



# Influence of Photoperiod and Shelter on Some Phenotypic Traits in African Catfish (*Clarias gariepinus* Burchell 1822) Larvae

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## ABSTRACT

Photoperiod and shelter have direct or indirect effects on phenotypic traits expression in different fish species. The present study was, therefore, intended to explore whether these light and shelter could influence some phenotypic traits of African catfish larvae under laboratory condition. Newly hatched larvae were stocked in plastic aquaria (10L) at a rate of 5 individuals/L and reared for one month under four treatments such as 24h light (24L), 24 h dark (24D), 12h light and 12h dark with PVC (12DL\_PVC), and 12h light and 12h dark without PVC (12DL) conditions. A total of 108 larvae were sampled for phenotypic traits analysis. The results revealed that complete darkness (24D) significantly improved the overall growth parameters (total length, standard length, caudal peduncle length, anal fin length, caudal fin length, total body depth, dorsal fin length, pre dorsal distance, pre anal distance, pre ventral distance, head width, head length, eye diameter, maxillary barbell length, inter orbital length, and specific growth rate) than all other treatments, while no significant variation was found among other treatments. Although shelter did not show any significant role in the overall growth, it significantly reduced the larval mortality than those reared without shelter. The survival rates were also significantly different among various light regimes in which the highest was found in 24D (86%) followed by 12DL\_PVC (74%), 24L (71%), and 12DL (60%). The study also revealed that larvae reared in 24D exhibited maximum dark brown body colouration (63%), while in 24L showed maximum light brown colouration (96%). On the other hand, maximum larvae were appeared as medium brown color (76%) in tanks having PVC, whereas many of them were light brown (61%) in non-PVC tanks. Taken together, the study suggests that *C. gariepinus* larvae should be reared in completely dark condition to enhance their overall production.

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## Authors' Contribution

ASA Conceptualization, supervision, project administration and fund acquisition. SMR Conceptualization, methodology, investigation, data curation, writing-original draft, visualization. RTM Conceptualization, software, investigation, writing-review and editing data, visualization. YA Supervision, project administration. MMR Formal analysis, data curation, writing-review and editing. MAP Investigation, resources.

## Key words

Growth, SGR, Survival, Body colouration, PVC pipe

## INTRODUCTION

Photoperiod works as a Zeitgeber (cue or synchronizer), controlling regular endogenous rhythms in fish (Simensen *et al.*, 2000). In most cases, photoperiod regulates fish growth, social behaviour, metabolic rates, locomotion, sexual maturation, survival, reproduction and body pigmentation (colouration) (Duston and Saunder, 1990; Silva-Garcia, 1996; Boeuf and Bail, 1999; Boeuf and Falco, 2001; Trippel and Neil, 2002; Biswas and Takeuchi, 2002; Biswas *et al.*, 2002, 2005; Mendonça *et al.*, 2009). Being an environmental stimulus, the photoperiod is

connected to the light length period over a day. The intensity and increase of this 'light time' takes place in accordance with the types of weather and seasons of the locality (Bromage *et al.*, 2001). The photoperiod, in association with other synchronizers (e.g. temperature, salinity, habitat, shelter, dissolved oxygen, pH, water level, etc.), can influence the biological rhythm (circadian and/or annual) of animals, and thereby affecting food utilization, weight, energy allocation, movement, and other physiological factors as well (Biswas and Takeuchi, 2002). According to Almazán-Rueda *et al.* (2005), photoperiod requirements are species-specific and differ for every developmental phase. Dark and light rotation is, in general, regarded as the key synchronizer of feeding movement (Hossain *et al.*, 1999). While photoperiod may influence larval stages (e. g. growth) positively, juvenile stages remain unaffected (Barlow *et al.*, 1995). However,

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photoperiod has no influence on growth and metabolic rates for yellowtail flounder (Purchase *et al.*, 2000), halibut (Hallaråker *et al.*, 1995), and turbot (Imsland *et al.*, 1995).

Photoperiod manipulation is a common and effective practice in order to acquire better growth rates, maturation, spawning and accelerated development of larval fish (Mustapha *et al.*, 2014; Brown *et al.*, 2014; Lundova *et al.*, 2019). In case of mature fishes, photoperiod influence has been noticed since there are variations in light dependant circadian rhythmicity, production of melatonin and its secretory ratios (Iigo *et al.*, 1991), anatomical and biochemical variations in the pineal organ the prime transducer of the environmental light in fish through melatonin (Srivastava, 2003). Artificial photoperiod regimes are changes in the natural light and dark cycles and any manipulation or modification of environmental factors like temperature or light causes sudden changes in the environment, which may induce stress and ultimately compromise the overall welfare and interests of the fish (Barton and Iwama, 1991; Wendelaar Bonga, 1997). Light intensity offers several crucial benefits such as assisting fish to escape from predators (Talanda *et al.*, 2018), locate feeds for nourishment (Boeuf and Bail, 1999), pursue suitable preys (Granqvist and Mattila, 2004) and discover habitats (Harvey *et al.*, 2012). Moreover, the skin color of certain fishes can be affected by the reflection, absorption and scattering of light by the microstructures and pigments within the fish integument (Leclercq *et al.*, 2010; Sugimoto, 2002).

Refuges or shelters (such as caves, crevices, holes, etc.), another important life regulating physical factor, not only provide protection from currents, predators, competitors, and strong illumination but also provide opportunity for reproduction and foraging (Holbrook and Schmitt, 2002; Johansen *et al.*, 2008). Any species of fish requires particular habitat, which is also a life cycle dependent stage (Beck *et al.*, 2009). Animals use various types of shelter, but the benefits of these are not restricted to the increase in safety or reproduction. Shelter functions as a feeding ground (Moring and Nicholson, 1994; Ishida *et al.*, 2008) and resting place (Johnson and Stein, 1979; Benaka, 1999) for many teleosts. Many intertidal animals use shelters in order to avoid thermal stress and dehydration during arid periods or low tide. For instance, intertidal crabs move to suitable shelter (like burrows, crevices or damp cracks) to decrease the risk of water loss (Nybakken, 1993) while cane toad (*Bufo marinus*) seeks suitable diurnal shelter to avoid dehydration and temperature stress (Seebacher and Alford, 2002) during dry periods. So far as the Brazilian lizard, *Tropidurus torquatus*, is concerned, the excess of microhabitats, which helps egg development, affects the duration of the spawning period

as low scales of moisture reduce egg viability and decrease embryo dimensions (Wiederhecker *et al.*, 2002). Several studies have shown a positive relationship between shelter and growth in some aquatic animals. For example, mud eel (*Monopterus Cuchia*) and Asian catfish (*Clarias batrachus*) grow well in water hyacinth (Narejo *et al.*, 2003; Sahiduzzaman *et al.*, 2018) while estuarine grouper (*Epinephelus salmoides*) did better in tires of vehicles (Chua and Teng, 1979). In another study, submerged aquatic plants for *Penaeus monodon* showed the highest growth and survival (Ali *et al.*, 1999).

The influence of photoperiod on growth and survival of African catfish at the larval stage has been observed by a number of authors (Britz and Pienaar, 1992; Appelbaum and McGeer, 1998; Appelbaum and Kamler, 2000; Almazán-Rueda *et al.*, 2004), while Hossain *et al.* (1988) and Boerrigter *et al.* (2016) have observed the effect of shelter on survival, growth, aggression and stress of African catfish fingerlings having different outcomes (i.e. positive and negative results). For example, *C. gariepinus* fingerlings (weight 0.79 gm and length 49 mm) stocked in plastic tanks with shelter have had higher growth than without shelter (Hossain *et al.*, 1998) while Boerrigter *et al.* (2016) did not observe any positive results with shelter materials for the same species (8-10 gm). However, the effects of photoperiod and shelter on the phenotypic plasticity in this species, mainly larval stage, are not well understood. Immediately after hatching, larvae undergo several crucial stages (like yolk sac absorption, fin and head development, burble, eye, pigmentation, etc.) and any sudden changes in environment causes severe abnormalities that reduces the larval quality afterwards. The major constraint for widespread farming of African catfish is poor and highly variable survival of larvae under the fluctuating environmental conditions (De Graaf and Janssen, 1996). In aquaculture systems, light directly affects the quality of egg and fitness of offspring (Endal *et al.*, 2000), development (Boeuf and Bail, 1999), movement (MacPhail *et al.*, 2009), metabolism (Appelbaum and Kamler, 2000) and body colouration (Denson and Smith, 1997). All the above-mentioned aspects are species-specific and light intensity-dependent (Tian *et al.*, 2015).

African catfish, *C. gariepinus* is one of the most well accepted aquaculture fishes at least in twelve African countries (Mathiesen, 2012; Hecht, 2013). The important characteristic features of this species are easy to breed and culture with high stocking densities. Moreover, this fish can tolerate a high range of salinity, low oxygen concentration, high temperature, and most significantly, it offers large profits with minimum production cost (Teugels, 1986; Bovendeur *et al.*, 1987). Despite its limited freshwater supplies, the Kingdom of Saudi Arabia

(KSA) has emerged as a role model for the development of this species by optimizing the use of water, and high levels of sunlight and temperatures as the ecological conditions in this country are phenomenal. As Saudi Arabia solely depends on marine fish, this species could be an alternative protein source for the nation, and the regular consumption of this fish may reduce the fishing load in the sea. Considering the importance of this species in Saudi Arabia, the Kingdom introduced this species in 1987 from Egypt. However, despite having lots of potentialities, only limited numbers of studies have been conducted considering the local conditions of the middle-east where the desert climate is especially characterized by extreme temperature during summer and very low retaining water capabilities by the land. The use of shelter in fish ponds might provide protection against heat shock as well as ensure the welfare of fish as catfish is nocturnal in nature (Rahman *et al.*, 2014; Britz and Pienaar, 1992; Barriga *et al.*, 2016). Therefore, the aim of this study was to evaluate the effect of variable photoperiods in the presence/absence of shelter on some important phenotypes of larvae in African catfish *C. gariepinus*.

## MATERIALS AND METHODS

### *Broodstocks maintenance*

Fingerlings (5 to 8 cm) were obtained from the research station of King Abdulaziz City and Science Technology (KACST), Al Muzahmiya, Riyadh, KSA and maintained in 5000 tons fiberglass tank with continuous water circulation at the Fish Resources Research Center, King Faisal University, Al Hasa, KSA. Animals were fed commercial pellets (ARASCO Company, KSA) twice daily (35% protein; 5% of their body weight) until the evidence of maturation was seen. Three pairs of mature female (1520 to 1744g) and male (1100 to 1350g) broods were selected for this study to obtain experimental larvae.

### *Induced breeding and incubation*

Spawning to obtain the gametes was induced following the procedure described by Adebayo and Popoola (2008). Briefly, selected mature female and male broods were injected with OvaprimR at a dosage of 0.5 and 1.0 ml/kg for males and females, respectively and held individually in a separate plastic tank (600L) to avoid cannibalism. Ovulation took place fourteen to eighteen h from the first time of injection. Eggs were obtained in a clean bowl by stripping the female gently along its abdomen. Since sperm of *C. gariepinus* can not be obtained by stripping, testis were collected surgically. Immediately after separation, adhered fat and blood from the testes were removed and testes were cut into small pieces and passed

through bolting silk. Artificial insemination was conducted instantly after gamete collection. After mixing with fresh water, fertilized eggs were placed in hatching beds made with nylon mesh and then submerged in glass tanks (30 cm depth) with continuous water flow and aeration at room temperature (28-30°C). Hatching of *C. gariepinus* larvae occurred 15 h after artificial insemination at an average temperature of 30°C.

### *Photoperiod management*

A total of 600 newly hatched *C. gariepinus* larvae were randomly distributed into four experimental groups (three replicates per treatment) and stocked in plastic aquaria (10L) for a month to assess the effect of photoperiod and shelter on their phenotypic plasticity. Each treatment group was exposed to four different experimental conditions, i.e. 24 h light (24L), 24 h dark (24D), 12 h light and 12 h dark with PVC (12DL\_PVC), and 12 h light and 12 h dark without PVC (12DL). A stand lamp with fluorescent tube lights 80 lx was installed 15 cm above the continuous light filtered tanks. The dark tanks were covered with two layers of black polyethylene sheeting (250 p) and placed cardboard box. Remaining tanks were placed in natural photoperiod conditions. In 12DL\_PVC treatment, 2 of 6 cm PVC pipes were used as shelter material.

### *Larval rearing*

Larvae were stocked in a plastic tank (10L) at a stocking density of 5L<sup>-1</sup> for a month for observation of growth, specific growth rate, survival, body colouration and some other important phenotypic traits (see below) under different photoperiod and shelter conditions. The experimental animals were fed *Artemia* nauplii until satiation (*ad libitum*) thrice daily (0800, 1500 and 2100 h) for the first ten days, and then introduced commercial pelleted feed (ARASCO Company, KSA; 32% protein; 5% of their body weight) specially prepared for catfish species larvae. During the entire experimental stage, the water quality parameters stayed optimal. The water quality in the tanks was maintained by applying some measures like siphoning out the bottom debris and waste (leftover food, fecal matter, dead fish, etc) and replacing one-third of the water every alternative morning (0700 h) prior to feeding.

### *Phenotypic analysis*

Twenty-seven (9 individuals × 3 tanks) larvae were randomly sampled from each treatment for selected phenotypic traits measurement at the final day of the experiment. Collected larvae were euthanized in a 200 ppm benzocaine solution followed by 4% Davidson fixation (Moore *et al.*, 1953). The selected phenotypic traits of the

experimental larvae treated with different treatments in the present study included: total length (TL), standard length (SL), total body depth (TBD), caudal peduncle length (CPL), dorsal fin length (DFL), anal fin length (AFL), caudal fin length (CFL), pre dorsal distance (PDD), pre anal distance (PAD), pre ventral distance (PVD), head length (HL), head width (HW), eye dia (ED), maxillary barbell length (MBL), inter orbital length (IOL). On the other hand, yolk volume was determined according to the formula developed by [Bagarinao \(1986\)](#) for a prolate spheroid,  $YV = \pi/6 LH^2$ , where L denotes the length of yolk sac (YL) and H denotes height of yolk sac (YH). Phenotypic data assessed during this study were analyzed using the standard protocols of biometric characterization frequently practiced in the morphological characterization studies of the African catfish ([Turan \*et al.\*, 2005](#)). The photographic measurements of larvae were done using Leica stereo microscope model EZ4 integrated with 5 Megapixel HD camera (Switzerland). Image analysis software, Leica LAS EZ (Leica Application Suite, Version 20.0) was used for phenotypic traits measurement. Prior to preserving in Davidson fixation, larvae (N=27) were randomly weighed (body weight, BW) using a digital balance (Sartorius CPA10035) at the precision level of 0.000g. Final weight, final total length, specific growth rate (SGR), and survival rate were calculated in accordance with the following equations:

Final weight (g) = Mean final weight – mean initial weight;

Total length gain (cm) = Mean final length – mean initial length;

Specific growth rate =  $\{(\ln \text{ final weight} - \ln \text{ initial weight}) / \text{culture period}\} \times 100$ ;

Survival rates =  $(\text{Number of live fish} / \text{total number stocked fish}) \times 100$

#### Statistical analyses

All analyses were done using 'R' version 3.6.1 ([R Development Core Team, 2019](#)). The descriptive statistics (means, SD, SEs, etc.) were evaluated using the 'psych' package. The Shapiro-Wilk test of normality and the Levene's tests for homogeneity of variance were checked with the 'onewaytests' package ([Dag \*et al.\*, 2018](#)).

The generalized linear model (GLM) with 'quasi-poisson' family option was applied using the 'pscl' package ([Jackman, 2017](#)) for the 'percentage data' (survival rate and body-color percentage) which did not comply with the assumptions of any parametric model. The 'quasi-poisson' regression is not only flexible with data assumptions but also allows for over-dispersion in the dependent variable ([Mangiafico, 2016](#)). The subsequent Tukey post-hoc test was done using the 'emmeans' package ([Lenth, 2019](#)).

In growth performance analysis, the Kruskal-Wallis test (K-W) was used as an alternative to a non-parametric one-way ANOVA model when the variable was not normally distributed even by any transformation, but homogeneous. The one-way ANOVA with Welch's correction or Welch test (WT) was applied using the 'onewaytests' package ([Dag \*et al.\*, 2018](#)) for the variable which was not normally distributed and also not homogenized. The subsequent post-hoc test was done to find out where the variation among treatments lied. Since SGR is a percentage data, the GLM was used to analyze it.

The Principal Component Analysis (PCA) was applied to reduce the number of measured morphological traits to a few composite measures to explore the variation among treatments according to the selected morphological characteristics. The PCAs were performed using the 'FactoMineR' package ([Sébastien \*et al.\*, 2008](#)). The eigenvalues of first (PC1) and second components (PC2) of PCAs were 11.81 and 0.83 which explained 78.7% and 5.5% of the total variability, respectively. Therefore, only these two PCs were used in this study.

The linear discriminant function analysis (DFA) was performed using the 'MASS' package ([Venables and Ripley, 2002](#)) to find out the differences among individuals of four treatments based on their measured morphological traits. The first linear discriminant captured 0.87 (LD1), while second one (LD2) contributed 0.092 of the total proportion of trace. Therefore, only these two linear discriminants were selected in this study. The calculated model accuracy was 0.80 which is high enough to accept it. Finally, all figures were prepared using the 'ggplot2' package ([Wickham, 2016](#)).

To explore the reason for significant variation among treatments, the non-parametric multivariate analysis of variance (MANOVA) was done using 'rank MANOVA' package ([Friedrich \*et al.\*, 2019](#)) because all the morphological traits did not comply with the assumptions of parametric MANOVA. The subsequent pair-wise comparisons were checked with Tukey test using the 'multcomp' package ([Hothorn \*et al.\*, 2008](#)).

## RESULTS

#### Survival rate

The analysis revealed that treatment had a significant effect on the survival rate of the experimental fish (GLM:  $\chi^2=33.62$  and  $P<0.001$ ). The subsequent post-hoc test showed that fish reared in 24L had a significantly lower survival rate than those kept in 24D ( $z=5.59$  and  $P<0.001$ ) and with 12DL\_PVC ( $z=4.09$ ,  $P<0.001$  and [Fig. 1](#)). Although fish stocked in 24D showed a marginally significant higher survival rate than those kept in 12DL

PVC ( $z=4.09$  and  $P=0.066$ ), there was no variation among other treatments (Fig. 1).

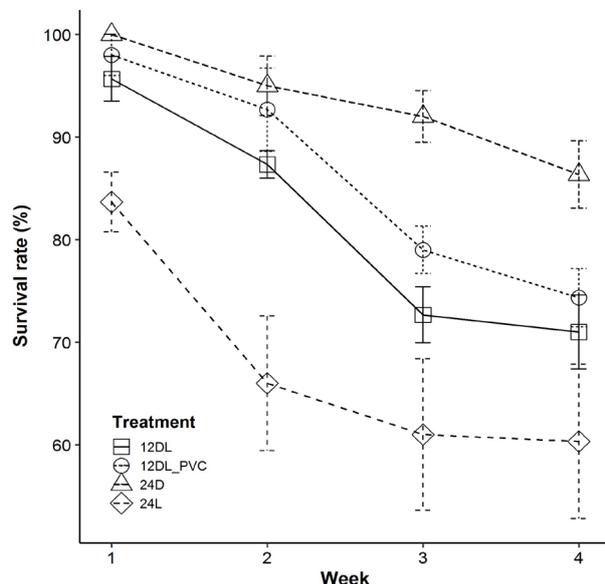


Fig. 1. Weekly percentage of survival rate (mean±SE) of fish reared in different treatments during one month of the experimental period.

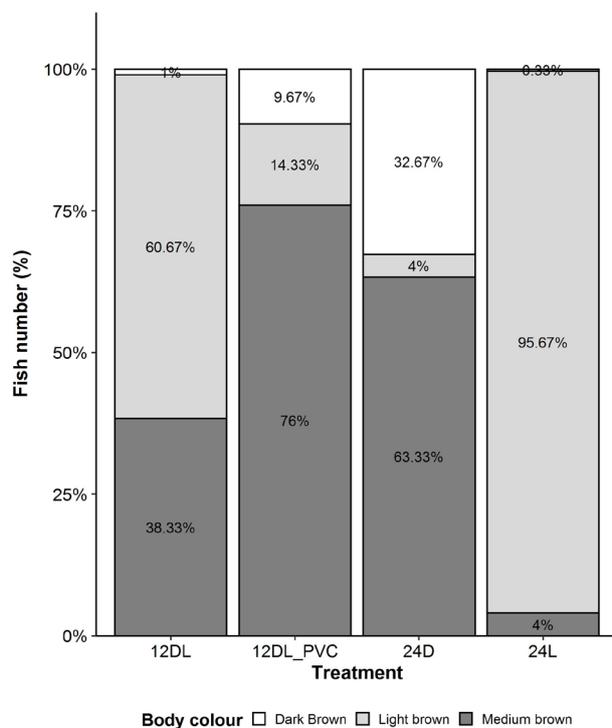


Fig. 2. Body colouration (percentage) of fish reared in different treatments after one month of the experimental period.

*Body colouration*

The observation of body colouration confirmed significant variations in light brown (GLM:  $\chi^2=1080.2$ ,  $P<0.001$  and Fig. 2), medium brown (GLM:  $\chi^2=668.51$  and  $P<0.001$ ) and dark brown (GLM:  $\chi^2=269.05$  and  $P<0.001$ ) appearances among treatments except no difference was found between 24L (0.03%) and 12DL (1%) reared fish ( $z=1.14$ ,  $P=0.66$  and Fig. 2) in case of dark brown body colouration.

*Growth performance*

The analysis of final growth data revealed significant variations among treatments in total length, body weight, and SGR (Table I). In all cases, fish reared in 24D showed significantly higher growth performance than other treatments, while only fish in reared 24L treatment had significantly higher SGR than those in 12DL treatment (Table I). However, no significant variation was found among other treatments in all measured growth parameters.

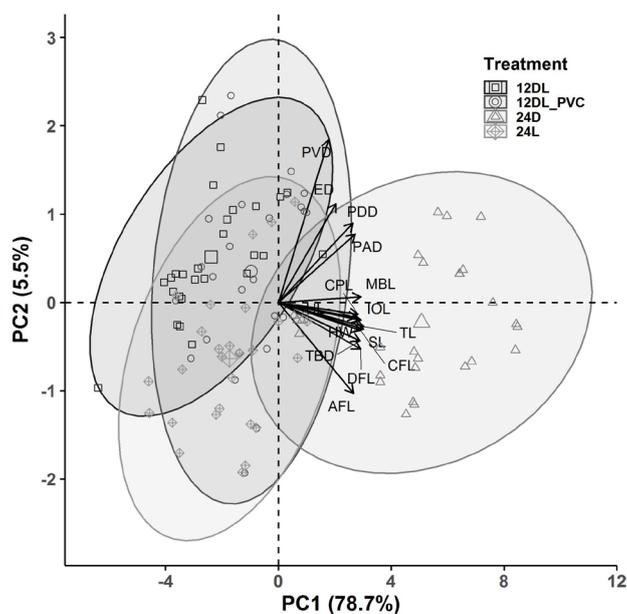


Fig. 3. Biplot of Principal Component Analysis (PCA) of 15 measured morphological traits in different treatments.

*Morphological traits*

The PCA analysis of 15 selected and measured morphological traits showed that the loadings of PC1 included the contributions of the most traits, while the PVD and ED were the major contributors in loadings of PC2 (Supplementary Figs. A1 and A2). The PCA variables also confirmed that these loading variables most effectively differentiated the 24D group fish from other groups (Fig. 3). The plotted scores of PC1 and PC1 clearly showed

**Table I. Growth performance (Mean±SEM) of fish reared in different treatments during one month of the experimental period. Superscript letters indicate significant differences (P<0.05).**

Traits	24D	24L	12DL_PVC	12DL	Stat- value	P	Model
FBW	0.08±0.00 <sup>a</sup>	0.05±0.00 <sup>b</sup>	0.05±0.00 <sup>b</sup>	0.05±0.00 <sup>b</sup>	113.48	0.001	WT
FTL	27.18±0.23 <sup>a</sup>	20.89±0.24 <sup>b</sup>	20.95±0.20 <sup>b</sup>	20.52±0.29 <sup>b</sup>	61.11	0.001	K-W
SGR	11.39±0.07 <sup>a</sup>	9.85±0.09 <sup>b</sup>	9.82±0.10 <sup>bc</sup>	9.44±0.14 <sup>c</sup>	188.78	0.001	GLM

FBW, Final body weight (g); FTL, Final total length (mm) and SGR, Specific growth rate (%).

that 24D treatment fish were grouped separately into the positive side of PC1, having very few overlapping with other groups, which mostly spread along the negative side of PC1 and overlapped each other (Fig. 3).

Consistent with the PCA analysis, the LDA outcomes confirmed distinct separation between 24D group and other treatments, which revealed two complete classes based on their measured morphological traits (Fig. 4).

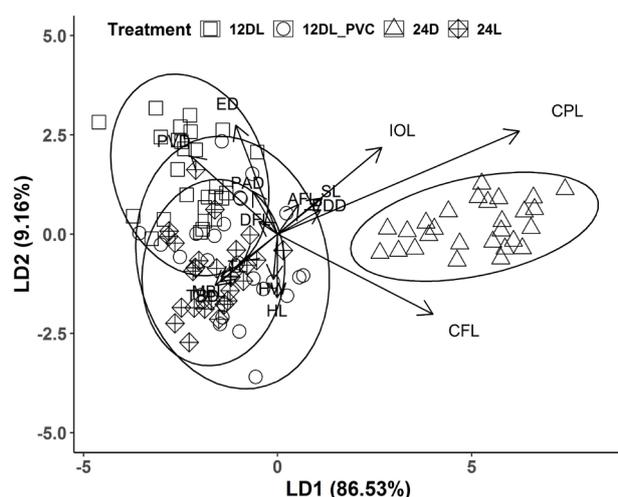


Fig. 4. Biplot of Discriminant Function Analysis (DFA) of 15 measured morphological traits in different treatments.

Finally, the non-parametric MANOVA model revealed a significant variation among the treatments (test stat=286.17 and P<0.001). The subsequent pair-wise comparison showed where the significant variation lay among different treatments based on the measured 15 morphological traits (Table II).

## DISCUSSION

Photoperiodism (photoperiod manipulation) is routine work in aquaculture industries for gonad maturation (Lundova *et al.*, 2019), sex alternation (Brown *et al.*, 2014), and somatic growth (Mustapha *et al.*, 2014; Lundova *et*

*al.*, 2019) in several commercially important fish species. The present study demonstrated that survival, growth, and body colouration of *C. gariepinus* larvae were directly influenced by the photoperiod manipulation. Prolonged exposed to dark condition resulted in better performance than that of prolonged light and natural conditions (12DL with and without PVC). However, the performances of larvae under shelter conditions are not as significantly conspicuous as in the dark condition.

**Table II. The outcomes of pair-wise comparisons of Tukey test for non-parametric MANOVA model. Superscript letters indicate significant differences (P<0.05). The values are shown as Mean±SEM.**

Traits	24D	24L	12DL_PVC	12DL
TL (mm)	26.62±0.40 <sup>a</sup>	20.90±0.25 <sup>b</sup>	21.05±0.23 <sup>b</sup>	19.92±0.26 <sup>d</sup>
SL	22.78 ±0.36 <sup>a</sup>	17.79±0.21 <sup>b</sup>	17.78±0.20 <sup>b</sup>	16.92±0.21 <sup>d</sup>
TBD	2.26±0.07 <sup>a</sup>	1.61±0.02 <sup>b</sup>	1.66±0.02 <sup>b</sup>	1.51±0.02 <sup>d</sup>
CPL	1.33±0.04 <sup>a</sup>	0.70±0.01 <sup>b</sup>	0.80±0.01 <sup>c</sup>	0.73±0.02 <sup>b</sup>
DFL	11.3±0.11 <sup>a</sup>	9.77±0.12 <sup>b</sup>	9.57±0.12 <sup>b</sup>	9.32±0.12 <sup>d</sup>
AFL	8.53±0.13 <sup>a</sup>	7.38±0.09 <sup>b</sup>	7.19±0.09 <sup>b</sup>	6.97±0.09 <sup>d</sup>
CFL	4.01±0.04 <sup>a</sup>	3.06±0.04 <sup>b</sup>	3.12±0.05 <sup>b</sup>	2.82±0.05 <sup>d</sup>
PDD	9.30±0.13 <sup>a</sup>	7.33±0.10 <sup>b</sup>	7.82±0.14 <sup>c</sup>	7.68±0.10 <sup>c</sup>
PAD	12.00±0.15 <sup>a</sup>	9.89±0.13 <sup>b</sup>	10.36±0.12 <sup>c</sup>	10.32±0.12 <sup>c</sup>
PVD	2.02±0.03 <sup>a</sup>	1.81±0.02 <sup>b</sup>	1.91±0.03 <sup>c</sup>	1.91±0.02 <sup>c</sup>
HL	8.18±0.11 <sup>a</sup>	6.74±0.09 <sup>b</sup>	7.02±0.10 <sup>b</sup>	6.06±0.13 <sup>c</sup>
HW	5.10±0.04 <sup>a</sup>	4.13±0.08 <sup>bc</sup>	4.26±0.07 <sup>b</sup>	3.97±0.05 <sup>c</sup>
ED	0.75±0.01 <sup>a</sup>	0.66±0.02 <sup>bd</sup>	0.72±0.01 <sup>c</sup>	0.67±0.02 <sup>d</sup>
MBL	6.45±0.18 <sup>a</sup>	4.87±0.08 <sup>bc</sup>	5.05±0.08 <sup>b</sup>	4.67±0.08 <sup>c</sup>
IOL	2.87±0.04 <sup>a</sup>	2.27±0.03 <sup>bc</sup>	2.32±0.02 <sup>b</sup>	2.25±0.03 <sup>c</sup>

TL, total length; SL, standard length; TBD, total body depth; BDA, body depth at anus; CPL, caudal peduncle length; DFL, dorsal fin length; AFL, anal fin length; CFL, caudal fin length; PDD, pre dorsal distance; PAD, pre anal distance; PVD, pre ventral distance; HL, head length; HW, head width; ED, eye dia; MBL, maxillary barbell length; IOL, inter orbital length.

The larval stages of fish are more sensitive than any of the juvenile and adult stages, as well as more susceptible to sudden changes in environmental factors (Pereira *et al.*, 2006). *C. gariepinus* larvae are photophobic in nature (Hogendoorn *et al.*, 1980) and prefer to hide in the protected habitats on the edges of water systems (Britz and Pienaar, 1992). Appelbaum and Kamler (2000) reported that the photophobic behaviour of *C. gariepinus* is initiated shortly after hatching, which supports the observation of the present study. Newly hatched larvae stocked in tanks with PVC materials showed that all larvae took shelter in PVC pipes and larvae were mostly visible while feeding. Such tendencies were continued until the second day. Behavioral studies of *C. gariepinus* by Britz and Pienaar (1992) revealed that this species is very sensitive to light and therefore, the preference of dark condition and escaping of light are the innate characteristic features of young *C. gariepinus*. Unlike various fish species, *C. gariepinus* are tactile and probably chemoreceptive predators rather than visual predators therefore the contribution of eyes in searching food is less significant (Hecht and Appelbaum, 1988). Thus, rearing in darkness is advantageous for the survival and growth of *C. gariepinus* larvae.

Light tolerance in fish varies with species and plays a significant role in survival (Britz and Pienaar, 1992; Silva-Garcia, 1996; Boeuf and Le Bail, 1999). It has been verified that continuous light can harm the interests and well-being of fish and cause high mortality in various species of fish at various phases in their life cycle (Huntingford *et al.*, 2006; Stevenson, 2007). This study proved that *C. gariepinus* larvae, exposed to prolonged light (24L), depressed their survival greatly compared to continuous dark (24D) or normal photoperiod (12DL), or shelter conditions (12DL\_PVC). Like the present study, similar analysis from Orina *et al.* (2016) observed a decrease in the survival rate in *C. gariepinus* larvae submitted to 24 h of light than the larvae exposed to 24 h dark or natural photoperiod (12 h dark and 12 h light). In another report, working with the same species, Britz and Pienaar (1992) did not detect any variations in survival rate until 13 days of rearing under continuous dark or light, with or without cover. Yet, their conclusion revealed that under dark conditions such factors like stress, aggression and cannibalism were reduced to a considerable extent. The high mortality under prolonged light could be the result of stressful conditions that ultimately promote the aggressiveness of fish. Coeval cannibalism (i.e. cannibalism among similar-aged individuals) due to aggressiveness is a common feature in larval and juvenile Atlantic cod (Folkvord, 1997). The prolonged light also caused high mortality in other catfish species, for examples, low survival rate in the European catfish (*S. glanis*) larvae (Kozłowski and Poczyczyński,

1999), stinging catfish (*Heteropneustes fossilis*) juveniles (Rahman *et al.*, 2020) and other finfish species such as *Scophthalmus maximus* juveniles (Imslund *et al.*, 1995) and *Salmo salar* (Sigholt *et al.*, 1995). Interestingly, low mortality was also detected in a number of fish species reared under natural photoperiods (Kiyono and Hirano, 1981; Tuckey and Smith, 2001; Canavate *et al.*, 2006), while different light conditions (prolonged light/dark or natural) did not show any deleterious effect on survival in tanbaqui Aride *et al.* (2006), marine pejerrey Sampaio *et al.* (2009), Tilapia Alvarez-Rosario *et al.* (2009). It might, therefore, be inferred that under different photoperiods, the mortality of fish species is associated with the environment in the tank, the adaptation of the species to those surroundings, life-span of the fish, and the fish species (Mustapha *et al.*, 2014).

Body colouration pattern of fish is a characteristic aspect that has fascinated biologists for a long time. External body colour is an important phenotypic trait and often used as a tool for predator avoidance (camouflage, bartesian mimicry, and palatability signal), prey capture (camouflage, aggressive mimicry) and conspecific communication (mating and agonistic signaling, shoaling preferences) (Cheney *et al.*, 2008; Mills and Patterson, 2009). Body colouration also contributes to market price and consumer choice (Yilmaz and Ergün, 2011; Mustapha *et al.*, 2012). *C. gariepinus* larvae reared in different exposure conditions (e.g., dark, light, and shelter) exhibited various body colourations or color differences. Dark brown colouration was mostly prevalent (predominant) in 24D photoperiod, probably due to the darkened nature of the facility, whereas lighter brown body colouration was noticed in the entirely illuminated medium (24L). Intermediate colors (light and medium brown) were observed for the larvae exposed to 12DL photoperiod conditions. There is also an evidence of color morph variations in another catfish group, for example in which juveniles of stinging catfish were reared for four months (120 days) under dark conditions and with shelter too (Rahman *et al.*, 2020). Melanophores, one kind of chromatophore containing melanized organelles, termed melanosomes, generate dark colours in the skin (Sugimoto, 2002). These pigments are liable for the integumentary colouration in fish, amphibians and reptiles because of having high cellular motility (Kaur and Dua, 2015). It has been also reported that integumentary colour changes have occurred through morphological as well as physiological means (light, hormonal regulation, social interaction, stress) (Sugimoto, 2002; Nilsson Sköld *et al.*, 2013). Melatonin is produced in the pineal gland, an endogenous time-keeping system in teleosts. Melatonin production profiles have been examined to determine

the diurnal habits of some vertebrate species, and the hormone formed by the retinal cones and pineal glands works like a natural timer regulating locomotor activity and endogenous rhythms (Boeuf and Bail, 1999). It has been observed in teleost such as *H. abdominalis* that there is a decrease in melatonin throughout the light phase and increase throughout the dark phase (Martínez-Cárdenas *et al.*, 2008; Leclercq *et al.*, 2010). Deep dark body colouration along with high body mass of fish species like *C. gariepinus* offers extra profits and promotes better market value.

*C. gariepinus* larvae exposed to continuous dark conditions resulted in a significant improvement of growth in contrast to continuous illumination. Comparison among the treatments reveals that catfish growth improved, in order, from 24D, 12DL\_PVC, 24L to 12DL treatments. Enhanced feed conversion efficiency, the reduction and absence of stress and aggressiveness, and suppression of locomotory activities in the dark may considerably increase the length, weight and other phenotypic traits (TL, SL, TBD, BDA, CPL, DFL, AFL, CFL, PDD, PAD, PVD, HL, HW, ED, MBL, IOL, YV, YL, and YH) of this larvae. Such activities allowed further energy that could be utilized on these metabolic actions to be transformed into physical development (Britz and Piennar, 1992; Appelbaum and Kamler, 2000; Almazan-Rueda *et al.*, 2005). Such negative photosensitive behavior is also evident in other catfish species (e.g. *Heterobranchus longifilis*: Kerdchuen and Legendre, 1991; *Silurus glanis*: Kozłowski and Poczyczynski, 1999; *Mystus nemurus*: Rahmah *et al.*, 2014; *C. batrachus*: Paramanik *et al.*, 2014; *Lophiosilurus alexandri*: Kitagawa *et al.*, 2015; *Hatcheria macraei*: Barriga *et al.*, 2016; *Heteropneustes fossilis*: Rahman *et al.*, 2020). Less movement, due to negative photosensitive behavior, means more energy for somatic growth and may contribute to the growth improvement. The present study also observed that under complete dark conditions, the high specific growth rate was a consequence of the full feeding and the utilization of the feed, all the more so considering the fact that these fish are nighttime feeders and photosensitive. It is predicted that the eye is not the major limiting factor for searching food; at least for *Clarias gariepinus* species other organ like barbels might have influence on finding food (Hecht and Appelbaum, 1988). Our results corroborate the outcomes of other fish species published elsewhere (Wilkens *et al.*, 1997 for paddlefish, Billard and Lecointre, 2001; Ali Bani, 2009 for sturgeon). Billard and Lecointre (2001) noticed that eyes of sturgeon are comparatively very small in respect to body, and therefore, it is predicted that the eye gives no contribution to capturing food. However, the possession of highly specialized organs such as the

rostrum, ampulla of lorenzini and barbels somehow make them light-independent for feeding.

Studies related to various fish species showed an extensive correlation between photoperiod (negative photosensitive) and larval development: an expansion in day-length improves the development rates for black porgy (Kiyono and Hirano, 1981), rabbit fish (Duray and Kohno, 1988), gilt-head sea bream (Chatain and Ounais-Guschemann, 1991), greenback flounder (Hart *et al.*, 1996), Atlantic salmon (Oppedal *et al.*, 1997), snapper (Fielder *et al.*, 2002), *Sparus aurata* (Ginés *et al.*, 2004), Atlantic cod (Taranger *et al.*, 2006), *Melanogrammus aeglefinus* (Davie *et al.*, 2007), *Oplegnathus fasciatus* (Biswas *et al.*, 2008), and *Pagrus major* (Biswas *et al.*, 2010). Working with Nile Tilapia, Elsbaay (2013) and Biswas *et al.* (2002) reported that long-day photoperiods improved body weight greatly in contrast to long-night photoperiods or natural light regime. Research performed by Biswas *et al.* (2006) illustrated that under long and consistent photoperiods, higher development rates are confirmed because of higher food intake and food transformation ability since visual feeder fish requiring light (Cox and Pankhurst, 2000). The reduction of metabolic rate by Nile tilapia during prolonged light periods also improves the somatic growth (Elsbaay, 2013) and this statement is also supported by Biswas *et al.* (2002) and Biswas and Takeuchi (2002). Larvae of *Clarias gariepinus* exposed to light for long periods of time caused a significant reduction of growth and survival because of increased locomotory activities (for searching shelter instead of capturing food), caused them stress and aggression and thus improve cannibalistic behavior among the fish population in the tank. Many studies revealed that fish spend more energy to find shelter, which reduces feed intake during prolonged light exposure (Verreth and Van Tongeren, 1989; Britz and Piennar, 1992; Appelbaum and Geer, 1998; Appelbaum and Kamler, 2000; Almazán-Rueda *et al.*, 2005). In European catfish (*S. glanis*), Kozłowski and Poczyczynski (1999) discovered that light caused a hostile attitude and more gathering of this species at the base, close to the walls and in tank corners, which eventually raised cannibalism and diminished overall growth. Yet, the manipulation of photoperiod had no impact upon the entire length and body weight of *Verasper moseri* (Amano *et al.*, 2004) and *Oreochromis niloticus* (Campos-Mendoza *et al.*, 2004), and female lambari (Navarro *et al.*, 2014).

Catfish are nocturnal in nature and prefer shelter to grow, develop and reproduce (Britz and Piennar, 1992; Rahmah *et al.*, 2014; Barriga *et al.*, 2016). The present study revealed that newly hatched larvae took shelter in tanks that were equipped with PVC pipes while larvae formed clusters and accumulated at the corner of tanks

where PVC pipes were absent. Installation of PVC pipes in tanks has shown better growth in case of Patagonian catfish (Barriga *et al.*, 2016), and Asian walking catfish (Sahiduzzaman *et al.*, 2018). This study did not notice any significant effect on growth in the case of shelter provided tanks. In a recent study with stinging catfish, Rahman *et al.* (2020) demonstrated that PVC itself in dark has no influence on growth, which corroborates the findings of the present study. Although we did not obtain better growth in the presence of PVC as shelter materials, the survival rate was higher compared to non-PVC tanks and this could be attributed to the fact that PVC provides shelter that increases the resting time and reduces stress and thereby influences survival (Hecht and Appelbaum, 1988; Rahman *et al.*, 2020).

### CONCLUSION

Together with early studies, the study showed again that colouration pattern, growth performances and survival of *C. gariepinus* larvae were directly influenced by photoperiod, while no significant influence was revealed by the shelter effect. Rearing under the prolonged dark condition resulted in several folds of phenotypic growth and increased survival. The highest survival was recorded in 24D followed by 12DL\_PVC, 24L, and 12DL, respectively. The complete dark brown body color (resembled as typical fish color) was observed in 24D condition and this shiny color may gain in consumer preference for regular consumption of this species. Shelter itself, which is not influencing but could be promising for better larval survival in dark conditions, needs to be studied further. Additional research is also required on the effect of other larval stages and environmental stimuli on growth performances of this and other commercially important fish species. Since the larval stage is more sensitive, rearing in dark conditions could be economical in terms of better aquaculture production by obtaining enhanced survival and growth.

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#### Ethics statement

All animal procedures were approved by the

Research Ethics Committee (REC) of the King Faisal University (KSA) and complied with the Guidelines on Ethical Treatment of Experimental Animals (KFU-REC/2020-02-01) set by the Ministry of Science and Technology, KSA.

#### Supplementary material

There is supplementary material associated with this article. Access the material online at: <https://dx.doi.org/10.17582/journal.pjz/20200507220534>

#### Statement of conflict of interest

The authors have declared no conflicts of interest.

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