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Impact of Paper Mill effluent on relative growth rate and oxygen consumption rate of freshwater catfish, *Mystus vittatus* (Bloch)

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Abstract
The present investigation was conducted to examine the chronic effects of sublethal concentrations of paper mill effluent on the freshwater fish *Mystus vittatus*, focusing on mortality/survival rate, relative growth rate (RGR), and rate of oxygen consumption. Based on the 96-hour LC_{50} value of 8.223% v/v for *Mystus vittatus*, two sublethal concentrations were selected: 0.8% v/v (≈1/10th of LC_{50}) and 1.7% v/v (≈1/5th of LC_{50}). No mortality was observed at either concentration, indicating 100% survival. However, RGR declined progressively with increasing concentration and exposure duration. Similarly, oxygen consumption rate showed a significant decrease in effluent-exposed fish, with the reduction being more pronounced at higher concentrations and longer exposure periods. These findings suggest that the chronic toxicity response of experimental is strongly dependent on both effluent concentration and duration of exposure.

Keywords: Paper mill effluent, Chronic toxicity, mortality responses, Relative growth rate, *Mystus vittatus*

Introduction
Water resources such as rivers, ponds, lakes, and wetlands are increasingly polluted by various solid and liquid wastes, causing severe damage to aquatic ecosystems. Globally, two critical processes influencing the environment are resource production and waste disposal, and maintaining a balance between them is essential for a healthy biosphere. Today, both rural and urban societies, being technologically and industrially advanced, release different waste products and by-products into the environment either directly or indirectly. The discharge of untreated effluents into natural water bodies and aquatic ecosystems, therefore, poses a significant threat to human health (Prakash and Verma, 2020a; Verma and Prakash, 2022; Prakash, 2023) [10, 22, 7].

Industrial growth has played a crucial role in the advancement of human civilization and is essential for the development and prosperity of any nation. However, industrial activities can be highly detrimental to aquatic life when toxic effluents are discharged without proper treatment. These effluents are complex mixtures of various chemical constituents and significantly influence the oxidation potential of aquatic ecosystems. Their direct or indirect release into freshwater bodies depletes dissolved oxygen levels and disrupts respiratory metabolism, often leading to high mortality rates in aquatic organisms. When discharged in large quantities without treatment, industrial effluents exert severe stress on water resources and alter the physicochemical characteristics of aquatic environments. Such alterations affect the behavioral, haematological, biochemical, histological, and physiological functions of aquatic organisms, including fishes (Prakash and Verma, 2020b; Prakash and Singh, 2020) [11, 8].

Growth in organisms is regulated by several biological processes, including food intake, digestion, absorption, metabolism, and excretion. The net available energy is distributed among metabolism, maintenance, reproduction, and growth. Consequently, if metabolic demands increase without a corresponding rise in food intake, growth will be limited (Verma and Prakash, 2020; Tiwari and Prakash, 2024) [21, 18]. Studies on oxygen consumption serve as an effective tool to assess the stress induced by toxicants in aquatic organisms and provide an index of energy expenditure mechanisms under environmental variations (Neelima *et al*., 2016; Verma and Prakash, 2019) [5,20]. It can serve as an early warning bioindicator of

pollution-related stress. Although respiratory responses are relatively less sensitive, they can still be effectively used in bioassay testing of treated industrial and municipal effluents prior to their discharge into water bodies.

Fish are reliable indicators of environmental conditions such as poor water quality and pollution, as they form an integral part of the aquatic food chain. They readily accumulate toxic substances, including pesticides and heavy metals, from their surroundings and thus serve as effective monitors of aquatic pollution. However, there remains a considerable gap in research on the effects of industrial effluents on freshwater fishes, which prompted the present investigation. A review of the literature indicates that industrial effluents adversely affect a wide range of non-target organisms, including fishes and other aquatic fauna.

No information is available on the direct effects of paper mill effluent on the growth of freshwater fishes. Therefore, the present study was undertaken to evaluate the impact of paper mill effluent on the survival rate, relative growth rate (in terms of body weight), and oxygen consumption of the freshwater catfish *Mystus vittatus*, with the broader aim of ensuring the health and safety of fish consumers.

Material and Methods

The healthy freshwater catfish, *Mystus vittatus* (Bloch) of uniform length (8.8±0.20 cm) and weight (9.5±1g) were collected from local fresh water bodies in and around Ayodhya. The collected fishes were washed with 1.0 % solution of KMnO4 for five minutes to remove any dermal infection. Then these fishes were brought to the laboratory in plastic container. The fishes were again screened for any pathogenic infections and transferred to the plastic jar containing 50L dechlorinated tap water.

Healthy fishes were acclimated to laboratory conditions for two weeksat room temperature (26±1.4 °C) prior to experimentation. For the first week, the acclimatized fishes were given artificial air by aerator.To keep the aquaria hygienic and free from mechanical disruptions, every effort was taken. All of the fish were given commercial food pellets on a regular basis, and the medium (tap water) was changed every two days to get rid of food remnants and faeces. Feeding was stopped 24hr prior to the toxicity test.

In the present study the sublethal concentrations of paper mill effluent were selected on the basis of the acute toxicity test. The 96 hours LC_{50} value of paper mill effluent was 8.223 (v/v) for *Mystus vittatus* (Jaiswal *et al*., 2024) [1]. The sub-lethal concentrations for chronic test were selected at i.e. 2.5% or 1/10 of 96h LC_{50} and 5.0 % 1/5th of 96h LC_{50}. In this experimental period test medium was changed daily.

Relative growth rate: The test fishes were exposed to two sub-lethal concentrations i.e. 2.5% or 1/10 of 96h LC_{50} and 5.0 % 1/5th of 96h $LC_{50}$of paper mill effluent; the observations were made at 10, 20 and 30 days exposure to note the number of fish survived in particular concentration. From this percentage of mortality in two sublethal concentrations were calculated at the end of the chronic exposure.

The weights of fishes were recorded by using electronic digital balance to the nearest 1.0 gram. Relative growth rate (RGR) of fish were calculated according to formula

$$RGR\ (\%) = \frac{\text{Final weight} - \text{initial weight}}{\text{Initial weight}} \times 100$$

Oxygen Consumption: Before beginning the experiment, the initial dissolved oxygen (DO) content of water in each glass jar was measured using a digital Water Quality Meter. A healthy fish was then introduced into each jar and allowed to respire for one hour. After this period, the DO content was again measured in the same manner. The amount of oxygen consumed by the fish was determined by calculating the difference between the initial and final DO values, with the glass jar serving as the respiratory chamber. The rate of oxygen consumption was expressed as milliliters of O_2 consumed per gram of fish body weight per hour (ml O_2/g/h).

Results and Discussion

In the present investigation, the test fish *Mystus vittatus* was exposed to sublethal concentrations of paper mill effluent ($1/10^{th}$ and $1/5^{th}$ of the 96h LC_{50}) for the duration of 30 days. The chronic effects of these concentrations were evaluated with respect to survival/mortality rate, relative growth rate, and oxygen consumption, and the findings are briefly summarized here.

In the present investigation, there was no mortality found i.e., 100% survival was observed in both the sublethal concentrations (Table-1). Similar 100% survival and 0% mortality were also observed in *Mystus vittatus* after chronic exposure period at two sublethal concentration of distillery effluent (Tiwari and Prakash, 2024) [18], in paper mill exposed fresh water fish *Puntitus stigma* (Sarwade, 2015) [16] and in pesticides exposed freshwater fishes, *Lepidocephalicthys termalis* (Kamble, 1983) [2].

In the present investigation, no mortality was recorded, and 100% survival was observed in *Mystus vittatus* at both sublethal concentrations of paper mill effluent (Table-1). Similar results of 100% survival and 0% mortality have been reported in *M. vittatus* exposed chronically to two sublethal concentrations of distillery effluent (Tiwari and Prakash, 2024) [18], in paper mill effluent exposed freshwater fish *Puntius stigma* (Sarwade, 2015) [16] , and in pesticide-exposed freshwater fish *Lepidocephalichthys thermalis* (Kamble, 1983) [2].

Table 1: Mortality of *Mystus vittatus* chronically exposed to sublethal concentrations of paper mill effluent

Exposed Period (Days)	Control	Distillery Effluent	
		0.8% v/v ($1/10^{th}$ of 96hLC$_{50}$)	1.7 % v/v ($1/5^{th}$of 96hLC$_{50}$)
0 day	0	0	0
10^{th} day	0	0	0
20^{th} day	0	0	0
30^{th} day	0	0	0
Total % mortality at the end of experiment	0%	0%	0%
Total % of survival at the end of experiment	100 %	100 %	100 %

To assess the chronic effects of paper mill effluent, the relative growth rate (RGR) of *Mystus vittatus* was evaluated based on body weight in both control and effluent-exposed groups of fishes. Changes in average body weight were recorded over different exposure periods. The results showed that in the control group, RGR increased progressively under normal conditions, whereas in effluent-exposed fishes, RGR declined compared to the control (Table 2).

According to Maruthi and Rao (2000) [4], increasing concentrations of industrial effluent reduce oxidative pathways and energy supply for various metabolic processes, ultimately resulting in reduced growth in effluent-exposed fishes.This reduction in growth in the present study may be attributed to the presence of toxic substances in paper mill effluent. Similar findings have been reported in effluent-exposed fishes such as *Mystus vittatus* (Tiwari and Prakash, 2024) [18], *Heteropneustes fossilis* (Prakash, 2023) [7], *Oreochromis mossambicus* (Varadaraj *et al*., 1997; Kaveri *et al*., 2018) [19, 3], *Puntius stigma* (Sarwade, 2015) [16], *Tilapia mossambica* (Noorjahan and Rohini, 2015) [6], and *Catla catla* (Ramesh and Nagarajan, 2013) [14]. The present investigation further demonstrated that the relative growth rate (RGR) of *M. vittatus* exposed to sublethal concentrations of paper mill effluent was dependent on both concentration and duration of exposure (Table 2). Specifically, RGR decreased as effluent concentration and exposure period increased. Higher RGR values were observed in fish exposed to 0.8% effluent compared to those exposed to 1.7%, likely due to the lower concentration of toxic substances in the diluted effluent.

Table 2: Relative GrowthRate of *Mystus vittatus* exposedto control and sub-lethal distillery effluent

Exposed Days	Control (Tap Water) Weight(g)	Paper mill Effluent/ Body weight	
		0.8% v/v ; Weight (g)	1.7% v/v ; Weight (g)
1st day	7.82±0.5	7.85 ±0.5	7.90 ±0.5
10th day	8.90± 0.7 (+13.81%)	8.70± 0.5 (+10.83%)	8.55± 0.6 (+8.23%)
20th day	9.55± 0.5 (+22.12%)	9.20± 0.6 (+17.20%)	8.80± 0.4 (+11.39%)
30th day	10.70± 0.4 (+36.83%)	9.75± 0.6 (+24.20%)	9.25± 0.6 (+17.09%)

Figure in the brackets indicate percentage of increase in Relative growth Rate of Fish

The rate of oxygen consumption in *Mystus vittatus* exposed to sublethal concentrations of paper mill effluent is presented in Table 3. A marked decline in oxygen uptake was observed at both concentrations, 0.8% v/v (≈1/10th of 96h LC_{50}) and 1.7% v/v (≈1/5th of 96h LC_{50}), over exposure periods of 10, 20, and 30 days.

The present investigation revealed a gradual decrease in oxygen consumption with increasing concentration and duration of exposure. The maximum reduction (−63.35% compared to control) was recorded at 1.7% v/v on the 30th day (Table-3). Almost similar results have been reported in *Cyprinus carpio* and *Mystus vittatus* exposed to distillery effluent (Prakash and Singh, 2000; Prakash and Verma, 2021; Tiwari and Prakash, 2024) [8, 13, 18]. Declines in oxygen consumption have also been documented in fishes exposed to pesticides and heavy metals (Sornaraj *et al*., 1995; Ray and Kumar, 2013; Prakash and Verma, 2019) [17, 15, 9].

Table 3: Changes in the Oxygen uptake of *Mystus vittatus* at different sublethal concentrations of distillery effluent (O_2 ml/g/hr)

Experimental group	Exposure Periods (Days)		
	10	20	30
Control (Tap water)	0.530 ± 0.55	0.525± 0.28	0.532 ± 0.45
0.8% v/v (1/10thof 96h LC_{50})	0.442 ± 0.45 (-16.60%%)	0.362 ± 0.50 (-31.05%)	0.311 ± 0.40 (-41.54%)
1.7 % v/v (1/5th of96h LC_{50})	0.306 ± 0.60 (-42.26%)	0.262 ± 0.32 (-50.10%)	0.195 ± 0.34 (-63.35%)

Figures in the brackets indicate %age decrease in the oxygen consumption or uptake

In the present investigation the decrease in oxygen consumption in distillery effluent exposed fish was due to degeneration of the gill epithelium that causes oxygen debt and also loses its effective mechanisms for "hypoxia anoxia" in which gill tissue not only suffers from oxygen debt but also loses its effective mechanism for removing carbon dioxide from blood. Anoxia or hypoxia increases carbohydrate consumption and thereby induces a sort of respiratory stress on organisms even at a sublethal level resulting in additional expenditure of energy (Prakash and Verma, 2020c) [12].

Conclusion

The findings of the present investigation suggest that the freshwater catfish *Mystus vittatus* can serve as a reliable bioindicator of water pollution. Industrial effluents exerted significant adverse effects on the survival, relative growth rate, and oxygen uptake of exposed fishes. These results highlight that monitoring growth and respiratory responses in fishes inhabiting polluted water bodies provides valuable insights into the physiological impacts of pollution. It can therefore be concluded that paper mill effluent poses a serious threat to non-target aquatic organisms such as freshwater fishes. Such investigations are also useful for comparing the sensitivity of different aquatic species, evaluating effluent toxicity through LC_{50} values, and determining safe concentration limits.

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