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RESEARCH PAPER

Photoperiodic Modulation on Growth and Behaviour of the Giant Gourami, *Trichogaster fasciata* (Bloch and Schneider, 1801)

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Abstract

The influence of different photoperiods was studied on the growth and behaviour of the giant gourami, *Trichogaster fasciata.* Fish (weight=1.1g; length=4.36cm) were subjected to photoperiod regimes of 0L:24 D, 8L:16 D, 12L:12 D and 16L:8 D for 90 days in triplicates. The growth rate was highest (weight=4g) and lowest (weight=2.36g) in the groups subjected to 16L: 8D and 0L: 24D photoperiods respectively. The mean body weight and mean total length were significantly higher (P<0.05) while standard length and caudal fin length (P>0.05) were not significant in the 16 hours light. Mean values of food conversion ratio and condition factor were lowest while various growth parameters were found to be maximum in the 16 hours light. 16L:8D photoperiod regime produced pronounced effects on the welfare of giant gourami as shown by the nonintrusive welfare indicators. The photoperiodic signals were found to be capable of modifying the behavioural activities where male aggression related to territory or defence and female selection were reduced in control group. The optimum photoperiod (16 L: 8 D) was an environmental cue for the better growth, welfare and behaviour.

Keywords: Aggression, female selection, growth, photoperiod, welfare indicators.

Introduction

Photoperiod acts as a zeitgeber which controls growth through endogenous rhythms and different circulating levels of growth hormones in body (Simensen *et al*., 2000). Specific photoreceptor cells of retina of the eyes and pineal gland produce melatonin in fish which modulates the production of growth hormone releasing factors (Moyle & Cech, 2000; Falcon *et al*., 2010; Reinecke, 2010). The appetite, food conversion and growth energy requirement are dependent on the secretion of growth hormones in fish (Donaldson *et al*., 1979; Bjornsson *et al*., 1989) and the feeding activity is regulated by the light: dark cycle (Boujard & Leatherland, 1992). Photoperiod was reported to increase the growth and survival of larvae (Boeuf & Baille, 1999) of fish particularly in temperate regions through photo stimulation which induced feed intake and improved the feed conversion efficiency (Woiwode & Adelman, 1991). Effects of different photoperiods on growth of various fishes have been reported by a number of workers, notable among them are Davies *et al*. (1986 a/ b); Imsland *et al*. (1995); Arvedlund *et al*. (2000); Purchase *et al*. (2000); Ergün *et al*. (2003); Rad *et al*. (2006); Taylor *et al*. (2006); Valenzuela *et al*. (2006); Bonnet *et al*. (2007); Askarian and Kousha (2009) and Yager and Yigit (2009). The developmental and maturation process are governed by genetic and environmental factors in fish. Yamamoto *et al*. (2001) reported the effect of temperature and photoperiod on the growth and maturation in fish. The other factors affected growth of fish were found to be feeding rate, feed, water quality, stock density and size (Trzebiatowwski *et al*., 1981). The optimum photoperiod can successfully be used to raise the yield of fish in aquaculture was concluded by many workers such as Saunders and Henderson (1970); Lundqvist (1980); Brauer (1982) and Saunders *et al*. (1985) who worked on relationship between photoperiod and growth rate in fishes. Ruchin (2004, 2006) investigated the influence of colour light on growth performance of *Carassius carassius*, and also on the effect of light on white blood cell count in *Cyprinus carpio*. Davies *et al*. (1986a/b) and (Davies & Hanyu, 1986) worked on the effect of temperature and photoperiod on sexual maturity and spawning of the common carp. Bhattacharyya *et al*. (2005) carried out the effect of photoperiod on gonadal activity of *Catla catla*. A reaction of environment determines the behaviour which acted as key element for fish welfare as reported by Martins *et al*. (2012). The welfare indicators must provide information on potential problems and the causes of impaired welfare (Rousing *et al*., 2001). The welfare of fish denoting the fish being healthy, comfortable, well-nourished, safe and able to express their innate behaviour and not suffering from unpleasant states such as pain, fear or distress. Mustapha *et al*. (2014) reported that the welfare can be greatly influenced by photoperiod in *Oreochromis niloticus* and *Clarias gariepinus*. The influence of photoperiodism on reproductive physiology and behaviour was reported by Fiszbein *et al*. (2010) in the cichlid fish, *Cichlasoma dimerus.* The specific behavioural signals for an early assessment of fish welfare are considered to be important for both farmers and researchers in a developing aquaculture industry.

The studies showed that behavioural changes can be interpreted as either good or poor welfare depending on the fish species. A survey of literature showed that the information on the influence of light on growth and behaviour of giant gourami is scanty. Hence, the present study was undertaken to evaluate the effects of different photoperiod regimes on the welfare, survival, growth and behaviour in the giant gourami, *T. fasciata.*

Materials and Methods

A total of 200 samples of giant gourami, *T. fasciata* (weight: 0.9 to 1.7 g; length: 4.0 to 4.9 cm) were collected using cast and drag nets from river Gomti situated at Lucknow region (26º 56” N 80º 43” E) and acclimatized in the laboratory for a month. The experiment was conducted in glass aquaria (0.45×0.22×0.30 m) for the duration of 90 days where individuals of giant gourami were classified into four groups namely A, B, C and D in triplicates. Each group containing 10 samples of fish (length=1.11cm; weight=4.36g) were subjected to different photoperiod regimes of 0 L: 24 D as control and the other three were 8 L:16 D, 12 L: 12 D and 16 L: 8 D respectively. All the aquaria were properly covered with black paper so that the light does not escape outside and also to avoid entry of external light. Artificial white fluorescent lamps of 40 watt and intensity of light of 200 lux were installed at a height of 14 cm above the water level in each aquarium. The experiment was carried out in dark room to avoid hindrance of natural light. The physico-chemical conditions such as ambient temperature, dissolved O_2, pH and volume of water were maintained constant in aquaria throughout the experiment. Fish were fed with artificially prepared balanced food comprised of 35% fish meal, 28% mustard oil cake, 28% rice barn, 2% sunflower and cod liver oils each, 5% carboxy methyl cellulose and multivitamin and multimineral tablets (‘Becozyme Forte’ Glaxo India Ltd, 25 tablets/kg foods) throughout the investigation. The faecal matter was removed daily before feeding the artificial food to the fish. The amount of food offered to each aquarium was kept constant (5% of body weight of stocked fish).

The weight of the fish exposed to the four different photoperiod regimes were taken using an electronic balance sensitive up to 0.001 g in the beginning and at a regular interval of 15 days till the end of experiment. The total length (tip of the snout to the tip of the longest caudal fin ray), standard length (tip of the snout to the base of the caudal fin) and caudal fin length (terminal vertical fin of a fish) were measured. The various parameters of weight and length of fish samples exposed to the three light regimes and darkness are given in Table 1.

Growth Parameters

Feed Conversion Ratio (FCR), Specific Growth Rate (SGR), Relative Growth Rate (RGR), Absolute Growth Rate (AGR), Weight Gain were calculated using equations as per Degani *et al*. (1989).

FCR=Weight of Dry Feed used/Wet Weight of Fish (g)

Percentage of SGR=[(Final Weight–Initial Weight)/(Days)]×100

Percentage of RGR=[(Final Mean Weight-Initial Mean Weight)/Initial Mean Weight]×100

Table 1. The effects of different photoperiod regimes on body weight (g), total length (cm), standard length (cm) and caudal fin length (cm) in *T. fasciata* after

Parameter	Photoperiod			
	Group A	Group B	Group C	Group D
Initial Body Weight	1.11±0.44	1.11±0.71	1.11±0.23	1.11±0.42
Final Body Weight	2.53±0.36	2.80±0.43	3.60±0.48	4.00±0.44
Initial Total Length	4.36±0.71	4.36±0.43	4.36±0.49	4.36±0.70
Final Total Length	4.63±0.74	5.10±1.07	5.99±0.88	7.02±1.66
Initial Standard Length	3.42±0.61	3.41±0.46	3.40±0.81	3.41±0.45
Final Standard Length	3.78±0.34	4.44±0.94	4.88±0.64	5.31±0.82
Initial Caudal Fin Length	0.90±0.79	0.90±0.59	0.92±0.72	0.94±0.60
Final Caudal Fin Length	1.20±0.62	1.66±0.96	2.00±0.24	2.47±0.60

Percentage of AGR=Final Mean Weight-Initial Mean Weight

Weight Gain (mg/g/day)=[(Final Mean Weight-Initial Mean Weight)/(Initial Mean Weight/Days)]×100

Condition Factor

The study of well being and fatness of fish was calculated through the Fluton's Condition Factor (Ricker, 1973) using the formula: $K=W \times 100/L^3$

Where K is Fluton's condition factor, W is net wet weight (g) of fish and L is length in cm. The factor 100 is used to bring K close to unity.

Evaluation of Welfare of the Fish

The welfare of fish in each photoperiod tanks was conducted visually daily through the use of non-intrusive signs and danger signals. Behaviour, swimming activity, injuries and colour were studied during the experimental period as per Mustapha *et al*. (2014). Winner status was assigned to the fish that were dominated and loser (subordinate) were defeated, suppressed and also stressed.

Behavioral Study

Behavioural study on *T. fasciata* was carried out to observe the female choice and male aggressive behaviour of different experimental groups such as control group (A) vs B, C and D as per Fiszbein *et al*. (2010) on cichlid fish, *Cichlasoma dimerus*. Altogether twelve observations were made to study the behavioural activity (female selection and aggressive interactions) at an interval of five minutes for duration of 1 h.

(A) Effect of Photoperiod on Female Selection

After 90 days of experiment, male and female gouramis were selected from three experimental groups namely 'B' (8 L: 16 D), 'C' (12 L: 12 D) and 'D' (16 L: 8 D). The female gourami were isolated from control group 'A' and placed in a neutral tank with males and females from different groups of the experiment i.e., females (control) vs group B (male & female); females (control) vs group C (male & female) and females (control) vs group D (male & female). The observation was made to analyse the selection of females (control or experimental) by males. The number of attack activity by males and females of three experimental groups towards the female of control group was counted.

(B) Aggressive Behaviour between Males

The male gouramis were selected from control group and from different experimental groups such as B, C and D and were placed in three tanks separately with a big stone in the middle as: males (control) vs B, males (control) vs C and males (control) vs D. Winner and loser status was assigned to the fish that performed more aggression and received more aggressive contacts respectively. The number of these interactions was counted.

(C) Time Spent

The time spent in each observation for female selection and aggressive behaviour was recorded.

Statistical Analysis

Mean body weight and mean total length were analysed using one-way ANOVA (Parametric test) while mean standard length and mean caudal fin length with Kruskal-Wallis one-way ANOVA (non parametric test). Statistical significances between behavioural activities (female selection and aggressive behaviour) and time spent in each activity were analysed using one-way ANOVA followed by post hoc Tukey´s tests. Data are presented as mean±SD and a probability level of 5% was used as the minimal criterion of significance. The Graph Pad Prism (version 5.01) and Paleontological Statistics (PAST, version 3.12) software were used for analysis.

Results

Maximum growth was observed in the group 'D' subjected to continuous light of 16 hours followed by groups 'C' and 'B' while minimum growth was noted in the group 'A' subjected to continuous darkness. The mean body weight (4.00±0.44), total length (7.02±1.66), standard length (5.31±0.82) and caudal fin length (2.47±0.60) were found to be maximum in group 'D' as given in Table1.

One-way ANOVA (Parametric) revealed that the total weight ($p = 0.0002$, $F = 6.614$), total length ($p = 0.0143$, $F = 4.322$) were found to be statistically significant whereas Kruskal-Wallis one-way ANOVA (non parametric test) depicted that the standard length ($p=0.0831$, $F=2.506$) and caudal fin length ($p=0.0951$, $F=2.376$) were not significant (Table 2).

Growth Parameters

The photoperiod significantly affected the fish growth parameters (Table 3). Maximum value of specific growth rate (3.22±1.38), relative growth rate (24.10±5.32), absolute growth rate (0.48±0.21) and weight gain (361.53±79.83%) was found to be in group 'D' subjected to photoperiod of 16 L: 8 D. Highest and lowest values of feed conversion ratio were observed in the groups subjected to continuous dark (FCR = 0.30±0.20) and continuous light of 16 hours (FCR = 0.14±0.09) in the present study. The survival rate was 100 percent in three groups (B, C and D) subjected of different photoperiod regimes while 20 percent mortality was observed in the group subjected to continuous darkness.

Condition Factor

Highest value of condition factor (K = 1.97±0.93) was observed in the group 'A' (control) but lowest (K = 1.96±0.82) in the long light phase of 16 L: 8 D (Table 3).

Welfare Indicators of the Fish

The observations of non intrusive welfare indicators on *T. fasciata* in the four different photoperiod regimes are given in Table 4. The differences in behaviour including swimming activity and different extent of injuries were noted in the various groups of *T. fasciata* subjected to different photoperiod regimes but no any colour differences were observed. The better survival, active swimming and absence of injuries were noted in the specimens of *T. fasciata* exposed to 16 hours light duration.

Behavioral Study

(A) Effect of Photoperiod on Female Selection

Females from the control group were driven to the upper part of the tank by the attack of males and females from other groups (B, C and D) in all cases within hours of beginning of the experiment. The attack was started by males and later on by females of treated groups of different photoperiod regimes over females of control group in the present study indicated that territory was established by males and females of treated groups (B, C, D), and defended by male of the respective groups. In all the three groups "selected" female were from photoperiod influenced group but not from the control group and found to be significantly different for group 'D' (p = 0.0043, F = 6.697) (Table 5). The non selected females from control group were found to be hidden among the plants.

Table 2. The results of ANOVA for the effects of different photoperiod regimes on mean body weight, mean total length, mean standard length and mean caudal fin length in *T. fasciata*

Parameter	'p' value	'F' value
Mean body weight**	0.0002	6.614
Mean Total Body Length *	0.0143	4.322
Mean Standard Length	0.0831	2.506
Mean Caudal Fin Length	0.0951	2.376

Values with different subscript in the same row are significant (P<0.05).
Values significant at the level of *less than 0.05, ** less than 0.01 and *** less than 0.001.

Table 3. Survival rate, growth parameters including feed conversion ratio, specific growth rate, relative growth rate, absolute growth rate, weight gain percentage and condition factor after 90 days under different photoperiod regimes (Mean±standard deviation)

Growth parameters	Group A	Group B	Group C	Group D
Survival (%)	80%	100%	100%	100%
FCR	0.30±0.20	0.23±0.16	0.16±0.12	0.14±0.09
SGR	1.58±0.69	1.88±0.85	2.77±1.20	3.22±1.38*
RGR	14.95±4.15	16.91±4.09	21.95±5.58	24.10±5.32*
AGR	0.23±0.10	0.28±0.128	0.41±0.1811*	0.48±0.21 *
Weight gain	224.3±62.27	253.66±61.35	329.33±83.77	361.53±79.85**
K	1.97±0.93	1.968±1.30	1.967±0.70	1.96±0.82 *

Values with different subscript in the same row are significantly different (P<0.05).
Significant level (* less than 0.05, ** less than 0.01 and *** less than 0.001).

Table 4. Welfare assessment of *T. fasciata* under different photoperiod regimes

Welfare indicators	Group A	Group B	Group C	Group D
Injuries	Found on the body and fins	Found on the body	None	None
Colour	Normal	Normal	Normal	Normal
Swimming activity	Suppressed	Active	Active	Very active

Group A = 0 L: 16 D, Group B = 8 L: 16 D, Group C = 12 L: 12 D and Group D = 16 L: 8 D

(B) Effects of Photoperiod on Male Aggressive Behaviour

After 19 minutes of the experiment the fish showed physical interactions. Two kinds of contact aggression were observed: bites (one fish bites another one mainly in the fins and lateral parts of the body) and mouth holding (one fish lock jaws with another fish and push against one another). A photoperiod induced males from groups B, C and D dominated in all the three cases and performed the first attack biting the males of control group. A stronger territorial and aggressive behaviour was observed in males of treated groups towards control males (p=0.0046, F=6.608) (Table 5). Behavioural inhibition was observed including decreased locomotors activity in males of control group found to be hidden behind the stone or in the top surface of the aquaria. Number of observations in female attacks and aggressive interactions were counted and given in Figure 1.

(C) Time Spent

The time spent was found to be significantly different for female selection (p = 0.0163, F = 4.811) and male aggressive interactions (p = 0.0433, F = 3.660). The details are given in Figure 2 and Figure 3.

Discussion

The findings of the present study showed that the growth of giant gourami, *Trichogaster fasciata* were affected by different photoperiod regimes, and maximum growth was recorded in the groups subjected to 16 L: 8 D photoperiod regime. The photoperiod regime of 16 L: 8 D hours was considered to be optimum for proper growth and survival in giant gourami because of increased feed intake and better feed conversion ratio. The present finding was similar to the work carried out by Tandler and Helps (1985); Folkvord and Ottera (1993); Imsland *et al*. (1995); Duncan *et al*. (1999); Ergün *et al*. (2003); Rad *et al*. (2006); Yager and Yigit (2009) and Kashyap *et al*. (2015) in various fish species, their reports also suggested that the fishes subjected to continuous photoperiod obtained highest growth. A number of workers such as Saunders and Henderson (1970); Lundqvist (1980); Brauer (1982); Saunders *et al*. (1985) reported that artificially increased day light

Table 5. Behavioural activity and time spent of *T. fasciata* between control vs different experimental trials. Values in the same row marked with different letters are significantly different (ANOVA results, Tukey´s post hoc test, P<0.05)

S.No	Behavioural activity	p value	F value
Exp.1	Female selection: Total number of attacks experienced by (control) female	0.0043 c***, b**,	6.697
	Time spent in Female attacks	0.0163 c*	4.811
Exp.2	Total number of male aggressive interactions between photoperiod influenced groups towards the male (control)	0.0046 c*	6.608
	Time spent in male aggressive interactions	0.0433 c*** b**	3.660

(Group A = Control), (Group B = 8L: 16D), (Group C = 12L: 12D), (Group D = 16L: 8D)
a = (A vs B) vs (A vs C)
b = (A vs B) vs (A vs D)
c = (A vs C) vs (A vs D)
Values significant at the level of * less than 0.05, ** less than 0.01 and *** less than 0.001.

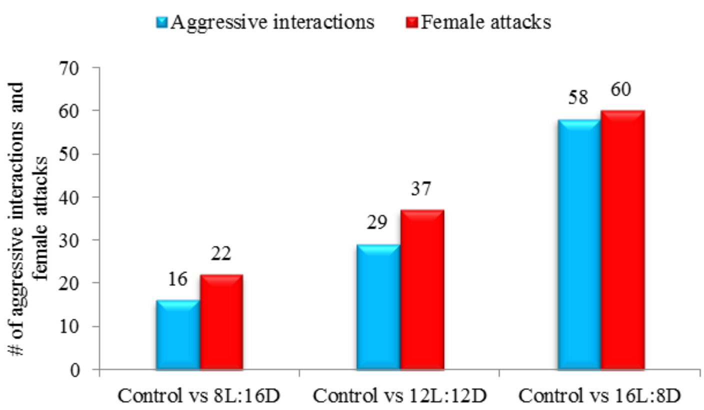

Figure 1. Total number of female attacks and aggressive interactions between control and three different experimental groups.

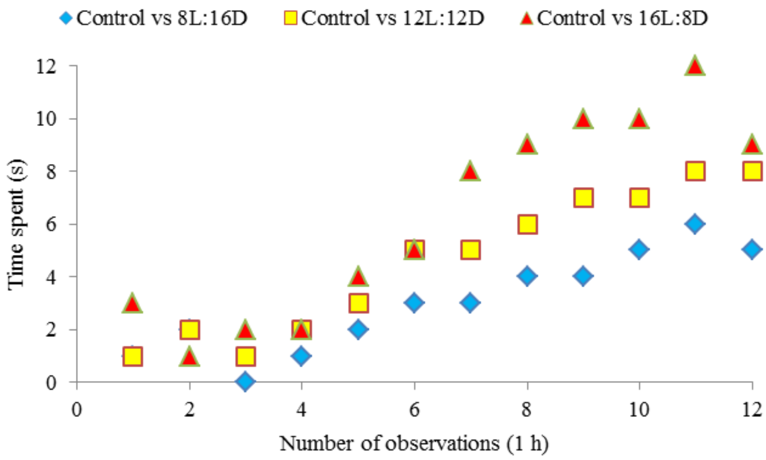

Figure 2. Time spent in each observation of female selection activity of 1 hour duration.

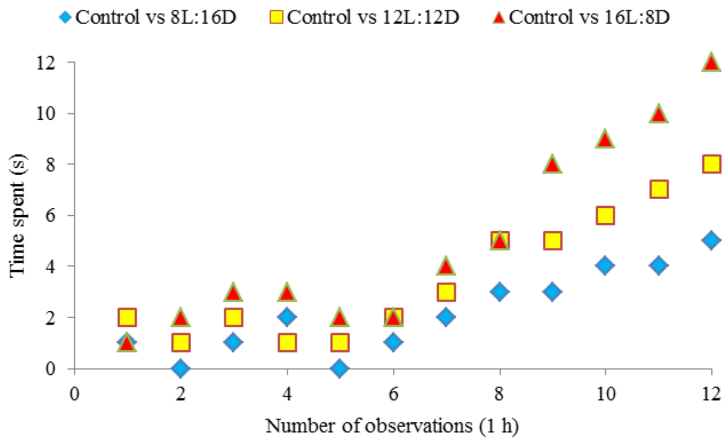

Figure 3. Time spent in each observation of male aggressive interactions in 1 hour duration.

is the good source for the enhancement of growth and development of fishes. In contrary to this, Arvedlund *et al*. (2000) reported slow growth under 24 L: 0 D as compared to 16 L: 8 D photoperiod regime in *Amphiprion melanopus* and emphasized that their developing juveniles had a period of inactivity during darkness and their growth was compromised. A numbers of workers such as Fuchs (1978) and Barahona-Fernandes (1979) reported different optimum photoperiod regimes for different fish species.

The requirement of photoperiod regime is considered to be species specific and play an important role for growth and survival of species (Britz & Pienaar, 1992; Silva-Garcia, 1996; Boeuf & Baille, 1999). The details of effect of photoperiod regimes on growth, survival and mortality are given in Figure 4. The less (2.80 g) and minimum (2.53 g) growth were recorded in the groups exposed to 8 L: 16 D hours photoperiod regime and complete darkness respectively in the current study. In contrary to this, the juvenile of *Clarius garpines* showed the high growth rate when reared under continuous darkness (Britz & Pienaer, 1992). Yamamoto *et al*. (2001) reported that shortening of light phase during the feeding trial affected feed intake of carp.

The differences in initial and pronounced growth were noticed after 45^{th} and 60^{th} day onwards of the experiment in the fish subjected to different photoperiod regimes. The same results were recorded in the case of AGR, SGR, RGR and weight gain which suggested that the juveniles of *T. fasciata* required several weeks to acclimatize to new rearing conditions. The similar results were reported by Kashyap *et al*. (2015) in *Catla catla*. Minimum value of FCR (0.139±0.090) was recorded in the group subjected to maximum duration of photoperiod which is similar to the finding of Rad *et al*. (2006) who pointed out that the duration of hours is inversely proportional to feed conversion (FCR) resulted into better growth. Sixteen hours duration of photoperiod was found to be suitable for the growth of giant gourami in the present study. Similar duration (16 L: 8 D) of photoperiod was reported by Gines *et al*. (2004) for maximum growth in Gilthead sea bream.

Condition factor is an important biological parameter which indicated the well being of fish in a specific water body where the fish grows. Moreover,

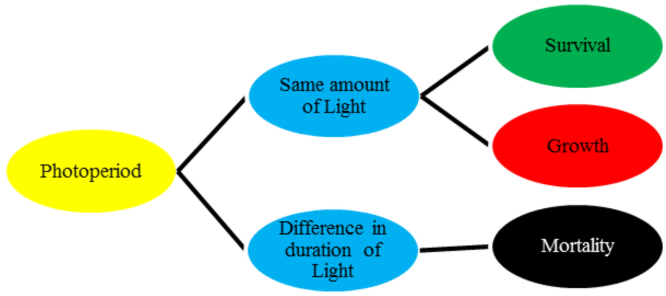

Figure 4. The effects of different photoperiod regimes on growth, survival and mortality of fish.

it is an index which indicates the physiological state of the fish and shows population welfare during the various stages of the life cycle. The value of condition factor was found to be lowest (K = 1.9635 ± 0.8203) in the group subjected to 16 L: 8 D photoperiod regime which was similar to the finding of Elsbaay (2013) who reported the lowest value of condition factor (K = 2.21) in long light phase (24 L: 0 D) while estimated the effect of photoperiod on growth rate of Nile Tilapia.

Herbert and Steffensen (2005) pointed out that the status of activity can be used as an indicator of poor or good welfare in fishes. The group ‘D’ of *T. fasciata* in present study was found to be very active as indicated by the swimming activity of the specimens which reflected that how a fish is sensing and responding to its environment. Maximum injuries were noted in the control group while no injuries were found in the groups subjected to 12 and 16 hours light duration. Almaza´n-Rueda *et al*. (2004, 2005) also validated the use of skin lesion frequency to assess the welfare of African catfish and showed that feeding methods, photoperiod and light intensity affected skin lesions frequency as well as other welfare indicators such as swimming activity, growth, plasma cortisol and free fatty acids. Continuous darkness (0 L: 24D) was found to be adverse effect on the welfare of *T. fasciata* which had resulted into the mortality of two individuals of fish. Chronic stress may be responsible for welfare impairment while acute stress might be a reason for the mortality of two fish in the present study as reported by Pickering (1998) and Damsgard *et al*. (2006) in their research works. The unfavourable photoperiods could have affected the immune systems of the species resulted in the observed deviations in the normal welfare behaviour of the giant gourami as reported by Mustapha *et al.* (2014). Pottinger and Pickering (1992) and Burgos *et al*. (2004) reported that artificial photoperiods affected the immune system of rainbow trout leading to mortality. The normal welfare condition was found to be in the photoperiod regime of 16 L: 8 D in *T. fasciata* in the present study which could be due to their innate behaviour being displayed.

The females “selected” by males in the present study were from photoperiod treated groups (B, C and D) than control (group A). The selection of a territory was found to be a characteristic behaviour in giant gourami as shown by the aggressive interaction between males from control group with other groups subjected to different photoperiod regimes led to the selection of a territory. Moreover, in aggressive interaction males of control were found to be hidden in order to escape themselves from the dominating ones. The present findings was found to be similar to that of the work reported by Fiszbein *et al*. (2010) where behavioural level of aggression related to territory selection and defence was reduced in control group, and females exposed to control were never chosen by males. Winners were those that keep most of the territory and subordinates (control) were exploited by the winners. A subordinate position within a group or social hierarchy may be a stressor (Schreck, 1981) which can be subjected to social stress resulting from the attacks of the more dominant individuals for access to resources (sexual partners and territory). Social stress caused to marked behavioural and physiological changes in subordinates, who often showed a general behavioural inhibition of food intake, locomotory activity and aggressiveness (Denight & Ward, 1982; Winberg & Nilsson, 1993a and O’Connor *et al*., 1999).

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