**Seasonal Variations of Actin –beta 2 gene expression in muscle tissue of *Labeo rohita* from the Kolar Reservoir, Bhopal District, Madhya Pradesh, India**

 **Abstract**

This study examines seasonal variations in the expression of the Actin-beta 2 gene in *Labeo rohita*, a vital aquaculture species inhabiting the Kolar Reservoir in Madhya Pradesh. Understanding how seasonal changes affect gene expression is crucial for optimizing aquaculture practices. We collected specimens during winter, monsoon, and summer periods corresponding to key environmental transitions by focusing on seasonal expression patterns, particularly of the Actin-beta 2 gene, the study links these genetic responses to environmental changes. The findings reveal significant upregulation of Actin- beta 2 during the monsoon, as shown by qRT-PCR amplification plots and ANOVA results. With about 57.21% of the variation in gene expression attributable to seasonal differences (P = 0.0219), these findings underscore the impact of environmental fluctuations. Tukey’s Multiple Comparison Test indicated significant expression differences between winter and monsoon, as well as between monsoon and summer, but not between winter and summer. This pattern suggests that monsoon conditions, such as increased water flow and nutrients, may boost metabolic activities and muscle development, while genetic or physiological mechanisms might stabilize gene expression across winter and summer. These insights into *Labeo rohita*'s adaptive physiology offer valuable implications for fisheries management and aquaculture practices, enabling adjustment to seasonal environmental changes to enhance growth and productivity in aquaculture settings.

Keywords: *Labeo rohita*, Actin-beta 2, Seasonal Variations, myogenesis

# Introduction

*Labeo rohita*, commonly known as rohu, is one of the most significant species in freshwater aquaculture across South Asia, valued for its adaptability to diverse environmental conditions and nutritional benefits. Understanding the genetic and physiological adaptations of this species to seasonal changes is crucial for optimizing aquaculture practices and ensuring sustainable fish production (Jhingran, 1991). Despite increased production, challenges like poor genetic management and disease necessitate research in selective breeding, biosecurity, and waste integration to ensure long-term sustainability and economic viability (Tynchenko et al., 2024). Muscle growth and development in fish are fundamentally driven by the expression of structural proteins, predominantly actin, and myosin, which play vital roles in muscle contraction and movement (Johnston, 2006). Seasonal variations can markedly influence the expression of these genes, corresponding with fluctuations in environmental parameters such as temperature, food availability, and breeding cycles. In temperate climates, fish are known to exhibit lower muscle gene expression during winter due to reduced metabolic activity, while increased expression is observed in warmer seasons, supporting more active metabolism and growth (Kinnby,2021).

The Kolar Reservoir in Bhopal District, Madhya Pradesh, provides an ideal setting to study these variations due to its distinct seasonal climate characterized by a cool winter, a wet monsoon, and a warm summer. Previous research highlights that fish populations in freshwater reservoirs often adjust their physiological responses to optimize survival and growth under varying seasonal conditions (Shuter *et al.,* 2012). However, specific data on the seasonal gene expression patterns of *Labeo rohita*, particularly in central Indian reservoirs, remains sparse.

This study aims to fill this gap by analyzing the seasonal expression patterns of Actin-beta 2 Gene in *Labeo rohita* from the Kolar Reservoir. By doing so, we hope to elucidate how seasonal shifts affect muscle development in this species, contributing insights that may guide fisheries management and improve aquaculture efficiency during different seasons.

# Materials and Method

Specimen Collection: *Labeo rohita* specimens were collected from Kolar Reservoir, located in Bhopal District, Madhya Pradesh. Muscle tissue samples were immediately frozen using liquid nitrogen and stored individually at −20 °C. RNA extraction from these tissues was conducted on the same day.

RNA Extraction and Reverse Transcription (RT): Tissue samples were homogenized in TRIZOL reagent (Invitrogen), and total RNA was isolated following the protocol described by Meng & Feldman (2010). The concentration and purity of the isolated RNA were evaluated using the Qubit RNA HS Assay Kit (Invitrogen) through spectrophotometry. RNA integrity was verified by assessing the 18S and 28S ribosomal RNA (rRNA) ratios using 1% agarose gel electrophoresis. To remove genomic DNA contamination, total RNA was treated with RNase-free DNase I (Promega, USA). Complementary DNA (cDNA) was synthesized from 1000 ng of total RNA using the iScript™ cDNA Synthesis Kit (Bio-Rad), as outlined in the study by Wang et al. (2011).

Quantitative PCR (qPCR): qPCR was conducted using the SYBR Green ExTaq II kit (TaKaRa) on the AriaMx Real-time PCR System (Agilent). Reactions were performed in a final volume of 20 μL, which included 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 employed to analyze SYBR Green I fluorescence intensity and determine the quantification cycle (Cq) values. The ΔΔCT method was used to convert Cq values into fold changes.

# Primers Used in the Study:

*Actin-beta 2 Primers* (primer designed in this study using Primer3web version 4.1.0): 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 process of RNA extraction, cDNA synthesis, and gene expression analysis using qRT-PCR, providing a detailed approach to studying gene polymorphism in fish species.

# Results and Discussion

**Gene Expression Analysis:** The amplification plot shown in Figure 1 highlights the differential expression of Actin-beta 2 and 18S rRNA. The Actin-beta 2 gene, indicated by blue peaks, and the 18S rRNA, acting as the housekeeping gene shown with green peaks, were successfully amplified, confirming the reliability of the qRT-PCR process and providing a foundation for subsequent analysis of gene expression variations across different seasons.

Seasonal Variation in Gene Expression: Figure 2 and Table 1 present the results of the one-way analysis of variance (ANOVA) and Tukey's Multiple Comparison Test concerning the expression of the Actin-beta 2 gene in the muscle tissue of *Labeo rohita* across different seasons. The ANOVA results indicate a significant difference in gene expression between the groups (P = 0.0219), with an F value of 6.016 and an R squared of 0.5721, suggesting that approximately 57.21% of the variation in gene expression is due to seasonal differences.

Figure 1:- The image showing the amplification plot of the studied genes Actin- beta 2 (blue peaks ) and 18S, Housekeeping gene (Green Peaks).

Figure 2:- 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 *Labeo rohita*.

Tukey's Multiple Comparison Test further elucidates these differences:

Winter vs. Monsoon: A significant decrease in Actin-beta 2 mRNA levels was observed during winter compared to the monsoon (Mean Difference = -0.3469, P < 0.05), suggesting that environmental conditions during the monsoon may upregulate Actin-beta 2 expression.

Winter vs. Summer: There was no significant difference in expression levels between winter and summer (Mean Difference = -0.02670, P > 0.05), indicating similar gene expression patterns during these two seasons.

Monsoon vs. Summer: A significant increase in Actin-beta 2 expression was found in the monsoon compared to summer (Mean Difference = 0.3202, P < 0.05), reinforcing the enhanced expression during the monsoon.

 Table 1: One-way analysis of variance and Tukey's Multiple Comparison Test test results on the mRNA of Actin-beta 2 genemuscle tissue of *Labeo rohita*.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| One-way analysis of |  |  |  |  |  |
| variance |  |  |  |  |  |  |
| P value | 0.0219 |  |  |  |  |  |
| P value summary | \* |  |  |  |  |  |
| Are means signif. | Yes |  |  |  |  |  |
| different? (P < 0.05) |  |  |  |  |  |
| Number of groups | 3 |  |  |  |  |  |
| F | 6.016 |  |  |  |  |  |
| R squared | 0.5721 |  |  |  |  |  |
|  |  |  |  |  |  |  |
| ANOVA Table | SS | df | MS |  |  |  |
| Treatment (between | 0.2981 | 2 | 0.1491 |  |  |  |
| columns) |  |  |  |  |  |  |
| Residual (within columns) | 0.223 | 9 | 0.02478 |  |  |  |
| Total | 0.5211 | 11 |  |  |  |  |
|  |  |  |  |  |  |  |
| Tukey's Multiple | Mean | q | Significant? P | Summ | 95% CI of diff |
| Comparison Test | Diff. |  | < 0.05? | ary |  |  |
| Winter vs Monsoon | - | 4.4 | Yes | \* | -0.6577 to - |
|  | 0.3469 | 8 |  |  | 0.03610 |  |
| Winter vs Summer | -0.0267 | 0.33 | No | ns | -0.3375 to |
|  | 0 | 92 |  |  | 0.2841 |  |
| Monsoon vs Summer | 0.3202 | 4.06 | Yes | \* | 0.009399 to |
|  |  | 8 |  |  | 0.631 |  |

The variation in Actin-beta 2 expression across different seasons could be attributed to environmental factors such as temperature, water availability, and nutrient levels, which are known to influence gene expression in fish ( The monsoon season, characterized by abundant rainfall and increased water levels, may create favorable conditions for increased metabolic activity and muscle development, thereby upregulating genes like Actin-beta 2. The lack of significant differences between winter and summer suggests that other factors, potentially genetic or species-specific regulatory mechanisms, may play a role in stabilizing Actin-beta 2 expression across these seasons (Storz & Wheat, 2010). Additionally, the use of 18S rRNA as a stable housekeeping gene supports the robustness of the data, ensuring that observed variations are indeed due to changes in Actin-beta 2 expression rather than experimental error (Suzuki *et al.,* 2000).

The study highlights the importance of environmental factors on gene expression in *Labeo rohita*, illustrating how temperature, nutrient availability, and hydrological changes influence muscle gene regulation. This underscores the value of further investigating the molecular mechanisms underlying these seasonal variations to better understand adaptive responses in aquatic organisms. Such insights provide a basis for understanding how climate and water quality changes could potentially impact muscle development in aquatic species, offering valuable implications for fisheries management and conservation strategies (Storz & Wheat, 2010; Harley *et al.,* 2006).

**Conclusion**

This study underscores the impact of seasonal variations on Actin- beta 2 gene expression in *Labeo rohita*, with significant upregulation during the monsoon. The findings suggest that environmental factors like increased water levels and nutrients during the monsoon foster enhanced muscle activity in fish. Understanding these gene expression dynamics informs strategies for fisheries management and conservation amidst climate changes.

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