INFLUENCE OF PHOTOPERIOD ON SOME HAEMATOLOGICAL PARAMETERS OF HETEROCLARIAS FINGERLINGS (HYBRID) UNDER LABORATORY CONDITIONS IN MINNA, NIGERIA

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ABSTRACT

A twelve-week experiment was conducted with Heteroclarias hybrid fingerlings under laboratory conditions to investigate the effects of different photoperiods levels: normal day and night period (control), 12hours of light and 12 hours of darkness (12L: 12D), 24 hours of light and 0.00hours of darkness (24L: 0.00D), and 0.00 hours of light and 24 hours of darkness (0.00L: 24D) respectively. The physiological responses of Heteroclarias fingerlings to different photoperiods and haematological parameters such as Mean Total Erythrocyte Count (MTEC), Mean total Leucocyte Count (MTLC), Mean Packed Cell Volume (MPCV), Mean Blood Glucose (MBG) and the Mean Blood Protein (MBP) were determined at the end of the study based on standard experimental procedures. The findings of the study showed that there were no significant differences ($P > 0.05$) between all the haematological parameters. The MPCV ranged from 12.75 $\pm$0.64% at 0L:24D to 15.00 $\pm$0.90% in the control treatment, MTEC ranged from 1.35 $\pm$0.05 x 10^{12}/L in 0L:24D to1.60$\pm$0.10 x 10^{12}/L under control and 12L:12D treatments, MTLC ranged from 14.40 $\pm$0.90 x 10^{9}/L at 24L:0D treatment to 15.30 + 0.90 x 10^{9}/L under 12L:12D treatment, MBGL ranged from 4.15 x 0.05 mMol/L at 0L:24D to 4.75 $\pm$ 0.35mMol/L under the control treatment while MBPL ranged from 6.40 $\pm$ 0.10g/dL under 12L:12D treatment to 6.60$\pm$0.20g/dL under 24L:0D respectively. The physicochemical parameters of the water such as Water temperature, pH, Ammonia concentration, Biological oxygen demand and Dissolved oxygen concentration were determined by standard methods and were within the optimum range for fish culture in the tropics. Different photoperiod had no significant effect ($P > 0.05$) on the physicochemical parameters of the water. The conclusion of this study showed that photoperiods had no effect on haematological parameters of Heteroclarias and physicochemical parameters of the culture medium. The findings obtained from this study will provide baseline information to fish farmers on the photoperiodic requirements of Heteroclarias fingerlings.

Keywords: Heteroclarias, hybrid, photoperiod, physiological responses, Haematological parameters, physicochemical parameters.

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INTRODUCTION

Haematological studies have been used as a road map in the diagnosis of many diseases and in evaluating their responses to therapy in both animals like fish and human beings (Solomon and Okomoda, 2012). Haematological

studies are also used routinely to assess the level of stresses due to environmental and nutritional factors. Haematological parameters such as haemoglobin undergo seasonal variation co-committal to climatic changes, light, water, temperature and to a lesser extent influenced by age (Hordig and Hoglundix, 1982). These parameters have also been reported to vary significantly with the application of different environmental stressors like temperature, laundry, pH, water currents and dissolved oxygen (Solomon and Okomoda, 2012). Physiological changes in the blood cells indices such as levels of lactate, glucose, plasma protein and cortical have been observed in fish exposed to altered photoperiodic conditions. However, the results were varied and species – specific in most cases and observed after the photoperiodic exposures usually ranged from 30days to 90days (Valenzuela, 2008). But, Davis *et al.* (2008) on their parts reported that when live adult specimen of *Clarias batrachus* were exposed to 24L:0D (24 hours of light absence of darkness) control and OL: 24D (absence of light: continuous darkness for 24hours). The total erythrocyte count, total leucocytes count were not significantly affected by photoperiodic changes.

The experimental fish used in this study is a hybrid from two African Cat fish, viz: *Clarias gariepinus* (female) and *Heterobranchus bidorsalis* (male) (Solomon and Boro, 2010).

Tsadu *et al.* (2008) documented that there was a rising increase in the demand for Heteroclarias or Clarias branchus hybrids than their pure breeds for aquaculture because of their fast and high growth rates. Heteroclarias has also been reported to be the most widespread and accepted hybrid fish in Africa, especially in Nigeria (Khaleg, 2000 and Ayanwale *et al.*, 2014).

Therefore, this study was designed to evaluate the influence of photoperiod levels viz: normal day and night period (control), 12hours of light and 12 hours of darkness (12L: 12D), 24 hours of light 0 hours of darkness (24L;0D), 24 hours of light and 0 hours of light (24D:0L) on some haematological and physicochemical parameters of Heteroclarias fingerlings under laboratory conditions.

MATERIALS AND METHODS

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

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Source of the experimental fish

One thousand eight hundred four weeks old Heteroclarias fingerlings with average weight of 0.89g were purchased from a private fish farm in Lagos, Lagos state, Nigeria.

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 (Adewolu *et al.*, 2008, Ayanwale *et al.*, 2014). During this period, the fish were fed on a commercial diet (Coppens) to satiation, morning and evening following the method of Ayanwale *etal.*, 2014. Water exchange was done when necessary in the morning. The left overfeed and faecal samples were siphoned immediately after feeding (Ghanbari *et al.*, 2012).

Experimental Design

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

Experimental set-up

The experiment consisted of four treatments with three replicates, each treament with stocking density of one hundred and fifty fingerlings. Photo periodic levels were determined using the 24 hours period in a day according to the method of Biswas *et al.* (2008). Treatment 1 was the control (normal day and night period), while treatments 2, 3, and 4 had 12 hours of light: 12 hours of darkness (12L:12D), 24 hours of light : 0.0hours of darkness (24L: 0D), and 24 hours of total darkness: 0.0hours of light (24D:0L) respectively. Twelve plastic indoor aquaria tanks with 15litres capacity (55 x 35 x 35cm^3) filled with borehole water up to the 25cm level were used for the set- up. The artificial lighting of treatments 2 and 3 were maintained throughout the duration of the experiment with the aid of an inverter as an alternative source of electricity in case of power outage. The aquarium tanks were completely wrapped with a black polythene paper to prevent light from any other source that may interfere with the set-up. The total darkness condition (24D: 0L) was achieved by covering the respective tanks with cardboard papers to simulate dark period while the continuous light treatment was also achieved with the aid of an energy saving bulb (26W) hung above the centre of the aquaria tanks (Solomon and Okomoda, 2012). The experimental tanks were covered with net to prevent the fingerlings from jumping out (Olufayo, 2009). The *fingerlings were fed on a commercial* diet (Coppens) to satiation, morning and evening following the methods of Ayanwale *et al.* (2014). These experimental units consisted of a closed system, without water recirculation. Therefore, tanks were drained twice a week and replaced with fresh bole water between 08.00 and 10.00hours. The left overfeed and faecal samples were siphoned immediately after feeding (Ghanbari *et al.*, 2012). The experiment was monitored for a period twelve weeks.

DETERMINATION OF SELECTED PHYSICO-CHEMICAL PARAMETERS

Water temperature, Dissolved Oxygen, Hydrogen Ion Concentration (pH), Biochemical Oxygen Demand (BOD) and Ammonia (NH_3) of the cultured water were determined based on standard methods (American Public Health Association, 1995).

Haematological Analysis

The haematological analysis was done at Pathology Department General Hospital, Minna, Niger state. Blood samples were taken from the fish at the end of the experiment (Adeyemo *et al.* 2003). The blood was allowed to flow freely into sample bottles containing 6 % EDTA (Ethylene Diamine Tetra Acetic Acid) solution, an anticoagulant and to the other plain sample bottles (without EDTA) according to the method of Haruna and Adikwu (2001).

Blood Glucose

The method of Sacks (1999) was used to determine blood glucose level. This involved the use of 10µl of sample mixed with 1,000 µl of the working reagent (a mixture of 250 mmol/L phosphate buffer, pH 7.5, 5mmol/L phenol, 0.5mmol/L 4-Aminoantipyrine, ≥ 10ku/L glucose oxidase, ≥ 1 ku/L peroxidase and stabilizer. It was then incubated for 10 minutes at 37^{0}C for 20 *minutes* at room temperature. Spectrometer at the wavelength 500nm was used to take the readings of the sample and the standard. Glucose level was calculated using the formulae:

$\frac{SA.OD}{ST.OD} \times 100 = mmol/L$ OR $mg/dl \times 0.55$

Where:

SA. O D = Spectrometer reading of the sample
ST. O.D= Spectrometer reading of standard

Total Protein

The serum total protein was determined using Biuret method (Johnson *et al.*, 1999). The process involved mixing 20.00µL of serum with 1000.00µL of Biuret solution into test tubes and incubated at 37.00^{0}C for 5 minutes in a water bath. Reading was taken at 540nm and at 1.00cm light path cuvette using spectrophotometer (GENESYS 10, Rochester NY USA). The total protein was calculated using the formula:

$$\frac{Test\ blank}{Standard\ black} \times Concentration\ of\ stana$$

Packed Cell Volume (PCV)

The packed cell volume was determined using the standard haematological procedures described by Svobodova *et al.* (1991). Whole blood was drawn in to a heparinised capillary tube, one end of which was sealed with plasticine and centrifuged for 5 minutes at 12,000 revolutions per minute. The PCV level was read using a haematocrit reader and was expressed in percentage.

Total Erythrocyte Count (TEC) and Total Leucocytes Count (WBC)

The TEC and TLC were determined using the method of Svobodova *etal.* (1993). Blood was drawn up to the 0.5 ml mark of pipette; it was then diluted to the 101ml mark using the diluting fluid as described by Svobodova *et al.* (1993). The counting chamber was filled with the mixture after placement of cover slip (charged) and the RBC counted under the microscope.
Total TEC = Number of TEC $\times 10^{12}$ / L
About 0.02ml of blood was added to 0.38ml of Turks solution (which destroys all TECs). The counting chamber was charged and the TLC counted under microscope.
Total TLC = Number of TLC $\times 10^9$ /L

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 of physicochemical parameters of the water medium of Heteroclarias fingerlings exposed to different photoperiods for a period of 12 weeks are presented in Table 1. Generally, there were no significant differences ($P>0.05$) in the Water temperature (range= 25.55 $\pm$ 0.45 from 24L:00D treatment to 26.75 $\pm$ 0.450c under 0L:24D regimen), Dissolved oxygen concentration (range = 4.53 $\pm$ 0.65mg/L from 24L:00D to 5.17 $\pm$ 0.42mg/L under 0L:24D), Ammonia concentration (range=0.29 $\pm$ 0.05mg/L from control,12L:12D and 24L:0D to 0.30 $\pm$ 0.05 mg/L from the fingerlings exposed to 0L: 24D), water pH (range= 7.38 $\pm$ 0.38 from 12L:12D and controlled fingerlings to 7.46 $\pm$ 0.36 under 0L: 24D treatment) and Biochemical Oxygen Demand Concentration (range=1.03 $\pm$ 0.37 mg/L from 24L: 0D exposure to 1.36 $\pm$ 0.29mg/L under 0L:24D during the experimental period.

Table 1: Mean Physicochemical Parameters Measured During Experiment on Influence of Different Photoperiods on *Heteroclarias* Fingerlings

Duration of Exposure to Light (Hrs)	Temperature (°C)	DO (mg/L)	Ammonia (mg/L)	pH	BOD (mg/L)
Ambient (Control)	26.19±0.64[a]	4.64±1.00[a]	0.29±0.06[a]	7.38±0.41[a]	1.19±0.21[a]
12.00	26.59±0.37[a]	4.94±0.36[a]	0.29±0.06[a]	7.38±0.38[a]	1.14±0.42[a]
24.00	25.55±0.45[a]	4.53±0.65[a]	0.29±0.05[a]	7.45±0.37[a]	1.03±0.37[a]
0.00	26.75±0.45[a]	5.17±0.42[a]	0.30±0.05[a]	7.46±0.36[a]	1.36±0.29[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.

The results of selected haematological parameters of *Heteroclarias* fingerlings cultured under different photoperiod levels for a period of 12 weeks are presented in Table 2. Generally, there were no significant differences ($p>0.05$) in the MPCV (12.75± 0.64% at 0L:24D to 15.00± 0.90% under ambient condition), MTEC (1.35± 0.05 x 10^{12}/L at 0L:24D to 1.60 ± 0.10 x 10^{12}/L under ambient light and 12L:12D respectively, MTLC (14.40 ± 0.90 x 10^9/L at 24L: 0D to 15.30 ± 0.60 X 10^9/L under 12L: 12D regime, MBPL (4.15± 0.05mMol/L under 0L:24D to 4.75 ± 0.35mMol/L under ambient light condition) and MBPL (6.40 ± 0.10g/dL at 12L: 12D to 6.65 ± 0.25g/dL under the ambient condition) respectively at the end of the study period.

Table 2: Influence of Different Photoperiods on Selected Haematological Parameters of Heteroclarias Fingerlings for a Period of 12 Weeks.

Haematological Parameters	Photoperiods			
	Normal day & night period (control)	12L:12D	24L:0D	0L:24D
Mean Packed Cell Volume (%)	15.00±0.90[a]	14.55±0.21[a]	13.80±1.27[a]	12.75±0.64[a]
Mean Total Erythrocyte Count (x10^{12}/L)	1.60±0.10[a]	1.60±0.01[a]	1.50±0.10[a]	1.35±0.05[a]
Mean Total Leucocyte Count (x10^9/L)	15.20±1.30[a]	15.30±0.60[a]	14.40±0.90[a]	14.90±0.10[a]
Mean Blood Glucose Level (mMol/L)	4.75±0.35[a]	4.55±0.25[a]	4.50±0.10[a]	4.15 x 0.05[a]
Mean Blood Protein Level (g/dL)	6.65±0.25[a]	6.40±0.10[a]	6.60±0.20[a]	6.50±0.10[a]

Values are mean ± standard deviation followed by the same superscript(s), in the same row, are not significantly different at ($p>0.05$) tested by DMRT.

DISCUSSION

The results of physiochemical parameters in this study were not influenced by the respective photoperiods and all were within the range approved for culturing fresh water fishes in the tropics. Water temperatures of 25.55±0.45 to 26.59± 0.37^{0}c were within the range of 25.00-32.00^{0}c acceptable for good fish growth (Ayanwale *et al.*, 2014). Dissolved oxygen concentration of the water media of Heteroclarias fingerlings; (4.53 ± 0.65 to 5.17± 0.42mg/L) were also within the recommended value of 5.00mg/L required for healthy growth, tissue repairs and reproduction of warm water fish as reported by Svobodova *et al.* (1993). The Ammonia concentration of 0.29± 0.05 to 0.30 ± 0.05mg/L were within the range 0.01 to 1.55mg/L for freshwater fingerlings as documented by Kohinoor *et al.* (2001). Water pH of 7.38± 0.38 to 7.4± 0.36 were also within the range of 6.50 to 9.00 as documented by Bryan (2004). Similarly, the Biochemical oxygen demand concentration of 1.03 ± 0.37 to 1.36 ± 0.29 mg/L recorded in this study were also within the acceptable range of 1.0 to 5.00mg/L recommended for fish growth in the tropics (CIESE, 2010). These results suggest no organic pollution from left over feed or faecal matter in the rearing media of Heteroclarias fingerlings throughout the experimental period which in turn increased the Dissolved oxygen concentration. These findings might be attributed to constant aeration and changing of water in all the experimental tanks (Ayoola and Fredrick, 2012). Therefore, to increase the productivity of Heteroclarias in captivity adequate aeration and refreshment (recirculatory system) of pond water must be put in place by such fish farmers.

The insignificant influence of photoperiods on the physicochemical parameters recorded in this study were in conformity with the findings of Campagnolo and Nuner (2008) who reported that *Pseudoplatystoma corruscans* fingerlings exposed to different photoperiod levels indicated no significant differences ($p>0.05$) in all the physiochemical parameters measured and also stayed within the acceptable range for fish culture.

Laboratory or field studies relating to the influence of photoperiod on haematological parameters are rather few in fishes and responses observed are quite variable (Srivastava and Sanjeer 2010). The insignificant influence of photoperiod irrespective of the treatments at the end of the study on the packed cell volume and total leucocytes counts respectively were in consonance with the findings of Ali Bani (2009) who also reported no significant influence of photoperiod on the PCV and TLC Juvenile Great Sturgeon (*Huso huso*) fish reared under different photoperiodic levels. The insignificant influence of photoperiod on the TEC recorded in this study was on the contrary to the findings of Martem yanov (1995) who observed that the erythrocyte number in fish blood decreases in responses to stress. These confirming results agreed with the earlier finding of adequate or minimum dissolved oxygen concentration in the water rearing media of Heteroclarias fingerlings. This also suggests that the fingerlings were not under any physiological stress due to oxygen demand as reported by Murugaian *et al.* (2008). To support the above submission, Biswas *et al.* (2004)

documented that Nile Tilapias exposed to different photoperiodic levels for 3months had their packed cell volume not significantly affected by photo periodical changes. Similarly, Davis *et al.* (2008) also documented that live adult specimen of *Clarias batrachus* exposed to 24L: 0D, Control and 00L: 24D had their total erythrocyte counts not significantly affected by photo periodical changes.

However, the findings of this study contradicted the reports of Valenzuela *et al.*, (2008) who observed that when rainbow trout fish were exposed to a photoperiod of 24L: 0D for a period of 14days the fish demonstrated an increase in the number of erythrocytes. The insignificant influence of photoperiod on the blood glucose and blood protein levels of Heteroclarias fingerlings observed in this study were in conformity with the works of Davis *et al.*, (2010) who opined that exposure of *C. batrachus* to artificial photoperiod (24L:0D, 0L:24D) for 24 hours did not indicate any significant changes in plasma glucose concentration. The findings of this study also confirmed that photoperiod alterations has been reported to stimulate or delay gonad maturation, and thus change spawning period, somatic growth, survival and social behaviour of fish such as *C. gariepinus* but not haematological variables (Rad et al., 2006 and Solomon Okoda, 2012).

CONCLUSION

Photoperiod had no effect on the selected haematological parameters of Heteroclarias fingerlings and physicochemical parameters of water media under laboratory conditions. However, farmers are advised to conduct routine evaluation of haematological and physicochemical parameters.

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