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Article *in* Egyptian Journal of Aquatic Biology and Fisheries · August 2020

DOI: 10.21608/ejabf.2020.109682

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Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 24(6): 47 – 58 (2020)
www.ejabf.journals.ekb.eg

Temperature effects on the opercular respiratory rates of *Clarias anguillaris* fingerlings reared under Laboratory conditions in Minna, Nigeria

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ARTICLE INFO

Article History:
Received: June 17, 2020
Accepted: Aug. 21, 2020
Online: Aug. 27, 2020

Keywords:
Clarias anguillaris ,
Opercular respiratory rate ,
Bodyweight,
temperature levels,
Fish size.

ABSTRACT

Effects of different temperature levels on the opercular respiratory rate of *Clarias anguillaris* fingerlings were investigated for a period of 6 weeks under laboratory conditions. Fifty fingerlings were raised in water temperatures of control (26.66±0.28), 30.00, 32.00, 34.00 ^{0}C with 2 replicates each respectively. Opercular Respiratory Rates (ORR), body weight, and physicochemical parameters were determined weekly based on standard methods. ORR was significantly ($p< 0.05$) reduced from 113.60 ± 7.67 to 105.50 ±10.23 opercular beats per minute from week 1 to 2 in the control temperature. ORR was also significantly ($p<0.05$) higher from 30 to 34 ^{0}C from week 1 to 2. A strong negative correlation was observed between body weight and ORR from all the treatments. Electrical conductivity (519.92±5.06 to 586.33±17.50 µS/cm) and Ammonia concentration (1.11±0.10 to 1.98±0.06 mg/L) were ($p<0.05$) higher from the control temperature to 30.00 ^{0}C. ORR of *C. anguillaris* fingerlings increased with an increase in temperature, while ORR decreased with an increase in fish size and duration of the experiment. The temperature had no effects on dissolved oxygen, biochemical oxygen demand and pH except bodyweight in weeks 1, 3 and 5. Ammonia concentration and electrical conductivity increased with an increase in temperature. The findings from this study revealed that higher temperature levels affect the opercular respiratory rates, ammonia concentration, and electrical conductivity of *Clarias anguillaris* fingerlings in captivity.

INTRODUCTION

Fish is the cheapest kind of animal protein and very important in diet requirement (**Allison and Ellis, 2001; Mgbemena *et al.*, 2020**). Catfish such as *Clarias anguillaris* are important to the sustainability of aquaculture industry in Nigeria (**Owodeinde and Ndimele, 2011**). Clarias anguillaris constitute an excellent food fish of high commercial value. Clariid catfishes are found in most freshwater bodies of South East Asia and Africa where they constitute a significant component of the catches. The highest generic diversity is found on the African continent where some 14 genera have been reported

(**Teugels, 1986**) against two in South East Asia. The aquaculture attributes of clariids include: ability to withstand handling stress, disease resistance, high growth rate, yield potential, fecundity and palatability. Clarias anguillaris are most readily acceptable in Nigeria, because they grow to large sizes.

As a result of rise in human population, aquaculture has become among the frontier industries in the world to meet the demand of animal protein requirement, which is even severely needed among the developing nations **(Msangi *et al.*, 2013).** The situation is being alleviated by the study of aquaculture environmental factors, such as temperature, pH, dissolved oxygen, turbidity and so on. Temperature is a major factor that determines the growth rate of aquatic organisms. To increase their production, good conditions which increase their production per culture units and increase growth rate, improved feeds must be applied **(FAO, 2008).**

Just like every other animal, respiration refers to the availability of oxygen and release of carbon dioxide in fish which may be carried out either through the skin, mouth or lungs as the case may be, even with organs like gills **(Jensen *et al*., 2003).** Most fish exchange gases using gills on either side of the pharynx. Gills are tissues which consist of threadlike structures called filaments. These filaments have many functions and are involved in ion and water transfer as well as oxygen, carbon dioxide, acid and ammonia exchange **(Randall, 1984).** Each filament contains a capillary network that provides a large surface area for exchanging oxygen and carbon dioxide. Fish exchange gases by pulling oxygen-rich water through their mouths and pumping it over their gills. The gills push the oxygen-poor water out through openings in the sides of the pharynx. Most species employ a counter-current exchange system to enhance the diffusion of substances in and out of the gill, with blood and water flowing in opposite directions to each other **(Andrews *et al*., 2010)**.

The temperature of the aquatic medium in which the fish is cultured determines the respiratory rate of the fish and consequently, its survival, productivity, distribution and normal biological activities **(Anita and Pooja, 2013)**. Inability of fishes to adapt to temperature fluctuations is responsible for the inability of fishes to respond physiologically to the environment and hence result to death **(Ayanwale *et al*., 2014)**, which is related to changes in the metabolic pathway **(Forghally *et al*., 1973)**. The oxygen is ultimately used in the oxidation of foods and other metabolic activities. As the degree of water temperature increases it produce highly stress conditions on fishes, the degree of toxicity produced is dependent upon environmental conditions such as temperature, pH of water, oxygen content and presence of residue molecules **(Tantanpale *etal*., 2009)**.

Therefore, this study focuses on the effects of different temperature levels on the opercular respiratory rate of Clarias anguillaris fingerlings under laboratory conditions.

MATERIALS AND METHODS

Source of the Clarias anguillaris fingerlings

Five hundred, four weeks old Clarias anguillaris fingerlings of average weight, standard length and total length of 1.30g, 5.01cm, and 5.73cm respectively were purchased from a private fish farm, Lagos, Lagos State.

Acclimatization of the fish

The fishes were allowed to acclimatize in the rearing tanks in the Laboratory of Biological Sciences of Federal University of Technology, Bosso Campus Minna (Latitude 9'31 and 400 North and Longitude 6'31 and 6'450East of the equator) for five days, during which dead and weak specimens were eliminated daily. They were fed to satiation with commercial fish feed (Coppens) in the morning and evening during the hours of 7am and 7pm respectively **(Ayanwale *et al*., 2017)**.

Experimental set-up

The aquaria were set up and maintained at four temperature levels namely, 26.66±0.28 (Control), 30, 32 and 34℃, using thermo-regulators (Model: LifeTech, 2009). The Control experiment had no thermo-regulator but maintained at normal laboratory temperature **(El-sheriff and El-feky, 2009)**. The aquaria tanks were filled with borehole water of 30 litres and stocked with 50 fingerlings each. The set-up was in two replicates for each temperature treatment including the control. The fingerlings were fed to satiation in the morning and evening of each day. The experimental diet was offered by hand-spreading at one side of the aquarium. Water exchange was done twice in a week during the morning hours **(Ayanwale *et al.*, 2017)**.

Determination of physicochemical parameters

Temperature

Water temperature of the control treatment was determined with mercury in bulb thermometer (10-1100C range).

Dissolved oxygen

Dissolved oxygen was determined using Winkler Azide method **(APHA, 2010)**. Water from each tank were collected into 250ml of dissolved oxygen bottle and fixed right in the laboratory with 1ml of reagent Manganous sulphate (A), 1ml of reagent (B) alkaline iodide solution (KOH+KI) and 2ml of concentrated sulphuric acid (H2SO4) acid added to each sample. Ten (10ml) of the sample was titrated with 0.025N Sodium thiosulpate using starch as an indicator. Calculation was based on the formula below (Boyd, 1979).

$$\text{Dissolved oxygen (mg/L)} = \frac{\text{Volume } (Na_2SO_3) \times \text{Normality} \times 8 \times 1000}{10\text{ml}}$$

Where, normality = 0.025ml of sodium sulphate (Na2SO3)
8 = Equivalent weight of oxygen in water.
1000 = conversion to mg/litre.

Ammonia (NH3)

One hundred (100ml) of the water samples were taken each from the experimental tanks and were pipetted into a Markham distillation apparatus (Kjeldal flask) after which 5ml of 40% NaOH was added. The flask was linked to the condenser and cooling water was switched on. 10ml of the 40% boric acid (H3BO3) solution was placed under the condenser and was distilled slowly until 50ml of the distillate was collected in the receiving flasks. The ammonia was obtained from the distillate by titrating with 0.05M HCL until the colour change from green to pink **(APHA, 2010)**.

NH_3 (mg/L) = $\frac{\text{titre value x 14 x 0.01 x 1000}}{\text{V}}$

Where 0.01 = Molarity of HCl used as titrant;
14 is the molecular mass of nitrogen;
1000 is conversion factor to mg/litre
V is the volume of sample used.

Biological oxygen demand (BOD)
Water samples were collected each of the experimental tanks and incubated for five days in the dark before the titration for oxygen using Winkler Azide method as explained above. BOD (mg/L) = Dissolved oxygen in day 1 – Dissolved oxygen in day 5.

pH
The pH of the water samples were determined with Jenway 3305 pH meter model standardized with buffer solutions of pH 4.0, 7.0 and 9.0 at room temperature for 5minutes before the readings were taken.

Electrical conductivity
The electrical conductivity meter probe (Jensway 4010) was inserted into the sampled water from the experimental tanks for 5 minutes before the readings were taken.

Measurement of weight
Five (5) Clarias anguillaris fingerlings were randomly selected from each of the experimental tanks weekly and were placed on a plain paper to absorb water. The specimen fish was then placed singly on a plastic petri dish cover whose weight was adjusted to zero and the weight of the fishes were determined using an electronic pocket scale; model EHA25 as described by **Kerdchuen and Lengendre, 1994** and cited by **(Ayanwale *et al.*, 2014)**.

Determination of opercular respiratory rate
This was determined according to the modified methods of **Ambrose and Ambrose (1995), and Ayoola and Fredrick (2012)**. One fingerling from each of the experimental tanks was randomly removed and placed gently in a similar aquaria tank filled with 30 litres of water. The fish were allowed to recover from stress incurred during handling before the number of opercular beats per minute was counted using a stop watch and repeated 5 times for each fingerlings between 7:00 to 10:00 am throughout the experimental period.

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 **(Duncan, 1955)** method was used to separate the means where there were statistically significant differences ($P < 0.05$).

RESULTS

The results (mean± standard error) of Opercular Respiratory Rate (ORR) of the C. anguillaris fingerlings exposed to different temperature levels are presented in Table1. There was significant ($p< 0.05$) reduction in the ORR from 113.60 ± 7.67 to 105.50 ±10.23 opercular beats per minute from week1 to 2 in the fingerlings cultured at control temperature. However, ORR was significantly ($p<0.05$) higher at temperature levels (30-340C) in weeks 1 and 2 ranged from 120.40±9.62 at 300C to 147.00±6.51 at 32 0C opercular beats per minute. There was significant ($p<0.05$) influenced in ORR of the fingerlings cultured at the highest tested temperature level (34.00 0C) in weeks 1, 4 and 6. In addition, the results also showed that ORR were not affected ($p> 0.05$) in the fingerlings cultured under 30.00 and 32.00 0C in weeks 2, 3, 4 and 5 respectively. Generally, in all the treatments the ORR decreased steadily in weeks from the commencement to the end of the experiment.

Table 1: Mean ±standard error of opercular respiratory rate (opercular beats per minute) of *Clarias anguillaris* fingerlings cultured under different temperatures levels for a period of 6 week

Temperature Level (^oC)	Week 1	2	3	4	5	6
26.66(Control)	113.60±7.67^a	105.50±10.23^a	101.00±10.80^b	64.40±7.19^a	59.00±6.57^b	56.00±5.42^b
30.00	120.40±9.62^b	142.00±7.42^b	84.00±7.77^a	68.00±4.89^a	54.00±4.27^a	51.00±3.14^a
32.00	133.00±6.33^c	147.00±6.51^b	84.00±7.02^a	68.00±3.27^a	54.00±4.23^a	56.00±4.00^b
34.00	144.00±4.99^d	144.40±7.26^b	118.00±9.04^b	101.00±7.52^b	90.00±5.37^c	71.00±6.23^c

Mean values with the same superscripts in columns are not significantly different at ($P > 0.05$).

The results (mean± standard error) of body weight of *C. anguillaris* fingerlings exposed to different temperature levels are presented in Table 2. The body weight of the fingerlings (ranged from 2.38±0.36 to 8.37± 1.46 g at 32.00^0C) was not influenced ($p> 0.05$) by all the treatments in weeks1, 3, and 5. However, there was significant reduction ($p< 0.05$) in the body weight (1.97± 0.33 g) of the fingerlings cultured under 32.00^0C in week 2. Also, the body weight (6.71 ± 0.73g) of fingerlings exposed to 34.00^0C was significantly ($P< 0.05$) reduced at the end of the study.

The results of correlation coefficient of the body weight and opercular respiratory rate of *Clarias anguillaris* cultured under different temperature levels are presented in Table 3. The correlation coefficient of the body weight and opercular respiratory rate of *Clarias anguillaris* fingerlings cultured under different temperature levels showed very strong negative correlation between the body weight and opercular respiratory rates of the fingerlings cultured in all the treatments.

Table 2: Mean ±standard error of weight (g) of *Clarias anguillaris* fingerlings exposed to different temperature levels for a period of six week

Temperature	Week					
Level (^{o}C)	**1**	**2**	**3**	**4**	**5**	**6**
26.66(Control)	2.27±0.4^{a}	3.32±0.27^{b}	4.41±0.61^{a}	8.54±1.78^{b}	7.60±1.63^{a}	8.10±1.21^{b}
30.00	3.41±0.8^{a}	2.93±0.35^{b}	4.16±0.55^{a}	8.54±1.33^{b}	8.06±1.41^{a}	8.32±1.21^{b}
32.00	2.38±0.3^{a}	1.97±0.33^{a}	4.76±0.79^{a}	6.71±1.17^{a}	8.37±1.46^{a}	7.57±1.17^{ab}
34.00	2.93±0.6^{a}	2.70±0.19^{b}	3.95±0.83^{a}	5.63±0.67^{a}	7.75±1.06^{a}	6.71±0.73^{a}

Mean values with the same superscripts in columns are not significantly different at (P>0.05).

Table: 3 Cross correlation coefficient between body weight and opercular respiratory rate of *Clarias anguillaris* fingerlings cultured under different temperatures for a period of 6 week

	T1B1	T2B2	T3B3	T4B4
T1R1	-0.97638	-0.89076	-0.94943	-0.95015
T2R2	-0.88603	-0.85617	-0.97496	-0.92611
T3R3	-0.89807	-0.82567	-0.97972	-0.92521
T4R4	-0.91366	-0.83325	-0.95106	-0.92571

T1B1, T2B2, T3B3 and T4B4 stand for body weight produced at 26.66 (control), 30·00, 32.00 and 34 .00 ^{0}C, respectively. While T1R1, T2R2, T3R3 and T4R4 represent the respiratory rate at 26.66, 30, 00, 32.00 and 34.00 ^{0}C respectively.

The results (mean± standard error)of physico-chemical parameters of *Clarias anguillaris* fingerlings exposed to different temperature levels during the experimental period are presented in Table 4.The physico-chemical parameters of the water in which *Clarias anguillaris* fingerlings were exposed to different temperature levels showed no significant differences (p>0.05) in the pH (ranged from 7.18±0.05 at 26.66 to 7.28±0.05 at 34 ^{0}C), Dissolved oxygen (ranged from 6.00±0.60mg/L at 26.66 to 8.20±0.60 mg/L at 34 ^{0}C) and Biochemical Oxygen Demand (ranged from 2.42±0.08mg/L at 30^{0}C to 3.42±0.08 mg/L at 26.66 ^{0}C) during the study period. However, there was significant reduction (p<0.05) in the ammonia concentration (1.11±0.11mg/L) at control temperature. On the other hand, there was significant difference (p<0.05) in the electrical conductivity of the water ranged from 519.92±5.08 µS/cm at 26.66 ^{0}C to 586.33±17.50 µS/cm at 30 ^{0}C.

Table 4: Mean± standard error of physico-chemical parameters of *Clarias anguillaris* fingerlings exposed to different temperature levels during the experimental period.

Temperature Levels(^{0}c)	pH	Dissolved Oxygen (mg/L)	Biological Oxygen Demand(mg/L)	Electrical Conductivity (µS/cm)	Ammonia (mg/L)
26.66±0.28	7.18±0.05^a	6.00±0.06^a	3.42±0.58^a	519.92±5.08^a	1.11±0.10^a
30.00	7.23±0.05^a	6.40±0.06^a	2.42±0.08^a	586.33±17.50^c	1.56±0.13^b
32.00	7.23±0.05^a	7.20±0.06^a	2.50±0.50^a	576.67±16.83^b	1.75±0.25^b
34.00	7.28±0.05^a	8.20±0.06^a	2.67±0.50^a	561.67±18.17^b	1.98±0.06^b

Mean values with the same superscripts in columns are not significantly different at (P>0.05).

DISCUSSION

The significant increase in the opercular respiratory rate of the fingerlings cultured at higher temperature levels (30.00 – 34.00 ^{0}C) in weeks 1 , 2 and similarly at the highest tested temperature levels (34.00 ^{0}C) in weeks 1, 4 and 6 respectively could be attributed to high temperature in the medium leading to a primary response to stress **(Sreeya and Lipton, 2011)**. To support the above submission, increase in temperature led to corresponding increase in opercular respiratory rates of the fish in an attempt to adapt to the environment, since temperature affects the metabolic activities, growth, feeding, reproduction, distribution and migratory behaviours of aquatic organisms **(Suski *et al.*, 2006)**. These findings were in conformity with the works of **Ayanwale *et al.* (2015)** and **Murugaian *et al.* (2008)**, they documented that increased opercular movement by the experimental fingerlings might be the reflection of an attempt by the fingerlings to extract more oxygen to meet the increased energy demand to withstand the rise in temperature but this too has a limit beyond, which the activity stops resulting in the death of the fingerlings. Similarly, **Murugaian *et al.* (2008)** also reported that *Mystus gulio* opercular beats increased with increasing temperature. However, these findings were contrary to the reports of **Tantarpale *et al.* (2012)** who reported significant reduction in the opercular beats of fresh water fish *Channa punctatus* reared at lower temperature.

Water temperatures at 30 and 32 ^{0}C in weeks 2, 3, 4, and 5 respectively have no effects on the Opercular Respiratory Rates (ORR) of the fingerlings because these temperature levels might be optimal for the normal biological activities of the fish. This might be due to the fact that fish are constantly involved in biological processes to regulate their internal body environment in order to optimize physiological processes necessary for survival and growth **(Bellgraph *et al.*, 2010)**. This is because temperature is a critical factor among many environmental factors affecting aquatic environment as well as the metabolism of internal homeostasis **(Sreeya and Lipton, 2012)**.

The decreasing trend in the opercular respiratory rate of the fingerlings cultured at control temperature in weeks 1 and 2 might be attributed to similar physiological responses and lack of stress exhibited by the fingerlings during this period **(Sreeya and Lipton, 2012**).

This observation was in consonance with the reports of **Tantarpale *et al.* (2012)** 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. Water temperature levels investigated in this study had no effects on the body weight on C. *anguillaris* fingerlings in weeks 1, 3 and 5 because phenotypic and genetic differences that usually influence fish populations and the expression of morphometric attributes have been found to be strongly influenced by fish species genetics not water temperature **(Turan, 2004;Yakubu and Okunsebor, 2011)**. The decrease in body weight of *C. anguillaris* fingerlings at 32.00 ^{0}C and 34.00 ^{0}C in weeks 2 and 6 respectively could be attributed to loss of appetite by fingerlings **(Woynarovich, 2011)**. This is because **Jobling (1994)** reported that water temperature has a major influence on the amount of food consumed by fish. The significant reduction in the body weight of fingerlings reared at 32.00 ^{0}C in week 2 confirmed higher ORR (147.00±6.51 operculum beats per minute) as reported by **Ayoola and Fredrick, 2012**. The decreasing trend in the ORR of the fingerlings from all the treatments with increase in weeks were in conformity with the works of **Ayoola and Fredrick (2012)** who observed that ORR decreased with increase in fish size. The strong negative correlation coefficient between body weight and opercular respiratory rate of *Clarias anguillaris* fingerlings were in agreement with findings of **Ayoola and Fedrick (2012)** who indicated that opercular respiratory rate decreased with increase in fish size.

Water temperature levels investigated had no effects on the Dissolved Oxygen (DO) concentration, Biological Oxygen Demand (BOD) and pH of rearing water of *C. anguillaris* fingerlings. DO concentration were within the recommended range (7.00-8.00 mg/L) for fish growth **(Saber *et al.*, 2004)**. BOD concentrations recorded from all the treatments were also within the recommended range (1.00-5.00mg/L) for aquatic organism **(CIESE, 2009)**. Similarly, the pH values of *Clarias anguillaris* fingerlings from all the treatments were within the tolerance range of 6.00 - 8.00 documented for juveniles of *Heterobranchus bidorsalis* and *Clarias gariepinus* **(Ivoke *et al.*, 2007)**. The reduction in the ammonia concentration of the control was expected because **Krishnamoorthy *et al.* (2008)** 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 (30 ^{0}C to 34 ^{0}C). However, values obtained from this study were above the range of 140 -160 mg/L **(Ovie *et al.*,2008)**.

The increase in the electrical conductivity of the fingerlings from control temperature to 30 ^{0}C was in agreement with the works of **Ayanwale *et al.* (2012)** who reported that water temperature might probably influenced or increased the mineral or ion concentration of the cultured water. The values obtained from the treatments (519.92±5.08 µS/cm at 26.66 ^{0}C to 586.33±17.50 µS/cm at 30 ^{0}C) were above the range of 140.00 to160.00 µS/cm reported by **Ovie *et al.* (2008)**.

CONCLUSION

The ORR of *C. anguillaris* fingerlings increased with increase in the highest temperature levels, while ORR decreased with increase in fish size. Water temperature had no effects on dissolved oxygen, biochemical oxygen demand, pH in all the treatments and

bodyweight of *C. anguillaris* fingerlings only in weeks 1, 3 and 5.However, ammonia concentration and electrical conductivity increased with increase in water temperature. The findings from this study revealed that higher temperature levels affects the opercular respiratory rates, ammonia concentration and electrical conductivity of *Clarias anguillaris* fingerlings in captivity.

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