



# Haematological Alterations in Common Carp (*Cyprinus carpio*) Infected by *Saprolegnia* spp.

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## Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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## ABSTRACT

The effect of Saprolegniasis on hematological parameters of *Cyprinus carpio* was studied in River Indus at Swabi, Khyber Pakhtunkhwa, Pakistan. The results showed that Saprolegniasis significantly decreased the total erythrocytes count, packed cell volume, and hemoglobin content, while the white blood cells and mean corpuscular volume were significantly increased in the infected fish as compared to the healthy fish. Mean corpuscular hemoglobin was found higher while mean corpuscular hemoglobin concentration was found lower in infected fish. Saprolegnia triggers a strong inflammatory response in its host by suppressing fish immunity. The Pearson linear correlation analysis showed a significant correlation for several parameters ( $P < 0.05$ ). It is obvious that Saprolegniasis seriously damage the population of freshwater fishes.

**Keywords:** *Cyprinus carpio*; saprolegniasis; hematology; histopathology.

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## 1. INTRODUCTION

The World is facing diseases, hunger, and malnutrition approximately 11% of the world population suffers from poverty and dearth of access to adequate nutritious food necessary for human health [1]. aquaculture provide foods and nutrients for billions of people that overcome diseases and malnutrition. Consumers often perceive that wild caught fish have higher nutritional value than fish produced through aquaculture, and this may be true for some nutrients, for example omega-3 fatty acid content [2].

In global waters, rivers support a wide range of aquatic flora and fauna [3]. River Indus is the longest and the most important river of Pakistan containing a great diversity of edible and commercial fishes [4].

Diseases and epidemics breakouts usually occur in the area of fluctuations in temperature, low water quality, and other factors. Therefore, freshwater fishes are under stress because of bacterial, viral, and other parasites including *Saprolegnia* parasite. Genus *Saprolegnia* belongs to the Family of Saprolegniaceae, Order Saprolegniales, Class Oomycota, Phylum Heterokonta, and Kingdom Protocista [5]. In past, *Saprolegnia* was classified as a fungus, commonly called 'cotton wool fungus' and 'water mold' [6]. *Saprolegnia* prefers water temperature between 15° C and 30° C while the mean water temperature of River Indus recorded at district Swabi was 20.25° C (Khan et al., [7], Ganguly et al., [8].

It is known that *Saprolegnia* attacks epidermal tissue; infection starts on the head or fins and can spread over the whole surface of the body. It can affect adult fishes of both genders and their eggs [9]. Poor water quality (e.g. water with low circulation, low DO, high alkalinity) and high organic matter, are connected with *Saprolegnia* contaminations. *Saprolegniasis* is frequently first recognized by watching fleecy tufts of cotton-like material hued white to shades of dim and dark-colored on the fish eggs [10]. Water causticity for the most part supports mycelial development and zoospore creation by *Saprolegnia* species [11]. *Saprolegniasis* can be in the form of primary as well as a secondary infection. It is noticed that most fishes which are subjected to stress because of handling wounds, lack of healthy sustenance, temperature stun, ectoparasites,

and continuous egg production are defenseless to *Saprolegniasis* [12].

Cultured fisheries of developing countries are continuously challenged by a number of pathogenic microbes. Among microbial diseases, fungal and fungal like pathogen outbreaks lead to negative social and economic impacts on stakeholders. Magray et al., [13].

*Saprolegnia* infection can be prohibited by suitable management practices, for example, good water quality and flow, keeping stocking density low to limit damage, and better feeding. When *Saprolegnia* is distinguished in a water body, sanitation ought to be assessed and revised. General medications are Copper nanoparticles 10 ppm (Kalatehjari, et al., [14], potassium permanganate, formalin, and povidone-iodine solutions. Bath treatment in NaOH (10-25 g/L for 10-20 min) or  $KmNO_4$  (1 g in 100 L of water for 30-90 min) should be given. Thorough cleaning and disinfection of tanks, raceways, or aquaria are good. Lakes with earthen or rock bottoms require a long duration of drying to absolutely killing the growth of fungi [15]. A gram-negative bacterium, *Pseudomonas aeruginosa* (Persian Type Culture Collection 1430) has been presented as a biological controlling organism against *Saprolegnia* [16].

*Saprolegnia* alters the hematological parameters in infected fish. In the study 27 healthy and 20 *Saprolegnia* infected Caspian salmon were examined for blood indices. Leukocytes were higher ( $P < 0.05$ ) in infected fish. The other parameters like hemoglobin content, packed cell volume, total erythrocyte count, lymphocyte, and monocyte were greater in healthy fish. The weight of healthy fish had a positive significant correlation with white blood cells. However, a negative correlation was observed between the length and weight of infected samples and white blood cells count [17].

Histopathological alterations have also been observed in the tissues of *Saprolegnia*-infected fish. Infected tissues showed various types of destructions due to hyphal penetration deep into the muscle tissues, spores were observed in the underlying musculature, and granulomas were formed with fibrillar layers. Infection was observed in form of white patches and lesions. Generally, the loss of epidermis, necrotized hypodermis with the growth of hyphae, and destruction of muscles were observed [18].

## 2. MATERIALS AND METHODS

The sampling was done from the river Indus at Hund village of district Swabi, Khyber Pakhtunkhwa, Pakistan in March and April 2017. By gross examination of fishes, it was found that among other fish species *Cyprinus carpio* had certain lesions and infections. After collection, twenty fishes (*Cyprinus carpio*) were transported to the Aquaculture and Fisheries Laboratory, Department of Zoology, PMAS- Arid Agriculture University Rawalpindi. The water sample was also collected from the sampling site to check the water quality.

The fish samples were carefully observed for skin infections. Five samples were found to be infected with Saprolegniasis. Skin infections were diagnosed by external examination, morphology, and previously available information. Regardless of gender, Control and infected groups were made comprising of five healthy and five infected fish respectively.

The total length and weight of both control and infected fish were measured. Then fish blood was obtained from the caudal peduncle vein or by cardiac puncture using 3 ml disposable syringes. The blood was refrigerated at 4 °C in Di Potassium EDTA containers for further analysis. Seven hematological parameters were determined viz, RBC count, WBC count, Hb. concentrations, PCV (hematocrit), MCV, MCH, and MCHC according to Schalm, 1986; Reddy & Bashamohideen 1989.

To study the histopathology of the skin and muscle tissues of *Cyprinus carpio*, the tissues of control and experimental fish were processed as, firstly the tissue was dehydrated with the ascending concentrations of alcohol 70 percent, 90 percent, and 100 percent alcohol, clearing process was done with the help of xylene,

infiltration, and embedding in paraffin wax was done. Fine thin sections were made with the help of rotary microtome, then the slides were dewaxed with the help of xylene, hydration process was done with the descending concentrations of alcohol like 100 percent, 90 percent, and 70 percent alcohol. The tissue was stained with Hematoxylin and Eosin stains, for clearing the tissue slides were treated with clove oil, clearing with xylene and in last the mounting of coverslip was done with the help of Canada balsam. The tissue slides of healthy and infected fish were comparatively examined for tissue damage.

## 3. RESULTS

The high values of these parameters in water samples are due to the stagnancy and low level of water in river Indus during the months of March and April. Low dissolved oxygen, TDS, pH, overcrowding, fish handling and injuries assist the parasite survival and spread of infection. So the above discussed factors are positively acting in the prevalence of disease.

The fish biometry was done to find the total length and body weight of control and infected fish. The results showed a non-significant decrease in both length and weight of infected fish (Table 2).

The hematological results revealed that Saprolegniasis significantly decreased the total erythrocytes count, packed cell volume, and hemoglobin content, while the white blood cells and mean corpuscular volume showed a significant increase in the infected fish. Mean corpuscular hemoglobin was increased and mean corpuscular hemoglobin concentration was decreased in infected fish but both were non-significant (Table 2).

**Table 1. Water quality parameters at sampling site**

| Parameters                    | Values  |
|-------------------------------|---------|
| Temperature (°C)              | 16.93   |
| Dissolved Oxygen (mg/L)       | 9.07    |
| Total Dissolved Solids (mg/L) | 499     |
| pH                            | 7.575   |
| Conductivity (µS/cm)          | 158.425 |
| Alkalinity (mg/L)             | 330.25  |
| Hardness (mg/L)               | 310.5   |
| Calcium (mg/L)                | 48.75   |
| Magnesium (mg/L)              | 51      |
| Chloride (mg/L)               | 70.25   |
| Arsenic (ppb)                 | 0.0057  |

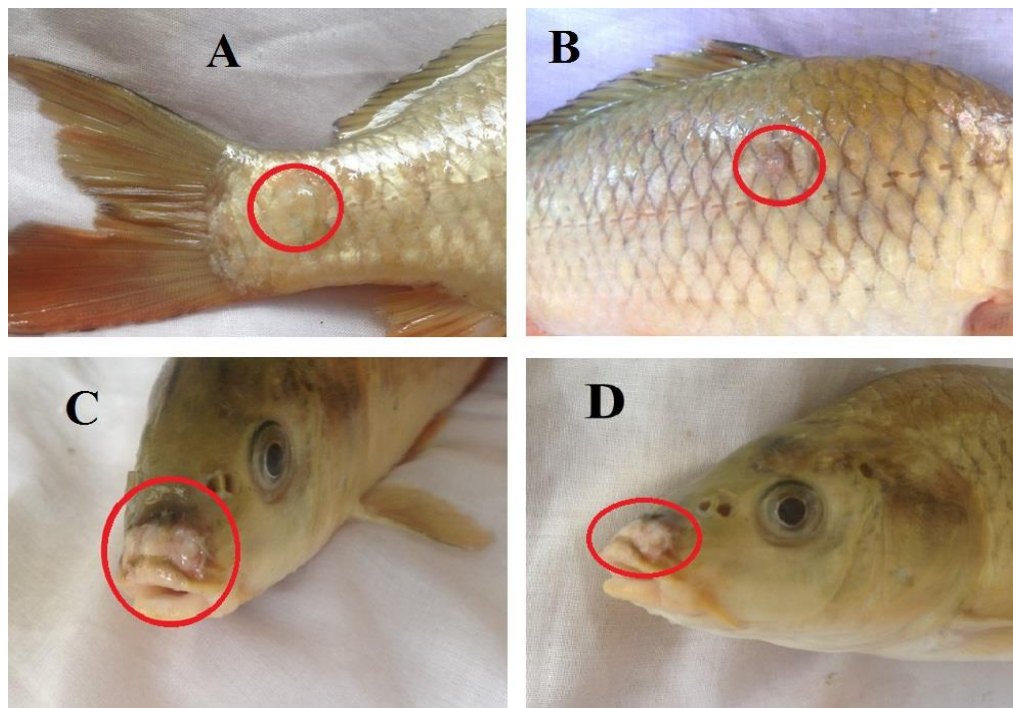
**Table 2. Biometric (length, weight) and hematological indices of control and experimental *Cyprinus carpio***

| Biometric & Hematological Parameters      | Control Fish   | Infected Fish | Significance P = 0.05 |
|---|----------------|---------------|-----------------------|
|   | Means ± SD     | Means ± SD    |                       |
| Total Length (cm)                         | 28.8±10.02     | 19.6±3.51     | P > 0.05              |
| Weight (g)                                | 421.7±489.91   | 103.8±52.68   | P > 0.05              |
| RBCs (×10 <sup>6</sup> /mm <sup>3</sup> ) | 1.742 ± 0.49   | 1.094 ± 0.37  | P < 0.05*             |
| WBCs (×10 <sup>3</sup> /mm <sup>3</sup> ) | 7.85 ± 0.92    | 9.486 ± 1.96  | P < 0.05*             |
| Hb. Conc. (g/dL)                          | 7.262 ± 1.21   | 5.124 ± 1.34  | P < 0.05*             |
| PCV (%)                                   | 26.843 ± 2.10  | 21.574 ± 3.15 | P < 0.05*             |
| MCV (fL)                                  | 161.56 ± 29.94 | 206.7 ± 36.35 | P < 0.05*             |
| MCH (pg)                                  | 42.85 ± 5.65   | 48.14 ± 8.47  | P > 0.05              |
| MCHC(g/dL)                                | 26.9 ± 2.85    | 23.54 ± 3.46  | P > 0.05              |

P < 0.05\* = Significant; P > 0.05 = Non significant

The tissues of infected fish showed varying degrees of histopathological deteriorations. Clinically, the fish infected by *Saprolegnia* species showed parasitic growths as white or grey threads resembling a tuft of cotton wool. The infections on the skin and mouth were common which are associated with conspicuous areas of hemorrhage, necrosis, and ulceration. Hyphae-like parasitic growth was observed on the hypodermal layer. The beneath musculature

was also disrupted, muscle cells lost their original appearance, and cells accumulated to form granulomas-like structures. In severely infected and smaller fish fungal hyphae were found penetrating deep in the muscular layer. The healthy fish' tissues were compact, robust, and composed of fine cells while the damaged tissues of the infected group showed ruptured cell membranes, damage, and degradation of cells.



**Fig. 1. Showing Saprolegniasis infection near the caudal fin of fish(A), Initial stage of Saprolegniasis lesion above the lateral line(B) Saprolegnia growing on the mouth of *Cyprinus carpio*(C&D)**

#### 4. DISCUSSION

The water quality of river Indus was not satisfactory; the value of dissolved oxygen was low as compared to normal freshwater while the alkalinity, hardness, and total dissolved solids are higher. The reason for extra turbidity and low water quality is the stagnancy and low level of water in the river during March and April. Infection spread is also supported by the low water quality and quantity of the river in the spring season.

The above hematological alterations derive support from the studies of Shah et al., (2015), Ali et al., [19], Chauhan et al., [18], Singh et al., [20] on *Oncorhynchus mykiss*, Salmon and Trout, *Clarias gariepinus* and *Cyprinus carpio* respectively. In various studies, the *Saprolegniasis* significantly decreased the total erythrocytes count, packed cell volume, and hemoglobin content, while the white blood cells and mean corpuscular volume showed a significant increase in the infected fish. The hematological esteem for *Cyprinus carpio* blood reported in the present examination was not altogether different from the qualities revealed by different analysts said above.

About *Saprolegnia* infection, evidence from the previous data showed that *Saprolegniasis* triggered suppression of the immune system, anemia, osmotic imbalance, haemodilution, the release of mucus, lethargy, and mortality in *Cyprinus carpio*. In different fish species also blood deficiency has been reported due to the breakdown of erythrocytes and hemorrhaging [21]. The reduction in leucocytes has been accounted for because of expanded discharge of corticosteroids and haemodilution (McLeay, [22], Gill & Epple, [23]. In another study reported that the number of white blood cells increases in infected fish due to the response of the cellular immune system to fungal infection. Fish's immune systems show a similar immune response to harsh conditions [24], and similar discoveries were found in Caspian salmon (*Salmo trutta caspius*) infected with *Saprolegnia*. Leukocytes are essential to the cell of the immune system in the defense mechanism of fishes. Due to the fungal infection, the numbers of leukocytes were increased, for improving a defense mechanism to reduce the stress caused by infection. In infected *C. carpio* and *O. mykiss*, the number of RBCs were decreased just like in Caspian salmon [17]. This reduction was due to fungal spreading on gills which reduce the

oxygen supply and can lead to the destruction of RBCs and or reduce the formation of RBCs due to lack of Hemoglobin in the cellular medium [25].

The significance of observing packed cell volume is to evaluate the effect of stressors on fish health and to determine the oxygen-carrying capacity of the fish blood. Mean Corpuscular Volume, Mean Corpuscular Hemoglobin, and Mean Corpuscular Hemoglobin Concentration is completely dependent on the levels of PCV, RBCs count, and Hemoglobin in the blood. In the present study, PCV, RBCs count, and Hb. concentrations were altered in the infected fish. Therefore, MCV, MCH, and MCHC were lower in the infected fish than in the control. These estimations could be a helpful analytic instrument for *Saprolegniasis* contamination in cultivable cool water fish species, for example in common carp and rainbow trout.

Skin and muscle tissues of infected fish showed heavy histopathological alterations, like loss of epidermis, necrotized hypodermis with hyphal development, and entirely wrecked muscles. Similar sorts of changes in the tissue of *Saprolegnia* contaminated fish have been documented by (Hatai et al., [26], Hussian et al., [27].

The frequent *Saprolegnia* infections in the region are due to low water temperature, fish reduced immunity in cold, stagnancy and low water quality, and limited water depth like stresses on fish. Studies suggest that Catfish were severely impacted by low temperature, at 15-25 °C temperature. The fish showed no antibodies and suffered high mortality [28-32].

#### 5. CONCLUSION

The *saprolegniasis* is world wide problem it occur in those fishes that were living in poor water quality conditions. Author suggest that to improve water quality for wild fishes by using different methods such controlling the wastes product coming into the natural water bodies from various factories. Government should be take a serious and strict action against all those who contaminated and exploited the natural water bodies.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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