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Effect of thermal stress on Haemoglobin concentration of Indian major carp Catla catla (Hamilton)

Dr.D.Sujatha[1] , Dr. D.Umamaheswari [2] , Dr.D.Vijayalakshmi [3]
[1]*Associate Professor of zoology, Visvodaya Government Degree College, Venkatagiri*
[2]*Lecturer in physics, S.P.W. Degree & PG College, Thirupathi*
[3]*Lecturer in HR, engineering college, Srikalahasti*
Orcid ID : 0009-0000-4564-5732

ABSTRACT
To differentiate thermal-stress (het-stress and cold-stress) from thermal-adaptation with in the fishes *Catla catla* were readapted to an abrupt rise of temperature from 22 ºc to 32ºc at the rate of 1ºc ⁄ hour (heat-stress) and to a slow rise of temperature at the rate of 1ºc ⁄ 60 hours (heat-adaptation). In haemoglobin content the heat-adapted fish exhibited a gradual elevation and the cold-adapted fishes exhibited a gradual decrease. However the %recovery is higher during heat-adaptation than that of cold-adaptation. On the other hand the stressed fish heat as well as cold exhibited initially fluctuations in haemoglobin content. The per cent recovery in stressed fishes is relatively much lower when compared to the 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*, Haemoglobin content, Temperature-Stress, Temperature-adaptation

INTRODUCTION
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).In recent times it is found necessary and possible to differentiate thermal-stress from thermal-adaptation. 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 anew 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 Effect of thermal stress on Haemoglobin concentration of Indian major carp Catla catla (Hamilton)

MATERIAL AND METHOD
The experimental male fish *Catla catla* weighing 20+2 grams were collected from local Government Fisheries Department, Anantapur and stored in large glass aquaria in the laboratory at

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room temperature (27°C±0.5°C) and exposed to natural photoperiod. Only male members of the fish *Catla catla* is used throughout the experimentation in order to avoid the effect of sex.

Haemoglobin content was determined in six individual fish samples and mean of six is taken for the account. The estimation is known as cyanomethaemoglobin method (Dacie and Lewis, 1971 and Brown 1976). Whole blood when added to Dabkin's solution which contains Potassium cyanide and Potassium ferricyanide. Ferricyanide in the solution converts haemoglobin iron from ferrous (++) to ferric(+++) state to form methaemoglobin which then combines with potassium cyanide to form stable pigment called cyanmethaemoglobin.

Reagents-Drabkin's reagent
Sodium bicarbonate NaHCO3 =1.00gm
Potassium ferricyanade K2Fe(CN3)= 0.02 g
Distilled water=100 ml

Procedure : 0.02 ml of whole blood was added to 4 ml of Drabkin's reagent in a test tube and mixed well. After being allowed to a stand at room temperature for a period time to ensure the completion of the reaction (30 minutes), the sample is read in a spectrophotometer at 540 nm using Drabhkins solution as a blank haemoglobin. This method is known as "Cyanomethoemoglobin method'

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)

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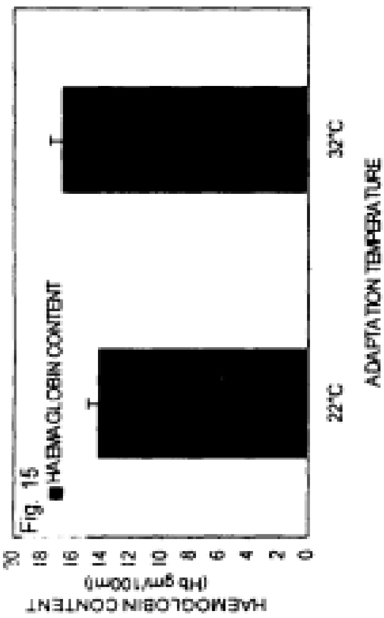

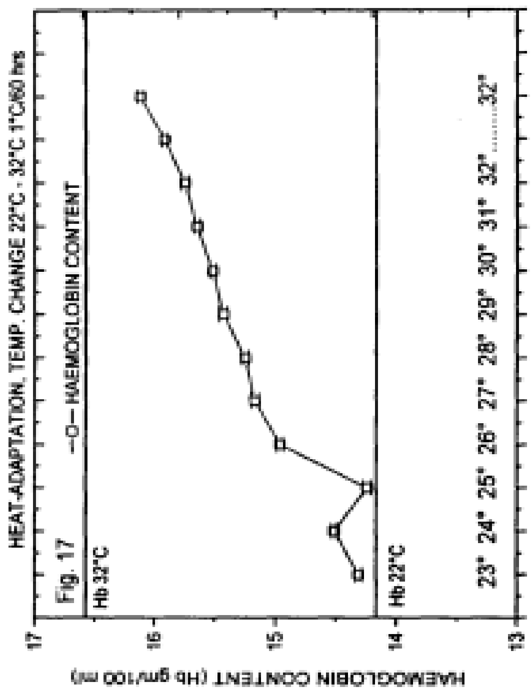

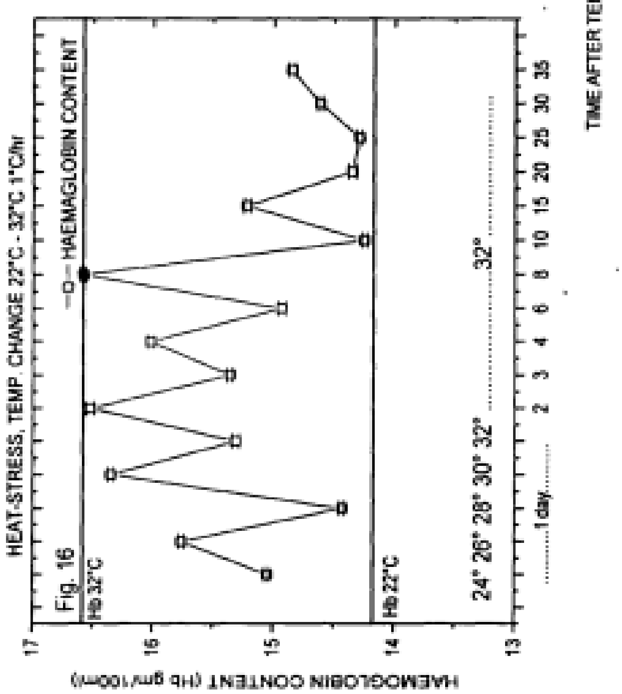

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FIGURE - 15

Histograms showing the level of haemoglobin content (Hb gm/100 ml) in *Catla catla* adapted to 22°C and 32°C temperatures. Each histogram is a mean of six individual measurements.

FIGURE - 16

Haemoglobin content (Hb gm/100 ml) (□) 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 - 17

Haemoglobin content (Hb gm/100 ml) (□) in *Catla catla* subjected to a 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.

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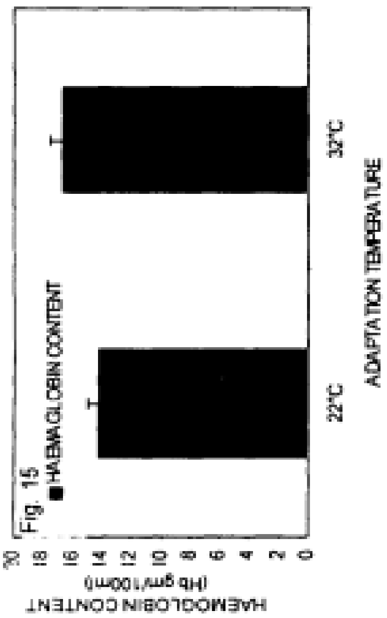

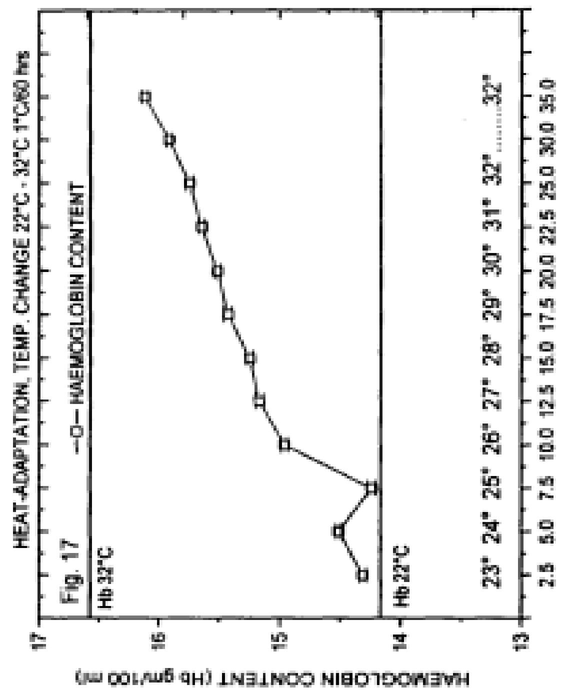

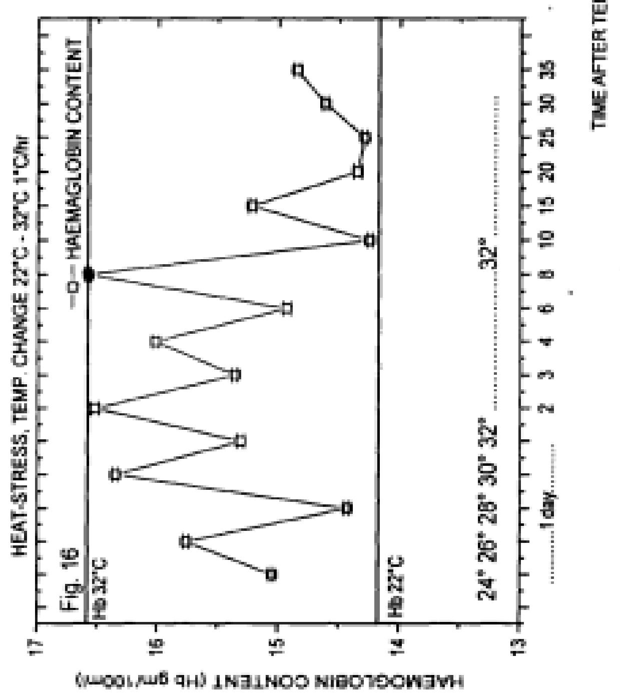

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FIGURE - 18

Histograms showing the level of haemoglobin content (Hb gm/100 ml) in *Catla catla* adapted to 32°C and 22°C temperatures. Each histogram is a mean of six individual measurements.

FIGURE - 19

Haemoglobin content (Hb gm/100 ml) (□) 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 - 20

Haemoglobin content (Hb gm/100 ml) (□) in *Catla catla* subjected to a 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.

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TABLE 12

HAEMOGLOBIN CONTENT (Hb gm/100 ml) in *Catla catla* DURING HEAT-STRESS AND HEAT-ADAPTATION

Adaptation temperature 22°C and 32°C (Controls)

Temperature	22°C	32°C
Mean	14.24	16.66
S.D ±	0.023	0.026

HEAT-STRESS (Temperature change 22°C to 32°C 1°C/hr)

EXPOSURE TIME (DAYS)	1 Day					2	3	4	6	8	10	15	20	25	30	35
READAPTED TEMPERATURE	24°C	26°C	28°C	30°C	32°C	←		32°C						→		
MEAN	15.05	15.76	14.43	16.35	15.31	16.52	15.36	16.02	14.93	16.58	14.25	15.22	14.35	14.29	14.62	14.85
SD ±	0.021	0.024	0.023	0.026	0.031	0.032	0.025	0.031	0.034	0.025	0.022	0.026	0.032	0.021	0.024	0.023
% CHANGE (+)	5.98	10.67	1.334	14.81	7.51	16.01	7.865	12.5	4.845	16.43	0.070	6.882	0.772	0.351	2.668	4.283
% RECOVERY	90.33	94.59	86.61	98.13	91.89	99.15	95.64	96.15	89.61	99.51	85.58	91.35	86.13	85.77	87.75	89.13
'T' TEST	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001

HEAT-ADAPTATION (Temperature change 22°C to 32°C 1°C/60 hrs

EXPOSURE TIME (DAYS)	2.5	5.0	7.5	10.0	12.5	15.0	17.5	20.0	22.5	25.0	30.0	35.0
READAPTED TEMPERATURE	23°C	24°C	25°C	26°C	27°C	28°C	29°C	30°C	31°C	32°C	→	32°C
MEAN	14.31	14.51	14.84	14.96	15.17	15.25	15.43	15.52	15.65	15.75	15.92	16.12
SD ±	0.026	0.022	0.031	0.025	0.035	0.028	0.036	0.024	0.026	0.031	0.023	0.025
% CHANGE (+)	0.491	1.896	4.213	5.05	6.53	7.092	8.356	8.988	9.901	10.60	11.70	13.20
% RECOVERY	85.89	87.09	89.07	89.79	91.05	91.53	92.61	93.15	93.93	94.53	95.55	96.75
'T' TEST	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001

MEAN : An average of six individual estimations

SD : Standard deviation

% CHANGE : Calculated at different temperatures in relation to the level of 22°C adapted control fishes (+) increase.

% RECOVERY : Calculated at different temperatures in relation to the level of 32°C adapted control fishes, which is fixed as 100.

Highly significant ($P < 0.001$); ($P < 0.01$); Significant ($P < 0.05$); NS : Non significant.

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TABLE 13

HAEMOGLOBIN CONTENT (Hb gm/100ml) in *Catla catla* DURING COLD-STRESS AND COLD-ADAPTATION

Adaptation temperature 32°C and 22°C (Controls)

Temperature	32°C	22°C
Mean	16.66	14.24
S.D ±	0.026	0.023

COLD-STRESS (Temperature change 32°C to 22°C 1°C/hr)

EXPOSURE TIME (DAYS)	........1 Day........					2	3	4	6	8	10	15	20	25	30	35
READAPTED TEMPERATURE	30°C	28°C	26°C	24°C	22°C	←		→			22°C			←	→	
MEAN	15.42	14.05	16.25	14.94	15.62	15.14	15.51	14.75	15.91	14.34	16.43	14.8	15.75	15.26	15.44	16.23
SD ±	0.031	0.029	0.033	0.022	0.021	0.035	0.025	0.022	0.036	0.027	0.029	0.028	0.021	0.032	0.031	0.035
%CHANGE (-)	7.442	12.06	2.460	10.32	6.242	9.123	6.902	11.46	6.285	13.92	1.380	10.80	5.462	8.403	7.322	2.581
% RECOVERY	91.72	97.13	85.90	95.09	90.1	93.28	91.09	96.42	88.28	99.3	84.63	96.92	89.4	92.84	91.58	86.03
'T' TEST	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001

COLD-ADAPTATION (Temperature change 32°C to 22°C 1°C/60 hrs

EXPOSURE TIME (DAYS)	2.5	5.0	7.5	10.0	12.5	15.0	17.5	20.0	22.5	25.0	30.0	35.0
READAPTED TEMPERATURE	31°C	30°C	29°C	28°C	27°C	26°C	25°C	24°C	23°C	22°C	→	22°C
MEAN	16.46	16.28	16.09	15.81	15.64	15.45	15.30	15.05	14.96	14.94	14.92	14.82
SD ±	0.021	0.025	0.032	0.035	0.036	0.028	0.024	0.022	0.04	0.026	0.024	0.022
% CHANGE (-)	1.20	2.280	3.42	5.102	6.122	7.262	8.163	9.663	10.20	10.32	10.44	11.04
% RECOVERY	84.42	85.68	87.01	88.98	90.17	91.51	92.56	94.32	94.95	95.05	95.23	95.93
'T' TEST	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001

MEAN : An average of six individual estimations

SD : Standard deviation

% CHANGE : Calculated at different temperatures in relation to the level of 32°C adapted control fishes (-) decrease.

% RECOVERY : Calculated at different temperatures in relation to the level of 22°C adapted control fishes, which is fixed as 100.

Highly significant ($P < 0.001$); ($P < 0.01$); Significant ($P < 0.05$); NS : Non significant.

RESULTS AND DISCUSSION

The Haemoglobin content which represents the average weight of haemoglobin (mg/100 ml) was measured in this investigation. The straight horizontal lines in Figs16,17,19 and 20 represent the haemoglobin content in *Catla catla* in 22°C and 32°C adapted fishes which are taken as the controls (Figs16,17,19 and 20) .The haemoglobin content is found to be higher in 32°C adapted control fishes than in the 22°C adapted ones (Fig.15).To differentiate the stress phenomenon from the adaptation process, the fishes from22°C adapted ones were re-adapted to 32°C with a slow rise of ambient temperature at the rate of 1°C/60hrs(Fig.17 Table.12) The 32°C adapted fishes were re-adapted to 22°C with a slow change in ambient temperature at the rate of 1°C/60hrs (Fig.20, Table13). The fishes

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from 22°C adapted ones were re-adapted to 32°C with a quick change of temperature at the rate of 1°C/hr (Fig.16, Table.12) and 32°C temperature adapted fishes were re-adapted to 22°C with a quick temperature change at the rate of 1°C/hr for a period of 35 days subjected to a quick temperature change at the rate of 1°C/hr both towards heat from to 22°C to 32°C as well as towards cold from 32°C to 22°C(Fig.16 and 19 Tables 12 and 13).These heat-stresses and cold-stressed fishes however established the new levels of the haemoglobin content and the continuous stress operating on the fish resulted in the "stress –adaptation" (heat and cold). The per cent change and per cent recovery in this parameter is relatively much higher in temperature- adapted fishes than the temperature- stressed ones. The per cent recovery is relatively higher in the heat-adaptation than in cold-adaptation and percent recoveries are significantly different from each type of adaptation .

The initial increase in haemoglobin content is reflected and correspondent to the increase in RBC count was observed by Sastri and Siddique(1984) and a significant decrease in haemoglobin content was observed by Joycee Shoba Rani et.al, (1987).In the present investigation the haemoglobin content was found to be low in the 22°C temperature adapted fishes than in 32°C temperature adapted ones. Inthe re adaptation experiment ssubjected to slow temperature change at the rate of 1°C/60hrs from 22°C to 32°C heat-adaptation the haemoglobin content of the basic levels 22°C temperature adapted fishes exhibited gradually stepping up of haemoglobin content and reached the controls of 32°C temperature adapted fishes.In the case of cold-adaptation when fishes were subjected to slow temperature change from 32°C to 22°C at the rate of 1°C/60hrs. There is a gradually stepping down in haemoglobin content and reached the controls values of 22°C temperature adapted 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 (96.75%) where as in the case of cold-adaptation this recorded as (95.93%) the percent recovery of haemoglobin is relatively higher in the case of heat-adaptation than that of cold-adaptation (Table.12& 13). Thus in temperature–adapted fishes there is a fairly good amount of recovery in the haemoglobin content suggesting that a very slow temperature change enable the fish to adapt to a new temperature without physiological load on the part of the fish as seen in the almost complete recovery in the parameter (Fig.17& 20 Tables. 12 & 13)

The haemoglobin content exhibited in different way in the fishes subjected torapid temperature change as against the changes observed in the adapted fishes. In these sressed fishes (heat-stressed and cold-stressed). There is no gradual stepping up/down of the haemoglobin content and reaching of the control levels were not observed even after 35 days. In the stressed fishes the haemoglobin content decreased initially and there are many fluctuations during the time course in both heat-stressed and cold-stressed fishes. The haemoglobin content could not reached the control medium (22°C and 32°C)temperature-adapted fishes in both heat-stress and cold-stress respectively and continuous stress acting upon the fish *Catla catla* resulted towards a phase of (stress-adaptation). Which relatively very less amount of per cent recovery (Tables.12 & 13). The per cent recovery during heat-stress is (89.13%). But in the process of cold-stress it is recorded (86.03 %) recovery for the haemoglobin concentration (Tables.12 & 13).The per cent recovery ishigher in thecase of heat-stress than in cold-stress. Thus the haemoglobin content involving re-adaptation experiments indicate that temperature act asa stress and stress is aphysiological load acting upon the fish *Catla catla* when exposed abruptly at the rate o 1°C/hr from a temperature of 22°C to 32°C(heat-stress) and from 32°C to 22°C (cold-stress)

CONCLUSION

Thus the study on Haemoglobin content reveals the distinction between slow and abrupt transitory changes taking place in the range of ambient temperature from 22°C to 32°C and vice-versa. Thus studies of this nature are highly useful in the evaluation of rates of temperature which acts as stressors and induce stress situation, and on the other in the evaluation of "safe" and ideal rates of temperature which do not act as stressor but, result in the slow and easy compensation of adaptation without

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physiological load on the part of the animal and evaluation techniques concerned with economical rearing and conservation of useful fauna of the aquatic habitat.

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