



Photoperiod affects *Sinocyclocheilus grahami* growth during larval and juvenile stages

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Abstract: Two trials were conducted to investigate the effects of photoperiod manipulation on growth rate, feed conversion efficiency, survival and deformity rate in larvae and juvenile golden-line fish (*Sinocyclocheilus grahami*). Larval fish were exposed to five photoperiods (0Light:24Dark, 8Light:16Dark, 12Light:12Dark, 16Light:8Dark and 24Light:0Dark) and juvenile fish to three photoperiods (0Light:24Dark, natural photoperiod and 24Light:0Dark) in Trial 1 and Trial 2 respectively. Fish were fed with a commercial diet to apparent satiation. There were no significant differences in final body length and weight between light treatments ($P>0.05$) after sixteen days under five photoperiods for larval fish. The higher mortality occurred in treatments of continuous dark (90.00%), 8Light:16Dark (90.00%) and continuous light (84.29%). Juvenile fish exposed to natural photoperiod showed a significantly higher growth rate ($0.95\% \text{day}^{-1}$), feed conversion efficiency (61.04%) and survival rate (100.00%) compare to other two treatments ($P<0.001$). Continuous light caused a highest percentage of deformity (42.22%) and lowest survival rate (58.89%). During the trial, the mean body weight was always lowest in continuous dark and a small number of deformities and death occurred. However, the growth performance in each period was different. Furthermore, continuous light caused a stubby-body morphology and natural photoperiod and continuous darkness exhibited slender-body morphology. These results indicate that golden-line fish could be reared at natural photoperiod during larval or juvenile stages.

Keywords: Photoperiod; *Sinocyclocheilus grahami*; Growth; Deformity; Survival

Introduction:

Fish growth is influenced by several biotic and abiotic factors. As one of feature of light, photoperiod is known to be important to fish growth, feeding, locomotor activity, metabolic rates, body pigmentation, sexual maturity and reproduction (Ginés, et al., 2004; Biswas, et al, 2005). 'Receptivity' of fish to light profoundly changes according to the species and the developmental status as a directive factor (Brett, 1979) by stimulating the endocrine system, e.g., growth hormone (McCormick, et al., 1995; Björnsson, 1997). Long photoperiod manipulation has been used to improve the growth during early developmental stages in many fish species (Barlow, et al., 1995; Puvanendran & Brown, 2002; El-Sayed & Kawanna, 2004). Interestingly, it has been reported that total darkness also improves body growth in some species, such as African catfish and silver catfish (Britz & Pienaar, 1992; Piaia, et al., 1999). Regardless of numerous studies on photoperiod effect on fish growth, there is no consistent conclusion.

Golden-line fish (*Sinocyclocheilus grahami*) (*S. grahami*), which belongs to Cyprinidae, Barbinae, *Sinocyclocheilus*, is a troglophile endangered species that survives in Lake Dianchi (Kunming, Yunnan province of China), (Chu & Chen, 1978; Pan, et al., 2009). In this genus, parts of hypogean species spend their whole lives in caves, such as *Sinocyclocheilus anatirostris*, *Sinocyclocheilus hyalinus* and some are able to move in and out of caves regularly (Romero, 1985). Under this circumstance, photoperiods play a key role in the evolution of these creatures. Based on our surveys, we found that *S. grahami* moves in and out of caves during winter and spring, respectively. Thus, with this special

life history, it is very interesting to understand what will change in this species under different photoperiods.

Artificial fertilization is important for biodiversity protection and has long been used in freshwater fish, e.g., salmonids and cyprinids (Billard, 1988, 1990). Breeding project for golden-line fish started in 2004, and it was successfully bred in 2007 (Yang, et al., 2007). Since then, aquaculture management on this fish has been studied (Pan, et al., 2010; Pan, et al., 2011; Pan, et al., 2014). In order to protect the endangered species, more than one million fries had been released to Dianchi Lake. However, the low growth rate has been challenging as it inhibits the extension of this species. The practical and effective management must be established to protect highly endangered animals. In this study, we found important mechanisms of photoperiod that affects *S. grahami* growth during larval and juvenile stages. We described the different growth performance under specific photoperiod for this species. We also showed that weather photoperiod manipulation could be applied in aquaculture to promote growth of *S. grahami*.

Materials and Methods:

Collection and treatment of larval *S. grahami*

The same generations of larval and juvenile fish were obtained from the endangered fish conservation center, Kunming Institute of Zoology, Chinese Academy of Sciences. The larval fish were acclimated for five days in 150 L aquaria at 21 °C in pond water (12-h light: 12-h dark photoperiod [12 L: 12 D]). During acclimation period, the fish were fed

twice daily to satiate with egg yolk. After acclimation, fish were randomly allocated into ten 25 L tanks (35 fish [10.40 mm]; 0.005 g] per tank) in 2013. Five experimental treatments (Trial 1) were conducted, and two replicate tanks were prepared for each treatment condition. The treatment conditions were continuous dark [0L: 24D], 8-h light/16-h dark [8L: 16D], 12-h light/12-h dark [12L: 12D], 16-h light/8-h dark [16L: 8D] and continuous light [24L: 0D]. One third of water in each tank was changed with fresh water daily. The oxygen level and temperature of water were maintained near 100% saturation and 20±1 °C, respectively. Fish were fed to apparent satiation with *Brachionus plicatilis* twice a day during the experiment. Artificial lighting was supplied by two 36 W fluorescent tubes giving 800 lx through the tank water column in a dark room. Each tank was isolated from others and shield by black plastic to avoid external light. Body length and weight were measured every six days. Vernier caliper and electronic scales were used to measure body length and weight, respectively.

Collection and treatment of juvenile *S. grahami*

Juvenile fish were acclimated for five days in 300 L aquaria at 21 °C in pond water in 2014 under natural photoperiods. During acclimation, fish were fed twice daily to satiation with a commercial diet (compound premix, Tongwei Group Co. Ltd.). After acclimation, fish were randomly allocated into six 300 L tanks (45 fish [average body length 2.11 cm; average body weight 0.224 g] per tank) on the fifth day. The three different light conditions (Trial 2) were set up, and two replicates were used for each condition. The light conditions were continuous dark (0L: 24D), natural photoperiod (NP), continuous light (24L: 0D). Artificial lighting regime was same as described in Trial 1. The temperature was maintained at 17.0-20.0 °C throughout the trial. Fish were fed to apparent satiation with a commercial diet (protein 40%, lipid 18%), twice daily during experiment. Water and the oxygen level were maintained as described in Trial 1. Morphological characteristics and weight were evaluated and measured every two months. Body width, caudal-peduncle length and depth were measured after six months of rearing to avoid damage.

Calculation and statistical analyses

Data was analyzed to evaluate the growth rate (SGR), feed conversion efficiency (FCE), survival rate (SR) and deformity rate (DR) by using the following formula:

$$\text{SGR (\%)} = 100 \times (\ln W_2 - \ln W_1) / \text{time (days)},$$

W1; initial weight (g)

W2; final weight (g)

$$\text{FCE (\%)} = 100 \times [\text{wet weight gain (g)} / \text{dry feed intake (g)}]$$

$$\text{SR (\%)} = 100 \times (\text{final individual number} / \text{initial individual number})$$

$$\text{DR (\%)} = 100 \times (\text{deformity individual number} / \text{initial individual number})$$

SAS 9.1 was used for statistical analysis in this study. Data were expressed as the mean ± Standard deviation (S.D for Trial 1 and 2 and were compared using ANOVA. The means within each treatment and among treatments were compared using Tukey's test. Normality was evaluated before statistical analysis by conducting Kolmogorov-Smirnov test.

Results:

Effects of photoperiod on larval growth and weight

All larval fish in different photoperiod treatment grew into juvenile in 16 days, and no significant difference was found in final body length and weight between treatments (ANOVA, P>0.05). However, we found growth performance was slightly promoted in continuous light treatment (average body length 16.32±1.04 mm, weight 0.027±0.004 g). During the first few days of the trial, fish in the continuous dark condition grew slowly comparing to other treatments, but then grew more quickly (Fig.1). Based on the data, continuous light caused slightly higher feed conversion efficiency and shortened day-length against feed conversion. During the treatment, no fish was found as deformed in all treatments. As shown in Table 1, the higher mortality occurred in three photoperiod treatments; continuous dark, 8-h light/16-h dark and continuous light.

Effects of photoperiod on growth performance of juvenile fish

Significant differences were found between treatments in every parameter at each measured time (ANOVA, P<0.05). The maximum average values of each parameter were found in the treatment of continuous light, and continuous darkness condition always showed the minimum value during the first eight months. Interestingly, fish in the natural photoperiod treatment showed increased rate in growth in the last two months.

Table 1. Growth performance of larval *S. grahami* in different photoperiod conditions

Parameters	Photoperiods				
	24L:0D	16L:8D	12L:12D	8L:16D	0L:24D
Initial body length (mm)	10.41±0.78	10.42±0.37	10.40±0.57	10.40±0.52	10.41±0.61
Final body length (mm)	16.32±1.04	15.67±0.82	15.63±0.67	15.57±0.50	15.64±0.68
Initial body weight (g)	0.005±0.003	0.005±0.002	0.005±0.002	0.005±0.001	0.005±0.002
Final body weight (g)	0.027±0.004	0.026±0.003	0.025±0.003	0.023±0.003	0.024±0.003
Weight gain (g)	1.365±0.364	1.260±0.415	1.281±0.290	1.166±0.173	1.208±0.280
SGR (%)	9.225±0.955	9.003±0.745	8.896±0.733	8.392±0.740	8.638±0.650
Total feed intake (g)	2.250±0.377	2.195±0.152	2.269±0.317	2.060±0.309	2.161±0.208
FCE (%)	60.89±3.57	57.08±6.81	57.07±2.96	56.97±50	55.81±0.56
DR (%)	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
SR (%)	84.29±0.02	100±0.00	98.57±0.02	90.00±0.06	90.00±0.02

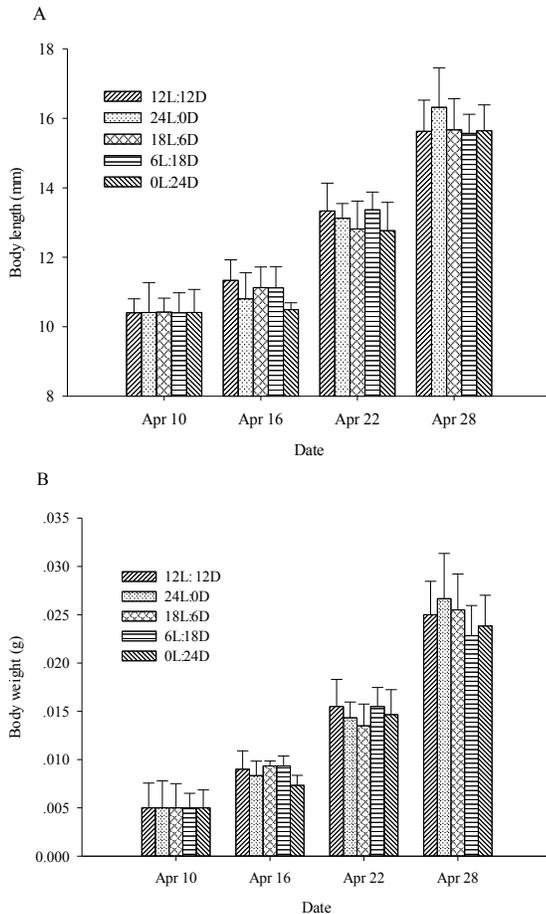


Figure 1. Changes in body length (A) and weight (B) of larval *S. grahami* in different photoperiod treatments. Significant differences ($P < 0.05$) between photoperiod treatments are denoted by '*'.

All values of parameters increased in continuous light except body depth (Fig. 2A, B and C; Table 2). As shown in Fig. 2, growth performance showed difference at different stages. In the first two months, juvenile reared in continuous light and natural photoperiod showed similar growth status, and were superior to fish in continuous darkness for all parameters. During the next two months, continuous light promoted growth rate distinctively and significantly than other two treatments ($P < 0.001$). From December to February, body growth in continuous light decreased conspicuously compare to other two treatments. During the last two months, fish reared in natural photoperiod showed the highest growth rate and the lowest growth rate found in fish in continuous light condition. After ten months of rearing, fish in treatment of natural photoperiod showed a higher SGR and FCE. Although continuous light caused a bigger body size than continuous darkness, the FCE was inferior.

The three different photoperiod conditions induced different morphological changes in fish. As shown in Table 2, values of final body length / width, final body length / width and final caudal-peduncle length / depth in continuous light were lower than that in other two groups. Continuous light caused a stubby-body morphology, however, in natural photoperiod and continuous darkness fish had slender-body morphology. Another interesting morphological characteristic was the high deformity rate in fish in treatments of continuous light and darkness, especially in continuous light condition. The deformed region was always found in the

head of fish. Furthermore, the highest mortality was recorded in continuous light. No death and deformity occurred in fish in natural photoperiod (Fig. 2D).

Discussion:

Photoperiod plays an important role during fish development. For many important commercial species, such as sea bass *Dicentrarchus labrax* (Barahona-Fernandes, 1979), turbot *Scophthalmus maximus* (Person-Le Ruyet, et al., 1991), and cod *Gadus morhua* (Puvanendran & Brown, 2002), extended daytime length can improve larval growth based on previous studies. It has been proposed that under continuous light conditions, larval fish may have greater opportunities to eat food and better growth performance (Litvak, 1999). To test the hypothesis, many factors need to be considered, and continuous food supply for fries is one of such factors. For instance, availability of food in the system is critical as the fries should be able to maintain enough energy to swim and grow (Downing & Litvak, 2000). In our study, although enough food was provided to fish at twice a day to avoid starvation, there was no significant difference in growth rate during larval stages. We suspect that although longer photoperiod provides more opportunity to capture food, larval fish still pay more energy in hunting. Another reason may be that larval golden-line fish require a period of inactivity to complete digestion and assimilation of energy, which is shown in haddock larvae (Downing & Litvak, 2000).

Table 2. Growth performance of juvenile *S. grahami* when exposed to different photoperiod conditions.

Parameters	Photoperiods		
	24L:0D	Natural photoperiod	0L:24D
Initial body length (cm)	2.11±0.04	2.10±0.04	2.11±0.06
Final body length (cm)	6.47±1.00	7.21±0.48	5.93±0.68
Initial body depth (cm)	0.42±0.02	0.42±0.02	0.42±0.02
Final body depth (cm)	1.36±0.19	1.30±0.13	1.15±0.18
Final body length / depth	4.78±0.46	5.56±0.34	5.21±0.40
Initial body width (cm)	0.24±0.02	0.24±0.02	0.24±0.02
Final body width (cm)	0.89±0.11	0.90±0.06	0.68±0.11
Final body length / width	7.32±0.83	8.02±0.63	8.76±0.78
Initial caudal-peduncle length (cm)	0.52±0.01	0.52±0.01	0.52±0.01
Final caudal-peduncle length (cm)	1.58±0.21	1.67±0.24	1.52±0.16
Initial caudal-peduncle depth (cm)	0.19±0.01	0.19±0.01	0.19±0.01
Final caudal-peduncle depth (cm)	0.73±0.09	0.70±0.05	0.61±0.07
Final caudal-peduncle length / depth	2.17±0.25	2.38±0.24	2.63±0.23
Initial body weight (g)	0.224±0.001	0.227±0.016	0.224±0.009
Final body weight (g)	2.920±1.189	3.988±0.623	2.073±0.724
Weight gain (g)	215.698±95.124	300.989±49.320	147.938±57.706
SGR (%)	0.826±0.152	0.952±0.047	0.722±0.117
Total feed intake (g)	518.611±96.148	496.552±74.516	277.324±43.235
FCE (%)	41.197±15.679	61.043±8.811	52.557±16.695
DR (%)	42.22±0.03	0.00±0.00	11.11±0.03
SR (%)	58.89±0.02	100.00±0.00	87.78±0.02

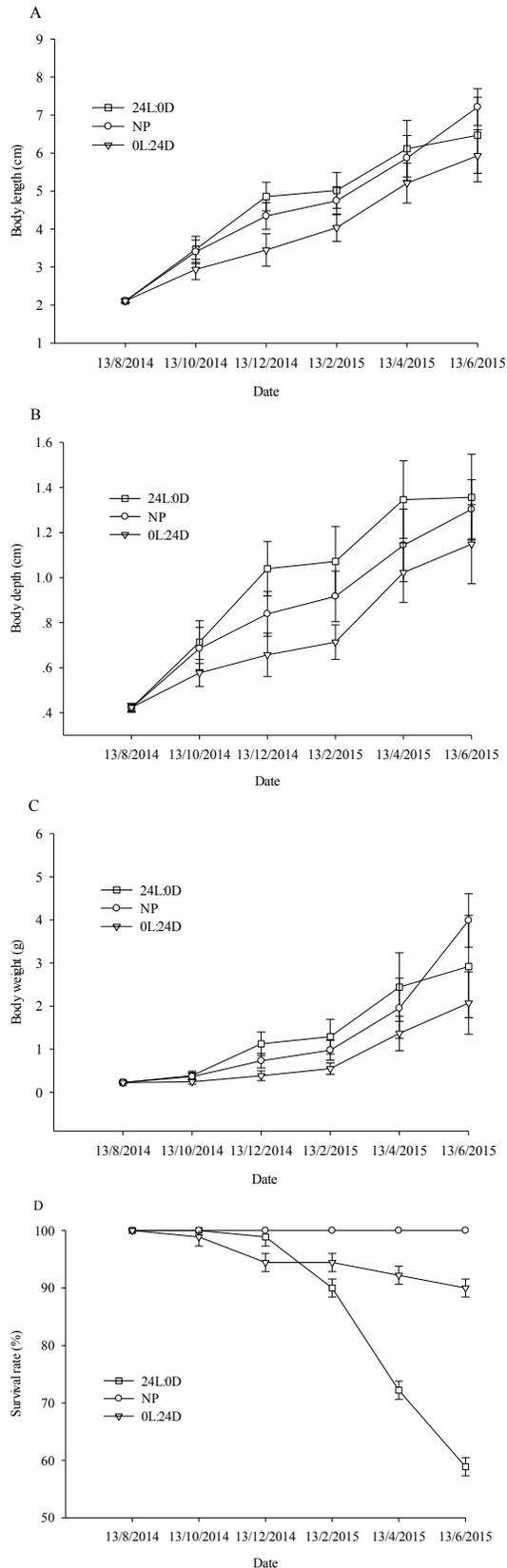


Figure 2. Changes in body length (A), body depth (B), body weight (C) and survival number (D) under different photoperiods of juvenile *S. grahami*; NP, natural photoperiod.

At the end of this trial, continuous light and reduced daytime length might cause higher mortality. Previous studies in rainbow trout and European sea bass have shown that extended photoperiod causes high mortality (Cerqueira, et al., 1991; Valenzuela, et al., 2012). However, it has not understood how the increased photoperiod can lead death of fish. In the present study, the death occurred in reduced photoperiods may be induced by feeding difficulty because dead fish are scraggy.

In nature, photoperiod is a critical signal that controls a variety of seasonally changing processes, including growth rate (Müller, 1978, Ricker, 1979). In artificial feeding, comparing to natural photoperiod, long photoperiods have been used to promote growth in many species such as green sunfish, *Lepomis cyanellus* (Gross, et al., 1965), Atlantic salmon *Salmo salar* (Saunders, et al., 1985), Atlantic cod *Gadus morhua* (Folkvord & Otterå, 1993), Barramundi *Lates calcarifer* (Barlow, et al., 1995a), Eurasian perch *Perca fluviatilis* (Jourdan, et al., 2000). However, in turbot *Scophthalmus maximus*, long term rearing on continuous light causes reduced growth rate, whereas short-term exposure to the continuous light condition stimulate growth which can be expressed by the final mean weights (Imsland, et al., 2013). In present study, short-term continues light has no significant effect on growth performance for four months of treatment, but began to show positive effects after the four month. It suggests that fish may need a period of time to adapt to new environment, which was shown in some other species (SilvaGarcia, 1996). Interestingly, from December 2014 to February 2015, the fries under continuous light conditions reduced their growth rate obviously when compared to the other two treatment groups. In the wild, golden-lined fish move into caves for overwintering or reproduction during the period (data is not shown). We speculate that long photoperiods might cause photo-toxicity to the juvenile fish in these two months because deformity and mortality rates were high distinctively during the period. However, in this period, growth rate in natural photoperiod decline slightly, which is likely caused by shorter photoperiod. As shown in other species (Imsland, et al., 1995; Trippel & Neil, 2003), it has been shown that short photoperiod inhibits body growth during winter. By contrast, growth rate slightly increases in the fish that are reared in continuous darkness condition. It is possible that sufficient food and low activity may lead to this consequence. In the last two months of experiment, the limitation to growth in continuous light should be the high percentage of deformity. In this period, high growth rate in treatment of natural photoperiod can be explanation by the gradual increase of day-length and normal morphology.

Besides of weight, another significant change caused by manipulated photoperiods is morphological phenotype. Long and short photoperiods caused stubby- and slender-body morphology, respectively. Previous studies showed that individuals that were stout in shape had higher absolute swimming costs than ones that were slender in shape. Slender-body has a higher swim speed compared to stubby-body (Webb, 1984; Boily & Magnan, 2002). Although we did not testswimming performance, it was found that fish in treatment of continues darkness escaped more easily than fish in other two treatments. It indicates that trade-off in the three photoperiods is different. In this study, before December 2014, fish

that were reared in continuous light showed a high growth rate, exhibiting advanced morphological changes at early developmental stages. However, these features may not be important for fish to escape photo-toxicity and avoid developmental deformity that was found in next periods. It suggests that fish that grow rapidly at certain stages, but no later or other stages as shown in coho and Atlantic salmon (Stead, et al., 1996; Johnsson, et al., 1997). Another distinctive feature is the deformed region was always found in the head, suggesting the possibility that continuous light and dark can disrupt photo transduction pathway. Indeed previous research in rainbow trout has shown that the application of continuous light photoperiod treatment may act as a chronic stressor by elevating plasma cortisol levels and thus leading fish to be prone to diseases (Leonardi & Klempau, 2003; Valenzuela, et al., 2012). Therefore, continuous light and darkness are likely to change hormone levels and decrease immune function in juvenile fish although more studies need to be done to support this.

Photoperiod manipulation including long-term or short-term photoperiod has been used to control somatic growth of certain species (Stickney & Andrews, 1971; Blancheton, 2000; Kissil, et al., 2001; Biswas, et al., 2005; Rad, et al., 2006). However, our study shows that, continuous light or short photoperiod regime use for larval *S. grahami* fish does not have significant effects on growth rate. Moreover, the manipulation was found to cause high mortality in fish. In our practice in aquaculture, it was found that natural photoperiod was the best rearing condition to produce healthier fish. It was also shown in *S. grahami* juvenile fish that natural photoperiod was the best condition for normal morphology and growth. In conclusion, our study reveals how the photoperiod affects *S. grahami* growth during larval and juvenile stages. Also, it will provide significant information for reproduction and early development of the species which is emerging as a model organism for evolutionary developmental biology.

Competing interests

The authors declare no conflict of interest.

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