Physiological Responses of the Heterobranchus Bidorsalis (Male) X Clarias Gariepinus (Female) Hybrid (Heteroclarias) Fingerlings to Different Temperature Levels under Laboratory Conditions

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Abstract— A twelve weeks experiment was carried out on Heteroclarias freshwater hybrid fish fingerlings under laboratory conditions to study the effects of different temperature levels, 26.91 (control), 28.00, 30.00, 32.00°C respectively and their physiological responses to oxygen consumption, ammonia excretion and opercular respiratory beats were evaluated. The oxygen consumption, ammonia excretion and opercular respiratory beats were determined weekly based on standard procedures. The findings revealed that the oxygen consumption of Heteroclarias hybrid fingerlings significantly ($p<0.05$) increased with increase in temperature. The ammonia excretion were not significantly different ($p>0.05$) in all the temperature levels. The opercular respiratory beats per minutes showed similar trend in weeks 1,2,4 and 8 but indicated significantly higher ($p<0.05$) opercular respiratory beats (range= 117.10±2.26 at 30°C to 142.75±3.04 opercular beat at 32°C in week 8) at highest tested temperature levels. However, there was a decreasing trend in the opercular respiratory beats per minute of the controlled fingerlings. Generally, the opercular respiratory beats per minute decreased with increase in fish size. The findings of this study confirmed that increase in water temperature affects the physiology of Heteroclarias hybrid and hence for effective rearing and for profit making, it is essential for the hybrid to be cultured in the temperature range between 26.91°C (control) to 28.00°C.

Keywords— Heteroclarias hybrid, Physiological Responses, Opercular beat, Temperature.

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A. Introduction

TEMPERATURE has been considered as the major factor among the array of environmental factors influencing the aquatic environment and the metabolism of ectothermic organisms [1]. Reference [2] reported that any rise in atmospheric temperature due to climate change (global warning), industrialization and urbanization would directly influence the water temperature. The authors also added that these factors could directly affect the ambient water temperature of the ectothermic fishes cultured in ponds. Therefore, there is need for the culturable freshwater fish to regulate their internal body temperature and utilize their physiological processes necessary for their survival [3]. Moreover, fish have developed physiological and biochemical adaptations to cope with abrupt changes in water temperature that minimize or eliminate the deleterious effects, which is called stress response[4]. Oxygen consumption rate is among the physiological responses that can be correlated with changes in environmental parameters, because it is related to the metabolic activities and energy flow that organisms must channel to homeostatic control processes [5]. Ventilatory responses are mainly responsible to increase the amount of oxygen supply into contact with gas exchange surfaces [6]. The author also observed that they are also good indicators of stress situation in fish. Lloyd [7] documented that the frequencies of opercular ventilation beats were frequently used as a measure of respiratory rates in fish.

Ammonia concentration affects gill morphology [8], respiratory activities [9] and oxygen consumption [10] in aquatic animals. In addition, health of cultured animals like fish in intensive aquaculture depends solely on avoiding accumulation of toxic waste products such as ammonia [1]. Similarly, to support the above submission, Reference [11] reported that ammonia excretion can be affected by factors such as species, body weight, water temperature, feeding and ration size.

Physiological mechanisms during thermal adaptation in poikilotherms in relation to temperature compensation were suggested by a few researchers [12] . The experimental fish in this research is a hybrid from two African cat fish, viz: *Clarias gariepinus* (female) and *Heterobranchus bidorsalis* (male) [13]. The hybrid (Heteroclarias) combines the fast growth traits of *Heterobranchus* Sp and early maturing rate of *C.* gariepinus [14], [15]. Heteroclarias has been reported to be the most widespread and accepted hybrid fish in Africa especially in Nigeria because of its disease resistance, tolerance to high stocking density and is the mainstake of family income [16] and [17]. The present study was designed to assess the influence of different temperature levels on oxygen consumption, ammonia excretion and opercular respiratory beats of Heteroclarias acclimatized at 26.91 (control), 28.00, 30.00 and 32.00^{0}c respectively under laboratory conditions.

B. MATERIALS AND METHODS

I. Experimental Site

The study was conducted at the Biology Laboratory of the School of Life Sciences, Bosso Campus, Federal University of Technology, Minna. Niger State, Nigeria.

II. Source of the Experimental Fish

One thousand eight hundred, four weeks old Heteroclarias fingerlings with average weight of 1.01g were purchased from a private fish farm in New Bussa, Niger State, Nigeria. The fingerlings were transported to the Biology Laboratory in 50 litres Jerican with well-aerated water through openings at the top for ventilation.

III. Acclimatization of the Fingerlings

The Heteroclarias fingerlings were acclimatized in rearing tanks for a period of seven days to allow them to recover from transportation stress. They were also visually observed to ensure that there were no infections from the source and also to select average weight of the fish to be cultured together [18], [17]. During this period, the fish were fed on a commercial diet (Catco fish concentrate) by coppens international, Holland. They were fed to satiation, morning and evening following the method of [4]. Water exchange was done when necessary in the morning. The left overfeed and faecal samples were siphoned immediately after feeding [19].

IV. Experimental Design

A Completely Randomised Design (CRD) with a total of 4 treatments replicated 3 times was adopted in this experiment.

1.0. Experimental Set-Up

The experiment consisted of four treatments with three replicates per treatment, each with stocking density of four hundred and fifty fingerlings. Treatment 1 was the control (26.91°C), while treatments 2, 3 and 4 had water temperature maintained at 28^{o}C, 30^{o}C and 32^{o}C respectively using thermo-regulator. Constant power supply was achieved with the aid of inverter. Twelve plastic indoor aquaria tanks, 25 litres capacity (55×35×35cm^3) filled with borehole water up to 20 cm level were stocked with 150 fingerlings each. The experimental tanks were covered with net to prevent the fish from jumping out [20]. The fingerlings were fed on a commercial diet (Catco fish concentrate) to satiation, morning and evening following the method of reference [4].

V. Determination of Some Physico-Chemical Parameters

2.0. Water Temperature

Water temperature of the control treatment was determined with mercury in bulb thermometer (10-110^{0}C range). Temperature was determined by lowering the thermometer into the tanks in an inclined position for about 5 minutes to allow for equilibrium before taking the reading at 10.00am in the morning throughout the duration of the experiment. The temperature levels of (28, 30, 32)^{0}C were maintained by the use of thermo regulators with a stand by inverter as an alternative source of power in case of power outage following the method of reference [21].

3.0. Dissolved Oxygen

This was determined by using Winkler Azide method of reference [22]. Water samples from the control and treatment tanks were collected by inserting 250 ml water sample bottles into the tanks and sampled water was fixed right in the laboratory with 1ml of reagent (I) (Manganous sulphate) and 1ml of reagent (II) Alkaline iodide solution (KOH + KI). About 2 ml of concentrated sulphuric acid was added to each sample and 10ml of the sample was titrated with 0.025N sodium thiosulphate using starch as indicator until it turns colourless. Calculation was based on equation 1.0 as described by [23]

Dissolved Oxygen (mg/L)

$$= \frac{\text{Volume}(Na_2SO_3) \times \text{Normality} \times 8 \times 1000}{\text{Sample volume (ml)}} \ldots\ldots\ldots\ldots\ldots\ldots (1)$$

Where, normality= 0.025 ml of sodium sulphite (Na_2S0_3)

8 = Equivalent weight of oxygen in water

1000 = Conversion to mg/litre

4.0. Hydrogen Ion Concentration (pH)

The pH of the water samples were determined with Jenway 3305 pH meter model at room temperature. The pH meter probe was inserted into the sampled water for about 5minutes until it stabilized before the reading was taken. The meter was standardized with buffer solutions of pH 4.0, 7.0 and 9.0 before the readings were taken.

5.0. Ammonia (NH_3)

100ml of the water sample from control and treatment tanks was pipetted into a Markham distillation apparatus (Kjeldal flask) and there after 5ml of 40% NaOH was added. The flask was connected to the condenser and the cooling water was turned on. About 10ml of 40% boric acid (H_3BO_3) solution was placed under the condenser ensuring that the tip of the condenser was immersed in the receiving solution and distilled slowly until 50ml of the distillate was collected in the receiving flask. The ammonia was determined from the distillate by titrating with 0.01M HCl until the colour at the end point changed from green to pink [22]. Calculation was based on the formular below

$$NH_3(mg/L) = \frac{\text{Titre value} \times 14 \times 0.01 \times 1000}{\text{Volume (cm}^3)} \text{ ………… (2)}$$

Where 0.01 = molarity of HCl used as titrant; 14 is the molecular mass of nitrogen and 1000 is the conversion to mg /litre.

H. Measurement of Growth Parameters

6.0 Standard Length and Total Length

At the end of every week, ten fingerlings from each tank were randomly sampled as described by reference [24]. Each fish was sampled one by one using a piece of fine mesh net and gently placed on blotting paper to absorb the adhering water. The Standard Length (SL) was determined by measuring the length between the mouth and the caudal peduncle while the Total Length (TL) was determined by measuring the interval between the mouth and the tail fin. They were individually measured with a graduated transparent meter ruler in centimeters. This method of manipulation and measurement was safe for the fish and they were returned to their respective tanks without any loss [24].

7.0 Weight

The weight of the fish was determined weekly by taking the individual weight of ten randomly sampled fingerlings. Weight was determined by using a sensitive compact scale model *CS 2000 HAUS* following the method of reference [24].

8.0 Weight Gain.

Weight Gain was calculated as: Weight Gain (WTG) = Final mean weight – initial mean weight [18].

8.0 Percentage Weight Gain

Percentage Weight Gain (PWG) = $100 \frac{Y-X}{X}$ …….. (3) as described by Adewolu *et al*. (2008).

Where:

Y= final mean body weight (g)

X= initial mean body weight (g)

9.0 Specific Growth Rate (SGR)

SGR was calculated as:

$$\textbf{SGR=} \frac{\log e\, W_2 - \log e W_1}{T_2 - T_1} \text{ …………………………………… (4)}$$

Where W_2 = weight of fish at time T_2 in days

W_1 = weight of fish at time T_1 in days

T_1 = Day Zero

T_2 = Eighty four (84) Days

Log_e = natural log to base e as described by reference [25].

10.0 Survival Rate

The experimental tanks were monitored daily to remove dead fish and the mortality was recorded; using the formular of reference[18].

Survival Rate (SR) was calculated as

$$SR = \frac{N_O - N_t}{N_O} \times 100\% \text{ …………………………….. (5)}$$

Where N_o = number at the start of the experiment

N_t = number at the end of the experiment

11.0 Respiratory Rate

The respiratory rate was determined weekly after the modified methods of [21] and [26]. One fingerling from each of the experimental tank was randomly and carefully removed and placed in a similar aquarium tank which was half filled with the same rearing water. Thereafter, thermo regulator was set at the desired temperature and inserted gently into the tank. The fish were allowed to recover from stress incurred during handling for five minutes before the opercular respiratory beat was counted individually per minute against each treatment using a stop watch. This procedure was repeated 10 times for 10 *Heteroclarias* fingerlings from each of the experimental tanks.

C. DATA ANALYSIS

The data collected were analysed for significant differences (P < 0.05) by the analysis of variance (ANOVA) using a Computer Statistical Package for Social Sciences (SPSS). Duncan Multiple Range Test method [27] was used to separate the means where there were statistically significant differences (P < 0.05)

D. RESULTS

The results of some physicochemical parameters of the water medium in which the Heteroclarias fingerlings were exposed to different temperature levels for a period of 12 weeks are presented in Table I. In general, the concentration of oxygen consumption increased with increase in temperature. The oxygen consumed (6.16 ± 0.91 mg/L) by the fingerlings under control treatment was significantly higher (P <0.05) than those of higher temperature levels (32.00 – 28.00^{0}c) and ranged from 4.58 ± 0.97 to 5.57 ± 0.73 mg/L. Moreover, the results also showed no significant reduction (P > 0.05) in the amount of oxygen consumed by the fingerlings reared at 30.00^{0}c (4.85 ± 0.01 mg/L) and 32.00^{0}c (4.58 ± 0.97 mg/L) respectively. Table I, also revealed that there were no significant differences (P > 0.05) in the ammonia concentration (range = 0.15 ± 0.09 to 0.17 ± 0.09 mg/L) of the Heteroclarias fingerlings cultured in all the temperature treatments. Similarly, the water pH of the fingerlings hybrid (range = 6.78 ± 0.9 at 26.91^{0}c to 6.95 ± 0.37 at 28.00^{0}c) did not differ significantly (P > 0.05) among the fingerlings cultured in the different water temperature levels.

The results of mean opercular beats per minute of Heteroclarias hybrid exposed to different temperature levels for a period of 12weeks are depicted in Table II. Generally, there was a decrease in the opercular respiratory beats of the experimental fingerlings as the fish size increased. The opercular beats per minute in the fingerlings increased as the temperature increased from week 1 to 8 in all the temperature treatments. It reached the highest beat of 143.05±6.01 in week 3 under 32^{o}C. The opercular respiratory beats of the controlled treatment $(116.40 \pm 2.13\ beats\ /minute)$ was significantly lower (P< 0.05) when compared with other temperature levels (28.00 – 32.00^{0}C).

TABLE I: INFLUENCE OF DIFFERENT TEMPERATURE LEVELS ON SOME PHYSICOCHEMICAL PARAMETERS OF HETEROCLARIAS FINGERLINGS WATER MEDIUM FOR A PERIOD OF 12 WEEKS

Temperature levels (^{0}C)	**Dissolved oxygen concentration (mg/L)**	**Ammonia concentration (mg/L)**	**pH**
26.91 (control)	6.6 1 ± 0.91^{b}	0.17 ± 0.09^{a}	6.78 ± 0.89^{a}
28.00	5.57 ± 0.73^{ab}	0.15 ± 0.09^{a}	6.95 ± 0.37^{a}
30.00	4.85 ± 1.01^{a}	0.16 ± 0.08^{a}	6.88 ± 0.27^{a}
32.00	4.58 ± 0.97^{a}	0.15 ± 0.09^{a}	6.82 ± 0.27^{a}

Values are mean ± standard deviation; values followed by the same superscript(s), in the same column, are not significantly different at ($P > 0.05$) tested by DMRT

TABLE II: MEAN RESPIRATORY RATE OF *HETEROCLARIAS* FINGERLINGS EXPOSED TO DIFFERENT TEMPERATURE LEVELS OVER A 12 WEEKS PERIOD.

Temperature	Weeks											
Levels (^{0}C)	1	2	3	4	5	6	7	8	9	10	11	12
26.91	116.60	117.10	116.40	117.10	108.60	118.20	96.70	100.50	113,25	102.65	105.75	94.05
(control)	±0.14^{a}	±0.99^{a}	±2.13^{a}	±0.71^{a}	±10.89^{a}	±0.28^{a}	±0.14^{a}	±0.85^{a}	±6.15^{b}	±3.61^{a}	±7.28^{b}	±0.21^{a}
28.00	116.50	126.60	139.40	125.8	110.25	111.65	100.85	107.85	111.20	103.95	108.90	96.40
	±7.07^{a}	±2.40^{a}	±6.93^{b}	±7.07^{a}	±2.47^{a}	±2.62^{a}	±0.64^{a}	±8.13^{a}	±2.40^{b}	±1.63^{a}	±4.81^{b}	±1.41^{a}
30.00	131.05	141.60	139.52	132.40	129.00	113.45	102.25	117.10	102.00	101.85	95.45	95.10
	±3.04^{b}	±0.85^{b}	±1.53^{b}	±3.54^{b}	±3.96^{c}	±7.14^{a}	±2.90^{a}	±2.26^{b}	±1.56^{a}	±3.75^{a}	±3.18^{a}	±0.85^{a}
32.00	133.70	142.25	143.05	142.75	121.60	115.7	108.00	119.50	112.55	103.65	103.50	103.45
	±1.13^{b}	±1.91^{b}	±6.01^{b}	±3.04^{b}	±1.41^{b}	±2.68^{a}	±0.57^{b}	±2.97^{b}	±2.19^{b}	±2.33^{a}	±2.83^{ab}	±1.63^{b}

Values are Mean ± Standard deviation, Values followed by the same superscript(s), in the same column, are not significantly different at ($P > 0.05$) tested by DMRT.

E. DISCUSSION

This present study revealed that oxygen consumption of Heteroclarias fingerlings increased with increase in temperature. This observation was in conformity with the reports of [28] who documented that oxygen consumption of *Oreochromis mossambicus was* enhanced with increasing temperature when cultured at 28, 33 and 38^{0}c respectively. To support the above submission, [29] opined that temperature accelerates metabolic activities of aquatic animals like fish, thereby, resulting into subsequent oxygen demand. This leads to decrease in the concentration of oxygen available to Heteroclarias fish in water due to high temperature. [30] The higher value of concentration of dissolved oxygen consumption recorded in the fingerlings reared at control temperature (26.91^{0}c) suggested that the fingerlings were not under any physiological stress. This is because the concentration of dissolved oxygen (6.16 ± 0.91mg/L) was above 5.00mg/L recommended as minimum dissolved oxygen required for healthy growth, tissue repairs and reproduction [31] and [30] . The finding of this study also showed that the ammonia excretion or concentration was not influenced by temperature. This could be attributed to daily removal of left over feed, and faecal samples from experimental tanks. Thus, preventing or reducing the risk of buildup of ammonia from all the temperature treatments [1], [17]. This observation was contrary to the reports of reference [29] who noted that ammonia excretion or concentration increased with increasing temperature in *Alepes djidaba* fingerlings showing that degradation of protein for energy was more at higher temperatures.

Similarly, the study revealed that changes in pH values of water from all the treatments were within the tolerance range of 6.0 to 8.0 documented for juveniles of *H. bidorsalis* and *C. gariepinus [15]*.

The decreasing trend in the opercular respiratory beat of Heteroclarias from all the temperature treatments were similar to the reports of reference [26] who documented that opercular respiratory rate decreased with increase in fish size. This observation is also in agreement with the reports of reference [32] who noticed that there was a physiological correlation of fish activity and opercular respiratory rate. This finding suggests that the correlation between fish activity and opercular respiratory rates decreased with increase in fish size.

Moreover, the similar trend in the opercular respiratory rates in weeks 1, 2, 4, & 8 from all the treatment including the control may be attributed to similar physiological responses exhibited by these fingerlings during this period [1]. During the same period, an increase in opercular respiratory beats with increasing temperature (30.00 – 32.00 ^{0}c) were in conformity with the works of [1] who observed increase in opercular movement to the temperature variations in the medium was a primary response to stress. To support the above submission, similar conditions were reported by reference [30] in *Mystus gulio* where opercular beats increased with the increasing temperature. The increased opercular movement by the exposed fish may be the reflection of an attempt by the fish (Heteroclarias) to extract more oxygen to meet the increased energy demand to withstand the rise in temperature [30].

The findings of this study also indicated decrease in the mean opercular respiratory beats of the controlled fingerlings (26.91^{0}c) only in week 3. This observation was in consonance with the reports of reference of [33] who documented that there was a decreasing trend in the respiratory rate and opercular beats of fresh water fish *Channa punctatus* reared at 15.00^{0}c as compared with higher temperature treatments.

CONCLUSION

Water temperature had no influence on the ammonia excretion and water pH of the Heteroclarias hybrid during the study period. However, increase in water temperature affects the opercular respiratory beats of the hybrid. Generally, the opercular respiratory beats decreased with increase in fish size. Therefore, for effective rearing and profit making, it is essential for the fish to be reared in ideal temperature between 26.91 and $28.^{0}00^{0}$c.

ACKNOWLEDGMENTS

This study was part of a Research Project funded by the TETFUND Institution based research intervention (TETFUND/FUTMINNA/ 2014/26 Federal University of Technology, Minna, Nigeria. We are thankful to the Laboratory Technologists of the Department of Biological Sciences, Water Resources Aquaculture and Fisheries Technology, especially Mr. B.M. Baba, Mrs. O. J. Olorunfemi and Mr. D. C. Mohammed for their technical assistance during the study period.

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