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TOXIC EFFECTS OF SODIUM LAURYL SULPHATE ON RESPIRATORY PHYSIOLOGY AND GILL STRUCTURE IN *AMBLYPHARYNGODON MELETTINUS*

*Dr. Jilna Alex N.

Associate Professor, Department of Zoology, Little Flower College, Guruvayoor, India.

*Corresponding Author

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ABSTRACT

The materials released from farms, industrial sites, and urban drainage have significantly harmed water quality and disrupted aquatic habitats. Numerous chemicals display acute toxicity to aquatic life, while some, despite being less immediately dangerous, are very persistent and bioaccumulative, leading to prolonged physiological and ecological harm. The current study examined the harmful effects of Sodium Lauryl Sulphate on the respiratory functions and gill anatomy of *Amblypharyngodon melettinus*. The study analysed physiological changes in breathing rate, histological variations in gill tissues, and the degree of respiratory damage induced by various concentrations of Sodium Lauryl Sulphate. The current study clearly shows that exposure to sub-lethal levels of SLS has a significant impact on the respiratory physiology and biochemical makeup of *Amblypharyngodon melettinus*. The noted rise in mouth opening frequency and opercular movement suggests respiratory distress, whereas the decrease in oxygen consumption rate indicates compromised metabolic function. The research further validates that Sodium Lauryl Sulphate causes both structural and functional impairments in fish gills. The histological alterations, including lamellar fusion, epithelial hypertrophy, and necrosis, result in diminished gas exchange efficiency and lower oxygen absorption. Moreover, the reduction in muscle protein levels indicates a disturbance in biochemical functions and heightened metabolic requirements under toxic stress situations. These results highlight that the ongoing discharge of detergents with SLS into water ecosystems significantly endangers aquatic wildlife, especially fish species dependent on gill function for their survival. Recognizing these effects in *Amblypharyngodon melettinus* is essential, as it offers fundamental information for evaluating detergent toxicity in freshwater environments and emphasizes the necessity for sustainable cleaning options and enhanced wastewater treatment strategies.

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Keywords: *Amblypharyngodon melettinus,* Fish gills, Respiratory impairment, Sodium Lauryl Sulphate,

INTRODUCTION

A fundamental element necessary for sustaining life on Earth is water. Pesticides, cleaning agents, heavy metals, waste, and industrial discharges are some of the numerous pollutants that are progressively blocking rivers and lakes. These substances have significantly harmed water quality and disturbed aquatic ecosystems because of their release from factories, farmlands, and urban runoff. Sodium Lauryl Sulfate (SLS), known as Sodium Dodecyl Sulfate (SDS), is a man-made anionic surfactant commonly utilized in soaps, detergents, shampoos, and toothpaste. It is a lesser-known yet widespread pollutant. It is commonly utilized as a component in both household and industrial cleaning products due to its potent foaming and emulsifying properties. Nonetheless, untreated industrial discharges and domestic sewage ultimately transport considerable quantities of SLS into water bodies. Once introduced into the environment, SLS alters water surface tension, reduces oxygen solubility, and interferes with the normal breathing functions of aquatic organisms. Fish serve as highly sensitive indicators of water pollution, as their physiological and anatomical traits rapidly react to environmental stressors.

In embryos of zebrafish (Danio rerio), exposure to non-lethal levels of SLS led to developmental deformities and chromosomal irregularities (Rahimi et al., 2020). Increased DNA fragmentation levels were associated with lipid peroxidation and lowered activities of antioxidant enzymes like catalase (CAT) and superoxide dismutase (SOD). These findings indicate that oxidative stress acts as a crucial link between SLS exposure and genotoxic harm. Research has validated the genotoxic effects of SLS in marine life. Yeltekin et al. (2022) found that fish hepatocyte cultures subjected to SLS showed considerable oxidative DNA damage and decreased activity of antioxidant enzymes. Likewise, Rahimi et al. (2020) noted chromatin compaction, nuclear fragmentation, and pyknosis in fish tissues exposed to detergent, signaling apoptosis induced by DNA damage. The Comet assay (single-cell gel electrophoresis), often used to identify DNA strand breaks, has shown elevated DNA tail moments in fish subjected to surfactant-polluted water, indicating genotoxic stress. Rejeki et al. (2008) investigated the long-term impacts of linear alkylbenzene sulphonate (LAS), a surfactant akin to SLS, on Lates calcarifer (sea bass) and found reduced survival rates and physical malformations in gill filaments. They noted epithelial elevation, cell death, and layer fusion, which greatly diminished the effectiveness of gas exchange. The results showed that even minimal levels of detergents might affect respiratory function and disturb metabolic balance.

The main way that SLS causes DNA damage is by producing reactive oxygen species (ROS). Ivon et al. (2020) reported hematological abnormalities and stress behaviors in Clarias gariepinus exposed to water contaminated with detergent. They noted a considerable decrease in hemoglobin

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levels and red blood cell numbers, suggesting diminished oxygen transport capacity and compromised breathing. Histological examinations of the gill showed significant lamellar distortion and vascular congestion, indicating the beginning of oxidative stress and respiratory impairment. Yeltekin and colleagues (2022) offered additional understanding of the cellular processes involved in detergent toxicity. Their in vitro research showed that exposure to SLS induced oxidative stress, lipid peroxidation, and cellular degeneration in epithelial cells of fish gills. Moreover, research conducted by Moniruzzaman et al. (2021) indicated that extended exposure to SDS modified respiratory enzyme activity and blood gas levels in different freshwater fish species. The noted rise in opercular beat frequency and decrease in oxygen consumption demonstrated that surfactants create respiratory stress and metabolic fatigue in fish. These results emphasize the relationship between gill histopathological damage and respiratory physiological impairment. Rejeki et al. (2008) examined the long-term impacts of linear alkylbenzene sulphonate (LAS), a surfactant akin to SLS, on Lates calcarifer (sea bass) and noted reduced survival rates and morphological deformities in gill filaments. They noted epithelial detachment, necrosis, and lamellar fusion, which greatly diminished the effectiveness of gas exchange. The results showed that even minimal levels of detergents could hinder respiratory function and disturb metabolic balance.

The present study examined the harmful effects of Sodium Lauryl Sulphate (SLS) on the respiratory function and gill morphology of *Amblypharyngodon melettinus*. The study provided an overview of aquatic toxicity caused by surfactants and highlighted the critical necessity for environmental regulations governing the release of detergents into natural water systems.

MATERIALS AND METHODS

Amblypharyngodon melettinus is a diminutive, silvery, omnivorous freshwater fish (reaching 8cm) typically located in shallow, slow-moving streams, ponds, and reservoirs in Kerala. It is a well-known, healthy local food source with a tapered shape, commonly located in stagnant waters with muddy bottoms. This small cyprinid fish contributes to aquatic food webs by consuming phytoplankton and tiny invertebrates, while also serving as prey for larger fish, birds, and other animals. Research on wetland ecosystems (like the Kole-wetlands of Thrissur) has documented it in high quantities, suggesting its abundance in fish communities within these environments and probable significance in nutrient cycles and food webs. Fish samples for the research were gathered from local ponds in Guruvayur municipality, and the weight of the fish was documented (1±0.64g). The study selected *Amblypharyngodon melettinus* as the model organism of the present study because of its responsive to ecological variations. Sodium lauryl sulfate was utilized to create the experimental sample water.

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Sub-lethal concentration (LC_{50}) of SLS is determined by dissolving 1 g, 0.8 g, 0.5 g, and 0.3 g of SLS in 10 L of fresh water and observed for mortality over 24 hours. All fish died in the 1 g and 0.8 g concentrations within 24 hours, while two deaths were recorded in 0.3 g concentration after 96 hours. Based on these observations, 0.1 g (Experiment I) and 0.2 g of SLS (Experiment II) in 10 L of water were selected as the sub-lethal concentrations for further studies. The fish are then exposed to these lower concentrations for 21 days to observe behavioral, physiological and biochemical changes without causing death. The results are compared with a control group kept in clean water to assess the effects of the sub-lethal doses. Experimental analysis was carried out in each seven days and the treatment water was renewed with the selected concentration.

Experimental Procedure

I: Measurement of fish activity

a) Frequency of water gulping through mouth

All the activities of an organism are directly dependent on its physiological status. In the present study the frequency of water intake was determined by counting the moth movements of the fish. The fish was placed in a glass tumbler of 1000ml for 10minutes. Then the frequency of mouth opening was counted for further 1 minute. Data was collected from both experimental and control group.

b) Opercular movement

Movement of gills in fishes can be considered as a measure of respiratory activity. Fish inhabiting in pure water will get plenty of oxygen and hence will be less stressed. Here the movement of gills will be relaxed. But as the water become hypoxic the fish will become stressed and show signs of asphyxiation. A major indication of asphyxiation in fish is the random movements of the gills. In the present study the difference in movement of gills was analysed by observing the fish treated with SLS added hypoxic water and control fish. Six fish were tested in each water sample for 10 minutes. Each fish was individually introduced in to the experimental jar. After ten minutes of acclimation data were recorded for 10 minutes.

II: Measurement of rate of O_2 consumption by fish

The ability of the fish to utilize dissolved oxygen was measured by introducing both the test fish and control fish to a respirometer containing fresh water. The data were taken separately. The metabolic rate in fish is usually estimated by oxygen consumption measurements. The apparatus used was a modification of Fry's respirometer (Kutty et al., 1971). The construction of the apparatus has been done following the principles described in the literature.

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Respirometer incorporates a chamber for the fish and a surrounding respiratory medium (usually water). In the present study the apparatus used was a closed static type respirometer where the water was allowed to flow out during the period of acclimation (10 minutes) and closed using stoppers during the period of experiment (90 minutes). Oxygen consumption was determined in closed chamber respirometers made of acrylic tubings sealed at one end and screw capped at the other. Two holes (15 mm in diameter) were made in its wall. The upper one was used to supply running water during acclimation. The lower one was used to allow excess water to escape when the large hole was closed. Volume of the respirometers was 2 Litres. The respirometers were big enough to allow the fish to move freely but not swim actively, characterizing an uncontrolled but minimum activity (routine metabolism). Experimental animaIs were gently introduced individually into the chambers that were then capped. Running water was allowed for 10 minutes, to diminish the stress of the animaIs due to handling. At the beginning of measurement, the water supply was stopped, the holes in the walls of the respirometers were sealed and the animaIs were allowed to take up oxygen for a period of 90 minutes. This period was established experimentally, in view of the size of the animal in relation to the volume of the respirometer, in order to prevent the oxygen concentration at the end of the experiment from falling to below 70% of the initial concentration. The respirometers were partly covered to protect fish from excessive light and from the movement inside the laboratory.

Samples of water were taken at the beginning and at the end of each experiment for the determination of dissolved oxygen. All measurements were made during the daytime and no fish were used twice. Bacterial oxygen consumption was negligible as demonstrated in control experiments under the same conditions. Dissolved oxygen was determined by Winkler's method modified by Moran *et.al*., (1980). Difference between the amounts of DO in the sample treated with detergent and that of control. Similarly, DO consumed by fishes in other water samples was calculated.

III: GILL STRUCTURE ANALYSIS

Prior to structural analysis of gills, fish were killed by severing the spinal cord behind the head. Gills were collected from the second gill arch. Tissues were fixed in Bouin's fluid for 24 h, transferred to 70 % alcohol and histological analysis is carried out.

IV: PROTEIN ESTIMATION

I) QUANTITATIVE ESTIMATION OF PROTEIN BY BIURETTE METHOD

One commonly used method for determining the total protein in a sample is the Biuret method (Layne, E., 1957 and Henry et al., 1974) The Biuret method is based on the complexation of Cu2+ to functional groups in the protein's peptide bonds. Sarcoplasmic proteins can be extracted from

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fish muscle with 0.15 M salt. The protein present reduces cupric ions to cuprous ions under alkaline conditions. The cuprous ions react with the biurette reagent to give a purple color that can be quantified colourimetrically.

All the data were analysed using one way ANOVA and Day-wise pair wise comparison was carried out using Tukey's HSD Test.

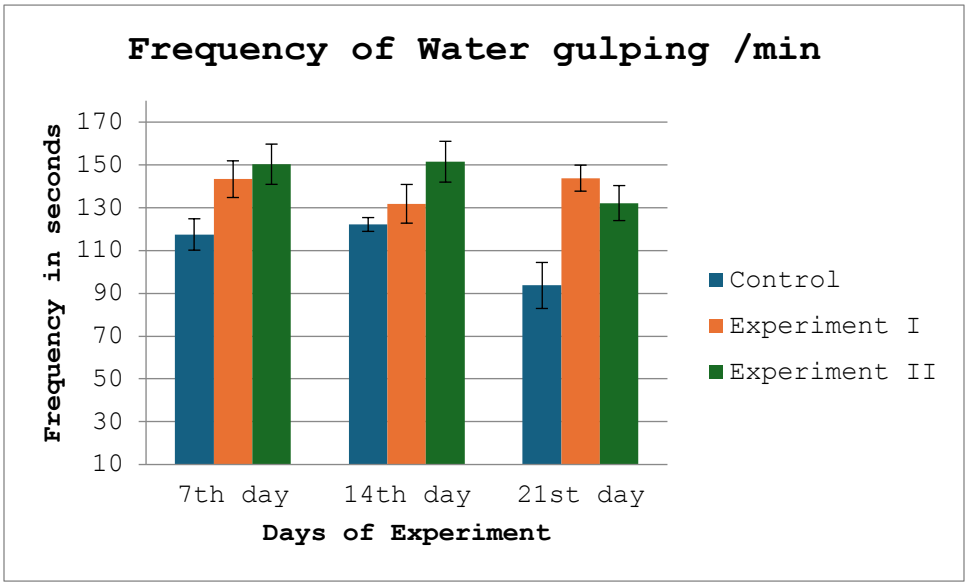
RESULTS

The results clearly showed that the sub lethal concentrations of SLS caused significant changes in both behavioural and physiological parameters of the fish.

Comparison of behavioral and physiological changes in control group and experimental group (Mean±SD)

a) Frequency of Water gulping (per minute in seconds)

Days of Experiment	Control	Experiment I	Experiment II
7th day	117.5 ± 7.32	143.33 ± 8.57	150.33 ± 9.41
14th day	122.17 ± 3.23	131.83 ± 9.04	151.5 ± 9.53
21st day	93.67 ± 10.75	143.83 ± 6.09	132.17 ± 8.18



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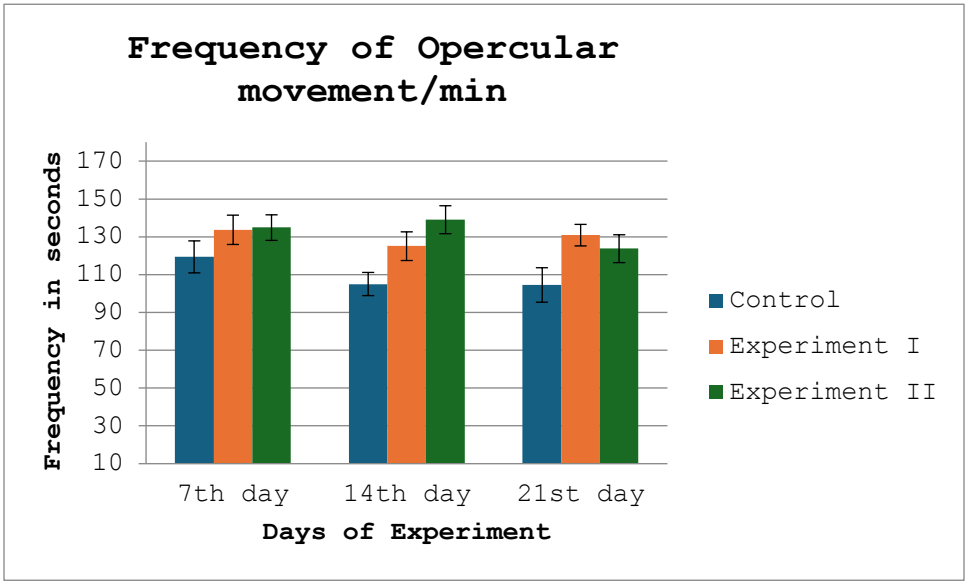
b) Opercular movement (per minute in seconds)

Days of Experiment	Control	Experiment I	Experiment II
7th day	119.33 ± 8.46	133.67 ± 7.76	134.83 ± 6.75
14th day	105.00 ± 6.12	125.00 ± 7.57	139.00 ± 7.39
21st day	104.50 ± 9.11	130.83 ± 5.71	123.67 ± 7.36



c) Rate of Oxygen consumption [mg/h/g (wet weight)]

Days of Experiment	Control	Experiment I	Experiment II
7th day	3.63 ± 0.66	2.35 ± 1.22	1.26 ± 0.88
14th day	7.40 ± 1.28	3.17 ± 0.63	1.45 ± 1.58
21st day	10.10 ± 0.52	3.70 ± 0.22	1.10 ± 1.06

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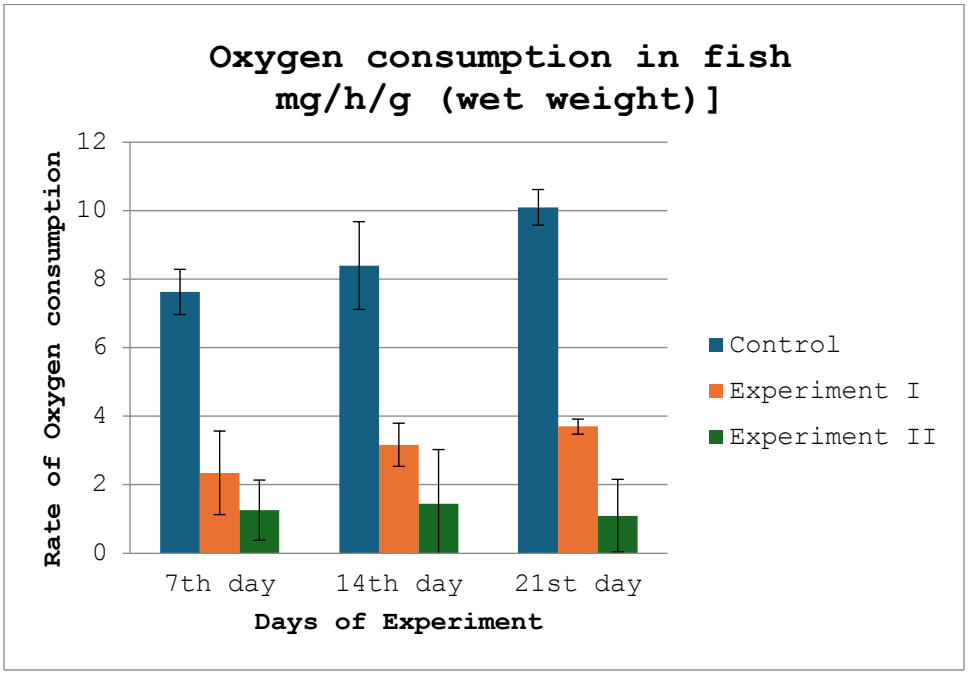

d) Protein analysis (mg/ml)

Days of Experiment	Control	Experiment I	Experiment II
7th day	5.48 ± 1.82	4.19 ± 0.38	3.87 ± 0.61
14th day	6.12 ± 0.98	4.19 ± 0.92	3.75 ± 0.85
21st day	5.48 ± 0.22	3.87 ± 1.22	3.75 ± 0.67

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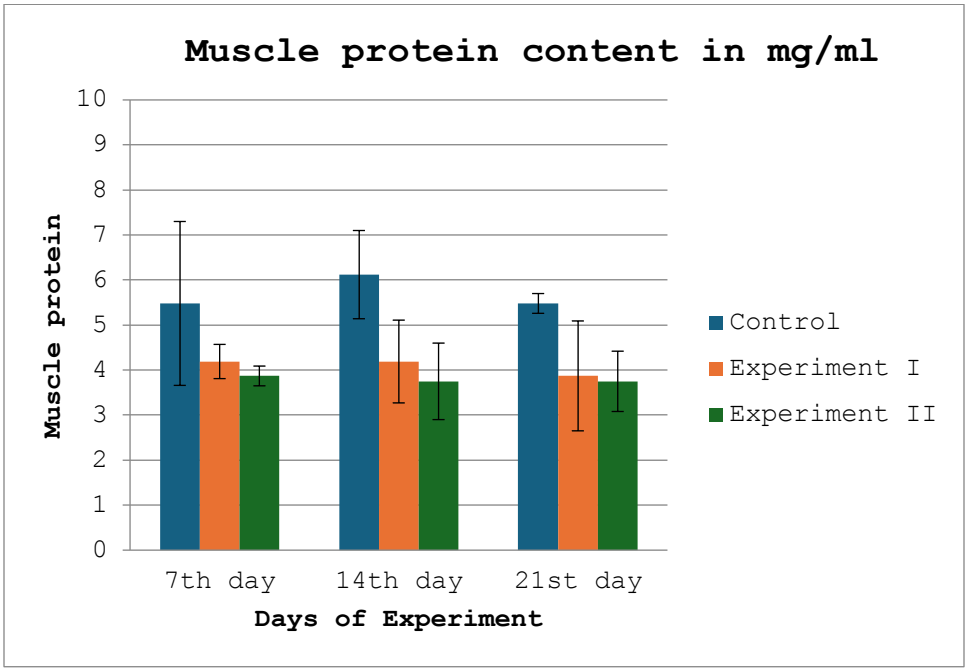

The air gulping tendency of the experimental groups was always higher than the control group in all experimental days. One-way ANOVA revealed statistically significant differences among the groups on all days, with F-values of 24.95, 22.00, and 56.50 respectively ($p < 0.05$). According to Tukey's HSD Test, both experimental groups differ significantly from control, but not from each other. The opercular movement of the experimental group was also high compared to the control group in all experimental days. One-way ANOVA revealed significant differences on the 7th ($F_{2,15} = 7.81$, $p < 0.05$), 14th ($F_{2,15} = 36.34$, $p < 0.001$), and 21st day ($F_{2,15} = 18.34$, $p < 0.001$). Tukey's post hoc analysis indicated that both experimental groups differed significantly from the control on all days. On the 14th day, all pairwise comparisons were significant, with Experiment II showing the highest opercular movement. However, on the 7th and 21st days, no significant difference was observed between the two experimental groups.

The fish's oxygen consumption rate was dramatically lowered after being exposed to SLS, providing a clear indication that the fish were under oxygen stress. One-way ANOVA revealed significant differences on the 7th ($F_{2,15} = 9.85$, $p < 0.01$), 14th ($F_{2,15} = 43.51$, $p < 0.001$), and 21st day ($F_{2,15} = 207.74$, $p < 0.001$). Tukey's post hoc test indicated that on the 7th day, only Experiment II differed significantly from the control. However, on the 14th and 21st days, all pairwise comparisons were statistically significant, with the control group showing the highest oxygen consumption followed by Experiment I and Experiment II. The results indicate a progressive decline in oxygen consumption under experimental conditions.

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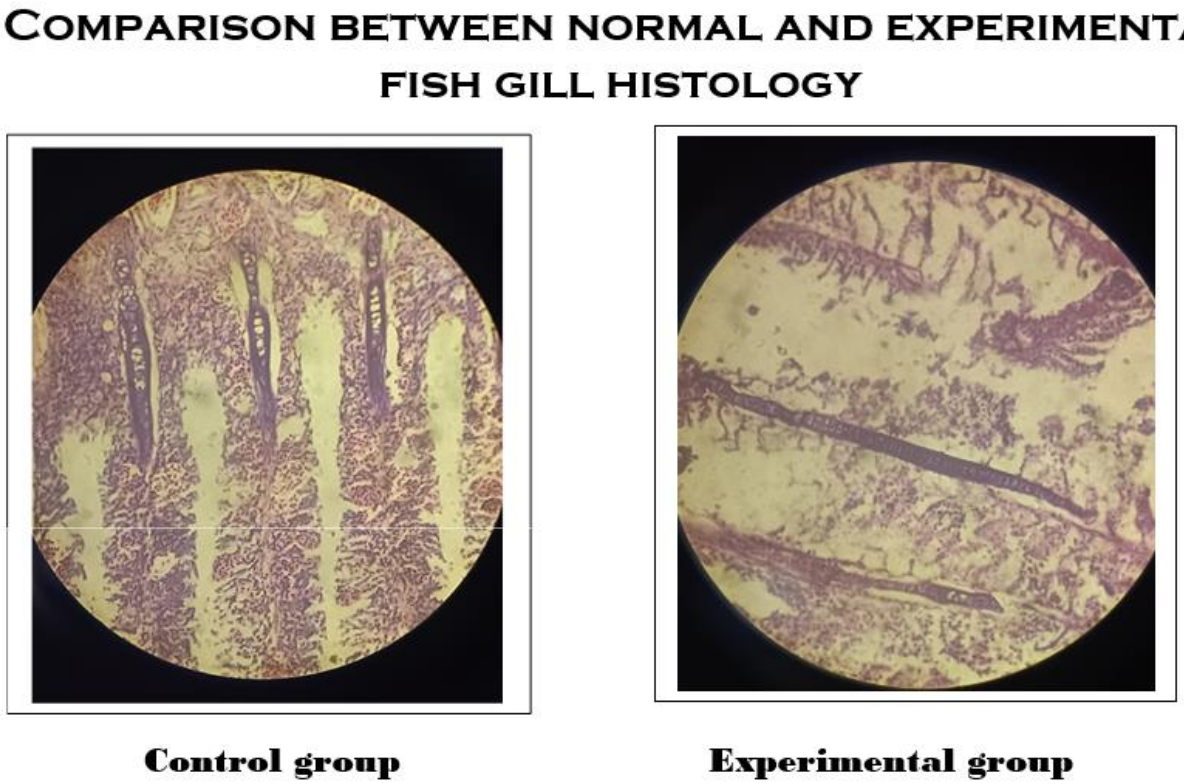
Protein content exhibited a declining trend in the experimental groups compared to the control. According to one-way ANOVA, on the seventh day, there was no significant difference ($F_{2,1^2}$ = 3.56, $p > 0.05$), but on the fourteenth and twenty-first days, there were significant differences ($F_{2,1^2}$ = 9.06, $p < 0.01$ and 9.83, $p < 0.01$, respectively). Tukey's post hoc test revealed that on the 14th and 21st days, the control group had considerably greater protein levels than both experimental groups, but there was no discernible difference between Experiment I and Experiment II. Overall, the findings show that the experimental groups' respiratory activity increased gradually, with the 21st day showing the greatest impact. The pattern indicates that the experimental treatment causes fish to experience prolonged physiological stress and that increased its ventilatory activity.

Microscopic analysis of the gills showed distinct structural changes in fish subjected to SLS. Control fish displayed typical gill structure with properly arranged primary and secondary lamellae and standard filament thickness. Fish subjected to 10 ppm SLS showed slight epithelial lifting and mucus build-up with filaments. At 20 ppm SLS, significant pathological alterations were noted, including lamellar fusion, epithelial hyperplasia, congestion in blood capillaries, and necrosis of gill tissue. These histological changes decrease the respiratory surface area and impede effective gas exchange.



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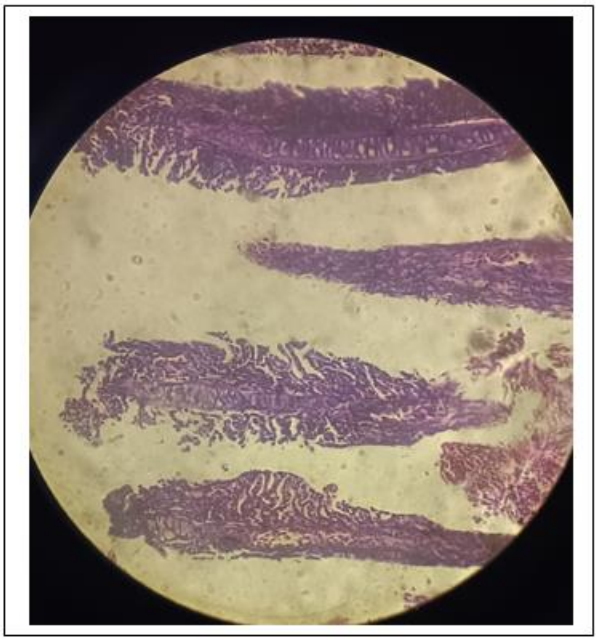

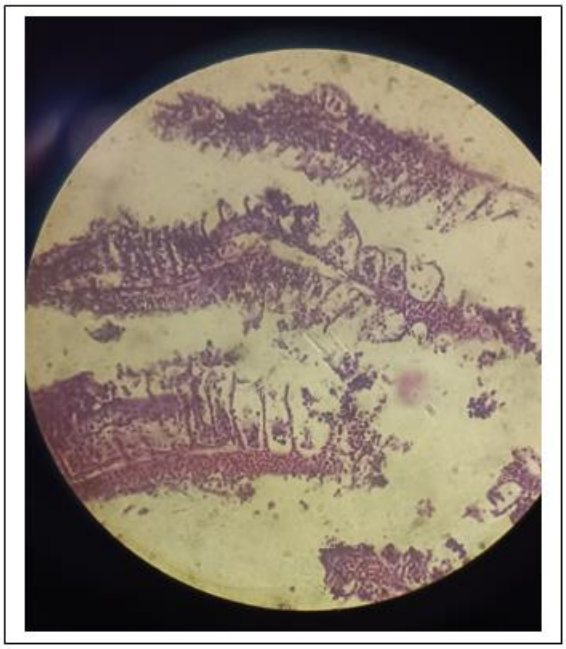

DISCUSSION

One of the initial and most delicate signs of toxic stress in fish is changes in behavior. In this study, the occurrence of mouth opening or water gulping significantly rose in the experimental fish subje cted to SLS. The higher frequency of mouth opening in fish subjected to surfactants can be linked to the decreased availability of dissolved oxygen and the disruption of normal gill operation. Surfactants like SLS lower water's surface tension and create foam barriers that hinder the diffusion of oxygen from the atmosphere into the water. Furthermore, SLS molecules engage with biological membranes because of their amphiphilic characteristics, resulting in harm to gill epithelial cells. As gills are the main organs for respiration in fish, any structural or functional disruption in gill tissues directly impacts oxygen absorption. Fish enhance their ventilation rate by more frequently opening and closing their mouths as a compensatory response. Comparable rises in respiratory activity have been documented in different fish species exposed to detergents and surfactants. Rahimi et al. (2020) found that exposure to anionic detergents led to more frequent mouth openings and unusual swimming patterns in Clarias gariepinus, suggesting respiratory distress. Mousavi and Khodadoost (2019) noted unpredictable behavior and heightened ventilation in fish subjected to detergent-polluted water. Alterations in water quality are also linked to this (Ogugba-Udume et al., 2020; Zakaria et al., 2024).

Opercular movement, indicative of the rhythmic motion of gill covers while breathing, serves as another crucial sign of respiratory function in fish. The pattern clearly shows that exposure to SLS enhances respiratory activity in fish as a reaction to environmental stress. The rise in the frequency

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of opercular movements is typically viewed as a compensatory reaction to hypoxic situations. When levels of dissolved oxygen drop or when gill function is compromised, fish strive to sustain sufficient oxygen intake by raising their ventilation rate. The swift motion of the operculum boosts water flow over the gill lamellae, which improves oxygen absorption from the external water. Nevertheless, extended periods of increased ventilation can result in metabolic fatigue and energy depletion in fish. Moniruzzaman et al. (2021) found that exposure to surfactants causes respiratory stress in fish by affecting gill permeability and disturbing ionic balance, resulting in heightened opercular beat frequency and irregular respiration patterns.

The rate of oxygen consumption, the oxygen consumption rate in the control group rose progressively. This rise could be linked to typical metabolic changes and the development of the fish throughout the experimental phase. Conversely, the experimental groups subjected to SLS exhibited a significant decrease in oxygen consumption rates. The findings clearly show that higher levels of SLS notably reduced the oxygen consumption rate in Amblypharyngodon melettinus. The decrease in oxygen uptake noted in the experimental fish could be linked to the harmful impacts of SLS on gill tissues and metabolic functions. Surfactants are recognized for disrupting the lipid bilayer of cell membranes, which results in heightened membrane permeability and harm to cells. In fish gills, such injury can lead to epithelial lifting, lamellar fusion, mucus production, and necrosis of gill tissues. These modifications in structure decrease the functional respiratory surface area and hinder the transfer of oxygen from water into the blood. As a result, fish's capacity to use dissolved oxygen declines, resulting in lower oxygen consumption rates. Rejeki et al. (2008) reported comparable results, noting that surfactant exposure led to substantial decreases in oxygen uptake and respiratory efficiency in fishre of metabolic activity in fish. Oxygen uptake indicates the energy needs of an organism and is strongly related to physiological functions like breathing, movement, and metabolic activities.

Another key factor examined in this study was the oxygen consumption rate, which acts as a direct measure of fish metabolic activity. Oxygen usage indicates the energy needs of an organism and is highly linked to physiological functions like breathing, movement, and biochemical metabolism. In the current study, the rate of oxygen consumption in the control group rose gradually. This rise can be ascribed to typical metabolic changes and growth of the fish throughout the experimental duration. Conversely, the experimental groups subjected to SLS demonstrated a significant decrease in oxygen consumption rates. The findings clearly show that higher concentrations of SLS notably reduced the oxygen consumption rate in *Amblypharyngodon melettinus.* The decrease in oxygen consumption seen in the experimental fish could be linked to the harmful effects of SLS on gill tissues and metabolic functions. Surfactants are recognized for their ability to break down the lipid bilayer of cell membranes, resulting in heightened membrane permeability and harm to cells. In fish gills, such injury may lead to epithelial detachment, lamellar fusion, mucus

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production, and necrosis of gill tissues. These structural changes diminish the functional respiratory surface area and hinder the diffusion of oxygen from water into the blood. As a result, fish find it harder to use dissolved oxygen, resulting in lowered oxygen consumption rates. Comparable results were noted by Rejeki et al. (2008), who found that fish exposed to surfactants experienced considerable declines in oxygen consumption and breathing efficiency

The reduced oxygen consumption may be alterations in gill epithelium due to oxidative stress. Surfactants such as SLS can induce oxidative stress by generating reactive oxygen species (ROS), which damage cellular macromolecules including lipids, proteins and nucleic acids. Excessive oxidative stress can impair mitochondrial function and disrupt energy metabolism, resulting in decreased oxygen utilization by tissues. Yeltekin et al. (2022) reported that exposure to SLS significantly reduced the activity of antioxidant enzymes such as catalase, superoxide dismutase and glutathione peroxidase in fish cells, indicating severe oxidative damage. In normal healthy fish, the gills show a well-organized structure that ensures efficient gas exchange. The comparison between normal and experimental fish gill histology clearly demonstrates that exposure to pollutants and toxic chemicals causes structural damage to gill tissues (Jeen *et al.,* 2018). While normal fish show a thin and organized epithelial layer suitable for efficient respiration, experimental fish exhibit pathological changes such as hyperplasia, lamellar fusion, edema, and necrosis. These histological alterations compromise respiratory efficiency and serve as important biomarkers for environmental pollution and aquatic toxicity studies (Simon, 2017; Susmi *et al.,* 2020).

Besides respiratory changes, the current study also examined the biochemical alterations in fish muscle tissue by measuring total protein levels. Proteins are essential for preserving cell structure, enzyme function, and metabolic activities. Any disruption in protein metabolism indicates physiological stress and harmful effects on the organism. The findings indicated that the protein level in the control group stayed fairly constant during the experimental duration. In the experimental groups subjected to SLS, the protein levels were notably reduced. The decline in protein levels seen in the experimental fish may result from enhanced protein breakdown and compromised protein production during toxic stress situations. Exposure to contaminants frequently results in increased protein breakdown to satisfy the heightened energy needs for detoxification and stress adaptation. Moreover, oxidative harm brought on by reactive oxygen species can disrupt normal metabolic processes and denature cellular proteins (Federica et al., 2025). As per Ivon et al. (2020), fish tissues experience a notable decline in protein levels from exposure to detergent pollutants because of heightened proteolysis and interference with amino acid metabolism. Additionally, the decrease in protein levels may be associated with harm to liver and muscle tissues, which are crucial for protein synthesis and storage. In stressful environmental situations, fish can utilize protein reserves for energy, resulting in a reduction of tissue protein

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levels. This occurrence has been extensively documented in fish subjected to heavy metals, pesticides, and surfactants. Rahimi et al. (2020) found that exposure to detergent contaminants led to notable decreases in muscle protein levels in freshwater fish, suggesting considerable metabolic strain.

CONCLUSION

The joint impact of behavioral, physiological, and biochemical changes noted in this study clearly indicates that even non-lethal levels of SLS can have considerable toxic effects on freshwater fish. The elevated respiratory activity noted in the test fish suggests that SLS exposure causes stress and interferes with regular respiratory function. Simultaneously, the decline in oxygen usage and protein levels indicates that the surfactant disrupts metabolic functions and energy generation in fish tissues. In natural aquatic environments, fish are constantly subjected to different pollutants found in water bodies that receive domestic waste and industrial discharges. While the levels of these pollutants might not consistently reach fatal thresholds, extended exposure to sub-lethal concentrations can result in ongoing physiological stress and diminished chances of survival. The respiratory issues identified in this study might diminish fish's capacity to endure environmental variations like temperature shifts and low oxygen levels. Likewise, biochemical changes like decreased protein levels can impact growth, reproduction, and immune responses, ultimately resulting in a decline in population. The findings of this research underscore the possible environmental hazards linked to the unregulated release of detergent contaminants into water ecosystems. Surfactants like SLS may not remain for extended durations in well-oxygenated water systems due to microbial breakdown, yet their ongoing discharge from domestic wastewater can sustain detrimental levels in the surroundings. Thus, efficient wastewater management and appropriate control of detergent discharges are crucial for safeguarding aquatic biodiversity and preserving ecological equilibrium.

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