A Comparative Study of Thermal Stress affecting the respiratory rate, physiochemical and behavioural responses of *Chhana punctatus* and *Mystus gulio*

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ABSTRACT: Climate change is the most detrimental effect that the human civilization is facing worldwide. Due to global warming the average annual global temperature has increased at a rate of 0.07^0 C per decade since 1880. The temperature is a very important abiotic physical factor that affects the life of aquatic fauna. The present study focuses on the effect of temperature on the respiratory rate, physiochemical and behavioural responses on two freshwater fishes *Chhana punctatus* and *Mystus gulio*. The present study was carried to evaluate the impact of different temperature range (15˚C, 20˚C, 28˚C, 30˚C and35˚C) on both the fishes. Both the fishes demonstrated increased opercular rate, increased tolerance leading to consumption of glycogen reserves in the muscle tissues and restlessness in the behavioural patterns. The tissue protein content showed a decrease with increased thermal stress. The number of surface visits of both the fishes increased to meet the need for extra oxygen uptake. The fishes exhibited jerky movements. Taken together the present study showed a significant alteration in the respiratory rate, physiochemical, and behavioural responses in both the fishes.

Index terms: Thermal stress, Temperature, Opercular beat, Behaviour, Fish

I. Introduction

Environment is under constant threat to cater the demands of urbanisation and human exploitation. Climate induced changes in the biotic and abiotic factors is a matter of great concern. Due to global warming the average annual global temperature has increased at a rate of 0.07^0 C per decade since 1880 [1]. Furthermore National Centres of Environmental Information (National Oceanic and Atmospheric Administration, NOAA) have reported the year 2016 as the warmest year in NOAA's 137-year series. To worsen the scenario different natural calamities, like El Nino have made the situation grave. The warming of global temperature not only causes the rise of sea level and occurrence of severe storms but also affects the flora and fauna [2-3]. The tolerance range of a particular species is a direct correlation of its adaptation in a certain habitat. But due to changes in temperature the tolerance range is affected and the species become sensitive. This is often manifested as behavioural, physiological and cellular changes in the animal [4-6].

Water is an important physical factor which supports life. Water quality is a good bioindicator. Increased human interference, industrial development, use of pesticides and fertilizers have led to the pollution of the water bodies affecting its chemical composition. Such changes in the water quality affects the fishes [7-9]. Fishes are also potent indicators of water quality as they accumulate the chemical toxic substance efficiently from the water [10]. The alteration of the different physical and chemical parameters of water like temperature, pH, turbidity, dissolved oxygen and carbon dioxide affects the fishes both physically and physiologically [11-15]. Fishes being ectothermic animals gradually adapt and acclimatise to thermal stress after an initial period of exposure. Alteration in the behaviour like swimming patterns, surfacing, cellular and physiological attributes of several fishes has been reported under different conditions [13-14]. The degree of toxicity has a dose dependant relationship with the stress [16]. Existing reports have documented the response of fishes to different physical and chemical factors. Studies also report changes in haematological parameters, behavioural and physiological changes in different fishes [17-19].

Fish farming is one of the most important livelihood of the people of Durgapur-Raniganj (D-R) area of West Bengal, India. Water quality is indeed a very crucial parameter that must be well acquainted to the farmers [20]. Even under natural seasonal conditions in the absence of significant level of pollution the physical and physio-chemical parameters of the rivers and lakes change [21]. However due to globalisation and industrialization, pollution and thermal conditions of water bodies are changing rapidly. Temperature, pH, rainfall, dissolved oxygen, salinity and carbon dioxide are important physico-chemical parameters affecting aquatic environment which are supposed to be the limiting factors for the healthy existence of fishes [22]. Interestingly, existing reports have documented the effect of such parameters on the growth of fishes. However the effects of a particular factor in a comprehensive manner taking into account the behavioural, physiological and biochemical attribute is lacking.

With this background information the study attempts to assess the comparative effect of temperature of water on *Channa punctatus* and *Mystus gulio* fishes in a comparative manner. The study unravels their behavioural changes, opercular activity, tissue proteinand glycogen content under different conditions of temperature.

II. RESEARCH METHODOLOGY

A. Fish

The fishes *Chhana punctatus* and *Mystus gulio* and was collected from a local fish farm of D-R area. All fishes used for the study were of the size (80-200mm) and weight of (20-80gm). The fishes were sorted according to size and respective species and transported in polythene bags to the laboratory. In the laboratory they initially disinfected with 0.05 % of potassium permanganate and transferred to glass tanks and acclimatised for 10 days before individual experiments. The tanks were properly aerated. The fishes were fed with commercial fish food (GFC) daily to avoid the accumulation of food residues in the tanks. Water was changed and faecal matter was removed daily. The surface was covered with a wire gauge to prevent the fishes from jumping out.

B. Experimental procedure

The fishes of each species were divided into 4 batches (A-D) of 10 numbers each. Each batch of fishes was exposed to the following temperatures: 15, 20, 28, 30, 35 ^{0}C respectively. Water temperature was measured with a calibrated mercury thermometer. The fishes were subjected to varying time periods of 24, 48, 72and 96 hours (hrs.) for collection of preliminary observations and to get an idea of the behavioural patterns of the experimental animals. The fishes were also divided into different groups depending on size.

The behavioural patterns likes surfacing behaviour was studied. The respiratory rate was studied by counting the number of opercular beats per minute.

The oxygen consumption rate (OCR in mg/gm/hr) was calculated by = A1-A2/N/T

Where A1 and A2 represent the amount of dissolved oxygen in the control and test flasks (mg) respectively, N = number of individuals in the flasks, and T=respiration time (hr).

The level of protein and glycogen in the tissues of fish in each set of experiment were also analysed using standard methods.

C. Statistical analysis

Results are reported as mean ± SEM. Statistical analyses were performed using the Graph-Pad Prism statistics software (Graph-Pad Software Inc., San Diego, CA, USA). Each experiment was performed three to four times and results are representative of each set of experiments. Student's paired t-test was performed and $P<0.05$ was considered to be statistically significant at 95% confidence interval.

III. Results and Discussions

3.1. The opercular beats demonstrated a negative correlation with the length of fishes

The fishes showed variability in opercular beats with their length. Both the types of fishes were categorised in different groups according to their length and their opercular beats were measured per minute. All the fishes demonstrated a decrease in opercular rate with increased length as shown in Table 1. The opercular rate of both *Channa punctatus* and *Mystus gulio* showed a similar trend with varying length. The increase in the opercular rate in fishes of smaller length indicates higher metabolic activity as compared to those of larger size which have a slower metabolic rate. Thus, the study further attests the fact that increased activity requires higher respiratory rate to fulfil the physiological need. The opercular rate is an experimental index of the respiratory activity assessed in the experimental fishes.

Table 1: Effect of fish length on opercular rate in different fishes. # Respiratory rate was calculated by counting the number of opercular movements per minute. Each data is a representative of 3- 4 individual experiments.

Group No.	**Length of fishes (mm)**	***Channapunctatus***	***Mystusgulio***
		Respiratory rate# (beats/min)	Respiratory rate (beats/min)
1.	80-90	151± 0.96	156 ± 0.94
2.	90-100	149 ± 0.93	155 ±0.96
3.	100-110	146 ± 1.32	151 ±1.02
4.	110-120	142 ± 0.84	150 ± 1.15
5.	120-130	138 ± 1.15	148 ± 1.11
6.	130-140	133 ± 0.96	145 ± 0.84
7.	140-150	130 ± 1.12	142 ± 0.66
8.	150-160	128 ± 2.5	140 ± 0.94
9.	160-170	125 ± 0.45	135 ± 0.94
10.	170-180	120 ± 0.86	133 ±1.02
11.	180-190	119 ± 0.94	125 ± 1.12
12.	190-200	118 ± 1.02	120 ± 0.94

3.2. Increased opercular rate in fishes with increase in temperature

Both the fishes demonstrated an increased opercular rate with increased temperature. The study was conducted in size matched fishes of length 190-200 mm. Interestingly the increase in the opercular rate was insignificant or remained unaltered when the time of exposure to a particular temperature was prolonged (Table 2). The experimental set performed at 28^{0}C (room temperature), served as control. The increase in opercular rate at 35^{0}C after 24 hours of exposure indicated the preliminary stressed condition. During this initial period the fishes (both *Chhana punctatus* and*Mystus gulio*) exhibited their primary response to adjust to the stressed condition. Many similar studies have reported the increase in opercular rates of many fishes where they were subjected to different stressed conditions. Similar findings were reported during the exposure of *Channa striatus* and *Oreochromis mossambicus* to a carbamate pesticide and increasing concentrations of a fertilizer respectively [23, 24]. Increased opercular rates of pink salmon after exposure to Crude Oil Prince William sound, Alaska as a result of Exxon valdez oil spill incident has also been reported [25].

3.3. Reduced oxygen consumption in both the fishes at higher temperature

Thermal treated fish showed an initial increase of Oxygen consumption at lower temperatures (15^{0}C and 20^{0}C) and a decrease with the increasing temperatures (35^{0}C; Table 2). Causes like gill damage or haematological abnormalities could be probable factors for the reduced oxygen consumption. Moreover at higher temperatures respiratory inhibitory factors become potent which also could serve as an instrumental factor. Thus it is evident that oxygen consumption is temperature dependent. The initial increase in oxygen consumption at higher temperature may be attributed due to initial adjustment phase of the fish to cope up with the introduced thermal stress. The fish simultaneously increases its opercular activity to procure more oxygen from the water to meet its physiological need. The increase of opercular activity also has a specific limit, beyond which the fish fails to survive. Hence, the temperature of an aquatic body is a significant factor that influences the survibility of fishes. Whereas, during a prolonged period of exposure time the oxygen consumption gradually reduces due to the onset of stress. These observations indicate that environmental factors like temperature play a probable role in the respiration rate of aquatic-ectotherms, like fishes. Both the fishes *Chhana punctatus* and *Mystus gulio* showeda similar trend of observations (Table 2).

Table 2: Effect of temperature on oxygen consumption of *Channa punctatus* and *Mystus gulio*. (Each value represents the mean ± SEM)

Sl. No.	**Temperature of water (^{0}C)**	**Time of exposure (hr)**	**Oxygen Consumption (mg/g/hr)**	
			Channa punctatus	*Mystus gulio*
1.	15	24	0.92 ± 0.03	0.91± 0.04
		48	0.91 ±0.03	0.91 ±0.04
		72	0.94 ±0.02	0.93 ±0.03
		96	0.96 ±0.04	0.94 ±0.06
2.	20	24	0.98 ±0.06	0.97 ±0.02
		48	0.97 ±0.06	0.97 ±0.05
		72	0.99 ±0.03	0.99 ± 0.04
		96	1.00 ±0.04	0.99 ± 0.04
3.	28	24	0.97 ±0.02	0.98 ± 0.03
		48	0.99 ±0.03	0.90± 0.05
		72	0.99 ±0.04	0.96 ± 0.03
		96	0.98 ±0.04	0.95 ± 0.04
4.	30	24	0.84 ±0.06	0.82 ± 0.03
		48	0.86 ±0.02	0.83 ± 0.04
		72	0.88 ±0.02	0.85 ± 0.02
		96	0.88 ±0.03	0.87 ± 0.03
5.	35	24	0.58 ±0.04	0.52 ± 0.04
		48	0.53 ±0.05	0.48 ± 0.02
		72	0.41 ±0.03	0.43 ± 0.06
		96	0.35 ±0.02	0.39 ± 0.03

3.4. Reduced glycogen and protein content with increasing thermal stress

Temperature is an important factor affecting the physiochemical processes of animals [26-28]. Muscle glycogen is a form of stored reserve that is utilized during physical exertion or to combat and confront stress. Both the fishes witnessed reduced muscle glycogen content when exposed to thermal stress. At increased temperature (i.e. 35^{0}C) the estimated glycogen content was reduced as compared to that observed at lower temperatures indicating that the reserve was utilised by the fish to settle and acclimatize the stress. The reduction was observed with increasing time of exposure. Similarly, the amount of protein content in the liver tissue showed a reduction when the fishes were exposed to higher temperature. This could be a result of denaturation or alteration of folding patterns of the functional structure of different proteins and enzymes at higher temperature (Table 3). Even the exposure at the same temperature for longer periods of time resulted in reduced protein content signifying towards probable proteolysis.

Table 3: Effect of temperature on the total glycogen and protein content of muscles of *Chhana punctatus* and *Mystus gulio* (Each value represents the mean ± SEM)

Sl. No.	Temperature of water (^{0}C)	Time of exposure (hr)	*Chhana punctatus*		*Mystus gulio*	
			Glycogen content (mg/g)	Protein content (mg/g)	Glycogen content (mg/g)	Protein content (mg/g)
1.	15	24	24±0.13	3.21±0.13	23±0.13	3.25±0.013
		48	23±0.09	3.01±0.12	22±0.11	3.11±0.012
		72	22±0.07	2.85±0.12	22±0.09	2.75±0.011
		96	19±0.08	2.5±0.08	21±0.07	2.45±0.07
2.	20	24	23±0.09	3.1±0.09	23±0.11	3.05±0.08
		48	22±0.12	2.8±0.07	22±0.09	2.91±0.06
		72	20±0.13	2.5±0.04	21±0.07	2.75±0.09
		96	20±0.08	1.8±0.05	20±0.09	2.60±0.06
3.	28	24	22±0.07	2.95±0.07	23±0.11	2.85±0.11
		48	21±0.09	2.8±0.06	21±0.12	2.75±0.11
		72	20±0.07	2.51±0.05	19±0.08	2.31±0.08
		96	19±0.12	1.75±0.06	19±0.09	1.55±0.05
4.	30	24	21±0.11	2.5±0.04	20±0.12	2.45±0.11
		48	21±0.08	2.2±0.04	19±0.13	2.1±0.07
		72	19±0.07	1.8±0.05	19±0.11	1.65±0.06
		96	18±0.09	1.4±0.06	18±0.12	1.32±0.08
5.	35	24	20±0.09	2.35±0.07	20±0.08	2.05±0.05
		48	18±0.11	1.8±0.11	17±0.09	1.65±0.05
		72	15±0.12	1.05±0.04	14±0.06	0.95±0.07
		96	14±0.09	0.55±0.03	13±0.07	0.42±0.01

3.5. Altered behavioural responses with increased thermal stress

Both, *Chhana punctatus* and *Mystus gulio* are facultative air breathing fish which visit the surface of water to gulp atmospheric oxygen under normal conditions. During exposure of the fishes to thermal stress they demonstrated hyperactivity and fast jerky movements. Longer exposure of the fishes to a particular elevated temperature resulted in restless movements, turning upside down and ultimately loss of equilibrium.

Facultative air breathing fishes often show alteration of their surfacing behaviour in response to stress which is directly correlated to their need of oxygen consumption [29]. As a result of stress there is an increased demand for oxygen to accommodate the physical hypersensitivity. A similar trend was observed for both *Chhana punctatus* and *Mystus gulio* when they were exposed to elevated temperatures as compared to the lower temperatures. The number of surface visits per hour showed a gradual increase with increasing temperature (Table 4).In conclusion it is very clear from the present study that temperature is an instrumental factor affecting the opercular beat, physiochemical and behavioural responses of fishes. The thermal stress is a potent factor as result of worldwide global warming that could affect aquatic life in the near future. Furthermore assessing the temperature of water is equally important in addition to the other standard parameters as it is a probable stress induction agent. Thus the study also indicates the need for preservation and maintenance of the temperature of the local fresh water bodies and the environment for proper growth of fishes.

Table 4: Effect of temperature on the surface visits of both Chhana *punctatus* and *Mystus gulio* respectively. (Each value represents the mean ± SEM)

Sl. No.	**Temperature of water (^{0}C)**	**Time of exposure (hr)**	**No. of surface visits** *Chhana punctatus*	**No. of surface visits** *Mystus gulio*
1.	15	24	15 ± 0.37	16 ± 0.31
		48	15 ± 0.34	16 ± 0.35
		72	16 ± 0.37	17 ± 0.38
		96	16 ± 0.41	17 ± 0.31
2.	20	24	13 ± 0.35	13 ± 0.41
		48	12 ± 0.38	13 ± 0.38
		72	13 ± 0.41	14 ± 0.33
		96	13 ± 0.35	14 ± 0.33
3.	28	24	14 ± 0.32	14 ± 0.35
		48	15 ± 0.37	14 ± 042
		72	15 ± 0.42	15 ± 0.41
		96	15 ± 0.35	15 ± 0.35
4.	30	24	22 ± 0.31	25 ± 0.36
		48	24 ± 0.45	26 ± 0.31
		72	24 ± 0.32	28 ± 0.32
		96	26 ± 0.33	28 ± 0.40
5.	35	24	28 ± 0.35	26 ± 0.45
		48	28 ± 0.37	28 ± 0.37
		72	29 ± 0.38	30 ± 0.39
		96	30 ± 0.41	30 ±0.34

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