

Research Article

Water quality and bacteriological analysis during newly practiced live fish transportation in Bangladesh

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ABSTRACT

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In Bangladesh, live fish transportation through a modified open truck system is relatively new. This system aids in the delivery of fresh fish to the farthest locations in the country, although it is considered a difficult task to keep the fish alive and maintain their quality while being transported. The present study aimed to assess the water quality and bacteriological status during live fish transportation through this modified open truck system. For this purpose, the physicochemical characteristics of the fish transporting water, as well as the bacteriological state of both the water and the fish, were analyzed at various time intervals when approximately one ton of Indian major carps (Rui, Catla, and Mrigal) was transported from the Natore district to Sylhet Sadar. During the 14-hour travel, the fish holding water was changed every four hours. Thus, the current study tracked all of the characteristics for the first four hours, from fish loading to water change. The initial values of pH, temperature, and dissolved oxygen were 7.41, 25.5°C, and 3.56 ppm, respectively, and observed a change to 7.62, 27.2 °C, and 2.91 ppm, respectively, at the end of the tracking period. An increasing trend was observed in the case of water conductivity and TDS, but these values did not differ significantly within the study period. On the contrary, when a microbial load of water, gill, and intestine samples were analyzed, it was recorded that the total viable count (TVC) increased several folds from the initial values. Initially, the microbial load was lower (10^3 cfu/ml) as the fresh underground water was loaded, which dramatically increased after 3 hours of transportation and reached 10^8 cfu/ml water. The mean Probable Number (MPN) was also found to increase, which was probably imposed due to the lack of personal hygiene. The findings revealed important information about live fish transportation.

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INTRODUCTION

Aquaculture is a diverse and rapidly expanding food industry worldwide. It provides access to aquatic food species, which is one of the major sources of animal protein for human consumption. Projections indicate that aquaculture will play a critical role in meeting the needs of animal protein of the growing global population by 2050, which is expected to drive an upward trend in production. From 2000 to 2019, production based on live weight has nearly tripled, encompassing contributions from 475 cultured species. Asia led in production volume, contributing between 90% and 92% during this period and maintaining consistent levels,

while contributions from Europe and Oceania declined. (FAO, 2024; Verdegem *et al.* 2023; Naylor *et al.*, 2021).

For Bangladesh, fish contribute to the national diet significantly. It is around 60 percent of animal-source food and 15 percent of total protein intake ([Bangladesh Bureau of Statistics, 2011](#); [Belton *et al.*, 2011](#); [Hussain, 2010](#)). Fish is connected with the culture and represents an important source of micro-nutrients ([Golub & Varma, 2014](#)). The fishing sector generates income and employs around 15 million people among 155 million, while aquaculture employs 73% of rural households ([Alam, 2011](#)). Fish, both cultured and captured, are considered an important crop and positioned second in the agricultural sector. Even resulted in a 9%

increase in production during Corona outbreak. Millions of people benefited from the increased fish production in terms of livelihood and employment ([Rahman, 2017](#)). For Bangladesh, this sector has been playing a crucial role in the economic sector for the last few decades. Bangladesh produced 42.77 lakh MT in FY 2018-2019 and ranked one of the world's leading fish-producing countries ([DoF, 2019](#)). According to [DoF \(2019\)](#), Last three decades, fish production has increased by about six times in Bangladesh (7.54 Lakh MT in 1983-84 to 43.84 Lakh MT in 2018-19).

Bangladesh is reported with 320 different fish species, indicating its diversity in fish. It sits at the core of the Ganges delta. The major river system is the Padma, Brahmaputra, Jamuna, and Meghna providing diverse and abundant fisheries. This enormous biodiversity provides potential and advantages in the fishery industry for the country. Bangladesh is the world's third-largest producer of inland captures and the sixth-largest aquaculture producer. ([Hussain, 2010](#); [FAO, 2017](#)). The major fish-producing areas are greater Mymensingh, Natore of Rajshahi, the Floodplain and riverine area of Cumilla, Barishal and Chandpur, the Haor area of Sylhet, Kaptai Lake, Cox's Bazar, Khulna, and Bagerhat ([Rahman *et al.*, 2013](#)).

Fish transportation is a global necessity, and it is commonly done to transport fish from a surplus-producing place to a non-surplus-producing place to fulfil the demand of that area ([Marcalo *et al.*, 2008](#)). Live fish transportation differs from transporting terrestrial livestock as it requires a life-support system during transportation ([King, 2009](#)). Stress due to handling loading, transport, and discharge are considered key factors during live fish transportation ([King, 2009](#)).

Fish production, consumption, and transportation, therefore, have play roles in the national food safety, security, nutrition, and poverty alleviation. Due to the surplus production of fish in the mentioned area of Bangladesh, the fish need to be transported from one place to another to reach the consumer and to optimize the supply-demand chain. Fish transportation is normally done to different parts of the country. Hence, live fish distribution channels are also affected by the demands and stress of the channels due to long distance from the producing area ([Rahman *et al.*, 2013](#)).

Fish are transported from around 100 to 500 km in any locality ([Rahman *et al.*, 2013](#)). It is reported that around 60% of fish are supplied locally, meaning they are transported within 50 km, and 40% are transported from another place in the country ([Alam *et al.*, 2010](#)). But in the fish market of the Sylhet region, around 55% of the fish are locally available, whereas 45% of the fish are transported live from different parts of the country ([Azam *et al.*, 2016](#)). In most cases, the live transported fish in the Sylhet region are Indian major carps, Pangus, Tilapia, Koi, Shing, Magur, and Pabda ([Azam *et al.*, 2016](#)). The consumers take fish as their ideal regular diet. The price of fish and degree of consumer preference are greatly influenced by the degree of freshness of fish ([Nguyen *et al.*, 2015](#), [Stubbe & Yang, 2011](#)). Qualitative losses occur as a result of delays in the transportation of fresh fish. Unexpected transportation delays lead to a reduction in quality. Delayed transportation hampered quality by 60-70% before it reached the consumer and resulted in the loss of 28% of fish during transportation ([Alam, 2005](#)).

It is very challenging to supply fresh fish to consumers because fish is more perishable than land animals. Maintaining water quality, stress factors, and microbial

quality are quite challenging during live fish transportation, which are the governing factors to keep fish alive during transportation ([Harmon, 2009](#)). After death, fish perished quickly, and the price dropped. Autolysis and bacterial decomposition play a combined role in fish spoilage, which is accelerated in tropical climates ([Ghaly *et al.*, 2010](#)). Fish muscles have less stoma protein, which is beneficial for consumers' health, but pass the rigor mortis quickly. Rigor mortis starts after the death of animals ([Venugopal & Shahidi 1996](#)). Hence, the spoilage is accelerated, which becomes worse with the spoilage activity of microbes. To minimize the spoilage effect, farmers have started to transport live fish as fresh to stop spoilage and to have a better price.

Transportation may be defined as the transportation of live fry, fingerlings, and marketable fish. The live transportation system is divided into two basic transport systems: the closed system and the open system. In a closed system, fish are transported in a sealed container where all necessary surviving conditions are maintained closely. For instance, sealed plastic bags filled with water and oxygen. A closed transportation system is sensitive and costly. In an open system, fish transporting containers remain open, and aeration is done continuously for oxygenation. Nowadays, open trucks are used for live fish transportation, where a shallow machine (water pump) is commonly used for oxygenation purposes. A plastic/tarpaulin is normally used to cover the bed of a truck where the water is retained as an artificial pond in the "open truck system.". The volume of water and fishes varies based on the sizes and capacity of the truck ([Hossain *et al.*, 2021](#)).

Water quality is a crucial factor during transportation of live fish. It can be deteriorated by different factors such as the initial quality of water, water holding time, starvation of fish, and amounts of fish in the system ([Metar *et al.*, 2018](#)). The success of the transportation depends on the water quality, the duration of the transport, water temperature, fish size, and species ([Conte, 2004](#)). Water is crucial during transportation, and it can easily be contaminated with the organic and inorganic toxin resulting from the bacterial breakdown of fecal waste. Hence, the carrying medium becomes harmful to the fish and may result in the death of the fish during transportation ([Washburn *et al.*, 1953](#)). Water quality might hampered by the mucus, ammonia, undigested feed particles and feces produced by fish during transportation. The change in water quality might stress fish to a higher degree ([Schumann *et al.*, 2017](#)). The decomposition of this waste even enhances the microbial load in the transport system. So, there is a relationship between changes in water quality and bacterial load, which is ultimately related to the success of transportation ([Portz *et al.*, 2006](#)). Decreased levels of oxygen affect the respiration of the fish. Also it increases the levels of carbon dioxide and ammonia in the transport medium ([Pavlidis *et al.*, 2003](#)). Ammonia levels increased by the excretion of fish during transport which affect the pH and carbon dioxide levels in the system ([Sampaio & Freire 2016](#)). Those combined affect live fish transportation, which needs to be addressed.

Live fish transportation is influenced by loading density, fish species, physicochemical parameters and its physical conditions ([Carneiro *et al.*, 2009](#)). Raw water and feces of fish are considered the primary route of microorganisms in the transportation system. The goal here is to better integrate these two approaches, relating physicochemical

consequences to water quality deterioration and microbial changes, especially considering oxygen deprivation and the enrichment of ammonia after transport (Hossain *et al.*, 2021).

Understanding environmental conditions, especially water quality and microbial changes during transportation, is key to establishing new protocols that could potentially reduce stress and slow down the loss of water quality during live fish transportation. As survival and healthy fish are dependent on water quality parameters during transportation, microbial load and stress factors need to be studied during transportation. Hence, this study aimed to investigate the physicochemical quality of water and bacterial load during live fish transportation.

MATERIALS AND METHODS

Study site selection

Natore district of Rajshahi division is well known for carp fattening. The fattened carp was then transported live to different parts via an open truck system. Sylhet region is well known for its natural resources of water. But, aquaculture is still underdeveloped in this region. Hence, the carp (Indian major) is mostly transported to Sylhet from different fish producing parts of the country. One of the transportation channels is the transportation of live fish to Sylhet from Natore. It is a 14-hour journey of the live fish. During the journey, the water has been changed every 4 hours. For the study, we tracked one water exchange time period for its change in water quality and bacteriological parameters. We collected samples for bacteriological analysis. The samples were preserved into ice and brought to the laboratory of fisheries microbiology of the Department of Fisheries Technology and Quality Control, Faculty of Fisheries, Sylhet Agricultural University, Sylhet.

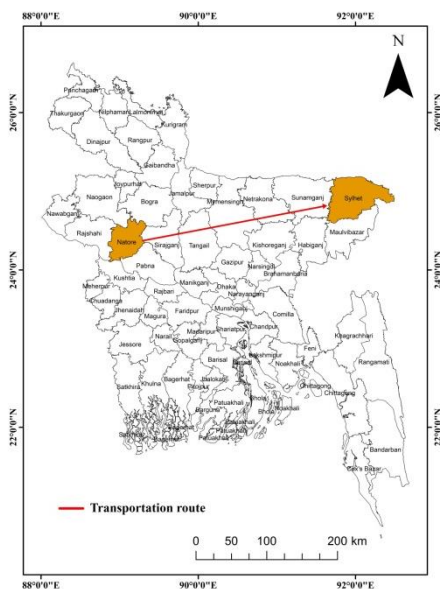


Figure 1: Map showing the study area.

Fish species selection

In most cases, the live transported fish in the Sylhet region are Indian major carps (Azam *et al.*, 2016). The following are the selected fish samples:

Table 1: Selected fish species for analysis in live transportation.

Local Name	Scientific Name
Rui	<i>Labeo rohita</i>
Catla	<i>Catla catla</i>
Mrigal	<i>Cirrhinus cirrhosus</i>

Water samples collection

Samples were collected every 1 hour (1 hour, 2 hours, 3 hours, and 4 hours) from the live fish transportation truck. Falcon tubes (50ml) were used to collect water and stored into ice. At the same time, water quality parameters dissolved Oxygen, pH, temperature, conductivity, TDS, and ammonia were continuously monitored using a portable water quality measuring kit (YSI ProDSS Multi-Parameter).



Figure 2. Portable water quality measuring kit (YSI ProDSS Multi-Parameter) and water sample preservation

Fish samples collection

Gills, intestines, and skin were collected at each hour (Fig. 3). The prepared samples (Gills, intestines, and Skin) from each time point were kept in a labelled 50 ml plastic tube and preserved in a portable icebox, transported to the lab where the samples were preserved in refrigerator for further study.



Figure 3: Photograph showing sample collection during live fish transportation and preservation of samples.

Microbiological Analysis

Qualitative and Quantitative microbiological analysis of the collected water and fish samples performed at the microbiology lab of Faculty of Fisheries. Total viable count (TVC) and total coliform count (TCC) by the Most Probable Number (MPN) method were performed at the microbiology lab of the Faculty of Fisheries.

Preparation of Sample

Firstly, 2gm of the samples (gills and intestines) collected from the live fish transporting truck were mixed with 10ml of distilled water individually. Then, the sample was shaken

properly to make a homogenous suspension. For skin, the sample was taken from an area of 1cm². One ml sample was transferred with a micropipette to a test tube containing 9.0 ml of distilled water, and the test tube was shaken thoroughly on a vortex mixture in order to get 10⁻¹ dilution of the original sample solution. Using a similar process, consecutive serial dilutions of 10⁻², 10⁻³, 10⁻⁴, 10⁻⁵, 10⁻⁶, 10⁻⁷ and 10⁻⁸ were made. A sample of 100µl of each tenfold dilution was spread on an agar plate using the spread plate technique. The plates were incubated in an inverted position in an incubator at 37°C for 24-48 hours. Only plates having 30 to 300 colonies were considered for counting in order to get acceptable values (Fig. 4). No. of bacteria per gram of the sample (CFU/g) was calculated by using the following formula:

$$\text{CFU/g} = \frac{\text{No. of colonies} \times 10 \times \text{dilution factor} \times \text{volume of the stock solution}}{\text{weight of the samples (g)}}$$



Figure 4: Photograph showing the bacterial colony in agar media and colony count from culture media.

Total coliform count (TCC)

MPN (Most Probable Number) method was used to count the total coliform in samples. It was based on the application of the theory of probability to the number of observed positive growth responses to a standard dilution series of sample inoculum placed into a set of culture media tubes (Cappuccino and Sherman, 2008).

Positive growth response after incubation indicated by observation of gas production in fermentation tube. The samples were diluted in such a manner that higher dilutions of the sample results in fewer positive culture tubes in the series. The number of sample dilutions to be prepared is generally based on the expected population contained within the sample.



Figure 5: Photograph showing gas bubble in Durham's tube during Total Coliform Count (TCC).

Procedure of total coliform test

Flow diagram for determination of Total Coliform (TC)

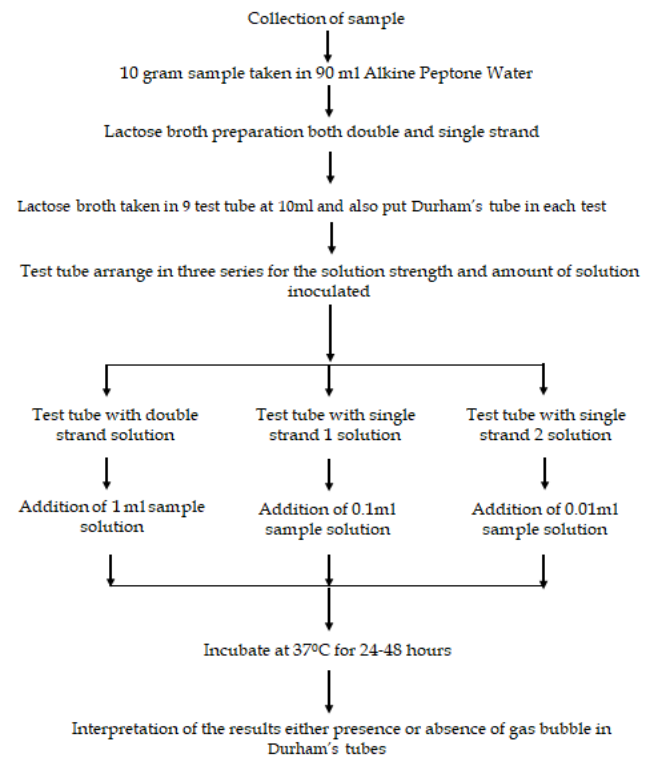


Figure 6: Diagram showing total coliform (TC) determination procedure.

Statistical analysis

Microsoft Excel 2019 was used to analyze both physicochemical parameters and bacterial viable count. Pearson's correlation was done to evaluate the significant relationship between physicochemical parameters and bacterial viable count at 5% level ($p < 0.05$) using statistical software SPSS version 24.0.

RESULTS

The aim of this study was to examine a recently implemented live fish transportation system in Bangladesh by monitoring the water quality parameters such as temperature, pH, dissolved oxygen, ammonia, TDS, and conductivity, as well as a bacteriological analysis of a fish holding water sample. We also look at the bacteriological changes in the skin, gills, and intestines of the Rui, Catla, and Mrigal species that have been transported.

Water quality analysis

Physicochemical parameters of water during live fish transportation system in Bangladesh

Microbial analysis of water in the live fish transportation system in Bangladesh

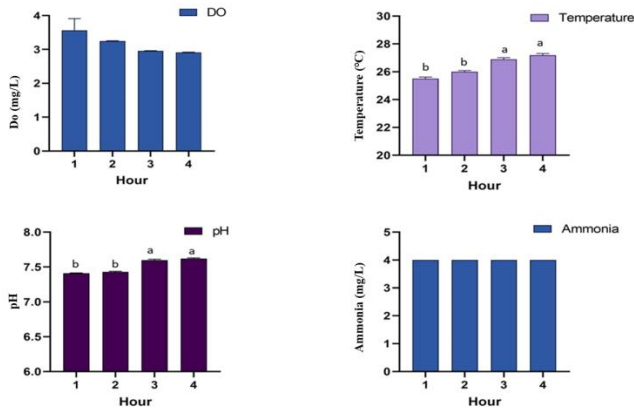


Figure 7: Changes in physicochemical parameters of water during live fish transportation.

We investigated the pH at the start of the transportation to confirm that it was suitable for fish physiology. The pH value was measured at four different times with a uniform time interval. There was a very slight rise from 7.41 to 7.62, as well as an average of 7.51, but within the range of neutral pH (Fig.7).

During the transportation period, the water temperature varied from 25.5 to 27.2 degrees (°C). These temperature changes demonstrate a sustained rise in temperature during live fish transit (Fig. 7).

The level of dissolved oxygen was in the minimal range, decreasing from 3.56 to 2.91. The average DO was 3.17; there were no significant changes in ammonia concentration during the observation period (Fig. 7).

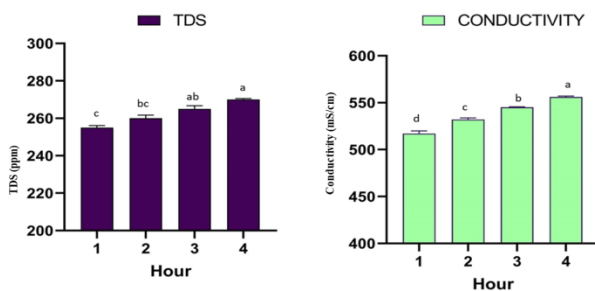


Figure 8: Total dissolved solids and conductivity of water during live fish transportation.

Two other important water quality factors, TDS (Total Dissolved Solids) and Conductivity, were investigated. During the sampling period, there was an increasing trend in both of these limiting factors. TDS increased from 255(ppm) to 270(ppm). The conductivity of the water used in the transportation of live fish was counted from 517 (mS/cm) to 556 (mS/cm).

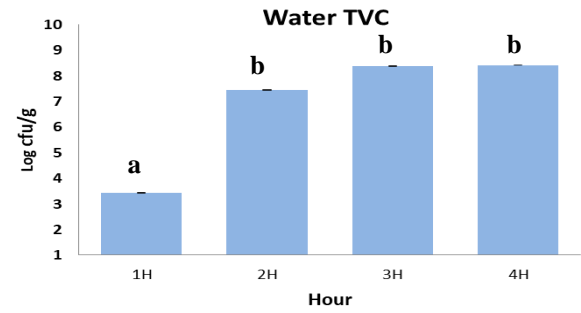


Figure 9: Microbial load in water during live fish transportation

The lowest bacterial load of water during this experiment period was observed at the 1st hour as the fresh underground water was loaded, which gradually increased with the increasing time interval. There was a significant increase in microbial load in water compared to first-time points (Fig. 9).

Total coliform count of the water sample

With the Most Probable Number (MPN) index, the total coliform was calculated. The presence of coliform bacteria in the water sample was determined through this test. The sample from 1st hour shows the lowest MPN, whereas the Sample from the last hour has the highest (Figure -10).

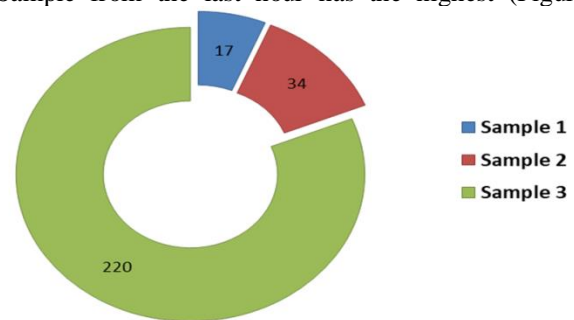


Figure 10: Total Coliform Count of Water Sample by Most Probable Number (MPN) Method

Microbial load analysis of Rui, Catla, Mrigal during live fish transportation

Microbial load analysis of skin samples (Rui, Catla, and Mrigal)

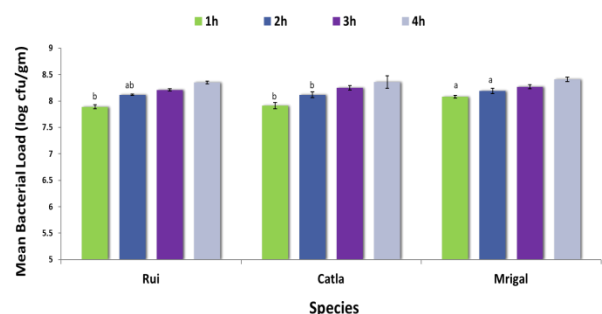


Figure 11: Changes in microbial load of skin sample from Rui, Catla and Mrigal.

The mean bacterial load was calculated on a logarithmic scale. The overall results of bacterial load of skin samples of 3 species during the sampling period were presented as mean \pm SD (Fig. 11). Microbial loads grew steadily as sampling time increased for every fish species. It was significantly ($p < 0.05$) lowest bacterial load of Rui skin sample was 7.89 ± 0.00577 found in 1st hour and the highest in last hour 8.35 ± 0.0133 (Fig. 11). There were significant differences between the microbial load of Catla and Mrigal skin samples. Overall, skin microbial load changed over time, but the change was significant for Rui.

Microbial load analysis of gill samples (Rui, Catla, and Mrigal)

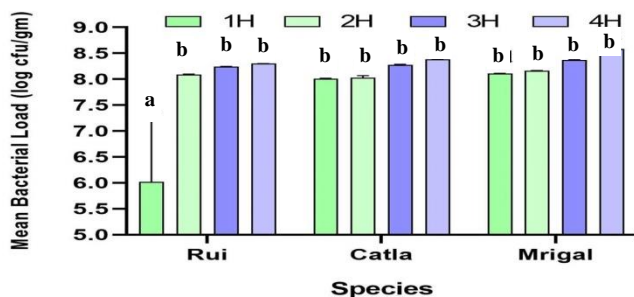


Figure 12: Changes in microbial load of gill sample from Rui, Catla, and Mrigal

The mean bacterial load was calculated on a logarithmic scale. The overall results of the bacterial load of skin samples of 3 species during the sampling period were presented as mean \pm SD (Fig. 12). There was a slight increase in microbial load over time, but the changes were not significantly different among species and time points (Fig. 12).

Microbial load analysis of intestine sample (Rui, Catla, and Mrigal)

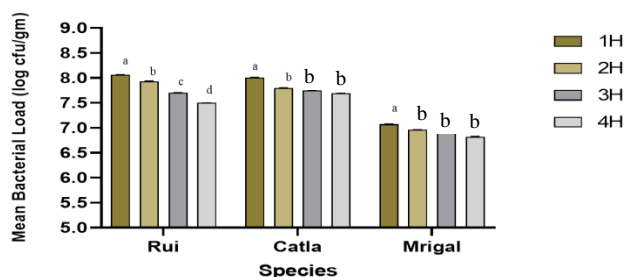


Figure 13: Changes in microbial load of intestine sample from Rui, Catla and Mrigal

The mean bacterial load was calculated on a logarithmic scale. The overall results of the bacterial load of skin samples of 3 species during the sampling period were presented as mean \pm SD (Fig. 13). The microbial load followed a decreasing trend in the intestine meaning the microbial load at 4th hour timepoints were relatively low compared to the microbial load of 1st hour time point. The decreasing trend was significant.

Table 2: Correlation between temperature and viable cell count of transported fish

Fish Species	Temperature Parameter	r	P
Rui	Gill	0.812	0.188
	Intestine	-0.983*	0.017
	Skin	0.879	0.121
Catla	Gill	0.974*	0.026
	Intestine	-0.919	0.081
	Skin	0.979*	0.021
Mrigal	Gill	0.956*	0.044
	Intestine	-0.969*	0.031
	Skin	0.972*	0.028

Pearson correlation analysis between the viable count of bacteria and temperature was carried out, and the relationship was considered for significance at $p < 0.05$. In all species, gills and skin have a positive correlation with temperature, while the intestine sample had a significant negative correlation (Table 2).

4. DISCUSSION

This study was conducted to assess the change of water quality parameters and bacterial load during live transportation of Indian major carp (*Labeo rohita*, *Catla catla*, *Cirrhinus cirrhosis*). The notable challenge of live fish transportation is to reduce transportation mortality by keeping the water quality parameter near the optimum range to minimize stress since a remarkable percentage of fish die before arriving at the targeted location. According to Engdaw (2014), temperature, DO, and pH are the most important that control the physical characteristics of organisms and reduce the death during transportation. In our investigation, we observed no mortality during the transportation, which indicates this open truck live fish transportation system as a positive way of fish transit.

The water temperature results were 25-27°C in all three supply channels (Figure 7), which was aligned with the findings of Cerreta et al. (2020), who observed temperature fluctuation during the transportation of live fish.

The water pH is an important water quality parameter for aquatic organisms to survive since most of the chemical reactions in the aquatic environment are controlled by any change in its value. We checked the pH at the start of the transportation to confirm that it was suitable for fish physiology. The pH value was measured in four different times with a uniform time interval. A very slight rise from 7.41 to 7.62 as well as an average of 7.51. The findings of Berka et al. (1986) state that CO₂ produced from fish respiration alters the pH of the water toward acidity as the transport time rises. Water pH levels of 7–8 are considered to be acceptable. Fish are stressed by rapid pH changes.

In this study, initially, the DO was found to decline slightly and reached a critical level in the last sampling hour (Figure 7). The level of dissolved oxygen was in the critical range, dropping from 3.56 mg/L to 2.91 mg/L. The average DO was

3.17. In live fish transportation, the initial 30–60 min in the transport container is critical, as observed by [Conte, \(2004\)](#). DO is a limiting factor at these high loading densities over an extended traveling period. In this study, although the DO level was reduced to the critical limit, most of the transported fish survived, which may be due to the continuous aeration during transportation.

A study conducted by [Golombieski et al., \(2003\)](#) examined that low oxygen levels and high levels of higher levels of NH_4^+ concentration may result in huge mortality of fish. Also, low oxygen levels may be strongly influenced by high NH_4^+ concentrations, of which around 4.3 mg of oxygen is necessary to oxidize 1.0 mg of NH_4^+ , as observed by [Golombieski et al. \(2003\)](#) and [Esteves \(1988\)](#). [Hossain et al \(2021\)](#) conducted a study that found almost 5mg/L ammonia concentration in a live transportation system where we observed ammonia concentration at 4mg/L. The fish survived throughout the transportation period though the ammonia level was higher.

TDS was higher, indicating that there were more inorganic salts or organic materials present. On average, we discovered 262.5 ppm of TDS in the water. As examined by [James \(2000\)](#), a TDS value of 400 mg/L is permissible in a fish transportation system with diverse fish.

Water conductivity can be used as a broad indicator of its quality. Significant variations in conductivity may indicate that saline was added to the water during transportation. In our current investigation, we noticed a change in conductivity from 517 to 556 (Ms/cm). As suggested by [Knaepkens et al. \(2002\)](#), water conductivity (as a surrogate of productivity) may influence fish condition.

During this experimental period, water temperature remained 25.5 to 27.2 degrees ($^{\circ}\text{C}$). The highest bacterial load of water during this experiment period was observed at the temperature of 27°C and the lowest at 25.6°C . The temperature was positively correlated with the water sample microbial load.

A study by [LeChevallier, \(1990\)](#) stated that there was 30°C temperature in water of all the supply channels, which facilitated the metabolic activities of microbes and hence increased growth.

With the Most Probable Number (MPN) index, the total coliform was calculated. The presence of coliform bacteria in the water sample was determined through this test. [Ahmed et al. \(2013\)](#) aimed to discover indicator bacteria in drinking water samples in order to rule out the possibility of faecal contamination, which might cause health problems, by the most probable number (MPN) approach.

According to [Berka et al. \(1986\)](#), the proportions of harmful ammonia and CO_2 contents are direct functions of pH. Thus, the pH of the water is a control factor. CO_2 produced from fish respiration alters the pH of the water toward acidity as the transport time increases. Water pH levels of 7–8 are considered to be acceptable.

[Hossain et al. \(2021\)](#) noticed that the higher the temperature correlated with the higher growth rate of bacteria. In Rui species, Gill and skin have a positive correlation with temperature. In the case of Catla, Gill and skin had a positive, significant correlation with temperature.

CONCLUSION

From the present study, it can be concluded that live fish transportation in a long route is stressful for fish. There are different stress factors available, which should be maintained in a minimal range for the quality of live fish. Time, temperature, pH, and DO were found to be the most critical stress factors during live fish transportation. There are significant relations between temperature with the total viable cell count of water and fish samples. MPN numbers were found to be higher with the increase of water holding time. It implies that there was a lack of maintaining personnel hygiene. Overall, the microbial load increased as time went on, which indicates that water should be changed within 4-4.30 hrs.

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