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# Influence of Photoperiod Regime on Hatchability, Growth and Ontogeny Digestive Tract During the Larval Development of Grass Carp, *Ctenopharyngodon idella* (Cuvier and Valenciennes, 1844)

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**Abstract:** The fertilized eggs of grass carp, *Ctenopharyngodon idella* characterized by containing the emberyos inside perivitelline spaces. They remained inside these spaces for aperiod 96-120 hours. After hatching , the newly hatching frys continued to swim actively and depended on the yolk sacs for nutrition.The present study was conducted to detect effect of photoperiod on growth and survival rate of grass carp larvae.To achieve this purpose,the fertilized eggs were equally divided into four groups and exposed to four photoperiods (8D:16L , 16D:8L ,12D: 12L and 0D:24L ). The results indicated that the exposure of fertilized eggs in a long period of light (8D:16L) led to high mortality, hence the hatching rate was 31±0.506% and survival rate was 13.5±0.375%. The highest value of hatching rate was recorded after exposing the fertilized eggs to a long period of dark (16D:8L). Seven days after hatching, the light plays an important role in growth, since the larvae depended on the vision for searching the food. The survival rate was optimum in larvae exposed to a long photoperiod (8D: 16L or 12D:12L). The continuous illumination (0D: 24L) gave the lowest rates of survival and growth. The digestive tract of larvae developed early after exposured to a long period of light (8D: 16L, 12D:12L). It can be concluded that the photoperiod must be adopted for grass carp culture to improve the productivity in aquaculture system.

**Key words:** Photoperiod · Survival rate · Growth · Larval development · Grass carp · *Ctenopharyngodon idella*

## INTRODUCTION

The grass carp is a member of cyprinid family, which includes gold fish and common carp. It should not be confused with other nonnative carp such as big head carp, silver carp, black carp or mud carp. Grass carp was introduced to Egypt from Hong Kong during 1969 to control floating and submerged plants in the River Nile and tributaries [1]. Regarding to its importance as economic fish, grass carp can be reared in fresh water fish farms, but unfortunately, they did not spawn naturally in captivity ponds and hormonal induction is necessary. Few data are available on the early life stage of larvae and there is no available data describing how photoperiod can effect hatching and survival of grass carp larvae. Also, nothing is known about the influence of photoperiod on growth carp. In teleosts, it seems that the effect of photoperiod on growth differs according to the type of fish. In some fishes, there seems to be a synergistic effect between food availability and light that improves the trophic activity of larvae [2]. Maximization of photoperiod led to higher growth rates in some species such as *S. aurata* [3], *Lates calcarifer* [4], *Rhomosolea tapirina* [5] *and Pagrus auratus* [6]. However, in some other fishes larvae may have different response to light. In *D. labrax* larvae, continuous illumination was associated with abnormal in *Latrix lineata* after exposure to a long light [7]. Photoperiod may also stimulate hormones controlled by pineal organs which are responsible for circadian rhythms [8]. There is no available data concerned the relation between the effect of photoperiod and development of the digestive tract in grass carp larvae. Therefore, the present study aimed to throw light on the effect of photoperiod on hatchability and survival rate during the early larval stages. Also, the effect of photoperiod on growth and ontogeny of digestive tract of posthatching larvae was investigated to collect data about the composition of ingested food during the larval stages by using histochemical techniques.

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## MATERIALS AND METHODS

The experimental study was carried out at El-Serw Station for Fishes Researches during a period of spawning season of grass carp that restricted in May, 2008. The fertilized eggs were collected from the hatchery of San El-Hager and distributed into four glass aquaria. The capacity of water in each glass aquarium was 144L and the depth of water was not more than 40 cm.

**The Experimental Design:** The total number 1500 of fertilized eggs were equally divided and distributed into four groups.

- The first group was treated with 8D: 16L.
- The second group was exposed to 16D: 8L.
- In the third group, the total time for exposure to the light and the dark was equal 12D: 12L.
- The fourth group the continuous illumination was adjusted to become 0D: 24L.

To make a complete dark, a black plastic cover was used according to the determined time in each treatment. Also, the light period was controlled throughout the experiment by using fluorescent lamps to achieve an intense illumination of 30 lux on a surface of water. The characteristics medium at each experiment was recorded (pH, 7-7.5), dissolved oxygen was 6.0-6.9 mg/l and the water temperature was adjusted to become constant and ranged from 23-25°C. The percentage of survival rate of the embryos and the number of newly hatching larvae were recorded. After a period of 72-96 hours of fertilization, the white and unhealthy fertilized eggs were removed by using a fin plastic net.

For confirmation, each treatment was replicated three times. The average of lengths and weights of newly hatching frys were recorded and then the average was calculated. The period of experiment continued for 60 days post hatching.

**Feeding Larvae:** The newly hatching fry are mainly depended on the yolk sac for a period ranged from 3-4 days of posthatching. In addition, the eggs albumen were stirred and then spreaded on the water surface. After the alimentary canal was completely developed, the artificial food contained 30% of protein was supplied and the daily amount of supplementary food was about 5% of total weight of larvae for each treatment. The water in each glass aquarium re-changed at the least three times a weak and a gentle aeriation was adjusted by using electric air compressors.

**Sampling and Investigated Parameters:** Samples were collected after 3, 7, 15, 21, 30 and 60 days of posthatching. The total lengths in mm and the weights in mg were recorded. The intestine length in mm and the body length in mm were measured. The intestinal index was calculated [9], using the following equation.

$$\text{Intestinal index} = \frac{\text{Intestinal length (mm)}}{\text{body length (mm)}} \times 100$$

**Histological and Histochemical Studies:** In the experiment that gave the best growth (16 L: 8 D) samples were fixed directly in Bouin's fluid for about 48 hours, then dehydrated in series of ethanol, cleared in xylen and finally embedded in paraplast wax (m.p. 56-58°C). Transverse and sagittal section were cut at 5-6 micron and stained with hematoxylin and eosin [10]. In a large specimen either the intestine were isolated or decalcified a specimen as whole with 5% nitric acid for about 12 hours , then washed in distilled water and fixed in Bouin's fluid. Mallory triple stain was also used to detect the different layers that constitute the intestinal wall [11].

For histochemical studies, the procedures were used to determine the carbohydrate and protein materials in the alimentary canal until 30 days posthatching. The following techniques were used according the following procedures.

- The periodic acid Schiff (PAS) reaction to detect the carbohydrate material [12].
- The mercury bromophenol blue methods (hg bb) [13] for illustrating the total protein.

**The Statistical Analysis:** Data of the different treatments of photoperiods were statistically analyzed using Anova test [14], Microsoft excel 2003.

## RESULTS

**Fertilized Eggs:** The early fertilized eggs of grass carp were settled down on the bottoms of ponds. After the eggs absorbed the water, they became semipelagic and float on the surface of water for a period of 96 hours post fertilization. The embryos tried to get ride form the perivitelline space and the newly hatching fry appeared as slender shaped (Fig. 1a). The eyes of fry were not completely developed, so they become more sensitive to the light. (Fig. 1b). Three days later, most of yolk sac was absorbed and the pigments were condensed in the eyes and body surface. The mouth opening and the jaws were

movable, so the larvae are continued to swim actively. After that, they transformed from endogenous to the exogenous feeding (Fig. 1c).

**Hatchability:** The exposure to a long period of light led to high mortality as shown in treatment no. 1. The hatching rate was 31.0±0.506 and the survival rate was 13.5±0.375 under same circumstance of exposure. The hatching rate reached to the highest value either after exposure in a long period of dark as in treatment no. 2 (16D: 8L) or in treatment no. 3 (12D: 12L). The hatching rates were 72.33±0.112 and 58.66±0.380 respectively. On the other hand, the hatching rate was the lowest value (29.67±0.150) after exposure to a long illumination as in treatment no. 4. The survival rate was not observed under the same condition of this treatment as shown in Table 1.

On the other hand, the effect of photoperiod on frys 7 days post hatching indicated that the eyes were completely developed and the larvae were mainly depended on the exogenous feeding. The survival rate was optimum after the exposed larvae were treated in a long photoperiod (8D: 16L or 12D: 12) per day. The average of survival rate after a period of 30 days post hatching was 66.25±0.685 as in treatment no. 1, followed by 12D: 12L, since the survival rate was 62.50±0.665. On the other hand, the continuous illumination (0D: 24L) as shown in treatment no. 4 gave the lowest value of survival rate with abnormal shapes in some larvae as shown in Table 2.

**Growth:** The average increase in length was significantly differ (P<0. 05) after treating fish for a long photoperiod (8D: 16L, 12D:12L) 45 days post hatching. In the case of the exposed fish for 16D: 8L a moderate results of growth were obtained as shown in Table 3. After exposing fish for continuous illumination (0D: 24L) the lowest value in growth of length was recorded.

**Photoperiod and Metamorphosis of Digestive Tract:** The present results showed that the alimentary canal of newly hatching fry is a simple undifferentiated tube throughout its length. Three days later, the digestive tract differentiated into buccal cavity, pharynx, oesophagus and intestine. The intestinal length in relation to the total length ratio (intestinal index) increased significantly as a function of size, so that in fish 7.43±0.073 mm in length, it is 64.60, in 21.8±0.048 mm, it is 93.25 and in fingerling stages 61.66±0.278 mm, it is 155.96 as shown in Table 4.

Table 1: Effect of short and long photoperiod regimes on the hatching success and survival rate in grass carp, (*Ctenopharyngodon idella*) after 72-120 hours fertilization (Mean±SE)

| | Photoperiod (hours) | | | |
|---|---|---|---|---|
| Parameters | 8D:16L | 16D:8L | 12D:12L | 0D:24L |
| Fertilization rate | 60±0.215 (360)* | 77±0.136[a] (462) | 66.33±0.247 (396) | 38.33±0.189 (228) |
| Hatching rate | 31±0.506 (112) | 72.33±0.112 (333) | 58.66±0.380 (232) | 29.67±0.150 (69) |
| Survival rate | 13.5±0.375 (16) | 55.67±.359 (187) | 30.0±0.682 (70) | 0 |
| Period of hatching (hours) | 85-96 | 72-81 | 77-96 | >120 |

a-Significance differences was detected (p<0.05). *-Number of fertilized eggs

Table 2: Effect of short and long photoperiods on the average of survival rate in grass carp 7-30 days post hatching (Mean±SE)

| | Period (days) | | | |
|---|---|---|---|---|
| Photoperiod (hours) | 7 | 14 | 21 | 30 |
| 8D:16L | 79.60±0.701 | 73.50±0.491 | 73.25±0.445 | 66.25±0.685 |
| 16D:8L | 68±0.447 | 64.0±0.532 | 45.750±0.722 | 52.50±0.491 |
| 12D:12L | 74.4±0.690 | 72.0±0.562 | 68.75±0.414 | 62.5±0.665 |
| 0D:24L | 58.50±1.044 | 52.25±0.810 | 49.25±0.472 | 46.25±0.650* |

* Some larvae appeared abnormal shaped

Table 3: Effect of Photoperiod (hours) on the average of growth in grass carp 45 days post hatching (Mean±SE).

| | Photoperiod (hours) | | | |
|---|---|---|---|---|
| Parameters | 8D:16L | 16D:8L | 12D:12L | 0D:24L |
| Growth in length (cm) | 3.766±0.069[a] | 3.20±0.032 | 3.514±0.035 | 3.035±0.024 |
| Growth in weight (gm) | 2.82±0.051 | 2.257±0.043 | 2.585±0.042 | 2.064±0.031 |

• Number of collected fish in each experiment was 25. a-Significance differences was detected (P<0.05).

Table 4: The correlation between the average of total length (mm) and the length of the intestine in grass carp exposed for 16L:8D throughout the period of 60 days posthatching (Mean±SE)

| Age of fish (days) | Parameters | | | |
|---|---|---|---|---|
| | Total length (mm) | Intestinal length (mm) | Intestinal index | Morphological of intestin |
| 3-4 | 3.80±0.036 | 2.33±0.027 | 61.13 | Straight tube |
| 5 | 7.43±0.073 | 4.80±0.079 | 64.60 | |
| 10 | 15.26±0.056 | 12.46±0.063 | 81.65 | |
| 25 | 21.8±0.048 | 20.33±0.105 | 93.25 | First swelling |
| 35 | 31.0±0.182 | 35.50±0.091 | 114.51 | Second swelling |
| 50 | 45.50±0.091 | 65.833±0.139 | 144.68 | Third swelling |
| 60 | 61.66±0.278 | 96.166±0.187 | 155.96 | Fourth swelling |

• Number of collected fish in each experiment was 20

**Histological Examination**

**Buccal Cavity:** At early life stage (3 days old) the buccal cavity of grass carp larva is consisted of a thin layer of stratified squamous epithelium. Few number of mucous cells and taste buds appeared in the roof and floor of buccal cavity which is positively stained with PAS reaction. As the development of larvae progress (21 days old) the wall of buccal cavity is consisted of many layer of stratified squamous epithelium. The mucous cells increased in both size and number containing neutral and acid mucosubstances.

**Pharynx:** The pharynx of early life stage (3 days old) is composed of a thin layer of stratified epithelium which is somewhat thinner in the anterior region than the posterior. As the larvae progress, the posterior pharynx can be distinguished from the oesophagus by increase number of mucus secreting cells. The submucosa is made of striated muscle fibers. As the larvae progress, the mucosae are higher in the roof than in the floor of the pharynx. The mucous cells increased and contained acid mucopolysaccharide.

The submucosa increased in thickness and the stratum compactum is more obvious (Fig. 2a). In the posterior pharynx of larvae (30 days old), the mucus secreting cells increased and the branches of mucosa are freely anastamosed, so that a sort of network is formed (Fig. 2b).

**Oesophagus:** The oesophagus can be distinguished from the other regions by the appearance of secretory products in the epithelial cells. The oesophageal wall of larvae (3 days old) are lined by simple columnar epithelial cells containing acid mucosubstance. The mucosa is not directly separated from submucosa, since the lamina propria is not differentiated (Fig. 2c). During metamorphosis of larvae (21 days old). The lamina propria is differentiated from the submucosa and the striated muscular layer is made of circular layer (Fig. 2d) and (Fig. 3a). As the fish progress (30 days old), the mucous cells increased and stained positively with alcian blue-PAS-indicating the presence of acid mucopolysaccharide. The mucosal folds increased in height and have the first secondary branching (Fig. 3b).

**Intestine:** The intestine of fry at early life stage (3 days old) is simple and the mucosa contained few number of mucus secreting cells. The walls of intestine are uniform and the sub mucosa is not differentiated from the mucosa. The general structure is almost the same throughout the whole length of intestine with, exception the some differences in the form and height of mucosa. The outer layer of intestinal wall is lined with a single layer of squamous cells of serosa (Fig. 3c). As the development of larvae progress (7 days old) the height of mucosae increased and separated from submucosae at the some regions. The circular muscle layer is difficulty differentiated and appeared as a thin layer of striated muscle fibers. (Fig. 3d).

In the intestine of larvae (15 days old), the mucosal folds increased in height and the mucous cells increased in number. The submucosa separated from mucosa by a thin layer of lamina propria. The intestinal wall is composed of a circular muscle which is lined by a thin layer of serosa (Fig. 4a). The mucous cells stained positively with alcian blue-PAS, indicating the presence of acid mucopolysaccharide (Fig. 4b). After 21 days of posthatching, the mucosa became broader and the secondary branches appeared from the primary ones. In turn, the first swelling of intestine appeared, except the remaining part of the hind intestine (Fig. 4c and d).

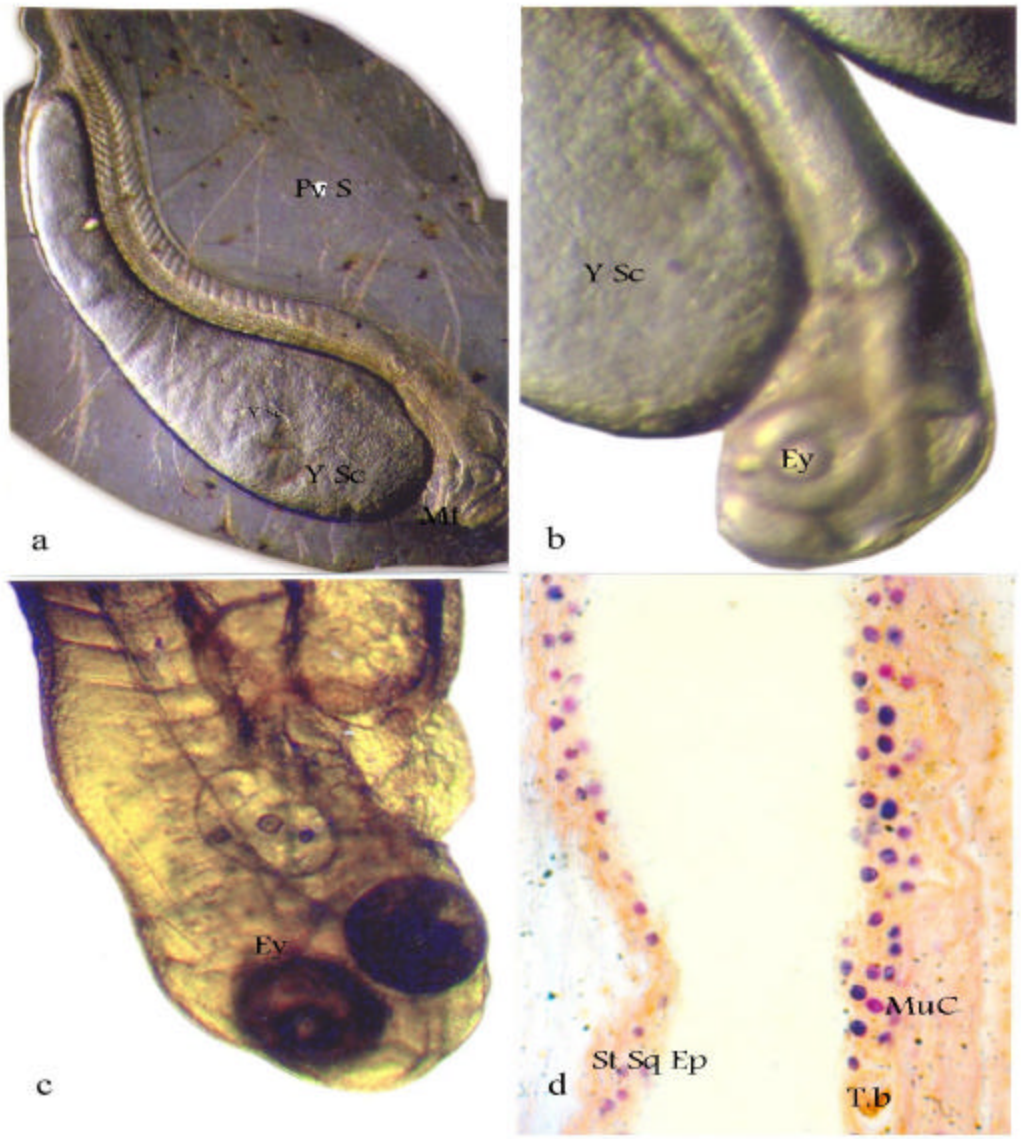


Fig 1: a: Photomicrograph of embryo of grass carp inside the perivitelline space, showing the yolk sac (Y. Sc), the mouth is closed (Mt), X100. b: Photomicrograph of newly hatching fry, showing the yolk sac (Y. Sc) and the eye (Ey) is transparent , X100 c: Photomicrograph of larvae after 7 days of posthatching, showing the black eye lens (Ey. Ln), yolk sac (Y. Sc) is reduced X100. d: Sagittal section in the buccal cavity stained with alcian blue PAS- orange G, showing the roof and floor of the buccal cavity are consisted of stratified squamous epithelium (St. Sq. Ep.) , taste buds (T. b) and mucous cells (Mu. C). X400

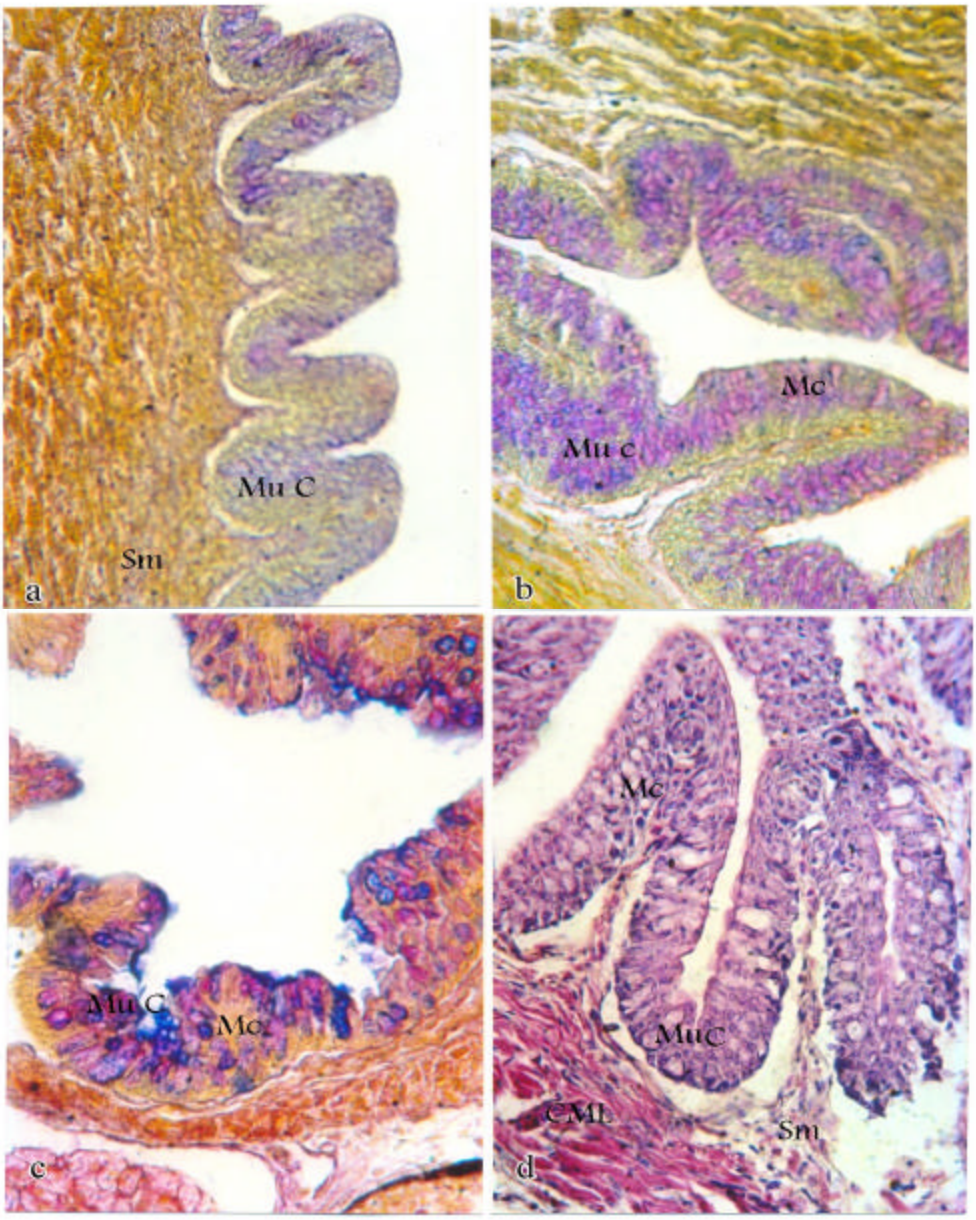


Fig. 2: a: Sagittal section in the pharynx stained with alcian blue-PAS-orang G, showing the mucosa contains mucous œll (Mu. C), submucosa, (Sm) and stratum compactum (Sr. Cp), X400. b: Sagittal section in the pharynx of larvae (30days old) stained with alcian blue-PAS-orange G, showing the mucosa are branched and the mucous cells increased (Mu. C.) X400. c: Sagittal section in the oesophagus of larvae (3days old) stained with alcian blue-PAS-orang G, showing the mucosa (Mc) contains alarge number of mucous cell (Mu. C) X400. d: Sagittal section in the oesophagus of larvae (21 days old) stained with Harris hematoxylin and eosin, showing the long mucosal folds (Mc), submucosa (Sm), circular muscle layer (C.M.L) and mucous cells (Mu. C). X400

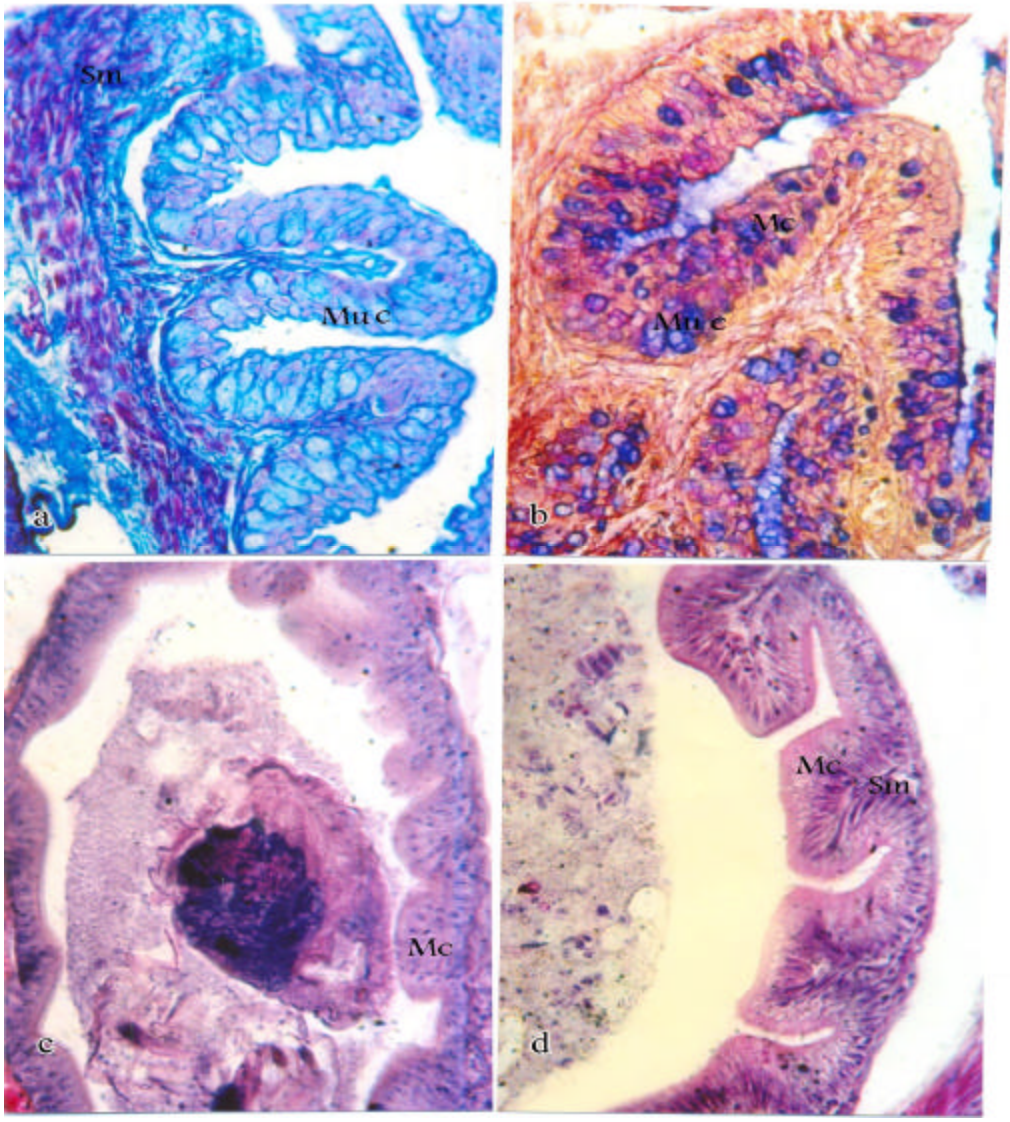


Fig. 3: a: Sagittal section in the oesophagus of larvae (21 days old) stained with Mallory triple stain showing the submucosa (Sm) appeared red and the mucous cells (Mu. C) stained faint blue, X400. b: Sagittal section in the oesophagus of larvae (30 days old) stained with alcian blue-PAS-orange G, showing the mucosa (Mc) contains alarge number of mucous cell (Mu. C) X400. c: Sagittal section in the intestine of newly hatching larvae stained with haematoxylin and eosin, showing the mucosa (Mc) and submucosa is not differentiated. X400. d: Sagittal section in the intestine of larvae (7 days old) stained with Harris haematoxylin and eosin, showing the mucosal folds (Mc), submucosa (Sm) . X400

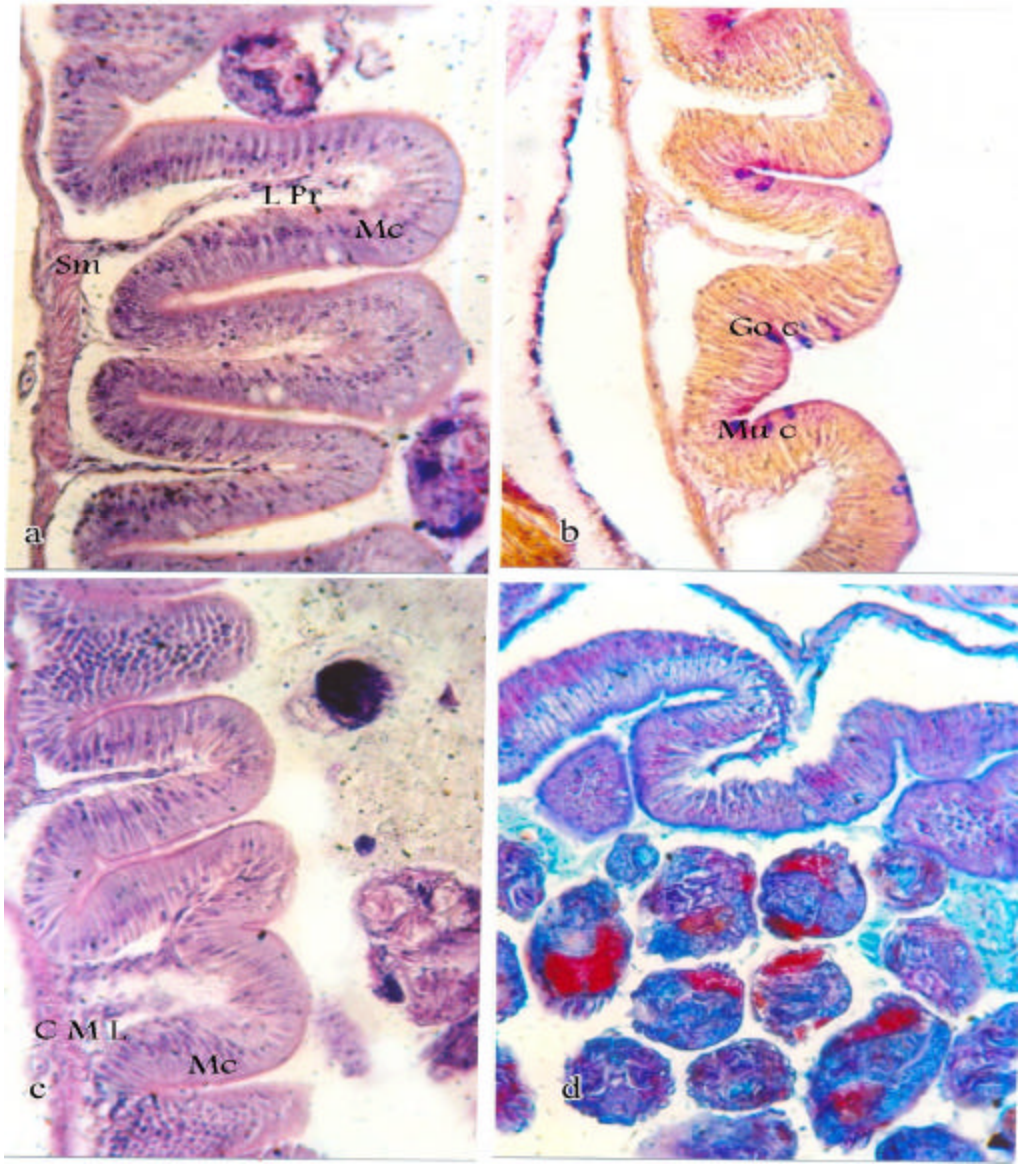


Fig. 4: a: Sagittal section in the intestine showing larvae (15 days old) stained with Harris haematoxylin and eosin, showing the submucosa (Sm) separated from Mucosa (Mc) by lamina propria (L. Pr) X400. b: Sagittal section in the intestine of larvae (7 days old) stained with alcian blue-PAS-orange G, showing the mucous cells (Muc) stained deep blue in color and appeared as goblet cell (Go.c) X400. c: Sagittal section in the intestine of larvae (21 days old), stained with Harris hematoxylin and eosin, showing the mucosa became flattened , (Mc), Circular muscle layer C.M.L. X400. d: Sagittal section in the intestine of larvae (21 days old), stained with Mallory triple stain, showing the lumen of intestine contained the remainder of food (Arrows) X400

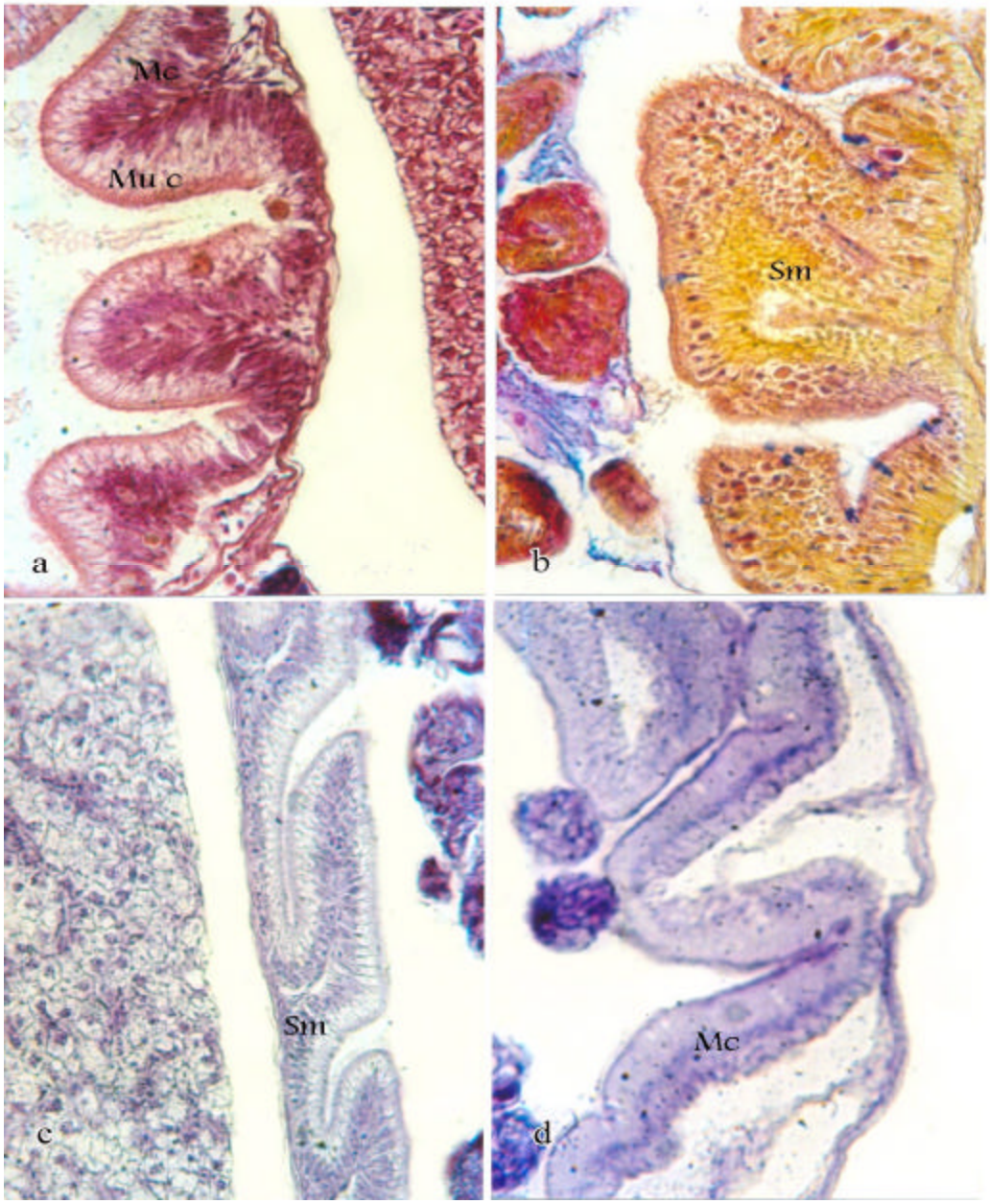


Fig. 5: a: Sagittal section in the intestine of increase number of mucous cells (Mu. C) at the outer border of mucosa (Mc) X300. b: Sagittal section in the intestine of fingerling fish (30 days old) stained with alcian blue-PAS-orange G, showing submucosa (Sm) extends towards the mucosal folds (Mc), beside a brush border towards the lumen of intestine X400. c: Sagittal section in the intestine of larvae (14 days old), stained with bromophenol blue, showing the protein material accumulated at the outer border of mucosa (Mc) and submucosa (Sm). X400. d: sagittal section in the intestine of larvae (21 days old), stained with bromophenol blue, showing the protein material is more concentrated in mucosa (Mc), submucosa (Sm) and lamina propria (L. Pr.), beside the remainder of food X400

As the fish progress (30 days old), the intestine was similar to the intestine of adult stage containing 6 number of swellings and the mucous cells increased and appeared as goblet cells (Fig. 5a and b).

After application with bromophenol blue staining to detect the protein material in the intestine of larvae (14 days old). The protein accumulated in the outer borders of mucosa and muscularis (circular muscle layer). However, the mucous cells were negatively stained (Fig. 5c). As the fish progress (21 days old), the protein accumulation became more concentrated in mucosa. (Fig. 5d).

## DISCUSSION

Grass carp, or white amur (*Ctenopharyngodon idella*) was imported from China in 1969 as most species of carp (i.e common carp and silver carp). It should not be confused with other nonnative carp and used for an effective biological control agent for some varieties of submerged aquatic plants and branched algae [1]. Few data were available on the early life stages of grass carp larvae and there is no references describing how the photoperiod can affect on hatchability of grass carp larvae. The present results described the effect of photoperiod on hatching, to metamorphosis (60 days of posthatching). Based on the results of the current study, it is obvious that the short photoperiod is important to complete the hatching success, since the hatching rate was 72.33±0.112 after the fertilized eggs exposed to 16D: 8L for a period 72-81 hours. Moreover, the long photoperiod gave the lowest value of hatchability (31±0.506) after the fertilized eggs exposed to 8D: 16L. There is no study concerned on how the photoperiod can affect on hatchability of grass carp larvae and needs further study. Extending photoperiod either 8D: 16L or 12D:12L improved the survival rate of grass carp larvae after 7-30 days of posthatching, since the eyes were completely developed. Visual feeding in fish larvae is well known as reported by [15].

The permanent illumination in the present study (0D: 24L) did not improve the survival rate of grass carp larvae. Since a 50% malformed individuals with complete eye migration when exposed to permanent illumination. Similar results were reported by [16]. In this respect, it should be considered that reducing day length from 24 to 12 hours was found to stimulate eye migration at the commencement of metamorphosis as in *Hippoglossus hippoglossus* [17]. Extending photoperiod either 8D : 16L or 12D :12L improve the growth of grass carp larvae for period 45 day, Since the increasing in duration of the visual feeding is generally associated to higher growth. Similar results were obtained on other species as it is the case of *S. aura*ta [3] *L. calcarifer* [4], *P. auratus* [6], *L. lineata* [7] and *Solea senegalensis* [16]. However, in *Melanogrammus aeglefinus* a long photoperiod did not improve the growth [18]. The permanent illumination as in the current study 24 L: 0D did not improve the growth of grass carp after 60 days of posthatching. Under such conditions, it is expected that the grass carp larvae continued to swim actively and expend extra energy that may result in reduced growth. Similar results were reported by [16]. On the contrary to the present results *L. calcarifer* larvae were found to feed 40% when exposed to permanent illumination in comparison to 12 hours of light regime [4]. Photoperiod may also stimulate hormones controlled by pineal organs which are responsible for circadian rhythms [8].In this respect, the larvae exposed to a continuous light (24L: 0D) may grow better than larvae exposed to reduced photo period [19]. Furthermore, the photoperiod of 18L: 6D with light intensity of 4 $\mu$ mol $s^{-1}$ $m^{-2}$ is the best combination on striped trumpeter larvae (*Latris lineata*) [7]. The effect of photoperiod on of metamorphosis of digestive tract of grass carp indicated that the best results obtained after the larvae exposed to 16L: 8D per day. The alimentary canal of grass carp larvae became completely developed after 30 days posthatching. The results suggest a photoperiod (16L: 8D) improved the growth and subsequently the alimentary canal. There is no study concerned with how photoperiod can effect ontogeny of digestive tract in fishes. However many of studies were carried out on the histology and histochemistry of the digestive tracts in other toleosts larvae under control conditions [20-24]. The activities of main digestive enzymes (Proteases, amylase and lipase) and animal husbandry (mainly growth and survival) were studied in common Pandora, *Pagellus erythrinus* larvae under three illumination levels 10, 30 and 100 lux [25]. In all treated groups as described by previous authors, trypsin activity was firstly detected on day 3 in related with mouth opening. The activities of digestive enzymes in the present study were not detected, but carbohydrate and protein materials resulting from the digestive enzymes were detected during the larval development by using histochemical techniques. A new trend on the interaction between the role of endocrinology and photoperiods in related to activities of main digestive enzymes during the larval development of grass carp will be carried out in the future study.

It can be concluded that photoperiod either of 16D: 8L or 12D: 12 L is important for hatching success for a period 72-120 hours of posthatching. However, after 7 days of posthatching, the eyes were completely formed and the larvae transformed from endogenous to exogenous feeding. Subsequently, the long photoperiod either 16L: 8D or 12 L 12D accelerated the growth and metamorphosis the digestive system. This technique may be applied for improvement the rearing fish larvae in aquaculture hatcheries.

## REFERENCES


1. Bishai, H.M. and M.T. Khalil, 1997. Fresh water fishes of Egypt (EEAA) Publication of National Biodiversity Unit. No.9, Egypt.
2. Boeuf, G. and P.Y. Le Baile, 1999. Does light have an influence on fish growth? Aquaculture, 177: 129-152.
3. Tandler, A. and S. Helps, 1985. The Effects of photoperiods and water exchange rate on growth and survival of gilthead sea bream *(Sparus aurata*, Linnueus, Sparidae from hatching to metamorphosis in mass rearing sytem. Aquaculture, 48: 71-82.
4. Barlow, C.G., M.G. Pearce, L.J. Rodgers and P. Clayton, 1995. Effects of photoperiods on growth, survival and feeding periodicity of larval and juvenile barramundi, *Lates calcarifer* (Bloch). Aquaculture, 138: 159-168.
5. Hart, P.R., W.G.J. Hutchinson and G. Purser, 1996. Effects of photoperiods, temperature and salinity on hatchery-reared larvae of the green back flounder (*Rhombosolea tapirina* Gunter, 1862). Aquaculture, 144: 303-311.
6. Fielder, D.S., W.J. Bardsley, G.L. Allan and P.M. Pankhurst, 2002 Effect of photoperiods on growth and Snapper, *Pagrus auratus* larvae Aquaculture, 211: 135-150.
7. Trotter A.J., S.C. Battaglene and P.M. Pankhurst, 2003. Effects of photoperiods and light intensity on initial swim bladder inflation, growth and post-inflation viability in cultured trumpeter (*latris lineata*) larvae. Aquaculture, 224: 141-158.
8. Ekstiem, P. and H. Meissi, 1997. The pineal organ of teleost fishes. Rev, Fish Biol. Fish., 7: 199-284.
9. Hofer, R. and F. Scheimer, 1981. Proteolytic activity in the digestive tract of several species of fish with different feeding habits. Oecologia, 48: 342-345.
10. Harris, H.F., 1900. On the rapid conversion of Hematoxylin into hemation in staininy reaction. Appl. Mikcrose, 3: 777-780.
11. Mallory, F.B., 1938. Pathological technique W.B. Saunders Company, Philadelphia teclinique W.B. Saunders Campany. Philadelphia
12. McManus, J.F.A., 1948. Histoiogical and histochemical uses of periodic acid stain. Technol., 23: 99-108.
13. Bonhag, P.F., 1955. Histochemical studies of the ovarian nurse tissues and oocytes of the milk-weed bg, Oncopeltus fusciatus (Dallas). 1-Cytology, nucleic and carbohydrates. J. Morphol., 96: 381-439.
14. Sokal, R.P. and E.J. Rohlf, 1969. Biometery San Francisco: Freeman and Campany, pp: 776.
15. Blaxter, J.H.S., 1986. Development of sense organs and behaviour of teleost larvae with special reference to feeding and predators avoidance. Trans. Am. Fish. Soc., 115: 98-114.
16. Canavate, J.P., R. Zerolo and C. Fernandez-Diaz, 2006. Feeding and development of Senegal sole (*Solea Senegalese's)* larvae reared in different photoperiods. Aquaculture, 258: 368-377.
17. Solbakken, J.S. and K. Pitmann, 2004. Photoperiodic modulation of metamorphosis in Atlantic halibut (*Hippoglossus hippoglossus* L.). Aquaculture, 232: 613-625.
18. Downing, G. and M.K. Litvak, 1999. The Effects of photoperiods, tank color and light intensity on growth larval haddock. Aquaculture International, 7: 369-382.
19. Puvanendran, V. and J.A. Brown, 2002 Foraging growth and survival of Atlantic cod larvae reared in different light intensities and photoperiods. Aquaculture, 214: 131-151.
20. Ferraris, R.P., J.D. Tan and M.C. De Lacruz, 1987. Development of the digestive tract of milk fish, *Chanon chanos* (Forsskal): Histol. Histochem. Aquacult., 61: 241-257.
21. El-gamal, A.E.E., 1992. Some anatomical and histological studies on the early life stages of Clarias lazera (C.8V.) in fish farms. M.Sc. T hesis, Faculty Science, Cairo University, Egypt, pp: 210.
22. Sarasquete, M.C., A. Polo and M., Yufera, 1995. Histology and histochmistry of the development of the digestive system of larval gilthead sea bream, *Sparus aurata* L. Aquaculture, 130: 79-92.
23. Baglole, C.J., H.M. Murray, G.P. Goff and G.M. Wright, 1997. Ontogeny of digestive tract during larval development of yollow tail Flounder: Alight microscopic and mucous histochmical study. J. Fish Biol., 51: 120-134.

24. Abdeen, A.M., E.K. Abdel Hady, A.E. El-Gamal and R.O. Barakat, 2007. Histological and histochemical studies on the alimentary canal of the the Nile fish *Oreochromis niloticus*. J. Environ. Sci., 33: 1-37.

25. Suzer, C., S. Saka and K. Firat, 2006. Effect of illumination on early life development and digestive enzyme activities in common pandora *Pagellus erythrinus* L. larvae. Aquaculture, 260: 86-93.

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