INFLUENCE OF ACUTE TEMPERATURE STRESS ON HEMOGLOBIN CONTENT IN SNAKEHEADED FISH, *CHANNA PUNCTATUS* GODAVARI RIVER, NANDED

Jagtap Ashwini Ravichandra

P G Dept. of Zoology, Yeshwant Mahavidyalaya, Nanded (Maharashtra), India

E-mail of Corresponding Author: ashu_anamica@rediffmail.com

Abstract
Temperature of aquatic environment is important for ensuring the normal metabolism of aquatic animals. It also affects blood vascular system of aquatic organisms. The present paper deals with the influence of temperature on the hemoglobin content of freshwater fish, *Channa punctatus*. The freshwater fish, *Channa punctatus*, were collected from Godavari River, Nanded (Maharashtra). The fishes were exposed to different temperatures viz. 15°, 20°, 25°, 30°, 35° and 40° C. The total hemoglobin contents were observed at different temperature stress. The values were compared with the fish's acclimated to ambient temperature. The haemoglobin content was found to be increased as temperature increases. The total hemoglobin content was found to be decreased as temperature decreases.
Keywords: Temperature Stress, Hemoglobin Content, *Channa punctatus*

1. Introduction

Temperature is considered to be the most critical factor among most of the ecological factors affects the aquatic environment[11, 12, 29]. Today temperature is being a serious problem throughout the world. Numbers of factors are responsible for the increase in temperature of the environment. The uncontrolled increased pollution and green houses are responsible for emission of enormous amounts of carbon dioxide and nitrous oxides[15]. The increase in concentration of gases in atmosphere results in the global warming trend in Earth's Climate[34, 19].
Numerous biochemical indices are used to assess the health of aquatic organisms exposed under various environmental stress conditions. Blood is a valuable diagnostic tool for the investigation of diseases and physiological or metabolic alterations[8]. The physical properties of blood are very sensitive to environmental changes[18]. Blood parameters are considered as patho-physiological indicator of the whole body and therefore important in diagnosing the structural and functional status of the fish under various stresses[5].
The circulatory system transports oxygen from the gills to oxygen requiring tissue cells. Oxygen from the inspired water diffuses across the gill epithelia into the blood due to an oxygen gradient. Oxygen tension of the blood is reduced due to binding of oxygen molecules by hemoglobin molecules within erythrocytes and carried in this form to the tissues. The binding capacity of oxygen per unit volume of blood depends on the number of erythrocytes, concentration of hemoglobin within the erythrocytes, prevailing oxygen partial pressures, and the oxygen binding affinity of the hemoglobin molecule. The arterial oxygen partial pressure (PaO_2) decreases in tissue capillaries where carbon dioxide is produced, which changes the oxygen affinity of hemoglobin by causing a rightward shift on the oxygen dissociation curve, so more oxygen is released allowing it to diffuse into the tissue cells [26, 27].
Hemoglobin is present in all vertebrates, which strongly decreases the circulatory requirement (i.e., volume of blood pumped by the heart for a unit quantity of oxygen consumed). Hemoglobin considered as a heat carrier which absorbs the oxygen in tissues. This absorbed oxygen in tissues liberated in the gills on oxygenation [35]. Changes in hemoglobin (Hb) have been identified as an efficient stress response mechanism, to compensate for a reduction in oxygen availability[2]. It is considered as an important diagnostic tool for determination of physiological status in aquatic animal under stress condition [28, 25].

2. Materials and Methods

The freshwater fish, *Channa punctatus* was collected from the Godavari River, Nanded (Maharashtra) with the help of local fisherman for the present investigation. They were brought to the laboratory and kept in glass aquarium with continuously aerated tap water. The physico-

chemical parameters of water were maintained and determined by standard methods (A.P.H.A, A.W.W.A., W.P.C.P., 1992). (P^H-7.0–7.2, Dissolved Oxygen-7.6 – 8.0 ppm, Carbondioxide-2.02 mg/L, Salinity 0.186 gms/L, Chlorinity - 0.112 gms/L). The fishes were acclimated at room temperature for 8-10 days prior to experimentation. Fishes were feed with the small pieces of earthworms to avoid the effect of starvation. The water in the aquarium was replaced daily with fresh tap water. The feeding was stopped one day before the starting of experiment to eliminate the effects of differential diet. The snake headed fish, *Channa punctatus* were subjected to above and below room temperatures to carry out experiment. The experimental set was designed to investigate hematological parameters including estimation of hemoglobin in blood of freshwater fish, *Channa punctatus*. The fishes were acclimated to different temperatures for 24 hrs, 48 hrs, 72 hrs and 96 hrs.

Blood sample in fishes were obtained directly by puncturing the caudal vessel [1]. The blood collected using 2 ml sterile disposable syringe with No. 21 needle. The use of glass syringes avoided because it may quicken coagulation [31]. The syringe rinsed with anticoagulant (potassium salt of ethylene diamine tetra acetic acid, EDTA). The needle inserted in the caudal vessel and very slightly aspirated during penetration. Blood was taken under gentle aspiration and then the needle withdrawn. After detaching the needle from the syringe, the blood mixed well in a vial containing anticoagulant (EDTA) at the concentration of 5 mg EDTA per ml of blood [10]. Each analysis repeated six times and results subjected to statistical analysis [7]. The collected blood of fish was used for the hemoglobin content.

Hemoglobin content of blood was determined by Sahil's Method [14]. The hemometer tube possesses the two acid haematin solution tubes on sides. These tubes are referred as standards. This standard acid haematin solution was used to compare with the sample for the determination of hemoglobin.

3. Result

The freshwater fish, *Channa punctatus* selected for the present research showed variations in the hemoglobin content when exposed to cold and warm temperature stress up to 96 hrs. The fish, *Channa punctatus* was exposed to different temperatures i.e. 15 °C, 20 °C, 30 °C & 35 °C. The hemoglobin contents were observed on 24, 48, 72 and 96 hrs of continuous exposure. The amount of hemoglobin content was found to be decreased at cold (15 °C & 20 °C) and increased at warm (30 °C & 35 °C) temperature stress. The results were compared with control set at 26 °C. Overall it was observed that, as temperature increases the hemoglobin content increases in *Channa punctatus* up to 96 hr period of exposure.

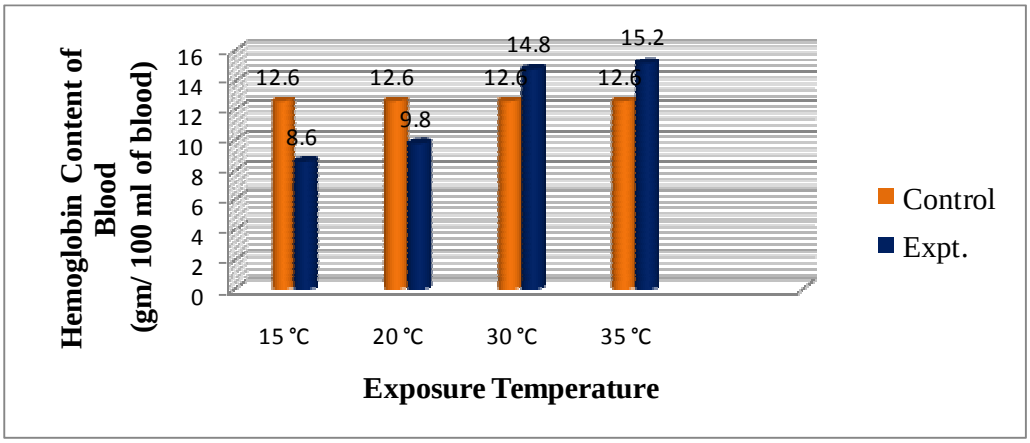
The values for hemoglobin content in *Channa punctatus* at 15 ± 1° C, 20 ± 1° C, 30 ± 1° C and 35 ± 1° C up to 96 hrs are listed on the table No. 3.1. Using 26 ± 1° C as the baseline temperature as it corresponds to the temperature of the natural habitat of *Channa punctatus*. The values obtained by exposure to cold (15 ± 1° C and 20 ± 1° C) and warm (30 ± 1° C and 35 ± 1° C) represents the remarkable changes relatively as compare to the control.

Table 3.1: Effect of Temperature on Hemoglobin content in *Channa punctatus* at cold and warm temperature stress

Sr. No.	Period of Exposure in hrs	Hemoglobin Content (gm/100 ml of Blood)			
		At 15° C	At 20° C	At 30° C	At 35° C
1	**26° C± 1° C (Control)**	**12.6 ± 0.32**	**12.6 ± 0.25**	**12.6 ± 0.27**	**12.6 ± 0.22**
2	24 Hrs	10.3 ± 0.14	11.9 ± 0.29	12.9 ± 0.29	13.8 ± 0.14
3	48 Hrs	9.8 ± 0.24	10.3 ± 0.14	13.3 ± 0.14	14.6 ± 0.24
4	72 Hrs	9.3 ± 0.16	10.1 ± 0.22	14.1 ± 0.22	14.9 ± 0.16
5	96 Hrs	8.6 ± 0.16	9.8 ± 0.16	14.8 ± 0.16	15.2 ± 0.16

Each Value represents Mean of Six Observations ± S. D.

Graph 3.2: Effect of Temperature stress on Hemoglobin content of Fresh Water Fish, *Channa punctatus* at 96 periods of exposure



Each Value is Mean of Six Observations ± S. D.

4. Discussion

Among the principal components of nature, water has the prime importance for the life of plants and animals [6]. The dissolved oxygen in water is used for metabolism in fishes. The circulatory changes are affected by many factors, on which water temperature is one of them[32], [23]. The dissolved oxygen concentration directly affects on the hematological parameters [24]. On investigation of hematological parameters of fish; the effect of temperature is an important aspect to determine the health condition of fishes. Establishing reference intervals for various hematological parameters of fish is important for evaluating effects of various environmental on the health of populations in aquatic biota.

The variations in hemoglobin content (Hb) have been identified as an efficient stress response mechanism, to compensate for reduction in oxygen availability [9]. Thus, experiment design directed towards investigating these hematological responses in *Channa punctatus* to determine whether temperature stress affected the hemoglobin content in fish. The results of the present investigation showed that as temperature increases the hemoglobin content was increased up to 35 ± 1° C. The decrease in hemoglobin content was observed and may be due to variations in the oxygen demand of the fish, *Channa punctatus*. The oxygen demand of the fish increases as temperature increases. The decreasing level of hemoglobin lead to a deteriorated oxygen supply [4]. The amount of haemoglobin content depends on the quantity of red blood cells. The variation in RBC's population leads to alterations of haemoglobin content. The present investigation showed incline in red blood corpuscles level with that of increased temperature. As temperature increases the combining capacity of oxygen with that of RBC's reduces. Due to this the capability to compensate under stress condition in animal reduces by increasing the RBC number in blood of fresh water fish, *Channa punctatus*. The overall result showed that, as temperature increases, the RBC content increases. Due to increase in RBC population, the haemoglobin content was also found to be increased [3].

Dheer exposed the freshwater fish *Channa punctatus* under three different temperatures (25, 30 and 35° C) and compared with a control group maintained at 14° C. He was found that Erythrocytic polycythemia accompanied by an increase in hemoglobin content indicates that the fish in thermal stress [20].

The overall data regarding the variation in hemoglobin content in freshwater fish, *Channa punctatus* exposed under varying thermal stress reveals that the hemoglobin tend to vary directly with temperature. The increase in oxygen capacity was seen at low temperatures as compared to high temperature of blood related to changes in temperatures. The normal variation of hemoglobin was mostly due to alteration of number of red cells and not due to any change in the absolute quantity of hemoglobin in each cell. Anything that alters the red cell count will alter the percentage of hemoglobin proportionally. The hematological study under influence of temperature carried on various fishes[17,22,16,30,13,33,21].

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