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Effect of Temperature on Growth and Proximate Composition of *Labeo rohita* (L.)

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Temperature has a major impact on aquatic organisms including fishes. It affects the vital physiological characteristics and metabolic functions. In this study, the results demonstrated that the fishes reared in outdoor culture system has a higher growth performance and biochemical synthesis over the fishes reared in indoor culture system and this may be attributed to the fluctuation in day to day atmospheric temperature; which inturn influenced the vital physiological and biosynthetic process of cultured fishes.

Keywords: Temperature; Labeo rohita; growth; proximate composition.

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1. INTRODUCTION

In the recent past, aquaculture sector has emerged an emerged as a key player in the food production landscape, providing a significant portion of the animal protein needed by all people, globally, with its growth rate of more than 7% [1]. This sector has been producing, 8.1 million tons (46%) out of the 179 million tons of fish produced globally. Also, it is anticipated that by 2030, aquaculture output would account for 53% of global fish production. surpassing 46% of earlier production [2]. According to [3 and 4] rising temperatures have the potential to accelerate the emergence of exotic diseases and increase the susceptibility of domesticated animals to heat-related ailments. The combined effects of these three interrelated factors may lead to variations in the frequency and intensity of the condition, as well as its growth and decline over time [5].

Temperature, precipitation, photoperiod duration, and other climatic factors are among the many that physically affect water bodies. Optimal fish production requires an understanding of the local environmental factors and how management tactics may be combined with them to enhance fish yield.

Furthermore, one of the primary environmental factors influencing fish development and metabolism is temperature. Aquaculture is concerned about the rising water temperatures brought on by global warming. Fish populations may decrease as a result of disrupted physiological processes by high temperatures and certain species may even become extinct [6]. Temperature of the aquatic environment has been found to affected to the fish survival, distribution reproductive and metabolism [7]. It feeds on organic stuff that has decomposed and vegetable waste, making it a bottom feeder. Physiological research can be used to anticipate how temperature changes would affect different fish species [8]. As fish are aquatic poikilothermic animals, increased water temperatures generate stress and change blood chemistry standards, which impact almost all biochemical and physiological activities. High temperatures, above the threshold of tolerance, accelerate the chemical reactions in fish bodies and have a significant impact on physiological processes [9]. High-quality protein and other organic compounds may be found in abundance in fish. They are the primary source of animal protein and are generally regarded as a nutritious food item that also contains other nutrients [10].

Due to its low cholesterol, great palatability, and soft meat, a significant portion of the population consumes fish, which is high in protein. It is the most affordable way to obtain animal protein and other vital elements that are needed in a human diet, especially for those with poor and moderate incomes. The sort of food an animal eats has a significant impact on the type and quality of nutrients it contains. Furthermore, the nutritional makeup of a fish's flesh is significantly influenced by its eating habits. Fish flesh contains all of the necessary amino acids for diet and around 85–90% of the digestible protein. A food's proximate composition, such as its protein and fat level, must frequently be measured to make sure it complies with regulations. Fish nutritional value has been the primary focus of biochemical component analysis in India. Due to its comparatively high digestion, fish protein is regarded to have a high biological and growth-promoting effect [11]. It also contains all 10 necessary amino acids in optimal quantities for human intake [12]. Understanding the biochemical makeup of various species, including fish, is very beneficial for determining their nutritional worth. It also aids in assessing the quality of these natural resources and maximizing their use [13]. Fisheries biochemical studies are useful in assessing environmental effects. Due to their specificity about the dietary values of fish and their ability to assess the physiological requirements of fish at various stages of life, biochemical investigations of fish tissues are of great interest. Species-specific responses to heat acclimation in tropical freshwater fishes, however, are comparatively little understood, particularly with regard to stress responses and the use and mobilization of energy stores. This viewpoint led to the current study's attempt to ascertain the effects of day to day atmospheric temperature fluctuation (Atmospheric temperature) on growth and biochemical variables, such as protein, carbohydrate and lipid in the muscle and gut tissues of freshwater Indian major carp *Labeo rohita.*

2. MATERIALS AND METHODS

2.1 Experimental Fish

Fresh water fish *Labeo rohita* weighing 5.15 ± 0.04g were collected from the Suriya Fish Farm, Kallidaikuruchi, Tirunelveli. The fishes were thereafter brought to the lab in polythene bags with low-temperature, oxygenated water with least disturbance. Then they were acclimatized

to the ambient laboratory room temperature range (27 ± 1.0°C) in 500*l* FRP tanks. During the period of acclimatization, which was lasted for two weeks, fed with lab prepared pellet diets and 30% daily water exchange was made to maintain the optimal water quality.

2.2 Experimental Setup

To assess the proposed hypothesis "Whether the fluctuation in day to day atmospheric temperature affects the growth and biochemical synthesis in fish *L. rohita*. Experiments were carried out in 500*l* FRP tanks in triplicate. The experimental fishes were kept in FRP tanks, each of which had aeration systems to keep the water quality at optimal level. The fishes were maintained at the rate of 10 nos./tank and the experiment was conducted for 30 days. During experiment, fluctuation in water quality parameters (Temperature, pH and Ammonia) were monitored and recorded following the method described in APHA [14]. The water exchange level was done at the rate of 30% per day. At the end and initial time of experiment, the growth and tissue samples were collected for biochemical analysis.

2.3 Experimental Diet and Feeding

The experimental pellet diet were prepared following the standard method. The conventional feed ingredients such as fish meal, groundnut oil cake, soya meal, rice bran, wheat bran, fish oil, vitamin and mineral mix were used in the diet. The proximate composition of the experimental feed was recorded as protein (37.85%), carbohydrate (17.84%), fat (10.62%) and moisture content (8.45%). The experimental feed was offer to the fishes twice daily to satiation (Morning 6.00 am and Evening 4.00 pm). The unfed remains were collected daily and measured.

2.4 Growth Parameter

The growth response such as weight gain (growth), feed intake, specific growth rate (SGR) and feed conversion ratio were calculated as per the formula given below:

Growth = Final weight (g) – Initial weight (g)

Percentage weight gain = Final weight (g) - Initial weight (g) / Initial weight × 100

Specific growth rate = Ln. final weight – Ln. initial weight / duration of (Experiment (days) × 100)

Feed conversion ratio (FCR) = Feed given (g dry weight)/Weight gain (g wet weight)

Survival (%) = Number of fish harvested/number of fish stocked × 100

2.5 Estimation of Total Carbohydrate

Carbohydrate in the experimental samples was estimated by Trevelyan and Harrison method. [15]. The freshly prepared anthrone reagent (5 ml**)** was pippeted into thick walled pyrex tubes (150 x 25 mm) and chilled in ice water. The solution under test (1 ml) was layered on the acid, cooled for a further 5 min, and then thoroughly mixed while still immersed in ice water. The tubes were fitted loosely with corks, heated to the appropriate temperature in a violently boiling, level water bath, then cooled in the water for five minutes. Then it was made up to 10 ml with water and optical density was determined in a spectrophotometer. The standard graph was plotted with D-glucose by using the above said method. The values were expressed as mg/g wet weight of the tissue.

2.6 Estimation of Total Protein

The total Protein content of the experimental fish tissue samples was estimated according to modified standard method of [16]. The Quantity of 5%homogenate of, muscle, and intestine tissues were isolated and precipitated with 5% trichloro acetic acid (TCA) and centrifuged at 3000 rpm for 15 minutes. The precipitate was dissolved in 1 milliliter of 1 N NaOH solution, and 0.2 milliliter of the extract was transferred into a test tube and combined with 5 milliliters of an alkaline copper solution (a 50:1 combination of 2% sodium carbonate and 0.5% copper sulphate). Then samples were allowed to stand for 10 min, at the end of which 0.5 ml folin phenol reagent (diluted with double distilled water in 1:1 ratio before use) was added. After 30 minutes, the optical density was measured at 540 nm in am spectrophotometer (Elico Model SL207) against a blank. The standard graph was plotted using bovine serum albumin (BSA) as standard. The values were expressed as mg/g wet weight of the tissue.

2.7 Estimation of Total Lipids

Lipids content in the tested tissue samples was estimated according to the method of [17]. 50 mg of test tissues were was homogenized with 10 ml water in a warring blender in chloroform: methanol mixture (2:1). The homogenates were filtered through Whatmann No. 1 filter paper and the residue was re-homogenized as before and then filtered. The non-lipid matter from pooled filtrate was removed by shaking vigorously with 0.88% KCl (added as one fourth of the volume). To a test tube containing 1 ml of filtrate, nitrogen was used to evaporate it. Then, 1 ml of concentrated H_2SO_4 was added, and the mixture was heated for 10 minutes. For estimation of total lipid, 0.2 ml of solution was taken and 2 ml of vanillin reagent was added. The developed color was read in spectrophotometer at 520 nm against reagent blank. The standard graph was plotted by the above method with cholesterol powder. The values were expressed as mg/g wet weight of the tissue.

3. RESULTS AND DISCUSSION

3.1 Water Parameter

Average water quality parameter register in the culture systems are given in Table 1. The data indicated the existence of variation between culture systems expect pH showed (7.16±0.05 7.33±0.5). Temperature was high (34.35 to 97°C) in atmospheric culture system and it was low in Controlled culture system (28.70 to32°C). Dissolved Oxygen shows very little variation (4.37 ± 0.16 to 5.23± 0.16 mg/L). Total alkalinity and ammonia content were low in Controlled culture system and it was high in atmospheric culture .It was reporter that a temperature range of 28 to 32°C is favorable for fish development (It was reporter that a temperature range of 28 to 32°C is favorable for fish development [18].

The temperature recorded in the present experiment falls within the optimal range of temperature that support the growth response and other vital physiological activities of the cultivable fishes [19]. Also the pH value recorded was at neutral level (7.16 to 7.33) which is conducive for cultivable organisms. In the aquatic environment ammonia irons play a major rule and it will adversely affect the fishes when it exceeds the optimal level. In the present study the ammonia level was high in atmospheric culture system (0.32±00 mg/L) and this raise may be attributed to the raise in temperature and related metabolic activities. However, the range of ammonia ions registered in this study fall within the safe level [20] reported that raise in temperature led to raise in pH and fall in dissolved oxygen in culture system. In this study, the pH and dissolved oxygen recorded was not deviated much and it may be due to the low range of variation in temperature between culture systems. Here, the total alkalinity in atmospheric culture system was high and it was low in Controlled culture system and this may be due to the variation in temperature between culture systems. Also this minimal variation is enough to bring out the change in alkalinity [21,22].

3.2 Growth Performance

Growth response of *L. rohita* showed culture systems dependent variation and the results recorded are given in Table 2. The weight gain (%) and FCR values were high in atmospheric culture system and it was 99% high compared to Controlled culture system. Accordingly the SGR of *L. rohita* cultured in atmospheric culture system was high (1.14 ± 0.02% per day) against the low value (0.63±0.03%/day) registered in Controlled culture. The present study result find support from the earlier report [20,23], who have also reported the temperature depend growth response in the experiment fish species. The high weight gain, FCR and SGR displayed by *L. rohita* in the present experiment is due to the variation in temperature between culture systems employed. Obviously the temperature recorded in the atmospheric culture system was

Table 1. Average water quality parameters (Mean ± SD) recorded in the indoor and outdoor active systems during the experimental period.

Water quality parameter	Indoor culture system	Outdoor culture system
Temperature	28.70±0.32	34.35±0.97
pH	7.33±0.05	7.16±0.05
Dissolved oxygen (mg/L)	4.37±0.16	5.23±0.16
Total Alkalinity (mg/L)	120.33±2.08	131.66±1.56
Ammonia (mg/L)	0.28±0.004	0.32±0.006

Table 2. Growth responses of *L.rohita* reared in indoor and outdoor culture systems during experimentation

Growth parameter	Indoor temperature	Outdoor temperature
Initial BW (g)	5.18±0.07	5.24±0.01
Final BW (g)	6.20±0.07	7.24±0.02
Weight gain (g)	1.01±0.05	1.99±0.02
% weight gain	19.50±1.19	37.98±0.61
Feed intake dry weight (g)	1.46±0.24	2.80±0.40
SGR (% / day)	0.63±0.03	1.14±0.02
FCR	1.45±0.02	1.40±0.01
Survival (%)	90.0± 00	90.0± 00

Table 3. Changes in proximate composition in the muscle and intestine tissues of *L. rohita* cultured in indoor and outdoor culture system

Biochemical constituents	Experimental tissues	Indoor culture system	Outdoor culture system
Total Carbohydrate (mg/g wet tissue)	Muscle	12.585±0.079	16.194±0.081
	Intestine	14.933±0.094	18.504±0.081
Protein (mg /g wet tissue)	Muscle	78.538±0.200	95.899±0.174
	Intestine	38.441±0.178	44.212±0.151
Total Lipids (mg /g wet tissue)	Muscle	32.478±0.030	34.218±0.223
	Intestine	24.38±0.192	30.173±0.016

Note: Each value is the mean±SD of three individual estimates.

high (34.35±97%) which favored the growth at this optimal level. However, in the present study, the survival *L.rohita* did not show much variation and this may be due to the fall of temperature variation within the optimal range of 28°C to 35°C.

3.3 Proximate Composition

The changes in biochemical constituents in muscle and intestine of *L. rohita* reared Controlled and atmospheric culture system are provide in Table 3. The tested parameters such as total protein, carbohydrate and lipid contents showed variation between the fishes reared in Controlled and atmospheric culture systems and it hold good for both the tissues tested. In muscle tissue of *L. rohita* culture in atmospheric culture system the protein, carbohydrate and lipid content register were 95.899±0.174, 16.194±0.081 and 34.218±0.223 mg/g wet weight and it was high when compared with Controlled culture fishes. More or less a similar variation was noticed in the intestine tissues of *L. rohita* reared in Controlled and atmospheric culture systems. This result indicated that the variation in water temperature between the adapted culture systems plays a significant role in synthesis of macro molecules in the experimental animal. Also the functional biological compounds including total protein, carbohydrate and lipid varies with variation in both abiotic and biotic factors [24,25]. Similar results were reported by peer researchers regarding biochemical parameters [26,27,28] .The changes in biochemical constituents such as glycogen, carbohydrates, proteins, free amino acids and lipids are important to indicate the susceptibility of organ systems to adverse conditions and toxicants by changing their function [29]. The present outcomes also revealed that the fish muscle contained richer amounts of protein than the intestine which corroborate with the results obtained by previous researchers [30,31] The lipid was deposited more in the liver, while muscle had a comparatively lower lipid level [30]. A similar result was founded in African catfish [31]. Hence, continuous monitoring of changes in the biochemical composition of different species is required [32,33].

4. CONCLUSION

In this study, *L. rohita* were comparatively reared in indoor and atmospheric culture systems with uniform density. The main variable differentiate these two culture system was variation in culture temperature in response with fluctuation in day to day atmospheric temperature. In these adopted culture system the temperature showed much variation and which in turn altered both the

growth responses and bimolecule synthesis in the experimental fish. The better growth responses and tissue biochemical synthesis recorded in *L. rohita* cultured in atmospheric culture system may be attributed to the variation in culture temperature within the optimal range.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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