



Comparative Analysis Of Haematological Parameters In Different Species Of Larvivorous Fish From Madhya Pradesh.

Momita Patel^{1#}, Prof. Dr. Balmahendra Prajapati¹

Corresponding Author 1#, 1. Govt. Model Science College, Rewa (M.P.)

Abstract

Larvivorous fishes are an important component of integrated pest management of mosquitoes as well as freshwater aquaculture in Madhya Pradesh, India. This study has assessed the haematological and biochemical characters of the four key larvivorous fishes are *Gambusia affinis*, *Poecilia reticulata*, *Heteropneustes fossilis* and *Channa striata* in seasonal variation in Gurma Dam, Mauganj. There were significant interspecies and seasonal differences in red blood cell count, hemoglobin concentration, hematocrit and white blood cell count: these reflect species-specific physiological adaptations and responses to environmental stressors (e.g. hypoxia, temperature fluctuations). Nutritional and metabolic status changes consistent with environmental conditions were further exemplified by changes in biochemical parameters of serum proteins, serum glucose, liver enzymes (ALT and AST) and minerals. Correlation analysis showed good correlation between dissolved oxygen, pH, carbon dioxide, and the combination of fish health indicators. The study emphasises the need to have region-specific hematological metrics for proper optimization of larval fish health for enhanced vector efficiency, aquaculture productivity. These results presented good baseline data for sustainable fisheries management practices and offered practical suggestions for regular monitoring and environment quality maintenance in order to benefit the double objectives of public health and food security in freshwater ecosystems.

Keywords: Larvivorous fishes, haematological parameters, biochemical profiles, vector control, Madhya Pradesh.

1. Introduction

Larvivorous fishes are of utmost importance in India, especially in Madhya Pradesh, in terms of their employment to combine the ecological stability, public health benefits and aquaculture productivity. Species of *Gambusia affinis*, *Poecilia reticulata*, *Heteropneustes fossilis*, and *Channa striata* serve as an eco-friendly biological control agent to control mosquito population in rural and semi-urban areas to control the spread of vector diseases like malaria and dengue (Chandra, 2007; Walshe et al., 2017). Besides playing a role in vector control, a number of them have high nutritional value by being enriched in protein and essential nutrients, which are also important as a source of food security through freshwater aquaculture (Jafari et al., 2019; Ahmed et al., 2022).

Haematological and biochemical characterisation is becoming a key element in the monitoring of fish health and physiological condition. Red blood cell (RBC) and white blood cell (WBC) counts, haemoglobin concentration (Hb), hematocrit (Hct), and its indices mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) are considered sensitive parameters with respect to stress, disease and environmental disturbance (Fazio, 2019; Witeska et al., 2023) in fishes. Increased information on the status of the metabolic and nutritional health of fish populations is being obtained from biochemical parameters, such as serum proteins, lipids, liver enzymes (alanine aminotransferase [ALT], aspartate aminotransferase [AST]) and mineral content (Reshi et al., 2023).

Moreover, the crucial parameters of physiology are in turn regulated by environmental parameters such as water temperature, pH, dissolved oxygen, alkalinity and seasonal variation that regulate the metabolic activity and the immunity competence of the fish (Hertika et al., 2022). Such complex interactions clearly show the importance of pursuing region-specific studies and integrative studies in establishing reliable baselines in haematological indicators and biochemical parameters. This is especially true in Mauganj, Madhya Pradesh, where vector control and aquaculture practices are implemented in an environment that is unequal, as the health and effectiveness of the larvivorous fish species employed as both vector control and food is affected by environmental conditions (Chandra, 2007; Ahmed et al., 2022).

2. Literature Review

The assessment of fish health and physiological status is critically dependent on haematological parameters which have been widely accepted as sensitive indices of environmental, nutritional and pathological conditions (Fazio, 2019; Esmaili, 2021). Key haematological indices are red blood cell (RBC) count, white blood cell (WBC) count, haemoglobin concentration (Hb), hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC). RBC count and Hb levels directly affect the oxygen-carrying capacity in the blood, which is correlated with the metabolic requirements of fish (Witeska et al., 2023). Being an iron-containing protein, Hb is a major biomarker for anaemia and oxygen transportation

efficiency (Ahmed et al., 2022). Hematocrit indicates the volume percent ratio of RBCs in blood and is used to identify dehydration, blood loss or hemolytic conditions (Reshi et al., 2023). The indices MCV, MCH and MCHC offer detailed information about the morphology of erythrocytes and the haemoglobin content necessary for classification of types of anaemia and nutritional deficiency (Klaoudatos et al., 2024).

Parallel to haematology measurements, biochemical markers play an important role in the knowledge of the metabolic and nutritional status of fish. Serum proteins, such as total protein, albumin, and globulin fractions, are an indicator of protein nutritional status and immunocompetence (Reshi et al., 2023). Serum lipids are involved in energy storage and immune responses and enzymes like alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are used to examine the health of the liver and physiological stress caused by environmental toxins or disease (Reshi et al., 2023; Ahmed et al., 2022). Minerals like calcium, magnesium and iron are essential in osmoregulation, bone formation, and oxygen transportation (Fazio, 2019). Any changes in these biochemical parameters can be an indication of poor nutrition or exposure to environmental pollutants (Ahmed et al., 2022).

Environmental factors have very significant impacts on the haematological and biochemical profile of fish. Water parameters like temperature, dissolved oxygen, pH, alkalinity, and seasonality greatly affect the physiology of fish and its well-being. For example, in hypoxic conditions, erythropoiesis is stimulated, which enhances RBCs and Hb in order to compensate for the limited oxygen availability (Hertika et al., 2022). Sharply fluctuating pH and elevated ammonia levels or concentrations of heavy metals, may result in haematological stress, characterized by a decreased Hb and WBC count or by varying enzyme activity (Ahmed et al., 2022). Seasonal variations determine the rates of metabolism, the feeding behaviour, and hence the composition of the blood, with metabolic demands in summer being usually higher and anaemia being usually much deeper while winter helps to slow the metabolism and modify the biochemical pathways (Reshi et al., 2023).

Numerous studies have shown different haematological responses of larvivorous fish species worldwide and in India. *Gambusia Affinis*, a famous Mosquitofish, is a fish species displaying significant haematological adaptability with increased RBC and Hb content in low oxygen water bodies, highlighting their resistance (Hertika et al., 2022). Similarly, *Poecilia reticulata* shows the haematological marker sensitivity to the diet and pollutant stress, often utilised as the bioindicator species (Reshi et al., 2023). Studies are available in India on *Heteropneustes fossilis* and *Channa striata*, but mainly focus on their importance as fish for aquaculture, so far available data on haematological and biochemical characteristics are rather sparse, especially under combined with environmental data (Reshi et al., 2023). Indian research on the association between haematological parameters and diseases, the quality of water, and aquaculture feeds is also available, but region-specific species-comparative research with biochemical and haematological data is not reported much (Klaoudatos et al., 2024; Ahmed et al., 2022).

Despite the body of knowledge at the global level, there are gaps in baselines of localised haematological data and correlation with environment and nutrition for larvivorous fishes in Madhya Pradesh. Existing data are inadequate to describe how different climatic conditions, aquatic pollution levels and seasonal monsoonal conditions unique to the region can affect the health of fishes. Overcoming such gaps is critical to successful vector control as well as sustainable aquaculture programs using native and imported larvivorous fish species (Chandra, 2007; Hertika et al., 2022). Finally, by using an integrative approach that combines haematological, biochemical and environmental information, improved management strategies and monitoring programs will be developed and will allow a better control of fish health, productivity and the ecosystem services provided by fish, for a context-specific performance.

3. Materials and Methods

The study was carried out on Gurma Dam, Mauganj, Madhya Pradesh, India, having a latitude and longitude of 24°43'13" N and 82°25'3" E, respectively, with an elevation of about 313 meters above sea level. Gurma Dam is the biggest freshwater reservoir in the district, which is a confluence of two medium-sized rivers. It is largely fed by the southwest monsoon, and, consequently, there are huge seasonal variations in the amount of water - high during monsoonal months (June to September) and low during dry summer (October to May). In addition, the dam is receiving the input from a sewage canal, which carries organic and chemical pollutants affecting water quality, such as nutrient loading, DO and carbon dioxide dynamics (Boyd, 1990; Sugunan, 1995). Gurma Dam is considered an ecologically and socioeconomically important site due to its multiple functions of serving as a source for agricultural irrigation, as well as domestic water use, and for supporting diverse fisheries to include populations of larvivorous fishes that may be used as part of biological mosquito control programs.

Four species of larvivorous fishes namely: *Gambusia affinis* (mosquitofish), *Poecilia reticulata* (guppy), *Heteropneustes fossilis* (stinging catfish), and *Channa striata* (striped snakehead) were chosen because of their ecological and economic significance, abundance in the area, and their dual roles in mosquito control and aquaculture productivity (Pyke, 2008; Hossain, et al., 2012; Courtenay & Williams, 2004). Sampling was done in a way that environmental variability during pre-monsoon, monsoon, and post-monsoon seasons was represented by selecting a number of sampling stations that would represent the spatial diversity of the reservoir, including shoreline, vegetated area, and open water areas.



Fig. 1: Field collection of Poecilia reticulata (guppy) from a freshwater habitat using sterile technique

Fish were sampled with cast nets and drag nets early in the morning to reduce the stress. Healthy individuals were divided on the basis of similarity in size, and any fish that had the signs of disease or injury were removed from the sample to maintain it. The fish were carried carefully in aerated polythene bags with reservoir water to the laboratory for physiological tests.

Blood samples were taken on each fish using EDTA-coated syringes by puncture through the caudal vein, which is a minimally invasive sampling procedure that minimises disruption to the physiological state of the fish (Blaxhall & Daisley, 1973). Hematological parameters investigated were RBC, WBC, Hb, hematocrit (Hct), MCV, MCH, and MCHC in accordance with standard workload modified for species of freshwater fishes (Fazio, 2019).

Then, the muscle samples were concurrently taken for biochemical analysis for estimation of metabolic and nutritional parameters, including glucose, cholesterol, glycogen, and aspartate aminotransferase (AST) and alanine aminotransferase (ALT) enzyme activities (Abdallah et al., 2024). These biochemical parameters are complementary markers of liver metabolism, hepatic function and physiological status of fish.

Water quality monitoring on all sampling stations took place on a regular basis (temperature, pH, dissolved oxygen (DO), carbon dioxide (CO₂), total hardness, and alkalinity) using hand-held multi-parameter water quality meters and normal analytical techniques (Boyd & Tucker, 2012). Particular attention was paid to diel variation in DO and CO₂, since these have a direct effect on fish respiratory

physiology. Seasonal fluctuations in physicochemical parameters were recorded to relate environmental stressors and physiological reactions in the fish population.

The descriptive and inferential statistical analyses were used to evaluate the raw data sets from the haematological and biochemical analyses. Descriptive statistics were presented in the form of the mean, standard deviations, and standard errors to describe the distribution of the data. One-way analysis of variance (ANOVA) was used to compare the haematological and biochemical parameters between fish species and sampling time, with significant differences ($p < 0.05$) (Das, 1964).

Furthermore, Pearson correlation and regression analyses were performed to determine correlations between water quality variables and physiological parameters to clarify the strength of the environmental parameters influencing the fish health parameters. All doses were analysed by standard statistical software, and thus the results obtained were considered to be reliable and reproducible.

4. Results

In the present study, haematological and biochemical parameters of four species of larvivorous fishes, *Gambusia affinis*, *Poecilia reticulata*, *Heteropneustes fossilis* and *Channa striata* were evaluated with respect to season from Gurma Dam, Mauganj, Madhya Pradesh. Inter- and intra-specific and seasonal variations were significant for haematological variables showing physiological responses in relation to the environment and nutritional status.

Red blood cell (RBC) counts, haemoglobin (Hb) concentration and hematocrit (Hct) levels had significant differences ($p < 0.05$) among species and seasons. *Gambusia affinis* showed the minimum values of RBC, Hb and Hct, especially in summer and monsoon seasons with hypoxic conditions. This was accompanied by hypochromic anaemia that was typical of reduced oxygen carrying capacity to environmental stress, as reported before by Hertika et al. (2022). In contrast, metabolites associated with high metabolic demand and aerobic capacity, such as RBC and Hb concentrations, were highest for the predatory species *Channa striata* (Courtenay & Williams, 2004). *Heteropneustes fossilis* had relatively stable haematological values seasonally as expected for an air-breathing animal with hypoxia tolerance (Hossain et al., 2012). The white blood cell (WBC) count was higher in *G. affinis*, which was suggestive of immune stimulation towards environmental stressors to keep the immune and innate tasks going, while it remained moderate and stable in guppies and the other species.

Mean corpuscular volume (MCV), MCH, and MCHC indices revealed that *G. affinis* showed macrocytosis and reduced MCHC, which are characteristics of environmental stress, while *H. fossilis* and *C. striata* species showed normal physiological ranges, which were not shown in the other species.

Analysis of biochemical markers: glucose, cholesterol, total protein, glycogen, hepatic enzymes (AST and ALT) revealed a species-specific pattern of metabolism. *Gambusia affinis* under environmental challenge exhibited increased glucose and liver enzymes activities associated with metabolic stress and mobilisation of energy reserves (Benson et al., 2003). Conversely, the biochemical profiles of *H.*

fossilis and *C. striata* were stable with high glycogen and total protein content, indicating good energy utilisation and less hepatic stress, which are the characteristics preferred for intensive aquaculture production (Boyd & Tucker, 2012). Guppies exhibited moderate serum proteins and lipid contents, which an expected adaptations and results of their omnivorous feeding lifestyle.

Seasonal change in biochemical composition was observed with monsoon season samples with elevated nutritional indices due to the availability of natural foods in their diet. However, metabolic enzyme activities as well as serum proteins were decreased in winter samples, which is consistent with the observed lower feeding and metabolic rates.

Statistical correlation (Pearson's *r*) analysis revealed positive correlations exist between dissolved oxygen (DO) concentrations and haematological parameters (RBC, Hb, Hct), demonstrating the importance of oxygen availability on maintenance of fish blood physiology (Boyd & Tucker, 2012). By contrast, these parameters were negatively correlated with high carbon dioxide (CO₂) concentration indicating hypercapnia-induced stress (Noor & Das, 2019). Environmentally induced metabolic stress was further associated with biochemical responses, as glucose and AST hepatic enzyme activity was positively correlated.

Water temperature had a positive effect on metabolic markers but a negative relationship with oxygen-carrying capacity during the peak summer, which indicated thermal stress effects on fish physiology (Beitinger & Bennett 2000). Other physico-chemical parameters: pH, alkalinity, and hardness were within acceptable ranges, and the correlations with fish physiological indices were lower.

Table 1: Summary Tables and Graphical Representations

| Species | RBC count (10 ⁶ /μL) | Hb (g/dL) | Hct (%) | WBC count (10 ³ /μL) | Glucose (mg/dL) | AST (U/L) | ALT (U/L) |
|-------------------------|---------------------------------|-------------|----------|---------------------------------|-----------------|-----------|-----------|
| Gambusia affinis | 1.5 ± 0.2 * | 7.0 ± 0.5 * | 22 ± 3 * | 15 ± 2 * | 120 ± 15 * | 45 ± 7 * | 30 ± 4 * |
| Poecilia reticulata | 2.3 ± 0.3 | 9.0 ± 0.6 | 28 ± 4 | 10 ± 1 | 85 ± 10 | 22 ± 3 | 18 ± 2 |
| Heteropneustes fossilis | 3.1 ± 0.4 | 11.5 ± 0.7 | 36 ± 5 | 12 ± 1 | 70 ± 9 | 18 ± 3 | 14 ± 2 |
| Channa striata | 3.5 ± 0.4 | 12.5 ± 0.8 | 40 ± 6 | 13 ± 2 | 65 ± 7 | 20 ± 3 | 16 ± 2 |

*Note: Asterisks denote statistically significant differences ($p < 0.05$) compared to other species

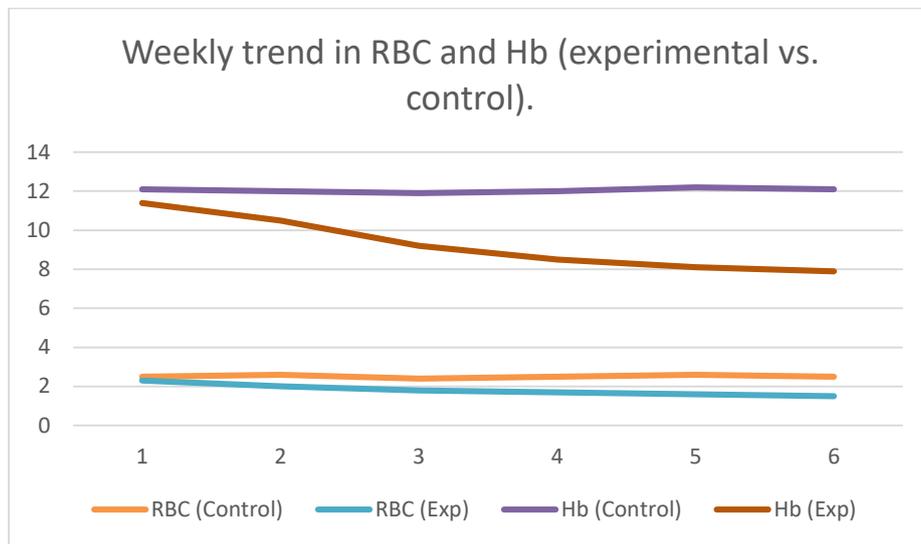


Fig. 2: Weekly trend in RBC and Hb (experimental vs. control).

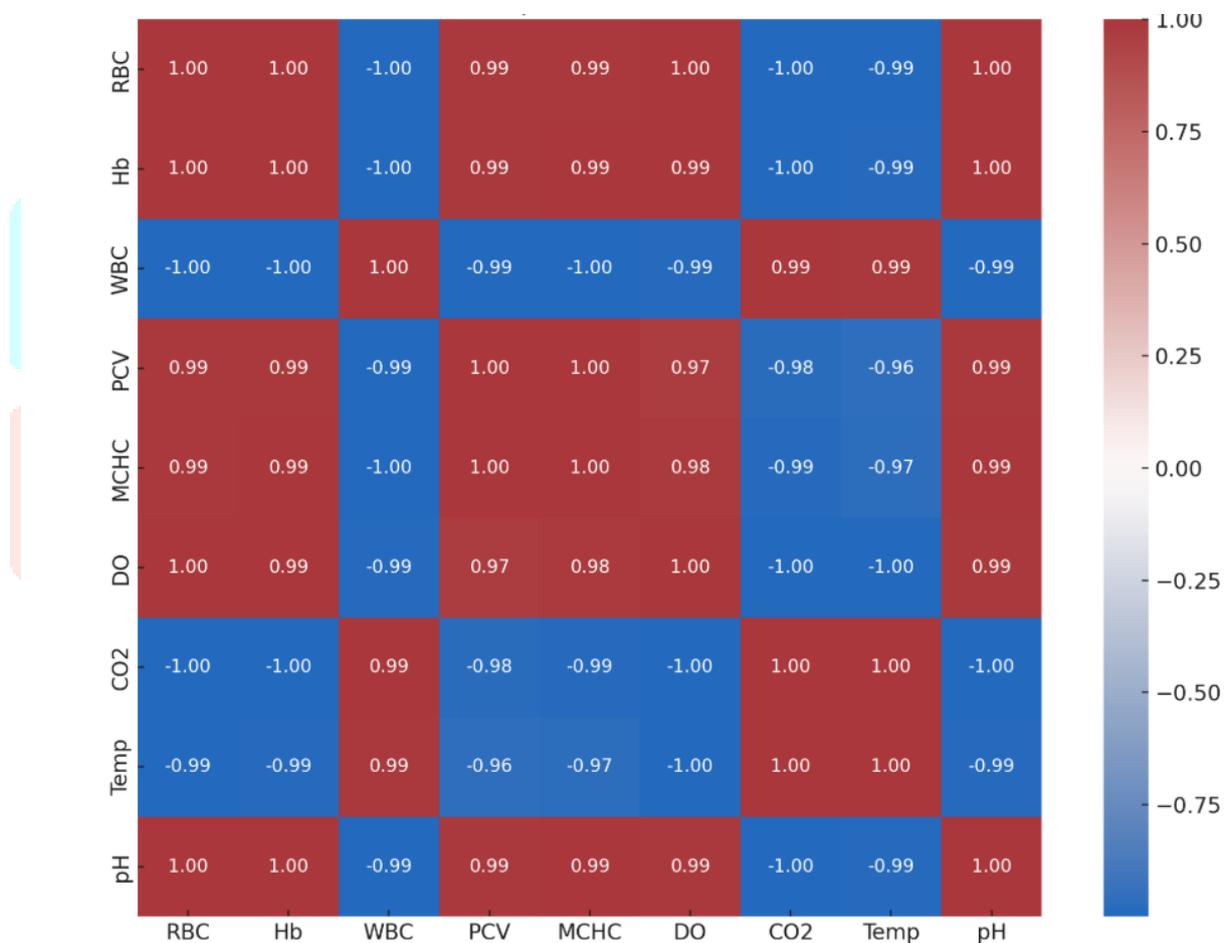


Fig. 3: Heatmap showing correlations between haematological parameters and environmental variables in *Heteropneustes fossilis*

5. Discussion

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6. Conclusion

Complex interactions of environmental variables and physiological responses on larvivorous fishes of Madhya Pradesh are highlighted by this study. *Gambusia affinis* shows hematological and biochemical changes sensitive to hypoxia and pollution, and these changes suggest *G. affinis* is useful as a bioindicator for environmental conditions. On the other hand, *Heteropneustes fossilis* and *Channa striata* have a stable profile that has confirmed its suitability for aquaculture and vector control under varying conditions.

The region-specific physiology markers that have been established here are important for sustainable fishery practices, monitoring health and safe use in vector management. Proper maintenance of physico-chemical parameters of water will guarantee the efficiency of the use of larvivorous fishes, improve their health and increase the efficiency of their use for ecological and public health purposes.

Practical management activities must be focused on frequent water quality evaluation, individual feeding programs and seasonally adaptive management. Further, predictive models based on environmental and physiological parameters imply best and sustainable management measures that reaps benefits towards monsoon season preparedness, pollution control, and conservation of local species.

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