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Seasonal Variation in Actin-Beta 2 Gene Expression in *Catla catla* from Kolar Reservoir, Bhopal District, Madhya Pradesh

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ABSTRACT: This study examines seasonal variation in Actin-Beta 2 gene expression in *Catla catla* (catla fish) from Kolar Reservoir, Bhopal District, Madhya Pradesh. Understanding how gene expression fluctuates with the seasons is crucial for insights into fish growth, health, and aquaculture practices. A total of 30 specimens were collected during winter, summer, and monsoon to assess the impact of changing environmental conditions on gene expression levels. Quantitative PCR (qPCR) was employed to analyze gene expression, revealing significant differences across the seasons. Notably, *Catla catla* showed markedly higher Actin-Beta 2 expression during monsoon compared to winter ($P < 0.05$). This increase is likely due to favorable conditions like enhanced food availability and optimal water temperatures during the monsoon period. In contrast, no significant differences were found between winter and summer, suggesting that these seasons may impose similar physiological stresses. The findings highlight the critical role environmental factors play in influencing muscle development in *Catla catla*. These insights are essential for fisheries management and optimizing aquaculture practices. By understanding how seasonal changes affect gene expression, this research can guide selective breeding strategies focused on improving growth rates and resilience in catla fish. Overall, this study provides valuable information on the physiological adaptations of *Catla catla*, with implications for enhancing aquaculture sustainability and productivity in freshwater ecosystems. Future research should further explore the underlying molecular mechanisms associated with these seasonal changes and their effects on fish health and development.

Keywords: *Catla catla*, Actin-Beta 2 gene, seasonal variation, gene expression, Kolar Reservoir, aquaculture, freshwater ecosystems.

INTRODUCTION

Catla catla, commonly known as catla, is a significant freshwater fish species belonging to the family Cyprinidae (Sarala & Bhuvana 2024), widely recognized for its importance in aquaculture throughout South Asia, particularly in India. Known for its rapid growth rate and palatability, catla is a staple in many communities' diets and plays a vital role in the livelihoods of fishers and aquaculture practitioners. Its nutritional benefits and economic value make it a crucial component in local and regional markets. Characterized by its large head and elongated body, *Catla catla* thrives in diverse freshwater environments like rivers, lakes, and reservoirs. As an omnivorous fish, it primarily consumes plankton, detritus, and plant matter, playing a key role in the aquatic food web. Its ability to efficiently utilize available resources enhances its survival in various habitats, ensuring widespread distribution and cultivation.

Despite its ecological and economic significance, the genetic diversity of *Catla catla* requires further exploration, especially concerning genetic polymorphism in contractile proteins such as actin and myosin. These proteins are crucial for muscle development and function, directly influencing swimming performance, growth rates, and overall fish health (Shadwick *et al.*, 2013). Understanding the genetic variations in these proteins can shed light on *Catla catla*'s adaptability to environmental changes and various aquaculture practices, aiding in improved management strategies. Genetic polymorphism significantly affects the phenotypic traits of fish, including growth and disease resistance. Identifying variations in the contractile protein genes of *Catla catla* can reveal economically beneficial traits essential for selective breeding programs to enhance aquaculture productivity. Knowledge of genetic diversity also enables better responses to environmental stressors, ensuring this species' resilience across aquaculture settings.

As freshwater ecosystems face increasing threats from

overexploitation, pollution, and climate change, studying genetic diversity in key species like *Catla catla* is vital for promoting sustainable fishery practices and conservation efforts (Mohanty *et al.,* 2013). Investigating genetic polymorphism in contractile proteins provides valuable information for effectively managing catla populations, ensuring ecological balance and economic viability. This research focuses on analyzing the seasonal expression patterns of the Actin-beta 2 gene in *Catla catla* from the Kolar Reservoir. By understanding how seasonal variations affect muscle development, we aim to offer insights that will guide fisheries management and improve aquaculture efficiency throughout different seasons, ultimately supporting sustainable practices and enhancing productivity (Soranganba, 2022).

METHODOLOGY

Specimens of *Catla catla* were collected from Kolar Reservoir in Bhopal District, Madhya Pradesh. Muscle tissue samples were immediately frozen in liquid nitrogen and stored individually at −20 °C to preserve RNA integrity. RNA extraction was performed the same day to ensure high-quality results.

RNA Extraction and Reverse Transcription (RT). Tissue samples were homogenized in TRIZOL reagent (Invitrogen) following the protocol outlined by Meng *et al.* (2010) to isolate total RNA. The concentration and purity of the extracted RNA were assessed using the Qubit RNA HS Assay Kit (Invitrogen) and verified through spectrophotometry. RNA integrity was confirmed by evaluating the 18S and 28S ribosomal RNA (rRNA) ratios using 1% agarose gel electrophoresis. To eliminate any genomic DNA contamination, the total RNA was treated with RNase-free DNase I (Promega, USA). Subsequently, complementary DNA (cDNA) was synthesized from 1000 ng of total RNA using the iScript™ cDNA Synthesis Kit (Bio-Rad).

Quantitative PCR (qPCR) was conducted using the SYBR Green ExTaq II kit (TaKaRa) on the AriaMx Real-time PCR System (Agilent). Each reaction was performed in a final volume of 20 µL, consisting of SYBR Green Premix Ex Taq™, 0.4 µM of each forward and reverse primer, and 2.5 µL of the RT reaction solution. Each sample was analyzed in triplicate using the following cycling conditions: initial denaturation at 95 °C for 30 seconds, followed by 40 cycles of 95 °C for 5 seconds and 60 °C for 30 seconds. The Agilent Aria 1.6 software was used to analyze SYBR Green I fluorescence intensity and determine the quantification cycle (Cq) values. The ΔΔCT method was employed to convert Cq values into fold changes.

The following primers were designed using Primer3web version 4.1.0 for this study: Actin-Beta 2 Primers Forward: 5′-CACCTTCTACAACGAGCTGC-3′, Reverse: 5′-GACACCATCACCAGAGTCCA-3′. 18S rRNA Primers (Housekeeping gene, from Duan *et al.* (2016) Forward: 5′-TAGCGACGGGCGGTGTGT-3′. Reverse: 5′-TGATTGGGACTGGGGATTGAA-3′. This methodology outlines the comprehensive steps taken for RNA extraction, cDNA synthesis, and gene expression analysis using qRT-PCR, providing a robust framework for studying the seasonal variation in Actin-Beta 2 gene expression in *Catla catla*.

RESULTS AND DISCUSSION

This study looked at how the Actin-Beta 2 gene in *Catla catla*, or catla fish, changes with the seasons in Kolar Reservoir (Bowden *et al.,* 2007). Our results show that there are significant differences in gene expression depending on whether it's winter, summer, or monsoon. In our analysis, we found that seasonal differences are very important; the statistical test showed a significant difference (P = 0.0014) in the gene expression levels among the three seasons (Table 1, Fig. 1). The F-statistic of 12.04 suggests that a good portion (around 66.74%) of the variation in gene expression was influenced by the seasons. We also checked if the variance among the groups was similar using Bartlett's test (Bartlett & Kendall 1946) as given in Table 3. The result confirmed that the variances were not significantly different (P = 0.4237), which means we can trust our comparisons of gene expression (Table 2).Using Tukey's Multiple Comparison Test (Keselman & Rogan 1977), we found that the expression of the Actin-Beta 2 gene was significantly lower in winter compared to monsoon (mean difference of -0.3847, P < 0.05) (Table 4). This makes sense because the warmer conditions during the monsoon may help the fish grow better. The confidence interval we calculated supports this finding, showing a clear difference. Looking at winter and summer, we saw no significant difference (mean difference of -0.02861, P > 0.05). This suggests that the conditions in these two seasons might be more similar for the fish. However, when comparing monsoon and summer, the gene expression was again notably higher in the monsoon (mean difference of 0.3561, P < 0.05). The results indicate that conditions during the monsoon are very favorable for the fish (Sreekanth *et al.,* 2019). In *Catla catla*. The fish seem to thrive better in the monsoon due to more food availability and favorable temperatures, leading to increased growth rates. On the other hand, during winter, the colder temperatures likely slow down their growth and reduce gene expression.

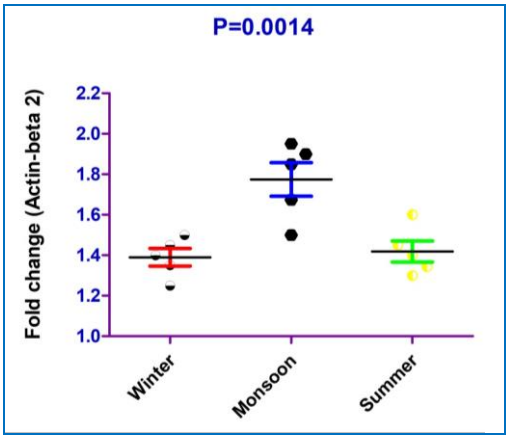

Fig. 1. The graph showing the One-way analysis of variance and Tukey's Multiple Comparison Test results on the mRNA of Actin-beta 2 gene muscle tissue of *Catla catla.*

Table 1: One-Way Analysis of Variance (ANOVA) Results.

Metric	Value
P Value	0.0014
P Value Summary	**
Are Means Significantly Different? (P < 0.05)	Yes
Number of Groups	3
F Statistic	12.04
R-Squared	0.6674

Table 2: ANOVA Table Summary.

Source	SS	df	MS
Treatment (Between Groups)	0.4593	2	0.2296
Residual (Within Groups)	0.2288	12	0.01907
Total	0.6881	14	

Table 3: Bartlett's Test for Equal Variances.

Metric	Value
Bartlett's Statistic (corrected)	1.718
P Value	0.4237
P Value Summary	ns
Do the Variances Differ Significantly? (P < 0.05)	No

Table 4: Tukey's Multiple Comparison Test Results.

Comparison	Mean Diff.	q	Significant? (P < 0.05)	Summary	95% CI of Difference
Winter vs Monsoon	-0.3847	6.229	Yes	**	-0.6177 to -0.1517
Winter vs Summer	-0.02861	0.4633	No	ns	-0.2616 to 0.2044
Monsoon vs Summer	0.3561	5.765	Yes	**	0.1230 to 0.5891

These findings highlight the important role of seasonal changes on the Actin-Beta 2 gene (Kocmarek *et al.*, 2014). Understanding these patterns is essential for effective fish farming and managing catla populations. The insights from this study can help breeders select strains that are better suited to various seasonal conditions, improving overall fish farming practices. For future research, it would be beneficial to dive deeper into the mechanisms behind these seasonal changes. Additionally, we should look at how these changes affect the fish's growth, nutritional value, and resilience to environmental challenges. This knowledge can help ensure the sustainability of *Catla catla* in various farming settings and support the livelihoods of those who depend on fishing and aquaculture

CONCLUSIONS

This study highlights the significant seasonal variation in Actin-Beta 2 gene expression in *Catla catla* from Kolar Reservoir, revealing how environmental factors influence muscle development. The findings underscore the importance of understanding these patterns for effective fisheries management and enhancing aquaculture practices. By identifying the impacts of different seasons on gene expression, we can optimize breeding strategies to improve growth rates and resilience in *Catla catla*. Future research is needed to explore the molecular mechanisms behind these changes and their implications for fish health and productivity.

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Conflict of Interest. None.

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