

Growth, Survival and Haematological Responses of *Heterobranchus bidorsalis* Juveniles to Changes in Photoperiod

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Abstract

The effects of artificial photoperiods on growth and haematology of *Heterobranchus bidorsalis* juveniles were assessed. *Hetrobranchus bidorsalis* (5.69±0.41g/100fish/m²) were randomly exposed to tanks representing different artificial photoperiods of 24L:0D (PD1), 18L:6D (PD2), 12L:12D (PD3), 6L:18D (PD4) and 0L: 24D (PD5) for 84 days. Feed intake, Mean weight gain (MWG), Feed Conversion Ratio (FCR), Specific Growth Rate (SGR) and Survival Rate were determined. Blood samples were analyzed for Packed cell volume (PCV), Red blood cells (RBC), Haemoglobin (Hb), White blood cells (WBC), platelets and white blood cell differentials. Data were subjected to one-way ANOVA and Fisher's LSD follow-up test was used to separate the means. Differences were considered significant at p < 0.05 and correlation analysis was used to test relationship between variables. Feed intake showed no significant difference (p<0.05) in treatments, but was marginally higher in PD5. However, MWG was highest in PD4 and PD5 groups $(22.30\pm 4.48g \text{ and } 20.40\pm 5.63g \text{ respectively})$ while it was least in PD1 and PD2 $(16.5\pm0.39$ sg and 15.0 ± 3.77 g). The FCR ranged between 1.2 ± 0.06 and 1.6 ± 0.72 , with the least values obtained in PD4 and PD5 while the highest value was in PD2. The PCV, RBC and WBC ranged from 20.00±200 to 29.00±1.00, 1.30±0.14 to 2.10±0.22^b and 15,000±200 and 27166.70±202 respectively. PD1 and PD2 had higher lymphocyte: neutrophil ratio, 68:24 and 67:27, respectively compared to the control (PD3) which recorded 71:21. The findings indicate that exposure of *H. bidorsalis* juvenile to continuous light for long duration could affect feed consumption and growth performance as well as haematological parameters while extended period of darkness achieves better performance.

Keywords: Heterobranchus bidorsalis, photoperiod, growth, haematology.

Introduction

The diurnal and seasonal light cycles, as well as changes in day length produce a predictable variation in the environment of organisms. Photoperiodism is associated with biological rhythms (Helfrich-Förster, 2003) and circadian rhythms usually have period lengths that are remarkably temperature sensitive. Knowledge of the optimal environmental conditions for fish growth is essential for all stages to maximize yields and reduce costs of production (Lambert and Dutil, 2001; Shan *et al.*, 2008). Manipulation of environmental factors such as temperature, salinity and photoperiod currently is used to modulate fish growth in culture (Jobling, 1994). This is because photoperiod manipulation can be used to regulate physiological functions such as growth, survival, gonadal maturation, reproduction (Björnsson et al., 2000; Bonnet et al., 2007) and metabolism in fish. Photoperiod has been used successfully to improve the larval (Hart et al., 1996), juvenile and adult growth of some species (Simensen et al., 2000; Petit et al., 2003). However, very little has been documented about the effects of photoperiod on the performance of Heterobrachus bidorsalis, an important cultured species in African aquaculture. It is evident that the increasing and decreasing components of the seasonally changing day length can be replaced by substituting day lengths or a combination of day lengths by artificial lighting/illumination (Bromage et. al., 2001; Taranger et al., 1998). This work therefore sought to determine the effects of different photoperiod regimes on the growth performances, nutrient utilization and haematological responses of Heterobranchus bidorsalis juveniles.

Materials and Methods

Two hundred juveniles of *H. bidorsalis* $(5.69 \pm 0.41g)$ were procured from the Fish Farm of the Department of Aquaculture and Fisheries Management, University of Ibadan, Nigeria and acclimated for two weeks at the Aquaculture Laboratory of the Department under 12hour light and 12hour darkness (Shahkar *et al.*, 2015).

Experimental Procedure

Fish were randomly stocked in five different photoperiod treatments at 100fish/m² as follows; PD₁: 24 hours light (24L: 0D), PD₂: 18 hours light and 6 hours darkness (18L: 6D), PD₃: 12hours light and 12hours darkness (12L: 12D), PD₄: 6 hours light and 18 hours darkness (6L: 18D) and PD₅: 24 hours darkness (0L: 24D). The PD₃ served as the control experiment. The experiment was set up in a dark room and light was provided by a 4 feet fluorescent tube placed 1.5m above the tanks. Black polythene sheets

were used to hood the bulbs to prevent light penetration at the periods the treatments were exposed to darkness. Fish were fed a commercial floating feed (2mm diameter, 45% crude protein) at 5% body weight for 84 days. Feeding rate was adjusted according to changes in weight occurring over a two week period.

Water Quality Analyses

Some physico-chemical parameters of the culture water were determined weekly. Dissolved oxygen, DO, was determined by titration using Winkler's method as described by Boyd, (1979). Alkalinity was determined with HANNA water test kit model no HI 3811. The pH was determined using a HANNA HI 98107 pH meter. Temperature was determined with the aid of mercury in glass thermometer.

Evaluation of Responses

Weight of the fish in each tank was measured biweekly by batch weighing using a sensitive weighing scale (Scout PRO 200x0.01, Model No: SPU 202) to the nearest 0.01g. Specific growth rate (SGR %), feed conversion ratio (FCR), gross efficiency of feed conversion (GEFC) and percentage survival were determined (Yanik and Aras, 1998).

Heamatological Analysis

Before the commencement of the feeding trial blood was taken from some fish sample for haematological analyses. At the end of the feeding trial fish were randomly selected for blood analysis across replicates in each treatment using a 2ml disposable hypodermic needle and syringe. Blood samples were taken from the caudal vein with heparinized syringes, put separately in 1ml heparinized tubes containing 0.5% EDTA anticoagulant and immediately centrifuged at 5,000rpm for 15 min to separate the plasma. The Packed Cell Volume (PCV) and Haemoglobin (Hb), Mean Corpuscular Volume (MCV) and Mean Corpuscular Heamoglobin Concentration (MCHC) were determined using methods of Koedprang et al., (2002). The plasma enzyme analyses were done as described by Thomas and Roberstson (1991)

Statistical Analysis

Data collected were subjected were subjected to descriptive statistic, analyses of variance and means separated using Fisher's LSD (least significant difference) test to determine significant difference among the means at p<0.05 confidence level.

Results

Water Quality Analysis Results

Results of the water quality analyses showed that the parameters were maintained at narrow ranges for all the treatments and replicates. This is shown in Table 1.

Growth Performance and Nutrient Utilization

The highest feed intake $(12.50 \pm 1.30g)$ was recorded for PD₅ followed by PD₄ with mean value $10.6 \pm 0.59g$. Fish in PD₂ had the least feed intake of $23.9\pm 1.38g$. As shown in Table 2, the feed intake of the fish in the treatment exposed to 24 hour darkness was higher and significantly different (p<0.05) from that of the other treatments. The highest mean weight gain 22.30 $\pm 4.48g$ was recorded in PD₅ followed by PD₄ with a mean weight of 20.40 $\pm 5.63g$ and PD₃ which has 19.10 $\pm 2.17g$. The least mean weight gain, $15.0 \pm 3.77g$ was recorded in PD₂. There were no significant differences (p<0.05) in the mean weight gain recorded in all the treatments except for PD₅. The FCR had least values of 1.3 ± 0.5 , 1.2 ± 0.6 and 1.2 ± 0.1 in treatments PD₃, PD₄, PD₅ respectively. These values were significantly different from those obtained from PD₁ and PD₂ with respective values of 1.5 ± 0.46 and 1.6 ± 0.72 .of SGR, GEFC and survival rate were not significantly different (p<0.05) but the higher weight gain. The percentage survival rate ranged between 83.3 ± 5.77 and 93.3 ± 5.77 .

Haematological Parameters

The PCV were significantly different (p<0.05) higher in PD₄ and PD₅ than the other treatments, with respective values of 29.00 ± 1.00 g/l and 25.00±5.00g/l. Fish in treatment PD, had the lowest mean PCV value of 20.00+2.00 g/l which was not significantly different from PD₁ and PD₂ (p<0.05). Values of Haemoglobin obtained in treatments PD₁ and PD₄ (7.00 ± 0.40 g/m³ and $7.80\pm$ 0.30 g/m³ respectively) which were and significantly different (p<0.05) and higher than the values obtained in PD₃ and PD₄ ($6.50\pm0.50 \text{ g/m}^3$ and 6.80 ± 0.60 g/m³ respectively) were significantly different from those obtain in other treatments. However, treatment PD₁ was also significantly higher than those of the other treatments. The highest RBC ranged between $1.30\pm0.14 \text{ g/m}^3$ and $2.11\pm0.22 \text{g/m}^3$. The highest value was obtained for treatment PD₅ while the least was recorded for treatment PD₁. As shown in

Treatment	DO(mg/l)	Alkalinity(mg/l)	рН	Temperature(⁰ C)
PD1	5.1±0.35	204±10.15	6.8±0.01	28±1.00
PD2	5.1±0.47	210±8.65	6.9±0.01	28±2.00
PD3	4.9±1.78	162±20.56	6.9±0.02	29±1.00
PD4	5.0±1.25	216±8.05	7.2 ± 0.03	27±1.00
PD5	4.9±1.35	234±10.84	7.2 ± 0.03	28±1.00

 Table 1. Water Quality Parameters

 Table 2: Growth Performance and Nutrient Utilisation of Heterobranchus bidorsalis Juveniles

 Kept under Different Photoperiod Regimes

Treatment	PD1	PD2	PD3	PD4	PD5
MWG(g)	16.5±0.39 ^a	15.0 ± 3.77^{a}	19.10±2.17 ^{ab}	20.4±5.63 ^{ab}	22.3±4.48 ^b
FI(g)	24.3 ± 0.39^{a}	23.9±1.38 ^a	$25.3{\pm}0.87^{a}$	25.3 ± 0.59^{a}	27.5 ± 1.30^{ab}
FCR	1.5 ± 0.46^{a}	$1.6{\pm}0.72^{a}$	1.3 ± 05^{b}	$1.2{\pm}0.6^{b}$	$1.2.\pm0.06^{b}$
SGR	$0.8{\pm}0.40^{a}$	$0.7{\pm}0.90^{a}$	$0.50{\pm}0.8^{a}$	$0.8{\pm}0.11^{a}$	$0.8{\pm}0.09^{a}$
GEFC	17.6 ± 4.78^{a}	166.3 ± 18.59^{a}	191.2±5.53 ^a	196.2 ± 41.28^{a}	177.6 ± 17.18^{a}
SR (%)	93.3 ± 5.77^{a}	$90{\pm}10.0^{a}$	90.3 ± 5.77^{a}	$90.0{\pm}0.00^{a}$	$93.3 {\pm} 5.77^{a}$

NOTE: Means with the same letter along the row are not significantly different (p<0.05)

Table 3, the values of WBC obtained from fish in all the treatments were significantly different (p<0.05) and ranged from 15,000±200 g/m³ to 27166.70±202 g/m³. The platelets also followed similar patterns to the WBC. The percentage lymphocytes in the fish in treatment PD₅ had the least value of 67.00±2.00% while fish in treatment PD₄ had the highest value of 74.30±1.53%. The percentage neutrophils also ranged between 21.00±1.00% and 29.00±4.00%

which were recorded in treatments PD_3 and PD_5 respectively. There were no significant differences in eosinophils and MCH value in all the treatments. The MCV values for PD_1 , PD_3 , PD_4 and PD_5 showed significant differences with one another but PD_4 and PD_5 were not significantly different from each other (p<0.05).

The correlation table (Table 4) also showed that there was positive relationship between WBC and

PD₅ Parameter PD_1 PD_3 PD_4 PD_2 PCV(%) 22.00±2.00^a 21.00±2.00a 20.00±200^a 25.00 ± 5.00^{b} 29.00 ± 1.00^{b} Hb(g/L) 7.00 ± 0.40^{ab} 6.50 ± 0.50^{a} 7.80 ± 0.30^{ab} 6.80 ± 0.60^{a} $8.80\pm0.40^{\circ}$ $1.60{\pm}0.02^{ab}$ 1.70±0.02^{ab} 1.40 ± 0.56^{a} 2.10b±0.22^b RBC(µL) 1.30 ± 0.14^{a} $WBC(10^9/L)$ $16,000\pm300^{ab}$ 15,066.70±1527^a 17100±200^{ab} 27166.70±202^c $15,000\pm 200^{a}$ $129,000\pm 300^{d}$ Plate($10^9/L$) 142000±2000^a 156.000 ± 1000^{b} 194.000±2000^c 233000±1000^e 68.00±1.00^{ab} 67.00±2.00^a 71.0 ± 2.00^{b} 74.30±1.53° 67.00 ± 2.00^{a} Lymph $(10^{\circ}/L)$ 24.00±2.00^{ab} Neutro($10^{9}/L$) 27.00 ± 1.00^{b} 21.00±1.00^a 20.30±0.58^a $29.00\pm4.00^{\circ}$ $Mono(10^9/L)$ 3.00 ± 1.00 3.00±1.00 3.00 ± 1.00 2.00 ± 0.00 1.00 ± 0.00 Eosin $(10^{9}/L)$ 5.00 ± 2.00 3.00 ± 0.00 4.00 ± 1.00 4.00 ± 1.00 3.00 ± 1.00 MCV(fl) 127.00 ± 2.00^{a} 132.00±4.00^{ab} $151.00\pm4.00^{\circ}$ 235.00 ± 5.00^{d} 137.00±4.00^b MCHC(g/l) 40.00±2.00^a 41±.00±2.00^a 51.00±1.00^b $73.00{\pm}4.00^{c}$ 41.00 ± 2.00^{a} 30.00±2.00^a 34.00±2.00^a 30.00 ± 2.00^{a} MCH (pg) 31.00 ± 1.00^{a} 31.00±1.00^a

 Table 3. Haematological Parameters of *Heterobranchus bidorsalis* Juveniles reared under different photoperiod regimes

NOTE: Means with the same letter along the row are not significantly different (p<0.05) PCV: Packed cell volume; Hb:Haemoglobin, RBC: Red blood Cell; WBC: White Blood Cell; Plat: Platelets; Lymp: Lymphocytes; Neut: Neutrophils; Monopils; Eosi: Eosinophils, MCV: Mean Corpuscular Volume; MCHC: Mean Corpuscular Haemoglobin Concentration

 Table 4. Correlation Analysis for Haematological Parameters of Heterobranchus bidorsalis

 Juveniles under Different Photoperiod Regimes

					1	0					
	PCV	Hb	RBC	WBC	Plat	Lymo	Neut	Mono	Eosi	MCV	MCHC
PCV()	1.00										
Hb	0.81	1.00									
RBC	0.64	0.52	1.00								
WBC	0.74	0.87	0.68	1.00							
Plat	0.81	0.84	0.51	0.85	1.00						
Lymp	-0.6	-0.02	-0.44	-0.32	-0.40	1.00					
Neut	0.32	0.31	0.71	0.60	0.42	0.81	1.00				
Mono	0.44	-0.63	-0.44	-0.71	-0.76	-0.20	-0.29	1.00			
Eosi	0.90	0.90	0.20	-0.21	-0.32	0.20	0.29	0.55	1.00		
MCV	0.16	0.23	-0.43	-0.11	0.27	0.79	-0.59	-0.16	0.8	1.00	
MCHC	0.12	0.18	-0.42	-0.19	0.15	0.87	-0.65	-0.12	0.17	0.97	1.00

PCV: Packed cell volume (%); Hb:Haemoglobin (g/L), RBC: Red blood Cell (μL); WBC: White Blood Cell (10[°]/L); Plat: Platelets (10[°]/L); Lymp: Lymphocytes (10[°]/L); Neut: Neutrophils (10[°]/L); Monopils (10[°]/L); Eosi: Eosinophils (10[°]/L), MCV: Mean Corpuscular Volume(fl); MCHC: Mean Corpuscular Haemoglobin Concentration (g/l)

platelet. Lymphocyte was negatively correlated with WBC. It was further shown that platelet was negatively correlated with only monophils. The PCV also correlated positively with haemoglobin RBC, platelet and WBC (Table 4). Lymphocyte values for PD₂ and PD₅ were not significantly different (p>0.05), PD₁ has an intermediate value but PD₃ and PD₄ were significantly different (p<0.05). Correlation analyses also indicated a positive relationship between lymphocyte and MCV, MCHC, monophils and eosinophils; MCV and MCHC. There were however negative correlation between neutrophils and MCV, MCHC, MCH. RBC and WBC, platelets and neutrophils were positively correlated.

Discussion

Water quality

The water quality parameters recorded from the experimental units were within the limits of tropical freshwater fish recommended by Boyd, (1979) and Omitoyin, (2007).

Growth Performance and Nutrient Utilization

This study showed that the fish in the treatments PD_4 and PD_5 exposed to prolonged and total darkness respectively (6L: 18D and 0L: 24D) had the highest feed intake and best efficiency in nutrient utilization compared to the other treatment groups. In contrast, the fish in treatments PD_1 and PD_2 exposed to total and prolonged light (24L: 0D and 18L: 6D respectively) fed least amount of feed and had the least feed utilization efficiencies. The mean weight gain recorded for treatments PD₅ and PD_4 were higher relative to PD_1 and PD_2 . The findings however contrasts the report of Jauro and Usman, (2015) who observed that Clarias gariepinus fingerling kept in 24 hour darkness consumed less feed, but utilized it more efficiently than those exposed to increasing light regime. This discovery is in tandem with the work of Ruchin, (2007) who also observed that the weight gain of European catfish Silurus glanis and African catfish Clarias gariepinus reared in aquaria was higher in the dark than in the light due to more efficient food utilization for growth. This agrees with the findings of Odunze, (2009) on Clarias gariepinus and Abdullah and Elsayed, (1998) on Oreochromis

aureus in which similar results were obtained. The inability of the fish to feed efficiently and consequently lower feed intake and weight gain could be attributed to stress of long photoperiod (Abdullah and Elsayed, 1998) as this also have considerable effects on other growth and nutrient utilisation parameters. The stress could cause the fish to spend more energy for homeostatic process (Schreck, 1982) in which the fish will not be able to utilize all the feed consumed for growth. Moshood et al., 2012 also opined that changesin photoperiod also make fish more aggressive with the attendant increase in swimming activity thus stressing the fish. The survival rates were not significantly different and the lowest value was recorded in PD₃ which occurred due to handling stress during the experiment. This report agrees with earlier report by Odunze, (2009) that the fish under photoperiod exhibited local adaptation syndrome (LAD) which may not lead to unreasonable mortality. This study has shown that there were significant differences in the blood haematological parameters of the experimental fish. There was increase in the level of haemoglobin (Hb) and decrease in mean corpuscular volume (MCV) under 24L: 0D photoperiod compared to 12L: 12D treatment (control). The increase in MCV may be due to swelling of RBC as a result of hypoxic condition of somatic stress in fish exposed to extended photoperiod (Wendelaar, 1997). The increase in neutrophils and decrease in lymphocyte recorded in 24L:0D is as a result of stress induced by extended period of illumination when compared with the control (12L:12D). It has been established that neutrophilia and lymphopenia constitute one of the consistent and characteristic changes observed in the haematological parameters of peripheral blood of fishes under stress regardless of the nature of stress (Strivastava, 2010). Closeness in MCH could be an indication of near uniformity in erythrocyte recruitment and water movement which had been indicated to be the source of variation in MCH under stressful conditions.

Conclusion and Recommendation

The impact of environmental conditions on physiological growth regulators need to be well understood. If physiological measurement reflect the growth of fish, evaluation of culture conditions could be accomplished much quicker and cheaper than lengthy growth studies. In outdoor culture situations, management for optimum conditions is limited by the changing of the seasons and cost constraints. Under controlled culture systems, environmental conditions such as photoperiod can be easily regulated. From this research it has been shown that growth, survival and haematological parameters of *Heterobranchus bidorsalis* Juveniles are affected by changes in photoperiod. It is therefore suggested that correct application of photoperiod may improve performance, profitability and sustainability of aquaculture practices.

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