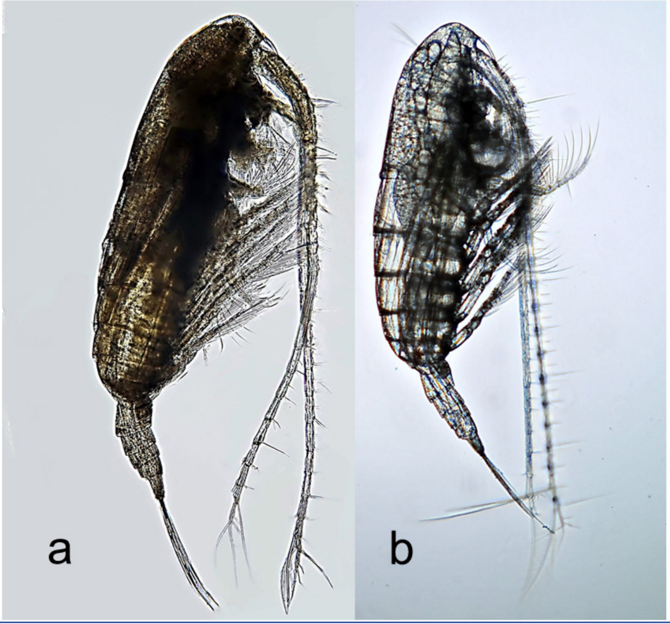

Figure 4. *Calanus finmarchicus* (a) and *Calanus helgolandicus* (b) females, lateral view. Both specimens were sampled on 5 April 2019.

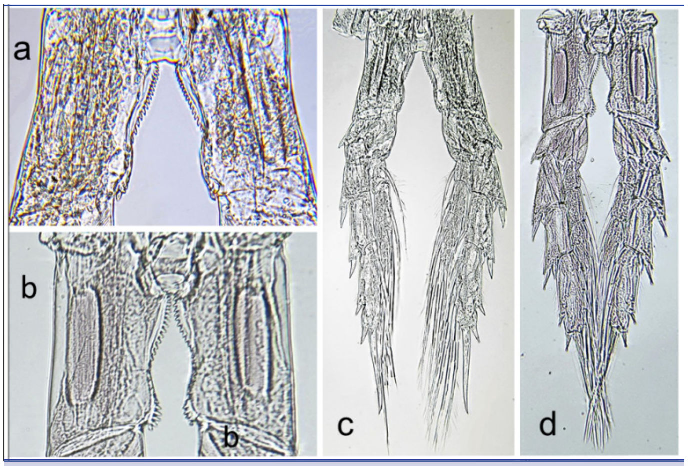

Figure 5. *Calanus finmarchicus:* coxopodite inner edge of P5 (a) and frontal view of P5 (c). *Calanus helgolandicus:* coxopodite inner edge of P5 (b) and frontal view of P5 (d).

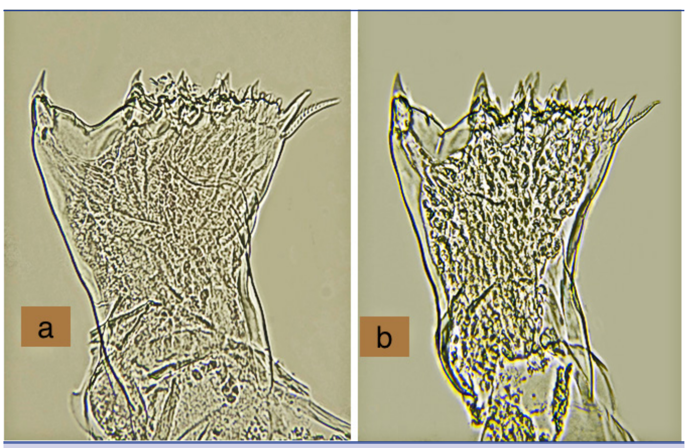

Figure 6. Gnathobase of left mandibula in *Calanus helgolandicus* (a) and *Calanus finmarchicus* (b) from the Sea of Marmara.

The population of *C. helgolandicus* is represented by two size groups of adults in the Sea of Marmara (Isinibilir *et al.* 2009). In spring, in the upper layers of the Bosphorus region of the Marmara Sea, predominantly large females with a prosome length of 2.67 ± 0.06 mm are found which are believed to be transported from the Black Sea by the strong surface currents, while in the area of the Princes' Islands and Sivri Ada, remote from the Bosphorus, small females with a prosome length of 2.23 ± 0.07 mm predominate. Experimental studies have shown that these two size groups of females lay eggs of the same size around 168–182 µm (Isinibilir *et al.* 2009), being similar to the typical ranges reported for the Black Sea *C. helgolandicus* population (Sazhina 1987) and *C. helgolandicus* from the North Atlantic (Guisande & Harris 1995; Poulet *et al.* 1995). Eggs laid by both size groups of females developed identical nauplii and early copepodites. However, starting from the third copepodite stage, individuals developing in the Sea of Marmara experienced growth retardation due to higher water salinity (Svetlichny *et al.* 2018). The average length of the prosome of *C. helgolandicus* females inhabiting the Aegean Sea is reported to be even shorter (2.04 ± 0.04 mm) (Fleminger & Hulsemann 1987; see Table 1).

The morphometric and morphological taxonomic characteristics (including those regarding the coxa of P5, the swimming leg basipodite and the mandibular gnathobases) of both individuals caught by us corresponded to the known morphological descriptions of *C. finmarchicus* (Brodsky 1950; Marshall et al., 1953; Frost 1974) and differed from those of *C. helgolandicus*. However, we also suggest that it will be important to utilize molecular tools for confirmation on discriminating between the two species as many authors have stated (Lindeque *et al.* 2004; Choquet *et al.* 2017). Unfortunately, we could not undertake molecular analysis since we only had two specimens which were dissected during the morphological analysis.

Both specimens were alive at capture, but inactive after being placed in the surface water of the Sea of Marmara with low salinity, probably due to osmotic shock. The discovery of the living *C. finmarchicus* in the Sea of Marmara indicates the possibility of very deep penetrations or transportation of the North Atlantic species into the eastern part of the Mediterranean basin. However, the vector for this transport is not clear, though it is believed to be via ballast waters of shipping. Because of dense population in the area, particularly in Istanbul which is one of the world's megacities, the Sea of Marmara has a very dense maritime traffic. In 2021, the number of vessels transiting between the Black Sea and Marmara alone was over 38 thousand (https://www.dailysabah.com/turkey/istanbul/vessels-swarm-turkiyes-marmara-sea-as-bosporus-traffic-heightens).

It has been reported that in the eastern Atlantic *C. finmarchicus* prefers colder (9 °C) and deeper waters, while *C. helgolandicus* dwells in warmer waters (16 °C) (Jonasdottir & Koski 2011). Based on long-term Continuous Plankton Recorder (CPR) data, several authors (Planque & Fromentin 1996; Wilson *et al.* 2016) suggest that *C. finmarchicus* is retreating north from the North Sea and *C. helgolandicus* is gaining ground due to warmer temperatures. However, Beare *et al.* (2002) observed that despite warming temperatures, *C. finmarchicus* abundance has increased in certain parts of the Atlantic Ocean, and it was therefore suggested that the lower abundance levels observed in shelf waters (North Sea, North Icelandic Shelf Region) are not a pan-Atlantic phenomenon. Similarly, Wilson *et al.* (2016) stated that temperature alone is not sufficient to explain the differences in the distribution of these two species in the eastern Atlantic and suggested that food quality be a key determinant on their population dynamics.

The Sea of Marmara is one of the most fertile seas within the Mediterranean basin with chl-a values as high as 20.0 µg L^{-1} (Yalçın *et al.* 2017) and production values of 100 gC/m^2/y (Ergin *et al.* 1993). Despite the high phytoplankton content of the Sea of Marmara, the establishment of *C. finmarchicus* in it seems difficult though not impossible. This is because *C. finmarchicus* is a cold-water species, but the waters of the Sea of Marmara below 25m are warm at 14-15 ^{0}C throughout the year. In order to understand and evaluate the dynamics of *C.*

Table 1. Prosome, urosome lengths (mm) and the ratio of *C. helgolandicus* and *C. finmarchicus*. (*urosome length calculated using the equation $L_{uro}=0.34\ L_{pr}^{0.82}$, by Isinibilir *et al.* 2009

Species	Region/Group	Season**	Sample size	Prosome length	Prosome Length Deviation	Urosome Length	Prosome/ Urosome	Reference
C. helgolandicus	Black Sea deep	W-SP	465	2.67	0.06	0.76	3.51	Isinibilir *et al.* 2009*
C. helgolandicus	Black Sea deep	S-A	376	2.57	0.09	0.74	3.49	Isinibilir *et al.* 2009*
C. helgolandicus	BS Shallow	A	59	2.57	0.16	0.74	3.49	Isinibilir *et al.* 2009*
C. helgolandicus	NE Marmara, Group1	SP	29	2.01	0.04	0.60	3.34	Isinibilir *et al.* 2009*
C. helgolandicus	NE Marmara, Group2	SP	46	2.23	0.07	0.66	3.40	Isinibilir *et al.* 2009*
C. helgolandicus	NE Marmara, Group1	A	23	2.01	0.10	0.60	3.34	Isinibilir *et al.* 2009*
C. helgolandicus	NE Marmara, Group2	A	64	2.44	0.12	0.71	3.45	Isinibilir *et al.* 2009*
C. helgolandicus	West N Atlantic		21	2.22	0.02	0.65	3.42	Fleminger & Hulsemann 1987
C. helgolandicus	Mid N Atlantic		20	2.34	0.04	0.67	3.49	Fleminger & Hulsemann 1987
C. helgolandicus	East N Atlantic Europe		20	2.36	0.07	0.68	3.47	Fleminger & Hulsemann 1987
C. helgolandicus	East N Atlantic-Africa		20	2.16	0.05	0.64	3.38	Fleminger & Hulsemann 1987
C. helgolandicus	Western Med.		20	2.31	0.06	0.69	3.35	Fleminger & Hulsemann 1987
C. helgolandicus	Adriatic Sea		20	2.20	0.12	0.69	3.19	Fleminger & Hulsemann 1987
C. helgolandicus	Aegean Sea		23	2.04	0.04	0.63	3.24	Fleminger & Hulsemann 1987
C. helgolandicus	Black Sea		42	2.61	0.03	0.78	3.35	Fleminger &Hulsemann 1987
C. helgolandicus	Off Tunisia		90	1.78	0.05	0.55	3.26	Drira *et al.* 2018*
C. helgolandicus	Marmara NE, 2018	SP	33	2.11	0.12	0.63	3.36	This study*
C. finmarchicus	Marmara NE, 2018	SP	1	2.84		0.76	3.74	This study
C. helgolandicus	Marmara NE, 2019	SP	46	2.66	0.14	0.76	3.51	This study*
C. finmarchicus	Marmara NE, 2019	SP	1	3.14		0.81	3.88	This study

**Seasons=W = Winter, SP = Spring, S= Summer, and A= Autumn)

finmarchicus in this new environment, this species should be closely followed in the Sea of Marmara through dedicated investigations.

CONCLUSION

A total of two specimens of live *Calanus finmarchicus* adult females (which is native to the north Atlantic) were found for the first time in the Sea of Marmara in 2018 and 2019. Certain morphological characteristics of *C. finmarchicus* differ from the sole *Calanus* species (i.e. *C. helgolandicus*) dwelling in the Sea of Marmara. These differences include an overall larger size, a higher ratio of prosome to abdominal length, the more evenly rounded frontal part of the female head, the shape of their genital segment and spermathecae, the dentate plate on coxopodite, different shapes and distances between the opal teeth of the gnathobases, etc. When more specimens are caught, it will be important to utilize molecular tools for confirmation of this species, also genetically. *C. finmarchicus* specimens may have been transported to the Sea of Marmara either via the lower current from the Aegean Sea or, more plausibly, through the ballast waters of vessels.

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Compliance with Ethical Standard: Authors declare that ethical approval is not require for this type of study.

Conflict of Interests: The authors declare that they have no financial interests or personal relationships that could affect this work, hence no conflict of interest.

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Bu Telif Hakkı Anlaşması Formu tüm yazarlar tarafından imzalanmalıdır/onaylanmalıdır. Form farklı kurumlarda bulunan yazarlar tarafından ayrı kopyalar halinde doldurularak sunulabilir. Ancak, tüm imzaların orijinal veya kanıtlanabilir şekilde onaylı olması gerekir.

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