

Impact of exposure of cold temperature on hematological parameters and plasma electrolytes of cold water fish *Shizothorax richardsonii*

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Abstract: The present study was conducted to determine the cold stress symptoms in fish blood during long term exposure of cold temperature stress condition. Blood is an indicator of physiological and hematological condition of an animal. Therefore, here we investigate the hematological parameters and plasma electrolytes level of wild population of *Shizothorax richardsonii* in cold stress condition. The following aspects were evaluated in blood: hemoglobin content, red blood cell, white blood cell and in plasma: Na^+ , K^+ level. For this purpose we designed the experiment and *Shizothorax richardsonii* divided in group I and group II. In group I, fishes were held in 5-6 °C and in group II; fishes were held in ambient temperature over a period of 96 hours. The effect of cold temperature on RBC, WBC, HB and Na^+ , K^+ level were determined in blood and plasma sample at interval of 24, 48, 72, 96 hours. Our result shows that RBC, WBC, HB content decrease in blood and Na^+ , K^+ level in plasma was significantly increase in the group I (5-6 °C) then group II.

Keywords: Fish; Cold Stress; Blood; hematological parameters; Plasma electrolytes

I: Introduction:

Snow trout (*Shizothorax richardsonii*) is an indigenous cold-water fish with large body size and restrictedly distributed in Himalayan and sub Himalayan streams, rivers along Jammu & Kashmir, Himachal Pradesh, Assam and Sikkim in India. Beside India, this species is also distributed in Bhutan, Nepal, Pakistan and Afghanistan (Talwar and Jhingran, 1991). They remain very active and forage for food (Sahoo et al., 2009; Sharma et al., 1989). During winter time they can sustain a temperature as low as 5-6°C and summer they thrive at 20-22°C.

Temperature is known to affect the enzyme reaction, growth efficiency, reproduction and immune response (Tanck et al 2000). Temperature also changes the hematological parameter and plasma electrolytes. Hematological parameters are good indicator which used in fish to assess the health of fish and monitor stressful effects in various conditions such as exposure of stress like toxicant, cold and so on. (Dick 1985, El-Domiaty 1987, Iwama 1986, McLeay 1973, McLeay 1975, McLeay 1977, Shah 2004, Wedemeyer 1983) Plasma electrolytes such as Na^+ , K^+ level can be used as blood osmolality stress indicators in fish (Eliassen et al., 1960 and

Doudoroff 1945). In fish cold temperatures increase the oxygen requirement, cardiac output, and blood flow and may result in increased pulmonary arterial pressure overload on the right ventricle (Julian et al., 1989). A number of studies have been carried out to determine the factors such as temperature of the environment, age, sex, and nutritional and reproductive status of the fish can modify the expression and activity of the detoxification enzymes, which could complicate the interpretation of induction responses (Jiménez and Stegeman 1990). Alternatively, limited studies are available on the hematological parameters in cold stress condition of wild population of fish. Although, the aim of my study was to determine the effects of cold stress on hematological changes and plasma electrolytes level (Na^+ , K^+) in *Shizothorax richardsonii*.

II: Material and Methods:

Experimental animal

Random live fish samples of *Shizothorax richardsonii* were collected from the river Kosi, near Almora, Uttarakhand using casting net and transferred to the laboratory where the experiment were conducted. The fishes were put in large aquarium and acclimated in the water for 7 days immediately before the exposure experiment.

Experiment design

Shizothorax richardsonii average body length and weight at the beginning of the experiment were 15.5 (14-16cm) and 31.5 (28-35 gm) respectively. The fish were equally distributed in two glass aquarium each filled with 20 liter water. The tanks were aerated with continuous aerator. One aquarium was maintained at approximately 5-6° C by adding ice flask and other at ambient water temperature.

Sample collection and preparation

At the end of experiment no changes observed in length and weight of fish. After 24, 48, 72, 96 hours exposure period sampling of blood were carried out. Blood samples collected using heparin zed syringe, half part of blood store in 4°C for hematologic study and half part of blood centrifuge at 5000 rpm for 10 min at 4°C. Plasma was frozen at 20°C until used for plasma electrolytes study.

Analytical techniques :

Determination of red blood cell

Total red blood cell (RBC) was counted immediately after blood collection in improved Neubaur haemocytometer (Shah and Altindag 2004a). Mix the blood thoroughly then taken in red pipette and diluted 1:200 with Hayem's fluid (Mishra et al., 1977) One drop of blood was loaded in haemocytometer chamber. RBC was counted and reported as $10^6/\text{mm}^3$ (Wintrobe 1934).

Determination of white blood cell

Total white blood cell (WBC) was counted immediately after blood collection in improved Neubaur haemocytometer (Shah and Altindag 2005; Mgbenka et al., 2003). Mix the blood thoroughly then taken in white pipette and diluted 1: 20 with Turk's diluting solution and loaded in 4 large corner squares of the haematocytometer. WBC was counted under the microscope at 640 X and reported as $10^6/\text{mm}^3$ (Wintrobe 1967).

Determination of Hemoglobin

For estimation of HB, routine Sahil's method was employed, by adding 20 μl of blood to 5 ml of Drabkin's solution using spectrophotometer at a wavelength 540 nm (Blaxhall & Diasley 1973).

Determination of sodium and Potassium level

Sodium and Potassium in the plasma were determined directly using a colorimetric detection kit (Coral Clinical Systems) at 530 nm and 630 nm respectively after 5 minutes incubation at room temperature.

Statistical Analysis:

Values of cold stress group were compared statistically with control group using Paired student t test. Significance was established at $P < 0.05$ using SPSS software.

III: Result:

Total Red blood cell count

The Red blood cell (RBC) count of controls showed a mean value $2.31, 10^6/\text{mm}^3$. The fish exposed to cold temperature showed as mean value of RBC 1.88, 1.86, 1.81, 1.79, $10^6/\text{mm}^3$ at 24, 48, 72, 96 hours

respectively. The exposure of cold temperature was found to be decrease number of RBC. The exposed of cold temperature at 24, 48, 72, 96 hours decrease RBC level showed in fig 1. The values showed a significantly decrease when compared to the control ($P < 0.05$).

Total White blood cell

The White blood cell (WBC) count WBC cell revealed that the blood of control fish showed a mean value $1.55, 10^6/\text{mm}^3$. The fish exposed to cold temperature showed the mean of WBC 1.50, 1.45, 1.42, 1.40, $10^6/\text{mm}^3$ at 24, 48, 72, 96 hours respectively. Fig 2. The values mentioned showed a significantly decrease when compared to the control. ($P < 0.05$)

Estimation of Hemoglobin content

The control fish showed value of 10.56 gram% for hemoglobin. Fishes were exposed to cold temperature showed the mean value of hemoglobin 9.38, 9.34, 9.31, 9.29, gram % at 24, 48, 72, 96 hours. Fig 3. The value for temperature showed a significantly decrease when compare to control ($P < 0.05$).

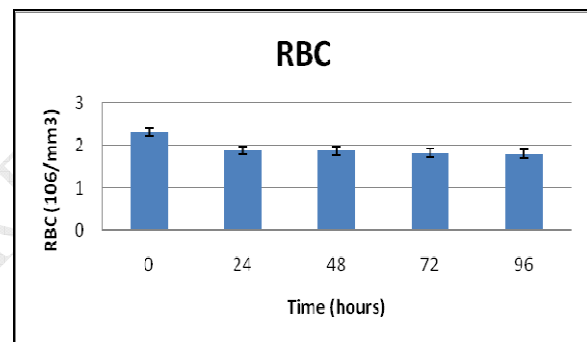


Fig 1. Blood RBC count at cold temperature in *S. richardsonii*.

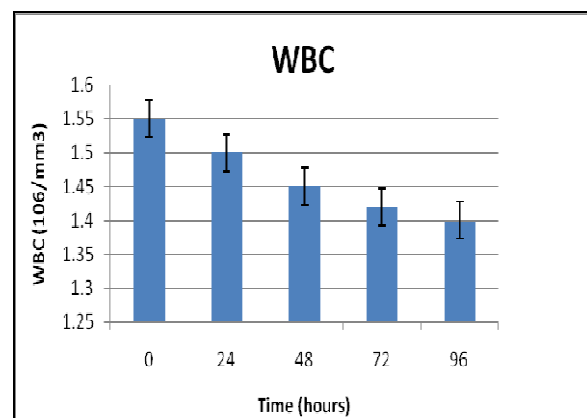


Fig. 2 Blood WBC count at cold temperature in *S. richardsonii*

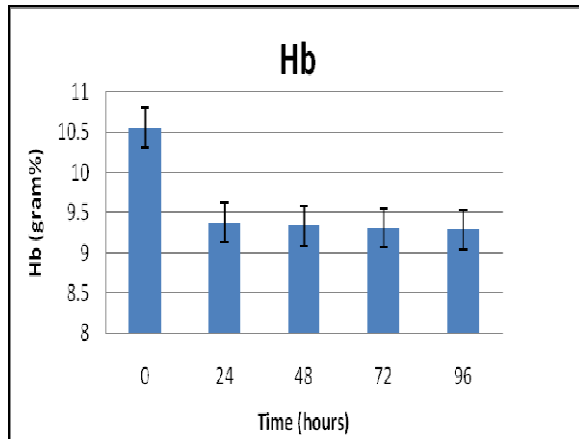


Fig 3. Hb count at cold temperature in *S. richardsonii*

Sodium and Potassium level

In Plasma sodium & potassium ion concentration showed a mean value of sodium 50.29 mmol/l and Potassium 2.27 mmol/l. The fish exposed to cold temperature showed a mean value of sodium as 61.25, 72.39, 81.26, 91.72mmol/l and mean value of potassium as 4.44, 7.34, 10.22, 14.17 mmol/l at 24, 48, 72, 96 hours respectively. Fig 4, 5. The value mentioned showed a significantly increase when compare to a control ($p < 0.05$).

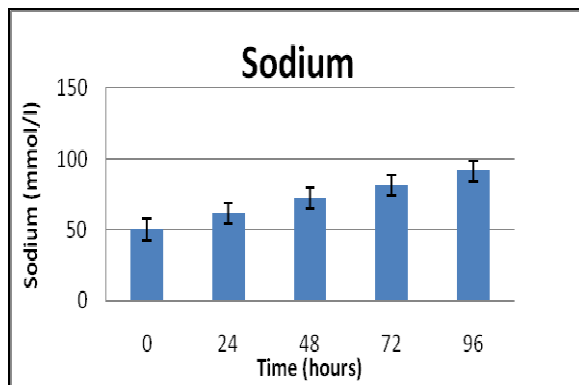


Fig.4. Plasma sodium level at cold temperature in *S. richardsonii*

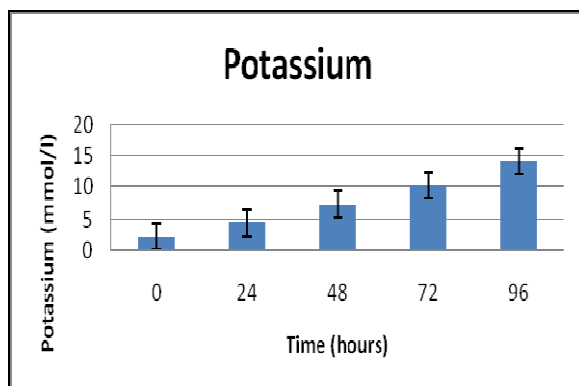


Fig 5. Plasma potassium level at cold temperature in *S. richardsonii*

Water temperature, insufficient food and low diet have been found to effect on fish growth (Fine. *et al.*, 1996). It is one of the major factors responsible for individual variation in fish hematology. Hematological indices are used to assess the functional status of the oxygen carrying capacity of the blood stream (Shan and Altindag 2004a) and assess the physiological and biochemical status of fish under the stressed condition (Fernandez and Mazon 2003). In recent years hematological indices have been used to determine the effect of stress (Wedemeyer and Yasutake, 1977). Hemolysis has been reported in common carp (*Cyprinus carpio*) during acute water temperature changes from 8°C to 4°C (Chen *et al.*, 1995).

The result of the present investigation show that the effect of cold temperature on blood parameter of *Shizothorax richardsonii* decreased in RBC, WBC and HB content. This result is supported by (Maxwell). RBC, WBC and HB decreased in cold temperature due to mechanism in which compensate for poor oxygen uptake in prevailing hypoxic condition. Cold temperature increases the oxygen requirement (Julian *et al* 1989). Water temperature decrease, the osmotic concentration and blood viscosity increase. Decrease in hemoglobin could be result of blood osmoconcentration, as shown by an increase in plasma osmolarity. HB level connected with number of RBC. When the number of RBC decreased, HB level decreased. The number of WBC one of the important indicator of health in fishes. When temperature decreased number of WBC decreased. Decreasing trend of HB during the cold temperature has been reported in many species (Chen. *et al.*, 1995 and Staurnes *et al.*, 1994). *Shizothorax richardsonii*, survive at low temperature by concentration of Na⁺ and K⁺ in plasma. Water temperature decrease, increase the osmotic pressure, increase Na⁺ and k⁺ content in plasma. Sodium and Potassium level increased during the initial decreased in water temperature in fish. (Doudoroff, 1945).

The present result demonstrate that *Shizothorax richardsonii* show the stress response to cold shock. The measuring of hematological parameter and plasma electrolytes, which are used in this study, has provides valuable information. This technique has provided valuable knowledge for fish health in monitoring stress response.

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VI: Discussion

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