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ARTICLE

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EFFECTS OF DISSOLVED OXYGEN LEVELS ON THE BEHAVIOR OF HILSA (*Tensualosa ilisha*) UNDER CONTROLLED CONDITIONS

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Dissolved Oxygen is a critical parameter for aquatic organisms, particularly those that obtain oxygen directly from water. The present investigation was conducted under controlled conditions with artificial aeration to maintain the optimum dissolved oxygen level and without aeration to evaluate the oxygen consumption. During the stress, the behavioural changes were assessed. This investigation focuses on the effects of DO on Hilsa, including swimming behaviour, survival rates, respiration processes, and metabolic functions in freshwater conditions.

Keywords: *Dissolved Oxygen, Ammonia, Fish Behaviour, Survival rate*

Introduction

Effective respiratory and metabolic adaptations to variations in oxygen availability are essential for aquatic life. Furthermore, there are wide variations in the oxygen requirements of aquatic organisms. As temperatures rise, fish's oxygen consumption rises as well; it usually doubles for every 10°C increase[1]. Dissolved oxygen (DO) refers to the quantity of oxygen (O_2) molecules that are present in water. Oxygen is crucial for all living organisms; therefore, dissolved oxygen is vital for the survival of aquatic life. Additionally, DO levels are important for assessing water quality, ensuring it is safe for human use[2]. Oxygen dissolves in water at only about 1/30th the rate it does in air, and its diffusion in water is 1/10,000th as fast as in air. Because of these properties, allow organic matter to decompose and organisms to breathe in water and sediments, which can drastically lower the oxygen content of freshwater bodies[3,4].

Oxygen consumption in fish is influenced by the oxygen concentration in the water and the temperature. Different species have varying levels of tolerance to these conditions. Low oxygen levels impact the rate and strength of their breathing movements, and the presence of carbon dioxide affects how efficiently they can extract oxygen from the water[5,6]. Various biotic and abiotic elements affect DO levels, including upwelling, respiration, photosynthesis, ice cover, mixing water bodies, air exchange, pollution, and physical components like temperature and salinity[7].

Exercise also causes a significant increase in fish oxygen intake; at ideal temperatures, ten times the typical oxygen consumption is the highest amount that can be consumed[1,8]. The process by which oxygen is transferred from the surrounding tissues where it is consumed involves several steps: I) fresh oxygen is continuously brought into touch with the respiratory surfaces by breathing motions, II) in the capillaries of the respiratory organs, oxygen diffuses from the surrounding water via its partial pressure gradient, III) erythrocytes contain haemoglobin, which binds to oxygen. The amount of haemoglobin bound per unit volume of blood is determined by the number of erythrocytes, haemoglobin concentration, current oxygen partial pressures, and the haemoglobin molecule's oxygen-binding properties, IV) from the gills, oxygen is then delivered to the tissues through the bloodstream, V) in

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the capillaries of the tissues, the oxygen partial pressure decreases, causing oxygen to separate from the hemoglobin[3].

According to Franklin[9], fish exhibit negative behavioral responses to elevated carbon dioxide levels and hypoxic conditions, as evidenced by their tendency to move away from affected areas, rise to the water surface, and engage in gulping behaviour. In contrast, fish do not display significant avoidance reactions to ammonia, even at concentrations that can lead to mortality.

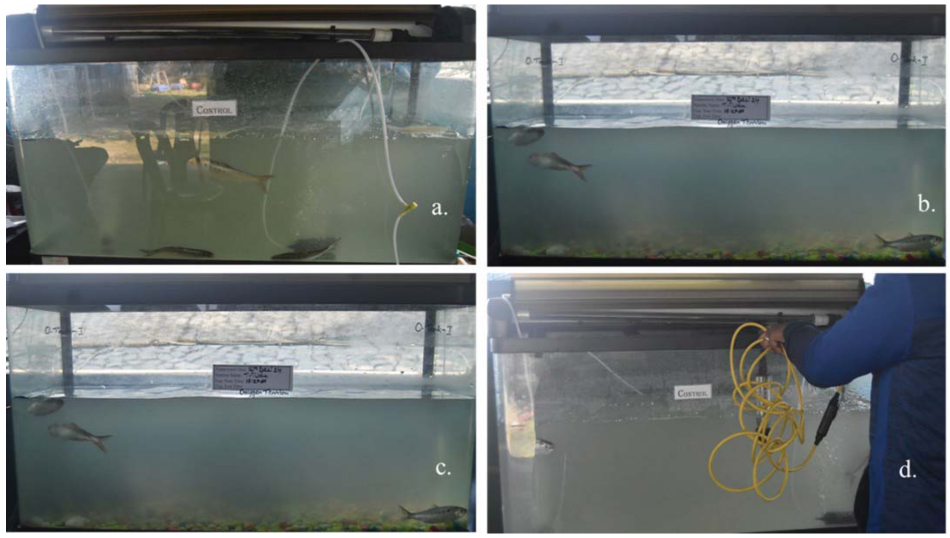

Fig. 1: Schematic diagram of the experiment: a) Control tank with artificial aeration; b) Experimental Tank-I, without artificial oxygen, c) Experimental Tank-II, without artificial oxygen, d) Do measurement during the experiment

Hilsa (*Tenualosa ilisha*) is a migratory fish species of significant ecological, cultural, and economic importance in South Asia, particularly India, and Bangladesh. This species is highly sought after as a food fish, leading to a pressing need for domestication. However, Hilsa populations face increasing threats from environmental stressors, notably hypoxia, which is characterized by low dissolved oxygen levels in aquatic environments[10]. Initial attempts to domesticate Hilsa within pond-based aquaculture systems have been documented[11,12], but these efforts have not yielded successful outcomes. This study aims to systematically assess the behavioral responses of Hilsa to decreasing oxygen levels under controlled conditions. The primary objectives are to quantify the species tolerance to hypoxia and to establish the critical oxygen thresholds essential for its survival.

Methodology

An experiment was conducted at the Hilsa Field Research Station, located on the banks of the Ganga River in Farakka, Murshidabad, West Bengal. Wild Hilsa were captured using hand lift nets and scoop nets to minimize stress. Healthy sub-adult Hilsa mean length of 15.72 ± 0.85 cm and an average weight of 72 ± 6.85 g were transferred to plastic tubs with Ganga water added with artificial aeration, and then transported to the experimental area. The fish were acclimatized for two hours to reduce stress from handling and transportation. The experiment was conducted on sub-adult Hilsa fish kept in a glass aquarium measuring 1 × 0.4 × 0.6 meters (Fig. 1). Each experimental tank contained five Hilsa fish. Fish of similar sizes were selected to ensure the results were comparable. The average stocking density of the fish was between 1 kg and 1.26 kg per cubic meter. The aquarium was equipped with artificial aeration to create waves and filled with filtered Ganga water using a double-fold 25mm plankton net. Physical water quality parameters were measured according to the guidelines set by APHA (2012).

The experiments were performed in duplicate, and results are reported as Mean ± SD (n = 5) unless otherwise stated. Arithmetic mean, two-way ANOVA, post Hoc Tukey HSD test for multiple comparisons using SPSS 18, and the

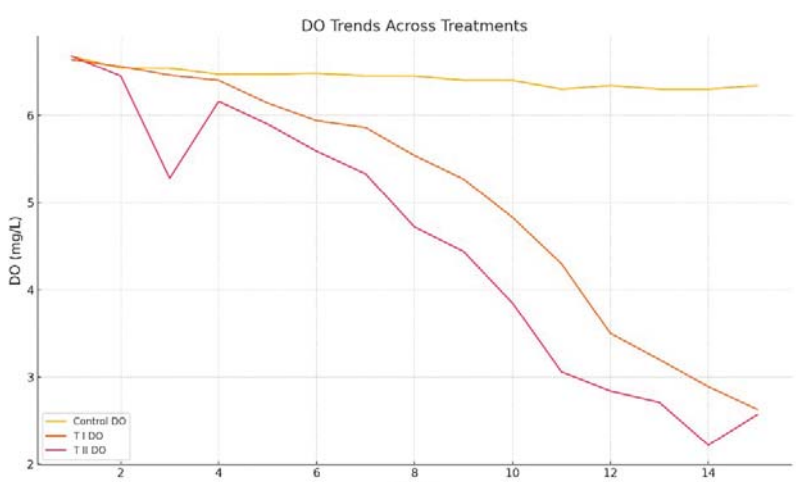

Fig. 2: Graphical representation of oxygen consumption in different system

Table 1 Mortality during Oxygen tension in 24 hr time exposure

Treatments	Mortality					% Mortality
	1st	2nd	3rd	4th	5th	
Control_I	350 min	-	-	-	-	20%
Control_II	-	-	-	-	-	0%
Experimental Tank-I	71 min	143min	554 min	602 min		80%
Experimental Tank-II	64 min	179 min	381 min	578 min	1237 min	100%

ranking technique were the statistical methods used to evaluate and interpret the results.

Results

The condition and behaviors of Hilsa fish were monitored hourly over a 24-hour period in both control and experimental tanks. The fish showed significant behavioral changes in response to low oxygen levels, including surface gulping, darting, increased mucus secretion, reduced swimming, balance issues, heightened responsiveness, bottom gulping, snout reddening, and scale loss. Mortality rates were also recorded at specific intervals. Among the both Control Tank I showed 20% mortality while in the duplicate Control Tank-II no mortality of Hilsa was recorded. while experimental tanks I and II had mortality rates of 80% and 100%, respectively, indicating severe effects of the altered conditions. These observations highlighted the adverse effects of hypoxia on Hilsa fish behaviour and overall health (Table. 1).

Oxygen, temperature, and ammonia levels were monitored throughout the study. Oxygen saturation dropped significantly, by up to 58.99% compared to the control tank (Table. 2), indicating reduced oxygen availability under experimental conditions. Temperature changes were minimal, averaging 18.8 ± 0.03°C, with only a slight 1-2°C increase in the afternoon. These small temperature fluctuations suggest they had little impact on the overall tank environment, unlike the notable changes in oxygen and ammonia levels.

The decreasing DO levels in T-I and T-II suggest that changes in conditions (e.g., higher NH_3 or temperature) are causing oxygen depletion. This phenomenon may be due to the biological activities (respiration and excretion) of the Hilsa that are kept in the Experimental Tank-I and Tank-II in hypoxic conditions (Fig. 2).

The relatively stable but slightly increasing temperature suggests controlled environmental conditions, with T-I and T-II possibly experiencing slight variations (Fig. 3). The increase in NH_3 in T-I and T-II suggests enhanced nitrogen release or accumulation in these treatments, likely due to biological or chemical processes. The control group, with minimal NH_3 changes, serves as a baseline (Fig. 4).

The graphs collectively reveal that dissolved oxygen (DO) levels decrease steadily in T-I and T-II treatments, with T-II showing a sharper decline, while remaining relatively stable in the Control. Ammonia (NH_3) levels rise significantly in T-I and T-II, correlating strongly with the observed DO depletion, indicating a likely causal relationship. Temperature increases slightly across all treatments but has a weaker impact on DO compared to NH_3. The Control group shows minimal changes in both NH_3 and DO, serving as a stable baseline. These findings

Table 2 Oxygen consumption changes (%) in 24 hr. time exposer

Exposer Period		Control (mg/l)	Experiment tank (mg/l)	Percent changes (%)
in Hour	in min			
0	0	6.68±0.00	6.66±0.01	0.30
0.08333	5	6.52± 0.02	6.48±0.03	0.54
0.16667	10	6.52±0.02	6.36±0.07	10.24
0.25	15	6.47±0.00	6.28±0.07	2.94
0.5	30	6.44±0.03	6.03±0.08	6.96
0.75	45	6.44±0.04	5.76±0.12	11.03
1	60	6.45±0.00	5.57±0.15	13.26
1.5	90	6.45±0.00	5.14±0.25	20.47
2	120	6.45±0.04	4.86±0.25	24.14
3	180	6.39±0.02	4.34±0.31	32.19
4	240	6.30±0.00	3.65±0.37	41.59
6	360	6.34±0.00	3.15±0.19	50.00
7	480	6.30±0.00	2.70±0.20	53.10
12	720	6.33±0.02	2.65±0.17	59.44
24	1440	6.37±0.03	2.61±0.03	58.99

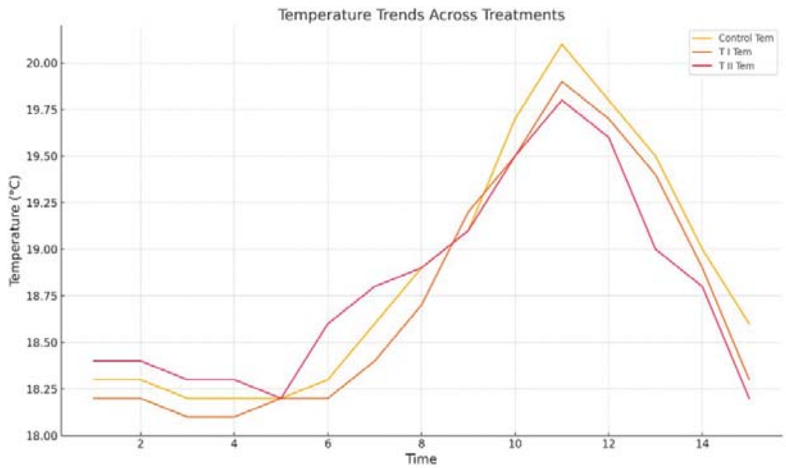

Fig. 3: Graphical representation of Temperature fluctuation in different systems during Oxygen tension

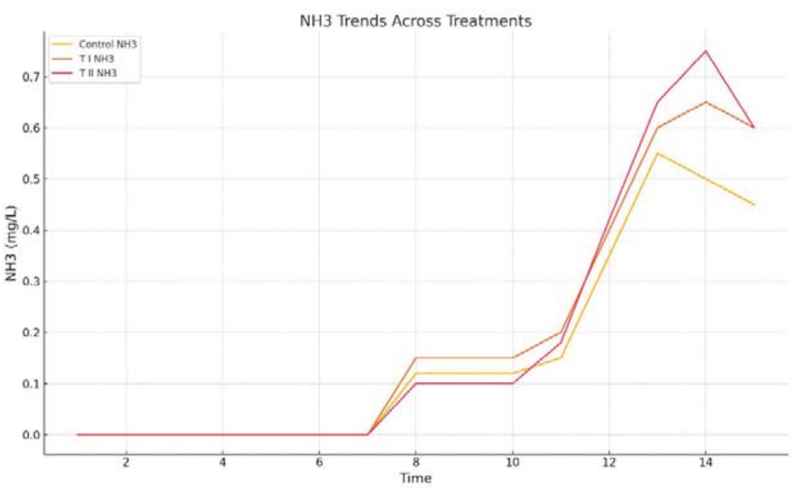

Fig. 4: Graphical representation of Ammonia (NH_3) fluctuation in different systems during Oxygen tension

suggest that increasing NH_3 levels in T-I and T-II are the primary drivers of oxygen depletion, with temperature playing a lesser role.

This is the result of an ANOVA and Tukey's HSD post hoc analysis for two variables: Dissolved Oxygen (DO) and Ammonia (NH_3). The analysis aims to determine whether between the three groups, there are statistically significant differences. (Control, T-I, and T-II).

ANOVA Results: For Dissolved Oxygen (DO), the ANOVA yielded an F-statistic of 9.837 with a p-value of 0.00031, indicating statistically significant variations among the groups (Control, T-I, and T-II). For Ammonia (NH_3), the ANOVA yielded an F-statistic of 0.457 with a p-value of 0.636, showing that there are no statistically significant differences between the groups. (Table 3).

Post Hoc Analysis (Tukey's HSD) for DO: To further investigate the significant differences observed in DO measurements, pairwise comparisons were conducted using Tukey's HSD test.

Every pairwise comparison between groups has a p-value less than 0.05, which indicates statistical significance. When comparing T-I and T-II to Control, a notable drop in DO is seen. Another significant decrease in DO is observed in T-II compared to T-I. In DO measurement, it is significant differences exist among all groups. This suggests that the treatment groups (T-I and T-II) caused changes in dissolved oxygen levels compared to the control group and to each other. There were no discernible variations in NH_3 measurements between the groups, suggesting that the therapy did not affect ammonia levels. These results suggest that while the treatments had a measurable impact on dissolved oxygen levels, ammonia levels remained unaffected. Further studies could explore the mechanisms driving these changes.

Discussion

In the case of juvenile largemouth bass (*Micropterus salmoides*), it is evident that if DO falls below 5-6 mg/l, their swimming performance significantly decreases over time[14], Similar findings are also observed in the case of Hilsa. When oxygen drops below 3-4 mg/l Hilsa shows abnormal swimming movement during the experimental study period. Hypoxic conditions are typically accompanied by hypercapnia situation that leads to increased CO_2 in the blood, as oxygen depletion generally results from the oxidation of organic matter and the respiration of living organisms, which produces carbon dioxide as a byproduct[15].

Table 3 Two-way ANOVA result between control and treatment group

Group 1	Group 2	Mean Difference	p-value	Lower Bound	Upper Bound	Reject Null
Control	T-I	-1.1933	< 0.05	-1.6747	-0.7119	Yes
Control	T-II	-2.0313	< 0.05	-2.5127	-1.5499	Yes
T-I	T-II	-0.8380	< 0.05	-1.3194	-0.3566	Yes

Similar observations are also recorded by Kutty and Saunders[16], declining oxygen levels also have an impact on young Atlantic salmon's (*Salmo salar*) swimming abilities. Hypoxia leads to increased stress, reduced swimming activity, and lowered immunity against diseases. Maintaining dissolved oxygen at saturation levels is crucial, as it ensures that physiological and metabolic activities remain unaffected. Oxygen is essential for respiration and metabolism in fish[17]. When dissolved oxygen decreases, respiration and feeding activities slow down, reducing growth and increasing susceptibility to disease. Low oxygen levels also prevent fish from properly digesting their food[18].

The current investigation, when compared with previous research, reveals that Hilsa fish undergo significant adjustments under hypoxic stress. Initially, they increase their oxygen-carrying capacity, but this ability declines as the stress persists. These findings highlight the species' sensitivity to oxygen fluctuations and its dependence on optimal conditions for maintaining metabolic balance. The results underscore the behavioural stress experienced by the fish under experimental conditions, as evidenced by the notable changes observed.

Conclusion

Dissolved oxygen is the main crucial factor for aquatic species to grow healthily, especially in aquaculture. For fish to survive, dissolved oxygen levels must be measured precisely. The main biotic and abiotic elements that affect DO fluctuations are respiration, photosynthesis, the breakdown of organic waste, aeration, air pressure, salinity, and temperature. Changes in these factors can lead to hypoxic (low oxygen) or hyperoxia (high oxygen) conditions, which significantly impact Hilsa biology. The response of Hilsa (*T. ilisha*) to hypoxic stress varies compared to other species, reflecting differences in their behavioral and biological characteristics. Hypoxic conditions can disrupt the homeostatic balance, causing the fish to struggle with maintaining equilibrium, swimming activity, physical appearance, responsiveness, and respiration. The findings of this study indicate that the oxygen demand in Hilsa is significantly higher than in other fish species. This heightened sensitivity can be attributed to several biological and physical factors, including their fast-swimming behaviour, specialized gill structure, gill surface area, and migratory nature.

Acknowledgments

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