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Effect of Stress on Haematological Parameters of Air Breathing Loach *Lepidocephalus thermalis* (Cuv&Val)

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Haematological parameters, Air Breathing Loach, *Lepidocephalus thermalis*

A B S T R A C T

It is well know that certain blood parameters serve as reliable indicators of fish health. So, in the present investigation we focus on hematological parameters as indicator for evaluation of the stress. Potential stressors that cultured fish are subjected to may be acute or chronic. So, same size and equal number of fish *Lepidocephalus thermalis* divided five tanks, two tank of fishes are exclusively aerial condition at 5h and 10h air exposure , another two tanks are5h and 10h submerged condition and next one is control groups. After that both 5h and 10h air exposure, also 5h and 10h submergence fishes were anaesthetized, blood samples and tissue sample were obtained from the control and experimental fish. Blood was assayed for selected hematological parameters ((RBC, WBC, Hct, Hb, MCV, MCH, MCHC, O_2 capacity, glucose and lactic acid) tissue sample were assayed glucose, glycogen and lactic acid. From the experimental group MCV, MCH, MCHC of blood content, liver and muscle tissue glycogen were depleted and other parameters are significantly increased. The result from this study reveals high mortality rate and deleterious consequences on the health of fish subjected to acute air exposure and submergence of fish *L. thermalis* physical stresses failed to evoke abnormal changes in the haematology and biochemistry. These modulations may bring some useful information in future aquaculture.

Introduction

Now a day's there are changing environmental factors leads to be affect the fish health by stress. Stress of the fish is nonspecific response and the fish need any demand made upon it. Stress in fish may be induced by various abiotic environmental factors such as changes in water temperature, pH, oxygen concentration and water pollutants including pesticides, insecticides (Meier et al., 1983; Lebelo et al., 2001). Biotic interactions such as predator pressure, parasitic invasions or strong competition with other organisms or among the fish in overcrowded areas and by human activities related to fish rearing and harvesting (manipulation, transport) can also

be a source of stress to fish (Witeska, 2005). Stress reaction involves various physiological changes including alteration in blood composition and immune mechanisms. Stress also induces changes in blood cell numbers and activities. An increase in red blood cell count and volume, and hemoglobin level usually has been reported in fish subjected to stress (Wendelaar Bonga, 1997) and decrease in white blood cell count, especially of lymphocytes usually occurs in fish subjected to stress (Ellsaesser and Clem, 1986). There are several definitions; most of them refer to an "altered state" which increases the energy demand. The response to stress in fish is characterized by the stimulation of the hypothalamus, which results in the activation of the neuroendocrine system and subsequently metabolic and physiological changes (Wedemeyer et al., 1990, Lowe and Davison, 2005). Under conditions of stress, the body of fish emits immediate responses recognized as primary and secondary responses. The primary response is the perception of an altered state by the central nervous system (CNC) and the release of the stress hormones, cortisol and catecholamines, into the blood stream by the endocrine system (Randall and Perry, 1992). Secondary responses occur as a consequence of released stress that causing changes in blood and tissue chemistry (Martinez-Porchas et al 2009). Tertiary or whole animals responses and measured by parameters such as growth, metabolic rate, and disease resistance.

Cortisol is the principal corticosteroid in teleost fishes and its concentrations in blood rise dramatically during stress (Mommsen, et al., 1999). On the other hand, the hematological parameters of fish can be used as indicators of physiological conditions and monitoring diseases and the stress caused by handling (Sebastiao et al., 2011). The study of blood parameters in fishes has been widely used for detection on physio-pathological alterations in different conditions of stress. The stress responses vary between fish species, the developmental stage of the fish, as well as between individuals within the same species (Pottinger at al., 1994). An important consideration inherent in these studies is the hyperglycemia usually returns to normal levels within 24 hours. Another closely related phenomenon is "asphyxiation hyperglycemia" (Natarajan, 1987) this occurs whenever fish are removed from water and the blood sugar level starts increasing within 15 minutes.

Water quality at the stocking locations may be extremely different from that in which fish were captured, and exposure to changes in environmental conditions often induces stress response in fish. Air-breathing fishes adopt various strategies to counter stress caused by environmental disturbances (Natarajan et al., 1987). There have been a number of studies on the physiological responses of air breathing fishes submerged in both anoxia and normoxia at various levels and durations. In those capable of aerial gas exchange in normoxic water, such as *Anabas scandens* (Natarajan, 1972) and *Channa striatus* (Rani, 1994) are also the least tolerant of submergence in normoxic water. For all the species, in normoxic submergence, the main problem is severe lactic acidosis, and it is the rate of lactate accumulation to which all other changes are keyed (Senguttuvan, 1996). Some plasma chemicals may be useful tools to evaluate the stress condition of the fishes (Campbell 2004). Because stress has been reported to elevate plasma cortisol (Pottinger *et al.* 2003, Haukenes *et al.* 2008**)** and glucose levels (David *et al.* 2005). However, there is a considerable range of tolerance to prolonged submergence among air-breathing fishes (Devika, 1999).

Lepidocephalus thermalis lives in fresh water rivers; it lives a normal life even in water having very little dissolved oxygen. Though some information is available on the respiratory patterns of *L. thermalis* (Natarajan, 1981), virtually no information is available on the air-exposure and submergence stress on the haematology and blood metabolites of this fish. In the present work, an attempt has been made to study the aerial exposure and submergence stresses on the haematology and tissue metabolites.

Materials and methods

*Lepidocephalus thermalis*is an air-breathing loach captured from palani hill streams using cast net and brought to the laboratory and maintained cement tanks (60x30x30cm) for over two months before using them in any of the experiments. There were regularly fed with boiled hen's eggs. The cement tanks were supplied with a continuous flow of tap water (Temp:28^{o}; hardness: 247 ppm as Ca Co_3; pH 7.6 ± 0.2; alkalinity 4.1 mmol/lit).

After the acclimatized period is over each individual weight was measured. 12 fishes in the weight ranges from 12-15g (average wt=13.5g) were kept under submerged water condition preventing surfacing for 5h and 10h. Again 12 fishes between 11.5-15.5g body wt (average 13.75g) were kept exclusively on aerial conditions for 5h and 10h. Immediately after the stress, they anaesthetized for 3min in a trough containing well aerated water and 100mg/l un-buffered MS-222 (Sandoz).

Blood sample collection

When inactivated the fish blood samples (0.2-0.3 ml) was collected from the exposed heart into a disposable syringe (No 16, 0.6x26mm) and the dead volume of the syringe were filled with about 0.08ml isotonic saline with a Na heparinate content of 50mg/ml which is prevent clotting. The samples were concluded within 2min, even if the size of the sample was not yet 0.5 ml. After thorough mixing in the syringe the blood was centrifuged and plasma stored at 20^{o}C until analyzed. Control fish were maintained under identical condition.

Tissue sample collection

Control and stressed fishes were killed as described above and liver and dorsal muscle tissues were rapidly excised and were processed for estimations of glucose, glycogen and lactic acid after homogenizing saline and centrifuged with 3000 rpm for 15min.

Red and white blood cell counts made were made using methods outlined by Hesser (1960) by using improved Neubaur haemocytometer. Heamoglobin content was estimated by Sahil method (Dacie and Lewis, 1969). Haemoglobin is converted to acid haematin by the action of hydrochloric acid.

The acid haematin solution is further diluted with the acid until its colour marches exactly that of the permanent standard of the comparator blank. The haemoglobin concentration is read directly from the calibration tube.

Haematocrit (Hct) (or) packed cell volume was determination followed by the method of Blaxhall and Daisely (1973). Haparinized capillary tubes were centrifuged for 10 minute at 3500rpm and percentages packed formed elements were read on a clay-Adams haematocrit reader. All Hct measurements were made immediately following 10 minutes of centrifugation.

Mean cell haemoglobin was calculated by the formulae

$$MCH\ (pgm) = \frac{Haemogloin\ (g/100^{-1}/10)}{RBC\ x(10^{-6}Cmm^{-1})}$$

Mean cell volume (MCV) was calculated by

$$MCH\ (\mu l\ x\ 10^{-9}) = \frac{Haematocrit\ (\%)x10}{RBC\ x(10^{-6}Cmm^{-1})}$$

and

Meancell Heamoglobin Concentration (MHCH) was calculated by

$$MCHC\ (\%) = \frac{Haemoglobin\ (g/100ml^{-1})\ x100}{Haematocrit\ (\%)}$$

O_2 Capacity was obtained bymultiplying the Hb content with the O_2 combining power of 1.25ml of O_2 per gram of haemoglobin (Johnsen, 1970).

Blood glucose was estimated by Folin-wu method and Blood lactic acid was analyzed by the enzymatic technique by Oser, 1976.Measurements were made in quartz cuvettes at 340nm with Bosch and Lomb spectrometer. Standard curves were made on the day of blood lactic acid determination. Tissue lactic acid were determined by the tissue were homogenized in TCA(7%) at 4^{o}C and centrifuged at 5000rpm (Long and Michal, 1974). Lactic acid concentration was measured spectro-photometrically (340nm) by means NADH produced in an enzymatic reaction with LDH (Oser, 1976).

Liver and muscle carbohydrate metabolites

Stressed fish were quickly killed liver and muscle tissues were removed and the metabolites weredetermined. Glycogen content was estimated with anthrone method (Seifter et al., 1950). liver and muscle tissues were homogenizes separately by adding 0.5ml KOH (60%) and 1ml KOH (30%). The mixtures were kept in boiling water bath for 30 min. 4ml of aliquot ethanol was added to the homogenate, stored into the fridge. Next day samples were centrifuged and the supernatant was saved for glucose determination. The pellets were re-suspended in 1ml of water and 0.25ml of aliquot ethanol was mixed with 1.75 ml of anthrone reagent for 15min at 100^{o}C. Glycogen and Glucose content was determined spectrophotometrically (Oser, 1976).

Statistics of the data was subjected to computer analysis (casio fx-100 vl Super – fx) following students‘t’ (Fischer, 1950).

Result and Discussion

The results obtained from the present investigation clearly shows that the fish *L. thermalis* at 5h air exposure stress, blood parameters were increased marginally except MCH and MCHC (Table. 1). However WBC, blood lactic acid and glucose increased significantly (ρ>0.05). While liver and muscle lactic acid and glucose content also increased (ρ>0.05) and the glycogen concentration reduced significantly.

In the 10h air exposure of stress of the fish *L. thermalis* blood parameters shows WBC, blood lactic acid, glucose and RBC alone increased slightly. The increase of Hct, Hb and O_2 capacities were insignificant. However, liver carbohydrate metabolites alterations of 10h stress similar to 5h stress, but muscle glucose and lactic acid of 10h stress compared to 5h stress very lesser percentage was increased and the glycogen content was decreased.

In the table.2 blood parameters of 5h submergence of stress blood lactic acid, MCHC, WBC, glucose, Hct and RBC level was significantly increased but the O_2capacity, Hb and MCV were insignificant and MCH declined minimally. At the same time 10h submergence of stress blood lactic acid, WBC, RBC, Hct, glucose and O_2 capacity increased significantly, but MCH, and MCV declined insignificantly. In the liver and muscle analysis of 5h and 10h submergence of stress more accumulation of lactic acid and glucose but severe depletion of glycogen content. (Table: 3 and 4).

Aquatic organisms are frequently exposed to stressors under different conditions like crowding culture, transport, loading and unloading, netting or pumping, Contamination / pesticide pollution etc., which cause a series of physiological responses, known as stress, which are divided in primary, secondary and tertiary responses (Wedemeyer, G.A., 1996, Barton, B.A., 2002). Some secondary effects of hormones, such as hyperglycemia, increase of total protein, hematological and physiological changes are important parameters to assess the fish health conditions. (Gusmao Affonso, et al, 2007). The hematocrit percentage, hemoglobin rate, MCV, RBC and WBC count are good indicators for oxygen transportation capacity of fish thus making it possible to establish relationships with the oxygen concentration available in the habitat and the health status of these fish (Lamas, J.Y, et al., 1994). Before stress the hematocrit, hemoglobin, MCH, MCV, MCHC and O_2 capacity values showed that significant results and after stress of fish these values are insignificants (Najafpour Babak. et al, 2012,). Now in the present results that air-exposure dares submergence cause adverse effects on *L.thermalis.* In our finding where similar results were observed for these types of stresses in *Macropodus cupanus (*Natarajan, 1987) and *channa striatus* (Rani, 1994). On the facultative air breathers have limited capacities to withstand the aerial stresses (Vijaylakshmi, 1996); *L.thermalis* being an obligate air breather (Natarajan, 1981) tries to modulate the heamatological and carbohydrate metabolites as much as possible to overcome the air exposure and submergence stresses. This is clearly visible from the changes seen in our factors.

The use of haematological parameters as indicators of fish health was proposed by Hesser (1960). Since then, researchers in their many studies have looked at the bio-chemical composition of fish blood. The effects of stress on the biochemistry of several teleosts have been studied under laboratory conditions. This work has included the effects of aerial exposure on the haematology (Vijayalakshmi, 1996) and fuel biochemistry (Senguttuvan, 1996). The use of haematological parameters as indicators of sub lethal effects of stress can provide information on the biochemical response fish make to changing external environment. This is a result of the close association of the circulatory system with the external environment and with every tissue.

Air exposure and submergence increase the RBC, Hb, and Hct at all periods of exposure for varying proportion in *L.thermalis.* In fish, erythrocytes and increase in Hb and Hct are regarded to be the result of impairment of gas exchange by gills (Natarajan, 1981) and the consequent “excitation” or “ stimulation” of erthropoiesis or “ compensatory erthropoiesis” (Ellis, 1977). However, this type of compensatory reaction usually stimulates erthropoiesis there by leading to the release of immature erythrocytes in the circulating blood (Ellis, 1977)

Table.1 Changes the haematological parameters of fish after 5h and 10h air – exposure.
Values are the mean±SD of 6 individual determinations

Hematological Parameter	5 h air exposed				10 h air exposed			
	Control	5h Air exposed	% change	ρ= 't' test	Control	10h air exposed	% change	ρ= 't' test
RBC (10^6/mm^3	2.80±0.71	3.06±0.82	9.29	>0.001	2.78±0.66	3.10±1.02	11.51	>0.001
Hb (g/100ml)	12.60±1.80	13.40±2.00	6.35	NS	12.81±0.90	13.20±2.20	3.04	NS
Hct (%)	31.50±3.42	35.20±2.90	11.75	>0.001	31.76±2.60	34.51±3.00	7.37	NS
MCH (ρg)	45.00±2.06	43.79±5.22	-2.69	NS	46.08±7.04	42.58±4.60	-7.6	NS
MCV(μ)^3	112.50±9.18	115.03±10.40	2.25	NS	114.24±10.52	111.32±8.05	-2.56	NS
MCHC (%)	40.00±5.06	38.07±4.19	-4.83	NS	40.33±3.71	37.67±5.66	-6.6	NS
O_2 capacity (Vol %)	15.75±3.21	16.75±2.98	6.35	NS	16.01±2.74	16.50±3.08	3.06	NS
WBC (mm^3)	12.64±0.43	16.51±1.82	30.62	>0.05	13.00±2.00	18.58±2.70	42.92	>0.05
Glucose (mg/100ml)	72.00±3.67	114.76±6.32	59.39	>0.05	69.08±9.00	98.00±8.10	41.86	>0.05
Lactic acid (mg/100ml)	41.20±2.82	69.00±5.40	67.48	>0.05	41.06±10.00	90.32±11.47	119.97	>0.05

Sign + or – indicate % deviation from the control, NS = Not significant

Table.2 Changes the carbohydrate metabolism of fish tissue after 5h air - exposure.
Values are the mean±SD of 6 individual determinations.

Parameter	5 h air-exposure							
	Liver				Muscle			
	Control	Experimental	% change	ρ= 't' test	Control	Experimental	% change	ρ= 't' test
Glucose	67.20±7.61	86.52±8.10	28.75	>0.05	71.40±8.06	91.36±7.50	27.90	>0.05
Glycogen	110.60±9.50	51.30±10.00	-53.62	>0.05	96.41±10.42	70.51±9.32	-26.86	>0.05
Lactic acid	0.32±0.01	0.60±0.02	87.50	>0.05	0.28±0.03	0.58±0.04	107.14	>0.05

Table.3 Changes the carbohydrate metabolism of fish tissue after 10h air - exposure.
Values are the mean±SD of 6 individual determinations

Parameter	10h air-exposure							
	Liver				Muscle			
	Control	Experimental	% change	ρ= 't' test	Control	Experimental	% change	ρ= 't' test
Glucose	68.41±6.58	76.30±6.12	11.53	>0.001	70.14±7.10	81.60±5.18	16.34	>0.01
Glycogen	114.30±10.40	67.20±7.10	-41.21	>0.05	95.30±9.17	80.31±10.20	-15.17	>0.01
Lactic acid	0.30±0.01	0.46±0.02	53.33	>0.05	0.29±0.02	0.42±0.03	44.83	>0.05

Table.4 Changes the haematological parameters of fish after 5h and 10h submergence.
Values are the mean±SD of 6 individual determinations

Hematological Parameter	5 h submerged				10 h submerged			
	Control	5h submerged	% change	ρ= 't' test	Control	10h submerged	% change	ρ= 't' test
RBC (10^6/mm^3	2.80±1.02	3.10±1.41	10.71	>0.001	2.76±0.99	3.26±1.76	15.94	>0.01
Hb (g/100ml)	13.00±2.01	14.00±2.28	7.69	NS	12.90±2.00	14.06±2.10	8.99	>0.001
Hct (%)	31.62±2.50	35.80±3.00	13.22	>0.01	32.00±3.00	36.20±2.05	13.13	>0.001
MCH (ρg)	46.43±7.60	45.16±6.00	-2.74	NS	46.74±6.04	43.13±7.11	-7.72	NS
MCV(μ)^3	112.93±11.40	115.48±9.52	2.26	NS	115.94±9.18	111.04±5.92	-4.23	NS
MCHC (%)	28.09±4.10	39.11±5.02	39.23	NS	40.31±4.17	38.84±7.06	-3.65	NS
O_2 capacity (Vol %)	16.25±2.10	17.50±3.05	7.69	NS	16.13±2.08	17.54±3.91	8.99	>0.001
WBC (mm^3)	12.90±3.40	19.80±3.16	37.98	>0.05	13.01±3.50	18.62±4.00	43.2	>0.05
Glucose (mg/100ml)	70.00±4.57	94.00±6.02	34.29	>0.05	68.40±5.17	76.41±6.71	11.7	>0.001
Lactic acid (mg/100ml)	40.40±5.00	59.00±3.40	46.04	>0.05	40.68±8.30	67.60±8.00	66.05	>0.05

Sign + or – indicate % deviation from the control, NS = Not significant

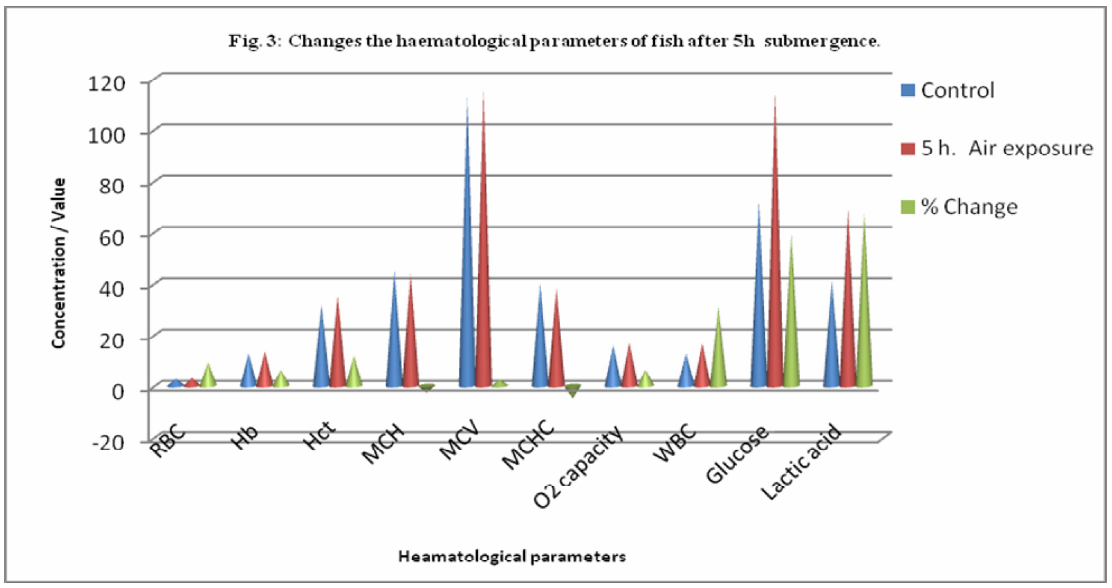
Fig. 3: Changes the haematological parameters of fish after 5h submergence.
120
100
80
60
40
20
0
-20
Concentration / Value
Control
5 h. Air exposure
% Change
RBC
Hb
Hct
MCH
MCV
MCHC
O2 capacity
WBC
Glucose
Lactic acid
Heamatological parameters

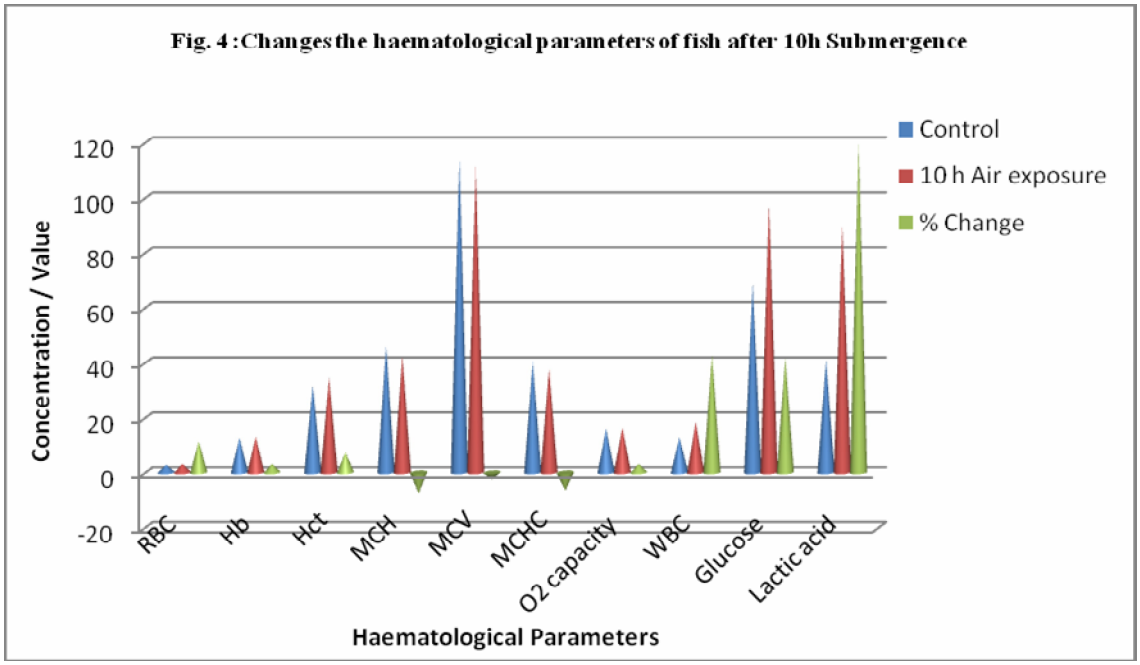
Fig. 4 : Changes the haematological parameters of fish after 10h Submergence
120
100
80
60
40
20
0
-20
Concentration / Value
Control
10 h Air exposure
% Change
RBC
Hb
Hct
MCH
MCV
MCHC
O2 capacity
WBC
Glucose
Lactic acid
Haematological Parameters

Table.5 Changes the carbohydrate metabolism of fish tissue after 5h submergence.
Values are the mean±SD of 6 individual determinations.

Parameter	5h submergence							
	Liver				Muscle			
	Control	Experimental	% change	ρ= 't' test	Control	Experimental	% change	ρ= 't' test
Glucose	68.21±5.32	88.46±9.10	26.69	>0.01	69.00±10.32	96.04±9.42	39.19	>0.05
Glycogen	106.30±6.14	65.18±8.20	-38.68	>0.05	110.38±10.21	74.50±8.56	-32.51	>0.05
Lactic acid	0.29±0.02	0.65±0.04	124.14	>0.05	0.30±0.04	0.70±0.06	133.33	>0.05

Table.6 Changes the carbohydrate metabolism of fish tissue after 10h submergence.
Values are the mean±SD of 6 individual determinations.

Parameter	10h submergence							
	Liver				Muscle			
	Control	Experimental	% change	ρ= 't' test	Control	Experimental	% change	ρ= 't' test
Glucose	69.04±7.08	79.66±5.11	15.38	>0.01	68.80±8.16	84.17±6.02	18.73	>0.01
Glycogen	111.40±13.00	91.82±10.00	-17.58	>0.01	108.50±11.03	88.18±10.06	-18.73	>0.01
lactic acid	0.29±0.07	0.40±0.08	37.93	>0.05	0.28±0.09	0.45±0.06	60.71	>0.05

The adaptive significance of increase of absolute values and WBC at air-exposure and submergence may be due to cope up the stress and it warrants further studies, Stress has also been shown in fishes to result in lymphocytopenia, monocytopenia and neutrophill (Ellis, 1977). Recently, the stress induced changes in the number of cells in the various populations of blood cells in *Cyprinus carpio* (Price et al, 1997) indicate that there are clear cut changes in the various populations of blood leucocytes.

Carbohydrate is the one of the important macromolecules which comes first to reserve fish from endow-ring stress. Glycogenolysis and or hyperglycemia appear to be a common response of the teleost to acute stress irrespective of the nature and duration of the stress. Stress induced breakdown of carbohydrate pool, supplies the growing energy requirement to meet the stress condition in general (Natarajan, 1985).

In order to meet the increased energy demand of stressed animals, glycogen, due its easy availability for energy production, is rapidly catabolized resulting in huge losses of this energy reserves. Reduction in glycogen content of liver and muscle observed in the present study supports this view. Catecholamines deplete glycogen reserves in fish (Nakano and Tomlinson, 1967). Thus, the marked glycogenolysis in the liver and muscle in this study after exposure to stress could possibly have been caused by a stress induced increase in circulating catecholamine. The depletion in glycogen stores should be accompanied by an increase in glucose content as observed in this study. The changes in the lactate levels also indicate metabolic disorders. Elevated lactate content suggests a sevier respiratory stress in the fish tissues. The upward trend in lactic acid in the issues may be taken to indicate that O_2 supply to the tissue in not adequate for the normal metabolic functions. Lactate as a measure of anaerobic metabolism has been widely used and increases of anaerobic metabolism have been shown to be a rapid and clear response of depletion of energy caused by lack of O_2. (Natarajan, 1985).

The abrupt decline in liver and muscle glycogen at air-exposure and submergence resulted in the hyperglycemia of the blood of *L.thermalis*. This consistent decrease in glycogen reserves suggests that glycogenesis was impaired. It is known that muscular extraction in fish is an accompanied by a marked rise in lactate (Mcleay and Brown, 1975) and the muscle glycogen is the source of blood lactate. In this study, it was found that the exposure of fish to air and submergence resulted in a decrease in muscle glycogen accompanied by an increase in muscle and liver lactate. The increased tissue lactate levels are possible due to the increased formation from pyruvate as a result of anaerobiosis. Lactate accumulation alters the permeability properties of cell resulting in disturbances in osmoregulation leading to degeneration of tissues (Rani, 1994).

Another consequence of high rates of anerobiosis is the need to process the resultant lactate and the replenish glycogen stores. Because blood lactate levels are elevated after stress in *L. thermalis*, other tissue could play an important role in rapid lactate clearance by removing lactate from the blood to oxidize it as an aerobic substrate and / or use it as a precursor for glucose or glycogen synthesis. Lactate may be removed from the blood to serve as an oxidative fuel (converted first to pyruvate). Gills of several species can oxidize lactate (Vijayalakshmi, 1996) but there have been no direct measurements of lactate oxidation in *L.thermalis*.

One of the first responses to stressor, such as air-exposure is the release of so called stress hormones: Adrenaline, Noradrenaline and cortisol. The releases of these catecholamines and cortisol trigger a broad suite of biochemical changes known collectively as secondary stress responses. The metabolic effects may include hyperglycemia and depletion of glycogen tissue reserves. The catabolic effects catecholamine and corticosteroids on the energy reserves stored in the body tissues may result in reduced growth in stressed fish. The changes seen in the muscle and liver tissue agree well with the general picture of secondary responses, whereas the catecholamine are thought to cause the initial elevation in plasma glucose levels by mobilizing the glycogen reserves (glycogenolysis) corticosteroids may contribute to the maintenance of hyperglycemia via the stimulation of gluconeogenesis (Espelid et al., 1996).

The hyperglycemia induced by air-exposure and submergence might be explained in part by inhibition of cholinesterase at neuroeffector sites in the adrenal medulla leading to hyper-secretion of adrenaline which stimulate the breakdown of glycogen to glucose (Rani, 1994). Stressful stimuli elicit rapid secretion of both glucocorticoids and catecholamine from the adrenal tissue of fish both hormones produces a rapid hyperglycemia (Folmar, 1993). In fresh water catfish *Heteropheustes fossils* (Sherwani and parweez, 2000) indicate that this fish is fairly hardy and not easily susceptible to stress by routine laboratory handling.

The present findings also indicate that *L.thermalis* is a hardy fish and physical stresses failed to evoke abnormal changes in the haematology and biochemistry. However, studies on the primary stress responses of plasma cortisol and immunological modulations may bring some useful information in the future.

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