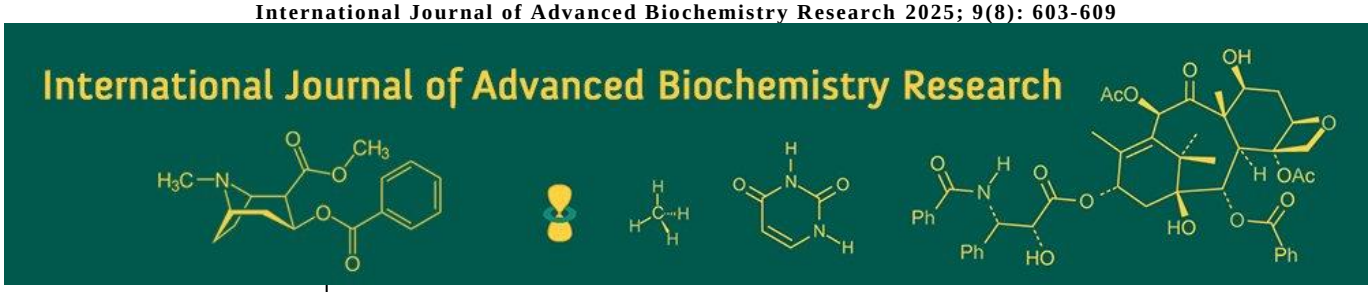

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First report on the impact of parasitic infection on the nutritional quality of banded gourami fish

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Abstract
This study explores the effects of parasitic infection on the nutritional quality of Banded Gourami fish (*Trichogaster fasciata*), focusing on changes in proximate composition, amino acid profiles, and fatty acid composition between non-infected and infected fish. Fish samples were collected from three wetlands in the Gandak River basin, India, and investigated for parasitic infections and analysed for morphometric characteristics, proximate composition (moisture, crude protein, crude fat, ash) using AOAC methods, amino acid profiles via HPLC, and fatty acid profiles using GC. The investigation revealed significant alterations in the nutritional profile of the infected fish. In particular, infected Gourami showed a notable decrease in moisture content, measuring 74.74% compared to 76.18% in non-infected fish. Conversely, there were substantial increases in crude fat (4.09% versus 3.14%), crude protein (15.45% against 14.80%), and ash content (6.32% compared to 5.69%). Amino acid profiling further highlighted a complex metabolic response where concentrations of glycine, histidine, and serine decreased, while levels of aspartic acid, glutamic acid, proline, lysine, and valine increased. Additionally, the study found distinct changes in the fatty acid profiles of infected fish. Notable alterations occurred among saturated, monounsaturated, and polyunsaturated fatty acids. Essential polyunsaturated fatty acids (PUFAs) such as linoleic acid (C18:2), alpha-linolenic acid (C18:3), arachidonic acid (C20:4), and docosahexaenoic acid (C22:6) were significantly more concentrated in infected samples. The observed metabolic changes necessitate further research on different fish species.

Keywords: Banded gourami, proximate composition, amino acid profile, and fatty acid composition

Introduction
Fish is an essential commodity for food security, particularly for vulnerable populations in developing countries (Pradeepkiran, 2019) [25]. Generally, it is considered a source of low-fat and high-quality protein, important micronutrients like vitamins A and D, phosphorus, magnesium, selenium, iodine, and omega-3 fatty acids, complementing primarily cereal-based diets (Garcia & Rosenberg, 2010; Mahanty *et al.*, 2014; De *et al.*, 2019) [22, 16, 19].
The Banded Gourami is a freshwater fish known for its vibrant colours and distinctive banded patterns, making it a popular choice in the aquarium trade (Mahanty *et al.*, 2014) [16]. Beyond its ornamental value, the Banded Gourami, scientifically referred to as *Trichogaster fasciata*, plays a crucial role in the aquatic ecosystems it inhabits, contributing to food web dynamics and overall biodiversity (Pérez *et al.*, 2020) [7]. Efficient digestion and absorption of nutrients are essential for the survival and reproduction of this species (Seth *et al.*, 2010) [9]. The nutritional composition of fish is vital not only for their growth, reproduction, and health but also influences their quality as a food source for both humans and other animals (Syandri *et al.*, 2023) [10].
A decline in nutritional quality can trigger ripple effects throughout the ecosystem, impacting both predator and prey populations (IRIANSYAH *et al.*, 2021) [15]. Thus, maintaining optimal nutritional levels in fish populations is crucial for ecological balance and economic stability. Several biotic and abiotic factors can significantly compromise fish health, with parasitic infections emerging as a particularly important concern (Gasparotto *et al.*, 2020) [3]. Parasitic infections can induce physiological stress, impair nutrient absorption, and disrupt metabolic processes, potentially leading to significant changes in the nutritional composition of fish (Song *et al.*, 2014) [17].

These changes can manifest as decreased protein content, altered fatty acid profiles, and reduced levels of essential micronutrients, which are vital for both the fish themselves and the consumers that depend on them.

Despite the acknowledged importance of fish nutritional quality and the potential impact of parasitic infections, there remains a gap in our understanding of how specific parasites and aquaculture systems affect the nutritional profiles of many fish species (Ramos *et al.*, 2022) [8]. Investigating these interactions is essential for promoting sustainable aquaculture practices and safeguarding both fish health and human nutrition (Turlybek *et al.*, 2025) [18]. Such research could provide valuable insights into the intricate relationship between parasitic infections and fish nutritional quality, thereby informing strategies to mitigate adverse effects and optimize the nutritional value of fish for both ecological and economic benefits.

Considering the importance of fish in human diets and aquatic ecosystems, this study aims to investigate how parasitic infections alter the nutritional composition of *Trichogaster fasciata.* Addressing this knowledge gap is crucial for developing effective strategies to minimize the adverse impact of parasites on fish nutritional quality and for optimizing aquaculture practices to ensure the production of nutritionally rich fish. This is particularly relevant in the context of sustainable aquaculture, where ensuring optimal fish health and nutritional value is paramount.

Materials and Methods

Study area and fish sample

The study was conducted in three different wetlands of the Gandak River basin, India. These are Kararia Lake, Sirsa Lake, and Majharia Lake. The selected sites were site 1 (S1) at (26.643766° N, 84.938853° E), Site 2 (S2) at (26.644592° N, 84.939101° E), and Site 3 (S3) at (26.602251° N, 85.000348° E). They differ in size and shape, with water spread areas ranging from 65 to 100 hectares, and depth varying from 2.2 to 8.5 meters. All three lakes are heavily infested with aquatic weeds, but support a rich diversity of fish species, especially SIFS. Kararia Lake is located in the periphery of an urban area and therefore receives municipal discharge. From these wetlands, live fish samples were collected from selected sampling sites in each wetland and brought to the laboratory, where they were maintained in a glass aquarium.

Parasitological examination

In the laboratory, we meticulously measured the morphometric characteristics of each fish, including total length and body weight, with precision, using a digital calliper to the nearest 1 mm and an electronic scale to 0.01 g. Following these detailed assessments, we conducted a comprehensive examination to classify each fish as either infected or non-infected. Both categories were securely stored in airtight zip-lock bags and preserved at a temperature of-40±1 °C. The isolated parasites were identified by following the morphological description of (Caffara *et al.*, 2011).

Analysis of nutritional profile

The proximate composition, amino acid profile, and fatty acid content were analysed using standard methods (Syandri *et al.*, 2023) [10].

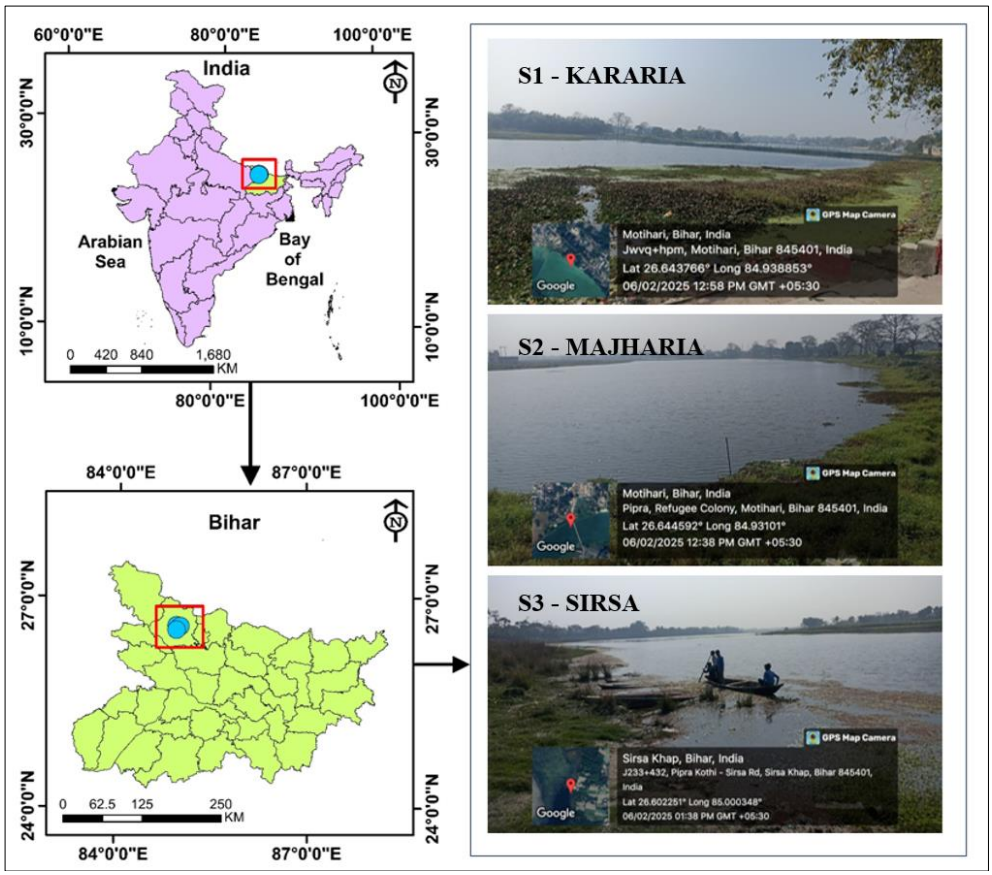

Fig 1: (A) Geo-location of the study area, (A) Site 1 = 26.643766° N, 84.938853° E, (B) Site 2 = 26.644592° N, 84.939101° E & (C) Site 3 = 26.602251° N, 85.000348° E

Proximate composition

Proximate composition analysis was conducted to determine the nutritional content of the fish flesh, focusing on moisture, crude protein, crude fat, and ash content (De *et al.*, 2019) [19]. The analysis followed standard Association of Official Analytical Chemists (AOAC) methods to ensure accuracy and reliability (Syandri *et al.*, 2023) [10]. Fish tissues were pooled for each analysis of both un-infected and infected fish. Moisture content was determined by drying the fish samples in an oven at 105°C until a constant weight was achieved (Mahanty *et al.*, 2014) [16]. The difference in weight before and after drying was used to calculate the moisture percentage (Ullah *et al.*, 2022) [27]. Crude protein content was estimated using the Kjeldahl method, which involved digesting the sample with sulfuric acid, neutralising with a base, and distilling the ammonia released into a boric acid solution, followed by titration with a standard acid (Mahanty *et al.*, 2014) [16]. Crude fat was extracted using the Soxhlet method with ether extraction, and the ash content was determined by incinerating the samples in a muffle furnace at 550°C for 16 hours (Syandri *et al.*, 2023) [10]. Ash content of uninfected and infected fish was determined by incinerating a known weight of dried sample at high temperature (600 °C for 6 h) in a muffle furnace.

Amino Acid Profile

Amino acid profiling was an essential method we used to measure the concentrations of individual amino acids in fish flesh samples. Before collecting samples, the fish fasted for 24 hours to ensure their metabolic state was consistent (Yu *et al.*, 2024) [29] in the laboratory. Once collected, we homogenised the pooled fish tissue and then hydrolysed the samples using 6N hydrochloric acid for 24 hours at 110°C. Next, we analysed the amino acid composition using a High-Performance Liquid Chromatography (HPLC) system, following AOAC guidelines (De *et al.*, 2019) [19]. We carefully selected and optimised the mobile phase to achieve the best separation and detection of amino acids (Mahanty *et al.*, 2014) [16]. To quantify the amino acids, we analysed the chromatograms, comparing retention times and peak areas to known amino acid standards to ensure the accuracy of our data. Finally, we compared the amino acid profiles of fish with parasites to those without. This comparison helped us understand how parasitic infections affected the nutritional value of the fish. Our thorough approach allowed us to determine whether parasitic infections significantly altered the fish's amino acid composition. In related experiments, we randomly assigned diets, ensuring each group received a control diet that met established amino acid requirements.

Fatty Acid Profile

The fatty acid profiles of both infected and non-infected Banded Gourami were compared to identify differences in lipid composition, which revealed how the infection affected fatty acid metabolism and storage (Stanley-Samuelson & Dadd, 1983; Vingering *et al.*, 2010) [34, 35]. Muscle tissue from each fish was pooled and snap-frozen at-40°C. Total lipids were extracted using a modified Folch method, which involved a chloroform: methanol: water solution, followed by centrifugation to collect the chloroform phase and nitrogen evaporation. To prepare fatty acid methyl esters (FAMEs), approximately 50 mg of the extracted lipids underwent transmethylation using 2M KOH in methanol and boron trifluoride in methanol at 80 °C. This was followed by n-hexane extraction, drying over anhydrous sodium sulfate, and filtration into gas chromatography (GC) vials. The FAMEs were analysed using a gas chromatograph equipped with a Flame Ionisation Detector and a capillary column, with specific oven temperature programming. The injector was set at 250 °C, the detector at 260 °C, and nitrogen was used as the carrier gas at a flow rate of 1.0 mL/min for 1 µL injections in split mode (1:50).

Individual fatty acids were identified by comparing their retention times with commercial FAME standards. They were quantified as a percentage of total fatty acids and categorized into saturated, monounsaturated, and polyunsaturated fatty acids.

Results and Discussion

Proximate composition analysis

The proximate composition of non-infected and infected gourami fish samples is summarised in Table 1. The table illustrates notable differences in moisture and proximate composition between the "Non-infected" and "Infected" samples. The "Non-infected" sample has a higher moisture content, recorded at 76.18%, while the "Infected" sample shows a lower moisture level of 74.74%. In contrast, the "Infected" sample presents higher values for key components: 15.45% crude protein, 4.09% crude fat, and 6.32% ash, compared to the "Non-infected" sample, which contains 14.80% crude protein, 3.14% crude fat, and 5.69% ash.

These differences in proximate composition highlight the significant effects that infection can have on the chemical makeup of the samples. The lower moisture content in the "Infected" sample indicates a more concentrated presence of dry matter. This increase in crude protein may suggest an intensified immune response, characterised by enhanced synthesis of protective proteins or increased activity related to cellular repair. The elevated levels of crude fat could point to alterations in lipid metabolism or changes in the composition of cellular membranes due to the infection. Additionally, the higher ash content reflects an increase in mineral concentration, which may be linked to immune activity or the presence of minerals associated with the infectious agent.

In comparison, the "Non-infected" sample serves as a reference point, representing a healthy biological state. These compositional changes signal the metabolic and structural modifications that occur during an infection. To gain a deeper understanding of the underlying mechanisms driving these observed variations, further specific analyses, such as histopathology, pathogen identification, and detailed biochemical profiling, are essential.

Table 1: (Proximate Composition Analysis of Non-Infected and Infected Gourami Fish Samples)

Sample No.	Moisture%	Crude protein%	Crude fat%	Ash%
Infected	74.74	15.45	4.09	6.32
Non-inf.	76.18	14.8	3.14	5.69

The present findings strongly affirm previous research demonstrating that infection status has a profound impact on the proximate composition of fish. For instance, it has been well-documented that parasitic infections lead to significant reductions in lipid content and alterations in protein levels within fish tissues. But the significant differences in the

content of moisture, protein and ash were not observed between the non-infected and infected fish. These changes can be attributed to the host's metabolic response to the parasite as well as the parasite's direct use of the host's nutrients. Such dynamics illustrate the complex interplay between host-parasite interactions, making it essential to consider nutritional composition when assessing fish health. These variations in proximate composition highlight the critical relationship between fish health and nutritional value, making it vital for both aquaculture and human consumption.

Amino Acid Analysis

The amino acid composition of both non-infected and infected samples was analysed, revealing a total of 17 amino acids quantified in each type. The results, outlined in Table 2, indicate significant variations in amino acid concentrations following infection. In the infected samples, notable increases were observed in the levels of aspartic acid (ASP), proline (PRO), and lysine (LYS). Specifically, the concentration of aspartic acid rose from 0.581 g/100 g in non-infected samples to 0.624 g/100 g in the infected counterparts. Proline levels increased from 0.036 g/100 g to 0.042 g/100 g, while lysine concentrations went from 0.266 g/100 g to 0.285 g/100 g. Additionally, glutamic acid (GLU) and valine (VAL) also exhibited slight increases in infected samples. The findings of the present study corroborate with previous studies.

Conversely, several amino acids demonstrated decreased concentrations in the infected samples. Tyrosine (TYR) showed a significant reduction, dropping from 0.009 g/100 g in non-infected samples to 0.007 g/100 g in infected ones. Other amino acids, including histidine (HIS), serine (SER), arginine (ARG), threonine (THR), leucine (LEU), and phenylalanine (PHE), also experienced slight declines. Glycine (GLY) saw a minor decrease from 0.326 g/100 g to 0.296 g/100 g. Amino acids such as alanine (ALA), cysteine (CYS), and methionine (MET) remained relatively unchanged between the two sample types, maintaining concentrations of 0.021 g/100 g, 0.014 g/100 g, and 0.002 g/100 g, respectively. A similar finding was also reported by Andersen *et al.* (2016). These alterations in amino acid profiles provide critical insights into the metabolic responses triggered by infection.

Table 2: (Amino Acid Analysis of Non-Infected and Infected Gourami Fish Samples)

Amino Acids	Non Infected (g/100 g of sample)	Infected (g/100 g of sample)
HIS	0.049	0.045
SER	0.12	0.119
ARG	0.062	0.06
GLY	0.326	0.296
ASP	0.581	0.624
GLU	0.725	0.736
THR	0.121	0.12
ALA	0.021	0.021
PRO	0.036	0.042
CYS	0.014	0.014
LYS	0.266	0.285
TYR	0.009	0.007
MET	0.002	0.002
VAL	0.065	0.066
ILE	0.055	0.054
LEU	0.095	0.093
PHE	0.022	0.021

Fatty Acid Analysis

The comprehensive analysis of fatty acid profiles uncovered pronounced and distinct differences between the non-infected and infected fish samples. In the category of saturated fatty acids, critical components such as lauric acid (C12:0), tridecanoic acid (C13:0), myristic acid (C14:0), and palmitic acid (C16:0) were observed reduced within infected samples. However, the saturated fatty acids pentadecanoic acid (C15:0), heptadecanoic acid (C17:0), stearic acid (C18:0), arachidic acid (C20:0), and behenic acid (C21:0) exhibited elevated levels in the infected samples, highlighting a potential shift in lipid metabolism, possibly linked to infection.

In addition to the changes noted in saturated fatty acids, the analysis of monounsaturated fatty acids revealed compelling differences. Notably, both cis-9-tetradecenoic acid (C14:1) and cis-9-hexadecenoic acid (C16:1) were found to be more abundant in the infected samples. In contrast, oleic acid (C18:1) was significantly reduced in the infected samples, suggesting that the metabolic pathways governing these fatty acids might be influenced by the presence of an infection.

One of the most striking findings from the analysis was the markedly higher concentrations of various polyunsaturated fatty acids (PUFAs) in the infected samples. Key PUFAs, including linoleic acid (C18:2), alpha-linolenic acid (C18:3), arachidonic acid (C20:4), and docosahexaenoic acid (C22:6), revealed substantial increases, with C18:3 and C22:6 showing the most pronounced elevation. In stark contrast, eicosapentaenoic acid (C20:5) was found in lower concentrations in the infected samples.

But previous studies reported that the monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA) of parasitised fish were also significantly reduced when compared to non-parasitised fish (Babu and Ravi Chandran, 2022). Further, the infected female population of European hake with larvae (L3) of *Anisakis* spp. showed decreased level of (arachidonic acid (C20: 4w-6), and eicosapentaenoic acid (C20: 5w-3) (Jouini *et al.*, 2023). Fatty acid analysis revealed significant differences in the percentages of individual fatty acids between anisakid

nematodes and adjacent European hake tissue. These observed alterations in fatty acid composition suggest a significant metabolic reorientation in response to the infectious agent.

Table 3: (Fatty Acid Analysis of Infected and Non-Infected Gourami Fish Samples)

Fatty acids	Infected	Non Infected
Saturated fatty acids		
C12:0	0.23	0.42
C13:0	1.62	4.69
C14:0	4.11	2.75
C15:0	0.82	0.66
C16:0	1.84	2.08
C17:0	1.41	1
C18:0	0.86	0.61
C20:0	0.13	0.1
C21:0	0.18	0.04
Monounsaturated fatty acids		
C14:1	0.52	0.37
C16:1	5.5	4.07
C18:1	3.63	4.37
C20:1	0.53	0.5
Polyunsaturated fatty acids		
C18:2	4.16	2.39
C18:3	5.91	2.98
C20:4	2.89	2.76
C20:5	0.39	0.87
C22:6	6.76	4.5

Conclusion

This study thoroughly investigated the nutritional changes in Gourami fish as a response to infection, highlighting significant alterations in their proximate composition, amino acid profile, and fatty acid composition. Infected Gourami fish showed a marked decrease in moisture content, alongside substantial increases in crude fat, crude protein, and ash. Notably, moisture content and crude fat emerged as the most distinct indicators of infection status. The parasite was identified based on the morphological and morphometric analyses as *Clinostomum* spp. belonging to the family Clinostomatidae. The incidence of *Clinostomum* infestation was first reported from the Kararia wetland (Kumar *et al.*, 2024) [1].

Amino acid profiling revealed a complex metabolic response, characterized by reductions in certain amino acids such as glycine, histidine, and serine. This decrease may be attributed to increased catabolism for energy or to support pathogen utilization. In contrast, levels of aspartic acid, glutamic acid, proline, lysine, and valine were elevated, indicating an altered metabolic flux that could be linked to gluconeogenesis or immune system support.

Additionally, the data presented unveil a compelling fatty acid signature that is closely associated with the infected state. These significant alterations in the profiles of saturated, monounsaturated, and polyunsaturated fatty acids underscore the profound metabolic shifts that occur during infection. It is imperative that we pursue further research to elucidate the specific enzymatic pathways and regulatory mechanisms driving these changes, as well as to assess their critical implications in host-pathogen interactions and disease progression. The insights gained from this research could not only serve as biomarkers for infection but also provide promising targets for therapeutic interventions aimed at effectively modulating lipid metabolism.

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