SERUM BIOCHEMICAL AND HISTOLOGICAL ANALYSIS OF *Heterobranchus bidorsalis* JUVENILES EXPOSED TO VARYING PHOTOPERIOD REGIMES

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Abstract

This study investigated the effects of photoperiods on the serum biochemistry and histological parameters of *Heterobranchus bidorsalis* juveniles exposed to artificial photoperiods for 84 days. Fish were randomly allocated into five different photoperiod treatments at 10 fish/m2 as follows; PD1: 24 hours light (24L: 0D), PD2: 18 hours light and 6 hours darkness (18L: 6D), PD3:12 hours light and 12 hours darkness (12L: 12D), PD4: 6 hours light and 18 hours darkness (6L: 18D) and PD5:24 hours darkness (0L: 24D) while PD3 served as the control experiment. Fish were fed twice daily with 45% crude protein commercial feed at 3% body weight. At the end of the experiment, blood samples were collected at random across treatments and in triplicates from the caudal vein with syringes and put separately in 1ml plain tubes and allowed to clot then centrifuged at 3000rpm for 15minutes to obtain serum for determination of serum biochemical parameters. There were increases across treatments in the aspartate amino transferase (AST) from 25 IU in PD3 to 35 IU in PD1 and 40 IU in PD2 as well as alanine amino transferase (ALT) from 17 IU in PD3 to 21 IU in PD1 and 32 IU in PD2. Fish were also selected at random and dissected for histopathological observations of some organs which revealed that fish in PD1 showed neuronal swelling and chromatolysis of the brain tissue, necrotic neurons in PD2 and PD3, spongiform vacuole in PD4 and PD5. The liver of the fish kept under PD1 showed autolysis, PD2 contained larger cytoplasmic vacuoles while PD3, PD4 and PD5 were normal without any visible lesions. The above suggested that fish in PD1 and PD2 were stressed. Therefore, culturing fish species such as *Heterobranchus bidorsalis* under extended period of darkness could minimize stress and enhance the health of the fish

Keywords: Photoperiod, *Heterobranchus bidorsalis*, serum biochemistry, histological parameters.

Introduction

Photoperiod appears to be one of several environmental stimuli that is related to the duration of light time over a day (Bizzaro et al., 2019). Animals also exhibit periodicity that occurrs in nature in diverse ways such as periodic movement, daily foraging and annual migration behaviours (Li et al., 2012). All physiological and metabolic processes in animals require a certain length of time which are rhythmic in nature (Sharma, 2003). Every organism has its own biological clock, which is reset by environmental cues to synchronize with the external environment (Helfman, 1993). The time measuring mechanism involved in photoperiodic responses appears to be based on a circadian rhythm of sensitivity to light. Although, the existence of circadian rhythms for measurements of day length remains hypothetical, the presence of internal time keeping mechanism is manifested by circadian rhythms at all levels of animal organization, for instance, cycles of cell divisions, hormone release, organismal level and behavioural patterns (Brady, 1982). These rhythms could be endogenous which may proceed in the absence of external stimuli, meanwhile they are entrained or reset by photoperiods which synchronize them to the diurnal light cycle (Teugels, 1990).

Intensive fish culture practices widely utilize photoperiodic manipulation for obtaining desired growth rates, spawning and early maturation of larval fish. Receptivity of fish to light profoundly changes according to the species and the developmental status (Boeuf and Le Bail, 1999). The light conditions that is associated with seasonal changes can regulate the endocrine and nervous systems of animals (The Free Library, 2019). Influence of photoperiod on adult fishes has been observed as changes in light dependent circulation rhythmicity, melatonin production and its secretory patterns (Iigo et al., 1991), anatomical and biochemical changes in the pineal organ (the chief transducer of environmental light in fish through melatonin) (Srivastava, 2003). Previous studies have shown that photoperiod influences fish growth (Osho et

al., 2016) and reproduction (Boeuf and Le Bail, 1999). The focus of this work is therefore to investigate the influence of photoperiod on the serum biochemical parameters of *Heterobranchus bidorsalis* subjected to varying regimes of light exposure as well as assessing the impacts of photoperiod on the histology of the brain, liver and kidney of the experimental fish.

Materials and methods

Two hundred juveniles of *Heterobranchus bidorsalis* (mean weight 5.69 + 0.41) were acclimated in four plastic tanks for three weeks before the experiment commenced. Fifteen experimental tanks of 60 litres capacity each were divided into five treatments. Each treatment was replicated thrice. Each replicate was stocked with ten juveniles of *Heterobranchus bidorsalis*. Before stocking, the fish were batch weighed to the nearest grammes. Fish were fed a commercial floating feed-Durante® feed-(2mm diameter, 45% crude protein) at 3% body weight for 84 days. Feeding rate was adjusted according to changes in weight occurring over a two-week period.

Experimental procedure

This work followed the procedure described by Osho et al. (2016). Fish were randomly stocked in five different photoperiod treatments at 10 fish/m2 as follows; PD1: 24 hours light (24L: 0D), PD2: 18 hours light and 6 hours darkness (18L: 6D), PD3:12 hours light and 12 hours 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.

Analysis of serum biochemical parameters

Prior to the commencement of the feeding trial, blood samples were taken from some fish for analysis of serum biochemical parameters. At the end of the feeding trial, fish were randomly selected for serum analysis across replicates in each treatment using a 2ml disposable hypodermic needle and syringe. Blood samples were taken from the caudal vein with syringes and put separately in 1ml plain tubes and allowed to clot. Collected blood samples were centrifuged at 3000rpm for 15minutes to obtain serum. The collected serum was used for the analysis of total proteins, albumin, creatinine, aspartate amino transferase (AST), alanine amino transferase (ALT), blood urea nitrogen and glucose. The total protein, albumin, globulin, blood Urea Nitrogen (BUN) were determined according to Coles (1986). Alanine amino transferase (ALT) and aspartate amino transferase (AST) were estimated using the colorimetric methods as described by Frankel (1957). Serum creatinine was measured as described by Harrison (1947).

Histopathological analysis

Three fishes were randomly selected from each treatment and across the replicates of the experiment. They were ventrally dissected to ensure a careful removal of the selected tissues for histopathological analysis. The organs (livers, kidneys and brains) were removed and prepared for histopathological observation. They were fixed in Bouin's fluid for 24hours and washed with 70% ethanol and dehydrated through a graded series of ethanol (Schalm et al. 1975; Kelly, 1979). They were embedded in paraffin, sectioned at 4-5 µm; thickness stained with hematoxylin and eosin and examined using light microscope and photomicrography (Kaneko, 1989).

Statistical analysis

Data collected were subjected to analysis of variance and means separated using Fisher's Least Significant Difference to test significant difference among the means at $p<0.05$ confidence level. Results were presented as means + standard deviation.

Results and Discussion

Results for Serum Biochemical Parameters

Results of the serum biochemical parameter among the treatments are shown in Table 1. The numerical higher total protein value was recorded in PD2 (3.8 +0.20g/dl) while the least was in PD1 (3.1 +0.15g/dl) but there was no significant difference among the treatments (p>0/05). Also no significance differences (p>0.05) in Albumin, Globulin, A:G Ratio and Creatinine levels among the treatments. Highest glucose level was recorded in PD4 (control) with mean value of 241+9.00mg/dl. There was no significant difference (p>0.05) between PD1 and PD5, PD3 and PD4 but these two groups were significantly different from each other and from PD2. The lowest ALT and AST values were noticed in PD 3, which is similar to PD1 but differ significantly from PD 2 and PD 4 ($p<0.05$). The highest BUN was recorded in PD5 which was not significantly different from PD2, PD3 and PD4 but significantly different from PD1 ($p<0.05$).

This study showed that plasma protein levels of *H. bidorsalis*. were within the recommended range of 2-8g/dl (Omitoyin et al., 2006) and values obtained were not significantly different from one another. Blood glucose levels have long been used as indicators of stress in fish (Hattingh, 1976; Wedemeyer and McLeay, 1981). Under conditions of stress, blood glucose either remained unchanged or took a longer duration of stress to show the change. In the current study, plasma glucose varied widely as there were significant decrease in glucose levels under 24L: 0D (PD1) and 18L: 6D (PD2) photoperiod as compared to 6L: 18D (PD3) and 12L: 12D (PD4 control). The observed lower glucose concentration recorded in PD1 and PD2 fish could be due to intense swimming activity and exhaustion due to continuous light, thereby causing increased energy demand and glucose utilization.

Table 1: Serum Biochemical Parameters of Heterobranchusbidorsalis Juveniles Reared under Different Photoperiods.

Treatment	PD_1	PD_2	PD_3	PD_4	PD_5
TP(g/dl)	3.10±0.15 [a]	3.80±0.20[a]	3.00±0.60 [a]	3.50±0.60 [a]	3.40±0.10 [a]
Albumin (g/l)	1.0±0.30 [a]	1.4±0.40[a]	0.80±0.30 [a]	1.20±0.3 [a]	1.30±0.10 [a]
Globulin (g/dl)	2.1±0.15 [a]	2.4±0.20 [a]	2.10±0.25 [a]	2.30±0.20 [a]	2.10±0.55 [a]
A:G Ratio	0.4±0.15 [a]	0.5±0.25 [a]	0.30±0.10 [a]	0.50±0.20 [a]	0.60±0.20 [a]
Glucose (mg/dl)	207±5.00 [b]	104±3.00 [a]	241±9.00 [c]	232±4.00 [c]	218±4.00[b]
AST(IU/l)	35±2.00 [ab]	40±7.00 [b]	25±3.00 [a]	36±6.00 [b]	32±2.00 [a]
ALT (IU/l)	21±3.00 [ab]	32±4.00 [c]	17±3.00 [a]	29±4.00 [c]	27±3.00 [bc]
BUN(mg/dl)	1.2±0.60 [a]	2.00±0.20 [b]	1.80±0.20 [ab]	1.8±0.10[ab]	2.40±0.40 [b]
Creatinine (mg/dl)	0.60±0.20 [a]	0.8±0.20 [a]	1.00±0.20 [a]	0.70±0.40[a]	1.10±0.20[a]

**Means with the same letter along the row are not significantly different (p▒0.05)*
Note: TP: Total Protein, A:G-Albumin:Globulin, BUN-Blood Urea Nitrogen, Creatinine

This result agreed with the submission of Strivastava and Sanjeev (2010) who reported that the increase in plasma glucose levels in *C. batrachus* was due to increased swimming and not due to the stressor effect of prolonged light. In *C. batrachus*, prolonged light is known to decrease locomotor activity (Srivastava, 2003) therefore not necessitating increase in plasma glucose levels. In line with our result, Bizarro et al. (2019) also observed that GIFT male Tilapia subjected to 24 h light showed lower glycemic levels.

Besides, the enzyme activities of the *H. bidorsalis* under the different photoperiods showed significant variations as the values of AST and ALT were highest in the 18hour photoperiod (18L: 6D). These enzymes can be used as biomarkers of stress in fish. Both the AST and ALT function as a link between carbohydrate and protein metabolism by catalyzing the interconversion of strategic compounds such as alpha keto-glutarate and alanine to pyruvic acid and glutamic acid respectively (Tiwari and Singh, 2004). These biomarkers therefore provide a tool for specific early warning sign for liver damage in fishes. Liver stress is generally known to elevate AST in fish (Tiwori and Singh, 2004) and this also conforms to the nature of function of AST where they respond to any liver-related stress or altered physiological condition.

As revealed by the correlation analysis presented in Table 2, there was positive correlation between total protein and globulin as well as albumin; r =0.70 and 0.76. Albumin showed a positive correlation with A: G ratio, ALT and Blood Urea Nitrogen, Globulin is positively correlated with AST and negatively correlated with A: G ratio. The A: G ratio did not show any relationship. Also, glucose showed a negative correlation with AST and ALT.

Histopathological analysis

The photomicrograph of the histopathological changes observed in the brain, kidney and liver of *H. bidorsalis* juveniles under different photoperiod regimes are as presented in Plates 1-3 and summary of the changes is presented in Table 3. The histopathological observation on the brain of the fish revealed the presence of well-defined aggregates of necrotic neurons and deep staining cells suspected to be inflammatory cells in PD1 and PD2. PD3 and PD4 showed numerous spongiform vacuoles while a few foci of neuronal swelling, degeneration and chromatolysis were observed in PD5. There were hepatocytes containing larger clear cytoplasmic vacuoles when compared with other sections of the liver in PD1 and PD2. PD3, PD4 and PD5 did not show any visible lesion revealing a normal parenchymatous appearance. There were no recognizable changes observed in PD2, PD3, PD4 and PD5. The kidney tissue of the fish exposed to 24-hour light (PD1) however showed a mild loss of the renal tubules indicated by empty spaces.

Histopathological studies of fish under the different photoperiods revealed that fish organs could be efficient indicators of stress occurring as a result of light inducement or photoperiod in fish (Harper and Wolf, 2009). This study has shown that the histopathological changes observed in the brain of *H. bidorsalis* under all the photoperiods showed one form of defect or the other ranging from well-defined aggregates of necrotic neurons and deep staining cells suspected to be inflammatory cells in (PD1 and PD2),numerous spongiform vacuoles in PD3 and PD4 as well as a few foci of neuronal swelling, degeneration and chromatolysis in PD5. The factors responsible for these may be due to other reasons than photoperiod or stress alone. Harper and Wolf (2009) reported that Jewel fish (*Hemicromis bimaculatus*) when they were exposed to chronic crowding stress, the

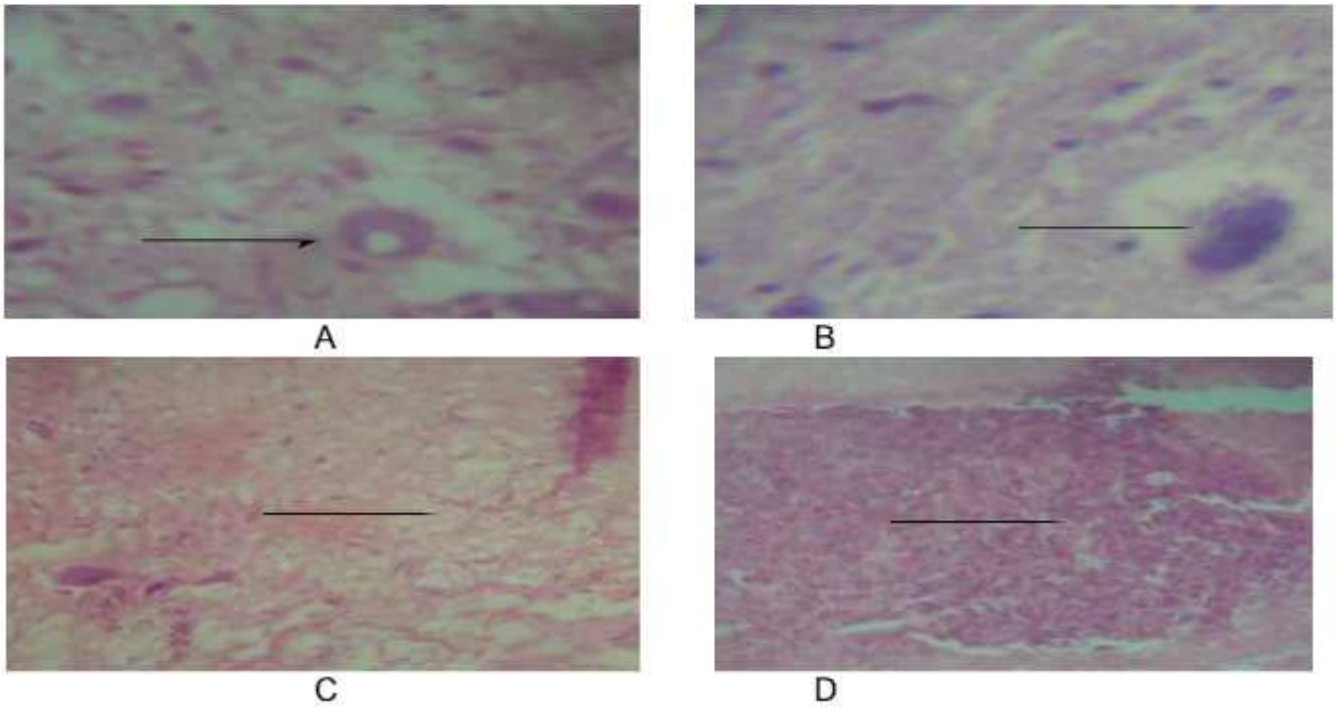
Table 2: Correlation analysis table for serum biochemical parameters of H. bidorsalis under different photoperiod regimes

	TP	Albumin	Globulin	A:G	Glucose	AST	ALT	BUN	Creatinine
T P	1.00								
Albumin	-0.07	1.00							
Globulin	0.70*	-0.10	1.00						
A:G Ratio	-1.6	0.83*	-0.53*	1.00					
Glucose	-4.6	-0.44	-0.34	-0.21	1.00				
AST	0.76*	0.07	0.61*	-0.10	-0.60*	1.00			
ALT	0.38	0.77*	0.34	0.54	-0.57*	0.39	1.00		
BUN	0.22	0.54*	0.15	0.36	-0.12	-0.02	0.35	1.00	
Creatinine	-0.41	0.37	-0.17	0.24	0.11	-0.66	0.25	0.46	1.00

Values with (*) asterisk are correlated
Note: BUN: Blood Urea Nitrogen, TP: Total protein, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase

crowded fish had structural cell alteration in the optic rectum, a major area of the brain concerned with processing and integrating sensory information as also reported by Burgess and Cos (1982). It can however be established that this was not truly stress response but instead a developmental adaptation caused by long term differences in patterns of sensory stimulation as reported by Harper and Wolf (2009). Barton and Iwama (1991) further noted that if the intensity of the stressor is overly severe or long-lasting, physiological response mechanisms may be compromised and can become detrimental to the fish's health and well-being or maladaptive, a state associated with the term distress.

Observation of the liver revealed that there were no visible lesions on sections of the liver investigated under 0L: 24D, 6L: 18D and 12L: 12D photoperiods respectively. There was however an entirely different observation under 24L: 0D and 18L: 6D photoperiod where autolysis and hepatocytes containing larger clear cytoplasmic vacuoles



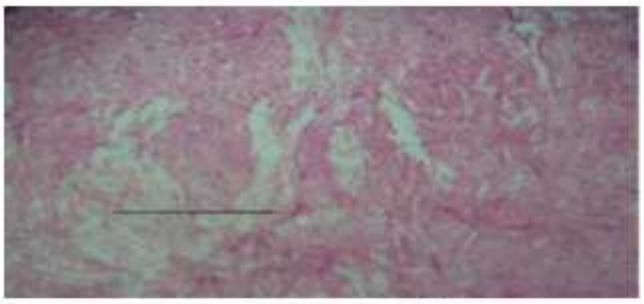


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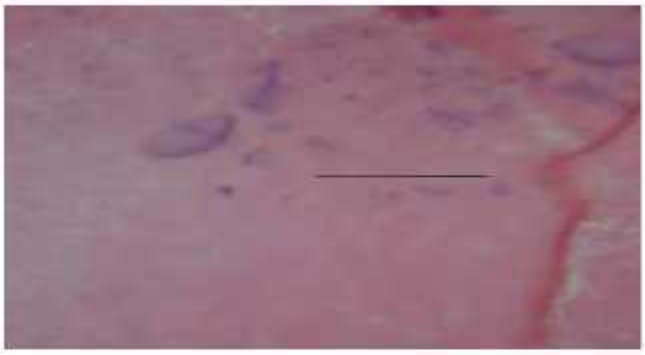
Plate 1: Brain sections under different photoperiods
A: 24L: 0D (PD1) Foci of neuronal swelling, degeneration and chromatolysis
B: 18L: 6D (PD2) Well-defined aggregates of necrotic neurons and deep-staining cells.
C: 6L: 18D (PD3) There were numerous spongiform vacuoles
D: 12L: 12D (PD4) Well-defined aggregates of necrotic neurons and deep-staining cells
E: 0L: 24D (PD5) Numerous spongiform vacuoles.



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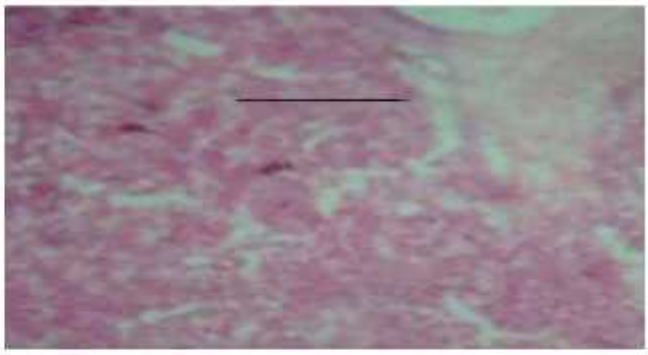

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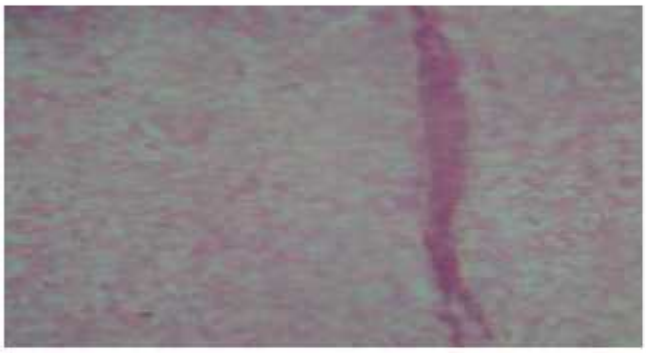

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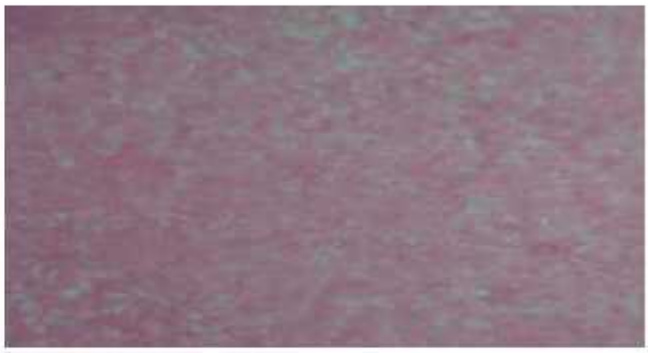

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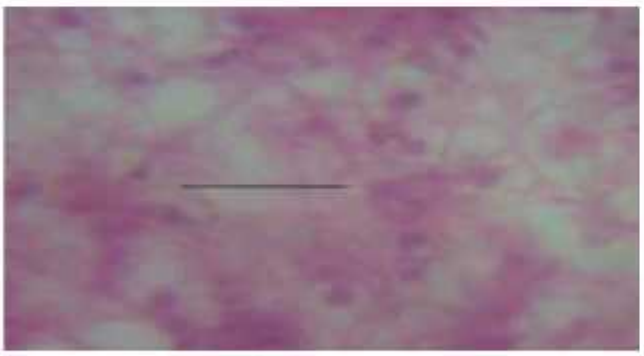

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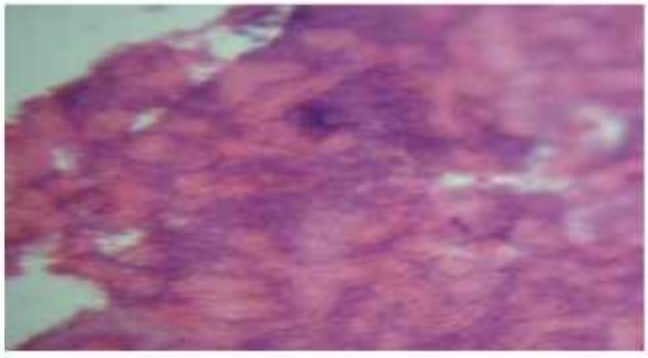
Plate 2: Liver sections under different photoperiods
A: 24L: 0D (PD1) Artefact/Autolysis
B: 18L: 6D (PD2) Hepatocytes contain larger clear cytoplasmic vacuoles compared with other sections.
C: 6L:18D (PD3) No Visible Lesion
D: 12L: 12D (PD4): No Visible Lesion
E: 0L: 24D (PD5): The hepatocytes contain larger clear cytoplasmic vacuoles when compared with other sections of the liver.

A



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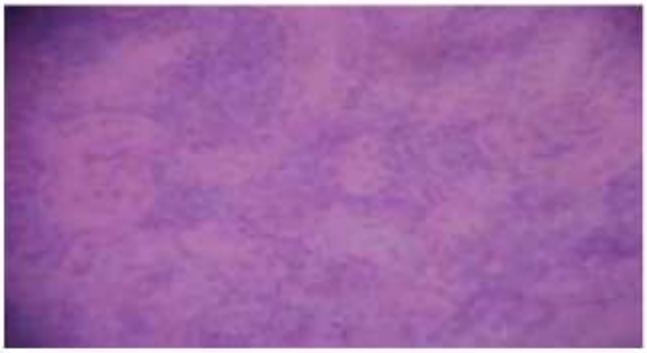

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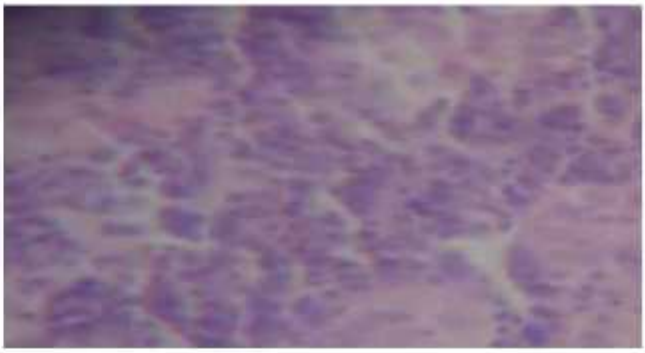

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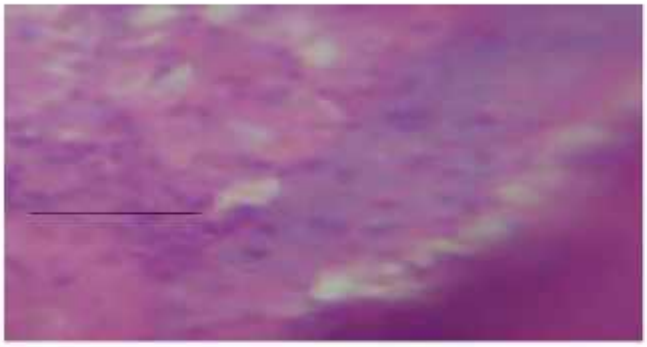

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Plate 3: Kidney sections under different photoperiods
A: 24L: 0D (PD1): No Visible Lesion
B: 18L: 6D (PD2): No Visible Lesion
C: 6L: 18D (PD3): No Visible Lesion
D: 12L: 12D (PD4): No Visible Lesion
E: 0L: 24D (PD5): There was mild loss of the renal tubules leaving behind empty spaces.

Table 3: Histopathological changes observed in the organs of H. bidorsalis juveniles under different photoperiods

Organs	Histological Changes	Photoperiods				
		24L:0D	18L:6D	12L:12D	6L:18D	0L:24D
Brain	-Well defined aggregates of necrotic neurons and deep staining cells	-	++	++	-	-
	-Numerous spongiform vacuoles	-	-	-	++	+
	-Few foci of neuronal swelling, degeneration and Chromatolysis	++	-	-	-	-
Liver	-Hepatocytes contain larger clear	-	++	-	-	-

Note: ++ = Presentand distinct with morphological changes of histological signs on the organ
+ = Present but less marked (mild) than usual
- = No lesion and morphological changes in organs

were detected. This may arise as a result of high demand on the liver occasioned by attempt to strike a balance between the fish state and the environmental challenge of extended light/illumination period. Meanwhile the main functions of the liver include processing nutrient from food, make bile, remove toxins from the body and build proteins (Ajani et al., 2011). Any interference with these important functions can lead to poor health (Daniel, 2009). Stress responses may be evident in liver because of its prominent role in energy storage and metabolism (Gingerich, 1982). This is consistent with a report by Teh et al. (1997) that channel catfish (*ictaurus punctatus*) showed hepatic necrosis and haemorhage as well as splenic changes such as edema, hyperemia and necrosis under the influence of

stress resulting in hypoxic conditions (Harper and Wolf, 2009). Wolf and Wolfe (2005) noted that quantitative alterations in hepatic energy storage are visible macroscopically as changes in liver size and coloration, and histologically as variations in hepatocellular vacuolation and tinctorial staining characteristics, increased hepatocellular vacuolation is more commonly associated with overnutrition or toxicity.

The kidney under the 24L: 0D photoperiod showed a mild loss of the renal tubules leaving behind empty spaces. Harper and Wolf (2009) reported that there were fewer reports of stress response involving the kidney except the hypoxia induced haemorhage, glomerular congestion and edema in the posterior kidneys of channel catfish. Photoperiods in other treatments were found to show no visible signs of distress even the effect in the 24L: 0D was mild. It can therefore be surmised that photoperiod has no significant effect on the kidney of *H. bidorsalis* but that an extended exposure such as 24 hours could bring about some visible signs of stress. Wolke (1992) noted that Pigmented Macrophage Aggregate (PAM) centers are variably sized constituent nests of phagocytic cells that can contain one or more intracytoplasmic pigments, such as ceroid, lipofuscin, melanin and hemosiderin. The kidney and spleen tend to be common locations for PAM which tend to increase in number and/or size as fish age, but reports indicate that proliferation of these structures may also occur as a non-specific response to various stressors, such as heat (Blazer et al. 1987), starvation and nutritional imbalance (Moccia et al. 1984).

Conclusion

Environmental factors such as photoperiod can influence the degree to which fish respond to stressors. As compared to terrestrial inhabitants, fish and other aquatic creatures are subject to a broader variety of stressors because their homeostatic mechanisms are highly dependent on prevailing conditions in their immediate surroundings.

Recommendation

The knowledge of the mechanisms of photoperiod will help scientists to predict phenology and the dynamics of the numbers of fishes in nature, to breed and monitor beneficial species and to direct the development of commercially important fishes under artificial conditions.

Declaration of interests

Authors have declared that no competing interests exist.

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