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To analyze the impact of Thermal- stress on the RBC number of Indian Major carp Catla catla (Hamilton)

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Abstract

To differentiate thermal-stress (het-stress and cold-stress) from thermal-adaptation (heat-adaptation and cold-adaptation), the fish species Catla catla were subjected to different temperature treatments - a rapid increase in temperature from 22°C to 32°C at a rate of 1°C per hour (heat-stress) and a gradual increase in temperature at a rate of 1°C per 60 hours (heat-adaptation) - During heat-adaptation, the fish showed a gradual increase in RBC number, while in cold-adaptation, a gradual decrease was observed. However, the percentage of recovery was higher in heat-adaptation compared to cold-adaptation. Interestingly, both heat-stressed and cold-stressed fish initially exhibited fluctuations in RBC number. However, the percentage of recovery in stressed fish was considerably lower compared to the fish underwent thermal-adaptation. Studies of this nature are highly useful in evaluating methods for the safe rearing and conservation of economically important Ichthyofauna of the aquatic habitat.

.**Key Words** : *Catla catla;* RBC number; Heat-stress;Heat-adaptation

INTRODUCTION

Temperature is one of the most important environmental factors with tremendous influence on the metabolism, activity and distribution of animals. A great deal of work has been done in poikilotherms in relation to temperature compensation (Kinne,1964a; Fry1964;Pampathirao1965; Hazel and Prosser 1974; Bashamohideen 1984). The rate of metabolic process in the form of activity of animals is influenced by the ambient temperature. The adaptation to a new temperature or a change in temperature involves a number of active processes and hence energy expenditure is needed for metabolic reorganization. In recent times it is found necessary and possible to differentiate thermal-stress from thermal-adaptation. “Stress” is used to mean the environmental factor itself and a stress factor or “stressor” is an environmental change that is severe enough to require a physiological response on the part of the animal. Otherwise the adaptation could be easily mistaken from the other phenomenon like Stress effects or Stress adaptation Kunnemann and Precht 1975; Grigo 1975; Bashamohideen 1984; In most of the animals including fishes blood being the medium of internal transport that comes in direct contact with various organs and tissues of the body. The physiological state of animal at a particular time was reflected in its blood.

Studies on blood parameters have become an important diagnostic tools in medicine over many years and they equally serve as diagnostic indices to investigate disease or stress in fish (Bensol et al,1979). Studies on fish haematology have drawn a new attention with reference to its basic physiology and also haematological responses to environment (Siddique et al 1970; Rao and Bohra,1973; Dub and Dutta,1974; Raizada and Singh,1982; Bashamohodeen,1984;) analysis of hematological parameters is one of the most valuable modern diagnostic tools to understand fish health. Recently, Anver (2004) established that the physiological values of hematological parameters are species specific and age dependent. With this background an attempt is made in this paper impact of thermal stress on the RBC numberof Indian major carp Catla catla (Hamilton)

Haemoglobin content of the fish adapted to 22°C and 32°C was measured separately and it was continued till the attainment of constant level in Haemoglobin content .These 22°C and 32°C fishes were re-adapted separately in the following pattern.

(1) The 22°C adapted fishes were re-adapted to a slow temperature change at the rate of 1°C/60hrs from a temperature range of 22°C to 32°C for a period of 35 days (heat-adaptation)

(2) The 22°C adapted fishes were re-adapted to an abrupt temperature change at the rate of 1°C/hr from a temperature range of 22°C to 32°C for a period of 35 days (heat-stress)

(3) The 32°C adapted fishes were re-adapted to a slow temperature change at the rate of 1°C/60hrs from a temperature range of 32°C to 22°C for a period of 35 days (cold-adaptation

(4) The 32°C adapted fishes were re-adapted to an abrupt temperature change at the rate of 1°C/hr from a temperature range of 32°C to 22°C for a period of 35 days (cold-stress)

RBC NUMBER

METHOD

In order to differentiate the stress phenomenon (heat-stress and cold-stress) from the adaptation process (heat-adaptation and cold-adaptation) the RBC count was determined in the carp, Catla catla in 22°C and 32°C adapted fishes (control fishes). The RBC number was counted separately in fishes subjected to a slow-temperature change and to rapid temperature change, both towards heat and cold, for a constant period of 35 days. The RBC count was made with neubeuer crystaline counting chamber as described by Devidson and Henry (1969). The RBC number (million/mm) was determined in six individual fish samples and mean of six is taken for the account. The procedure adapted for the counting of RBC number is as follows.

The blood was collected through the caudal incision and the blood was diluted with Hayem's fluid (5 gm of sodium sulphate, 1 gm of sodium chloride and 0.5 gm of mercuric chloride dissolved in 200 ml of distilled water and the RBC number was represented in millions per cubic millimeter (mm). The blood was drawn upto 0.5 mark in the RBC pipette by caudal incision of the fish and immediately the Hayem's fluid was taken upto "0" mark. The blood was mixed thoroughly by rotating the pipette and the mixture was allowed to stand for about 2-3 minutes for uniform mixing. The counting chamber and cover glass were cleaned and the cover glass was placed over the fortified area. Again the solution was expelled, and a drop of fluid was allowed to flow under the cover slip handling the pipette at an angle of 40°. It was allowed to stand for 2-3 minutes till the RBC settled in. The fortified area of the counting was focused under the microscope, and the number of RBC was counted in five small squares of the RBC columns (The RBC were counted in. the four comer squares and the central square), under the high power. The number of RBC was calculated and RBC count was represented in-million.-percubic millimeter (mm3). _

$$\frac{\text{NO. OF CELLS X DILUTION FACTOR (200) X DEPTH FACOTRS (50)}}{\text{AREA COUNTED}} = \text{MILLIONS/mm3}$$

FIGURE - 21

Histograms showing the levels of R.B.C. number (millions/mm^3) in _Catla catla_ adapted to 22°C and 32°C temperatures. Each histogram is a mean of six individual measurements.

FIGURE - 22

R.B.C. number (o-o) (millions/mm^3) in _Catla catla_ subjected to an abrupt temperature change from 22°C to 32°C (Heat-stress) at the rate of 1°C/hr. Each point is a mean of six individual measurements. Vertical bars represent standard deviation.

FIGURE - 23

R.B.C. number (o-o) (millions/mm^3) in _Catla catla_ subjected to slow temperature change from 22°C to 32°C (Heat-adaptation) at the rate of 1°C/60 hrs. Each point is a mean of six individual measurements. Vertical bars represent standard deviation.

Fig. 21

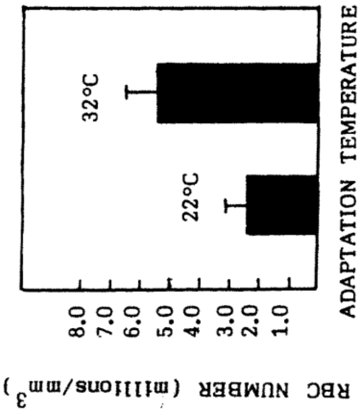


Fig. 22

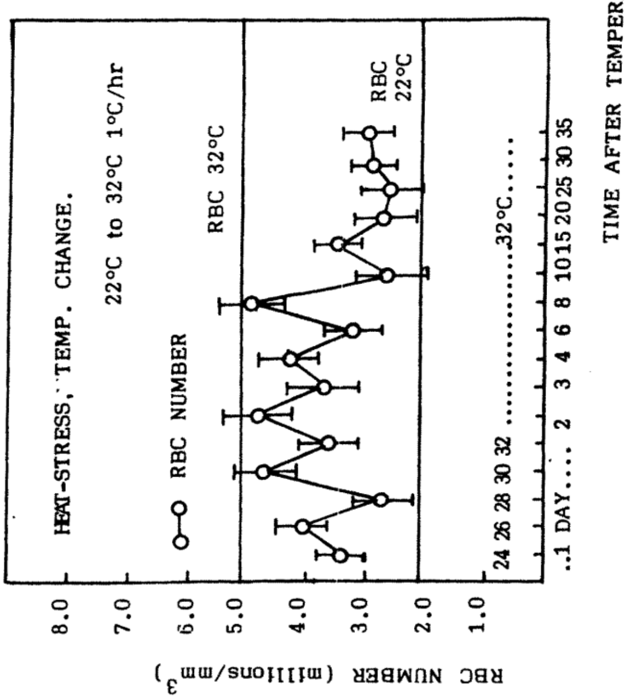


Fig. 23

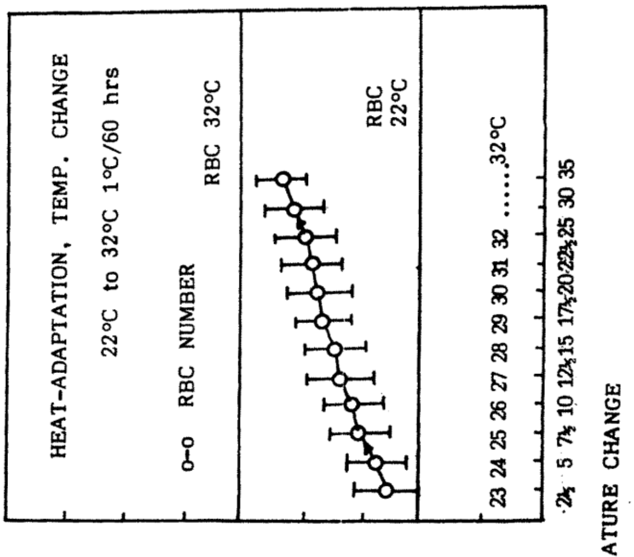


FIGURE - 24

Histograms showing the levels of R.B.C. number (millions/mm^3) in *Catla catla* adapted to 32°C and 22°C temperatures. Each histogram is a mean of six individual measurements.

FIGURE - 25

R.B.C. number (o-o) (millions/mm^3) in *Catla catla* subjected to an abrupt temperature change from 32°C to 22°C (Cold-stress) at the rate of 1°C/hr. Each point is a mean of six individual measurements. Vertical bars represent standard deviation.

FIGURE - 26

R.B.C. number (o-o) (millions/mm^3) in *Catla catla* subjected to slow temperature change from 32°C to 22°C (Cold-adaptation) at the rate of 1°C/60 hrs. Each point is a mean of six individual measurements. Vertical bars represent standard deviation.

Fig. 24

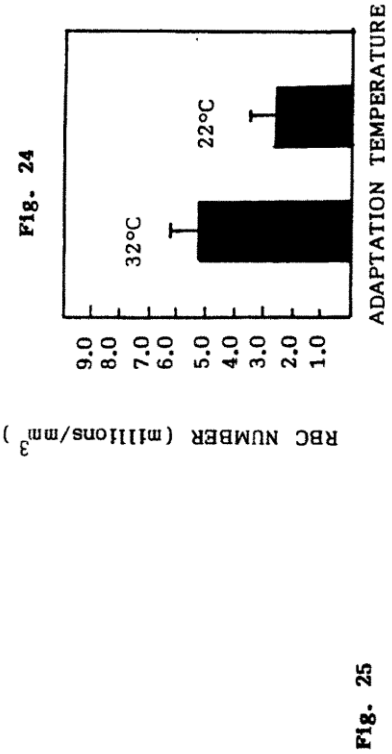


Fig. 25

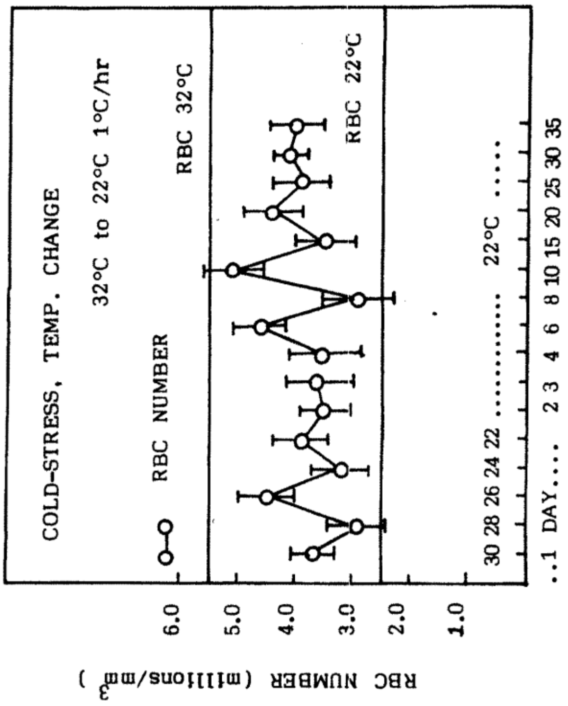
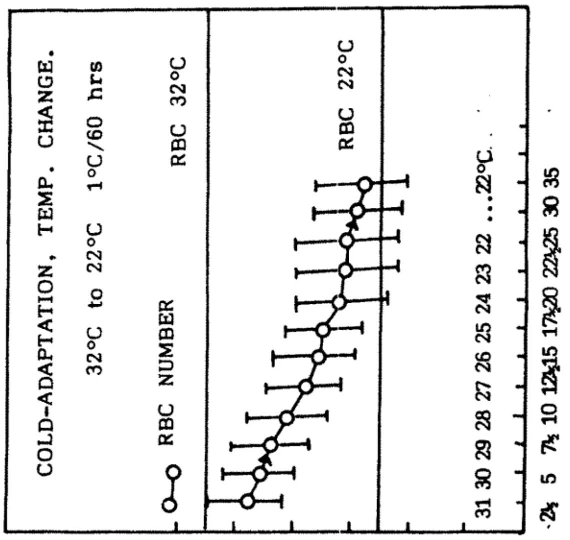


Fig. 26



TIME AFTER TEMPERATURE CHANGE

TABLE - 9

Comparison between heat-stressed, heat-adapted, cold-stressed and cold-adapted with reference to per cent change and per cent recovery in RBC count in *Catla catla* at 35 day exposure

RBC count	Per cent Change	Per cent Recovery
Heat-stressed	22.76	55.82
Heat-adapted	77.64	80.77
Cold-stressed	22.95	39.91
Cold-adapted	44.74	64.96

TABLE - 10

Comparison between heat-stressed and cold-stressed with reference to per cent change and per cent recovery in RBC count in Catla catla at 35 day exposure

RBC count	Per cent Change	Per cent Recovery
Heat-stressed	22.76	55.82
Cold-stressed	22.95	39.91

TABLE - 11

Comparison between, heat-adapted and cold-adapted with reference to per cent change and per cent recovery in RBC count in *Catla catla* at 35 day exposure

RBC count	Per cent Change	Per cent Recovery
Heat-adapted	77.64	80.77
Cold-adapted	44.74	64.96

RESULTS

The straight horizontal lines in figures 22,23,25 and 26 represent the rate of and RBC number in *Catla catla* in 22°C and 32°C adapted fishes which are taken as the controls. The RBC number was found to be higher in 32°C adapted control fishes than in the 22°C adapted ones (Figure. 23 and 26,Table-11)To differentiate the stress phenomenon from the adaptation process the fishes from 22°C adapted ones were readapted to 32°C with a slow rise of ambient temperature at the rate of l°C/60 hrs (Figure,23,) and the 32°C adapted fishes were re-adapted to 22°C with a slow change in ambient temperature at the rate of l°C/60 hrs (Fig. 26). The fishes from 22°C adapted ones were re-adapted to 32°C with a quick change of temperature at the rate of l°C/hr. The fishes from 32°C temperature adapted ones were re-adapted to 22°C with a quick temperature change at the rate of l°C/hr for a period of 35 days (Figure. 22, 25;). In Fig. 23, and 26 the two groups of heat-adapted and cold-adapted fishes which are subjected to a slow temperature change of l°C/60 hrs. Displayed a gradual change in the RBC number reached the original levels of control values of the 22°C and 32°C temperature adapted control fishes. On the other hand, the change in the RBC number is not gradual and reaching of the control values (22°C and 32°C adapted fishes) is not to be seen even after the period of 35 days in the fishes subjected to a quick temperature change at the rate of l°C/hr both towards heat from 22°C to 32°C as well as towards cold from 32°C to 22°C (Fig. 22, 25;). These heat-stressed and cold-stressed fishes however, established the new levels of the RBC number, and continuous stress operating on the fish, resulted in the stress adaptation (cold and heat). The per cent change and per cent recovery in these two parameters are relatively much higher in temperature - adapted fishes than the temperature—stressed ones (Table 9). The per cent recovery is relatively higher in heat-adaptation than in cold-adaptation (Table 11).

DISCUSSION:

Studies on haematological parameters have came into increasing used in assessing the condition of fishes and their response to environmental changes (Baynee et al., 1980). It is 68 known that, the blood glucose level corresponds to the standard metabolic rate (Umminger, 1977). It is also known that, the rate of oxygen consumption varies with the number of erythrocytes present and RBC account of 99% of oxygen uptake in the fishes (Lagler et al., 1977). In the present investigation, RBC number are found to be low in the 22°C temperature adapted fishes than in 32°Ctemperature adapted ones. In the re-adaptation experiments, subjected to a slow temperature change at the rate of l°C/60 hrs from 22°C to 32°C (heat-adaptation) the decreased rates of the RBC number of the basic levels 22°C temperature adapted fish gradually exhibited a gradually stepping up in these parameters are reached the levels of the controls of 32°C temperature adapted fishes. Thus, bradycardia with a low level of RBC number indicated lesser energy demand is eliminated in these adapted fishes when compared to stressed fishes. In the case of cold-adaptation, when the fishes were subjected to a slow temperature change from 32°C to 22°C at the rate of l°C/60 hrs there is a gradual stepping down in RBC number reaching the control values (i.e. 22°C temperature adapted control fishes). There is a fairly good amount of per cent recovery in both the heat-adapted and cold-adapted fishes. In the case of heat-adaptation the per cent recovery is for the **80.77%** RBC number, whereas in the case of cold-adaptation, it is recorded as for the **64.96 %** recovery for the RBC number the per cent recovery is relatively higher in the case of heat-adaptation than that of cold-adaptation (Table -9). Thus in temperature-adapted fishes, there is fairly good amount of recovery in the RBC number, suggesting that, a very slow temperature change enables the fish to adapt to a new temperature without physiological load on the part of the fish as seen in its almost complete recovery. RBC number, exhibited in a different way in the fishes subjected to a rapid temperature change as against the changes observed in the adapted fishes. In these stressed fishes (heat-stressed and cold-stressed) there is no gradual stepping up/down of the RBC number reaching of the control levels were not observed even after 35 days. In these stressed fishes, there are many fluctuations during the time course in both heat-stressed and coldsp-tressed fishes, and the continuous stress acting upon the fish *Catla catla* resulted towards a phase of stress-adaptation with relatively very less amount of per cent recovery (The per cent recovery during heat-stress **55.82%** recovery for the RBC number but In the process of cold-stress It Is recorded **39.91%** recovery for the RBC number . Thus the RBC number Involving re-adaptation experiments Indicate that, temperature acts as a "stress" and stress Is a physiological load acting upon the fish, Catla catla when exposed abruptly at the rate of l°C/hr from a temperature of 22°C to 32°C (heat-stress) and from 32°C to 22°C (cold-stress). During the stress situation the phenomenon of bradycardia Is retained as against its removal in temperature adapted fishes. The condition of bradycardia which has been noticed In the stressed fishes, leads to a decreased the velocity of blood circulation and volume of blood brought to the gills which in turn lowers the general metabolism of the fish. Thus, a very high level of per cent recovery in RBC number) is reflected in corresponding to high per cent increase in the same parameters, during adaptation process as against the stress condition in this fish *Catla catla*. Thus relatively low per cent recovery in RBC as observed in thermally- stress when compared to thermally—adapted , Catla catla might have been responsible for a decreased RBC number due to haemolysis as it is reached in a similar observation (Wood ward at al, 1979} where accelerated fibrinolysis in fingerlings of Coho Salmon subjected to decomprission stress and subjected‘that the rate of lysis might give some indication of the severity of stressors affecting the haemostatic system as indicated in the present study. Therefore, monitoring blood component like RBC can also provide information on the state of fish during stress situation

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