INTERNATIONAL JOURNAL OF APPLIED BIOLOGY AND PHARMACEUTICAL TECHNOLOGY
Volume: 2: Issue-4: Oct - Dec -2011 IJABPT *ISSN 0976-4550*

INFLUENCE OF PHOTOPERIOD AND TEMPERATURE ON REPRODUCTION AND GONADAL MATURATION IN GOLDFISH : *CARASSIUS AURATUS*

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ABSTRACT: There and great variety of reproductive styles in fish, usage of aquaculture in increasing fish production needs to have a proper understanding of endocrine physiology. In this study three different sets of experiment were designed 19L/5D at 20^0C, 12L/12D at 20^0C, 18L/6D at 20^0C photoperiod and temperature effects on these experimental regimes on reproduction are compared with control group. The gonadal maturation was assessed by microscopic and histological obervations. Gonadosomatic index (GSI) have been calculated. The results from our research revealed that at 19L/15D at 20^0C temperature the gonads are in more developed conditions than other photoperiodic regimes. GSI values is also high. Therefore,it was concluded that long photoperiod are useful to accelerate gonadal activity in goldfish *Carassius auratus.*
Keywords : *Carassius auratus,* gonadal, photoperiod, endocrine

INTRODUCTION

In teleost fish growth development and reproduction are influenced by daily and seasonally variations of photoperiod and temperature (Falcon *et al.,* 2003). Long day lengths stimulates body fattening and ovarian growth while short day lengths are inhibiting in stimulation if this response (Sarkar and Arora ,2001).Photoperiod plays an important role in the seasonal maturation of ovary in a substropical carp (*Catla catla* Maitra *et al* .,2005).

Ovarian recrudescence occurs at a time when day length begins to shorten and temperature reaches a seasonal minimum, while the ovarian recrudescence conincides with increase in day length and temperature. Phototperiodism is the ability of organism to assess and use the day length as an anticipatory use to time seasonal events in their life histories (Bradshaw and Helzafel, 2007). Many investigators have studied the influences of environmental factors on reproductive activity in fish particularly, the effects of photoperiod and water temperature on gonadal development. (Lam, 1983). In some fishes, the developmental and maturation of gonads is to a great extent dependent on day length, while in others, temperature appears to be the important factor; in still others, both day length and temperature are operative (Sundararaj, 1981;Baggerman, 1980; Poston ,1978).

Day length changes in combination with various temperature seem to be important in controlling reproduction in many fish species (De vlaming, 1972a).Involvement of photo thermal conditions in control of reproductive processes is well recognized in several teleosts (Thomas and Arnold, 1993). In some fishes, the development and maturation of gonads, is to a great extent dependent on day length, while in others, temperature appears to be important; in still others, both day length and temperature are responsible, each species has involved an annual breeding cycle which is responsive to a set of seasonal environmental cues (Garg and Jain, 1984). In many fish species gonadal recrudescence and regression are to large extent controlled by the day length of photoperiod (Baggerman, 1972).

Photoperiod strongly affected the timing of puberty and sexual maturation (Noberg, *et al.,* 2004). The role of photoperiod in control of the initiation and termination events, associated with seasonal reproduction is shown in number of species (Hau, 2001).

Developmental and maturational events in animals are influenced by environmental and nutritional factors, environmental factors are particularly important in the growth of ectothermic vertebrates such as teleost fish which rely on temperature and photoperiod (Imsland *et al.,* 1995).

Photoperiod alternations are used to stimulate or delay gonadal maturation and thus to stimulate or delay gonadal maturation which in turn is responsible for change spawning period or somatic growth (Lam, 1983). In cyprinids Carp, Catfish and other tropical and subtropical fish, high temperatures and long photoperiods appear to be important for the final maturation of the oocytes, ovulatio and oviposition (Rocha, 2008). In the temperate fish, photoperiod is the main proximal factor in the timing of maturation; although water temperature most likely also a modulating role to play,the absolute length of the photoperiod is not important for cueing the reproductive events. However the relative change from short to ling or vice versa seems to be critical for the entrainment of the true events. (Carillo, 2007).

MATERIALS AND METHOD

Sexually mature male and female goldfish were collected from commercial dealers and (weight 45 to 65gm) were kept in a aquarium until the experiment began in December, then fishes were transferred in glass aquarium size 4 x4 x 2 feet. Aerated and dechlorinated water with flow rate of 1.5 L min^{-1} 9 ppm dissolved oxygen, 7.8 pH and 102 mg as $CaCO_3$ total water hardness was used. Experiment was lasted for 3 months. The fishes were fed twice daily with commercial fish meal based extruded diet (diameter/mm; 55% Crudeprotein 14% Crude lipid, 4296 Cal g^{-1} diet gross energy) at 3.5% body weight at 10:00 am and 14:00 pm. After 1 hour after feeding uneaten feed were removed by the sandpipe at the bottom of the tank.

The effect of the photoperiod temperature regimes on reproductive function was assessed by gravimetric and histological techniques. Fish were sacrificed by severing the spinal immediately after sacrifice for histology gonads were fixed in bouin's solution and embedded in paraffin wax sections were cut at 5µm and stained with haemotoxylin and eosin.

Illumination was done by fluroscent light done by fluroescent light of 25 lux, Photoperiod was controlled by a timer and temperature was maintained with thermostat and heater. Effects of experimental regimes was examined by gonadal histology. The occurence of spawning was shown by the eggs attached to spawning nests kept submerged in the water. Egg hatchability was checked after each spawning by collecting 100 eggs and keeping them in a well aerated aquarium at room temperature spawned fish were identified after each spawning by individual check and confirmed later by histological observations.

Gonadosomatic index (G.S.I) weight of gonad is expressed as percentage of the total body weight.

$$\text{GSI (\%)} = \frac{\text{gonad wieght (g)} \times 100}{\text{total body weigh t (g)}}$$

RESULTS & DISCUSSION

The effect of various photoperiod and temperature regimes on gonadal activity were examined at the end of the experiment. At the maturity the range of total length of the fish was 8 -9 cm in males and 10-11 cm in females. Changes in gonads were studied in relation to different maturity stages and the following maturity stages at diffenent photoperiods have been observed (Table-1 and Table-2)

The male and female showed weight changes in the gonads, corresponding to the three different photoperiods. In the 19L/5D photoperiod there was a gradual increase in the gonadosomatic index in male 6.5±1.0 and in female 6.7±0.3 showed in (fig.-I). In this photoperiodic and temperature regime testis was fully mature and elongated bulged and dark pinkish in colour and in histologically nucleus was 10.2-1.4 µm in diameter in finely formed spermatids amongst the females, maximum number of fishes (8 out of 10 females) laid eggs. Complete mature eggs were seen in ovary. Ovary become yellow white in colour.(fig II, a).

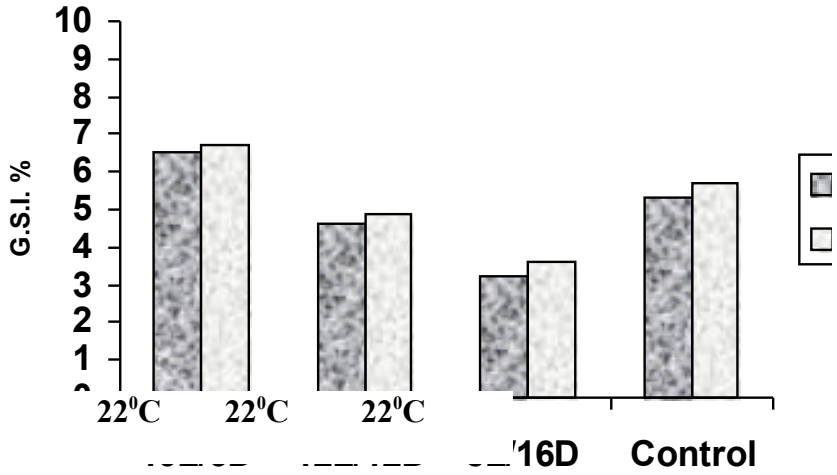

Fig-I Effect of different photoperiodic regimes on G.S.I.(%) in male and female goldfish.Data are showed in mean±$S_{EM.}$ Significant ($p<0.05$) effect of photoperiod on GSI of golfish

Table – I : Gonadal Conditions of goldfish at different photoperiod and temperature regimes

Photoperiod	Maturity Stage	Gonadal Condition	
		Male	Female
19L/5D at 20⁰C	Fully mature	Testis are elongate bulged and dark pink to creamish plus additional red spots.	Yellow-white vagina become light pink; one or two ripe eggs remaining in oviduct.
12L/12Dat 20⁰C	Maturing	Pinkish-white or creamy white left slightly longer than right one	Ovary considerably larger; white or yellowish white maturing eggs visible through wall under microscope; left ovary longer than right one.
8L/16D at 20⁰C	immature	Testis very fine, thin colourless, elongate, left slightly longer than right ore.	Right and left ovaries more or equal in size; colourless to whitish; eggs very minute distinct only under microscope.

Table – II : Histological differentiation of the gonadal maturation of goldfish at different photoperiods and temperatures

Photoperiod	Maturity Stage	Gonadal Condition	
		Male	Female
19L/5D at 20⁰C	Fully mature	Nucleus 10.2-1.4 µm in diameter) staining deeply with haemotoxylene and eosin finely formed spermatids.	Completely mature eggs, follicular tissue much reduced nucleoli reduced in number.
12L/12Dat 20⁰C	Maturing	Spermatogonia to primary spermatocytes 1.9-2.8 µm in diameter nucleus staining pinkish.	Oocytes growing rapidly; nucleus attached to inner border of nucleus membrane.
8L/16D at 20⁰C	immature	Nuclear diameter 3.2-1.7 µm nucleus of spermatogonium with centrally located nucleus.	Large stained nucleus, cytoplasm contained few primary oocytes 10-12 µm in diameter.

In the second photoperiod 12L/12D at 20^{0}C the gonadosomatic index was less in comparison to 19L/5D photoperiod. Male showed 4.6±0.6 and female showed 5.7±1.5 (fig-I). In this experimental regime testis and ovary was in mature stage testis was creamy white in colour longer than right one spermatogonia and primary spermatocytes were seen in histological preparations in female 6 out of 10 female fishes laid eggs. 2 male and 1 female were died and in 3 fishes egg laying was not seen. Ovary was considerably white or yellowish in colour and oocytes were growing rapidly.(Fig-II b and III b).

In third experimental regime 8L/16D gonads were in immature stage.

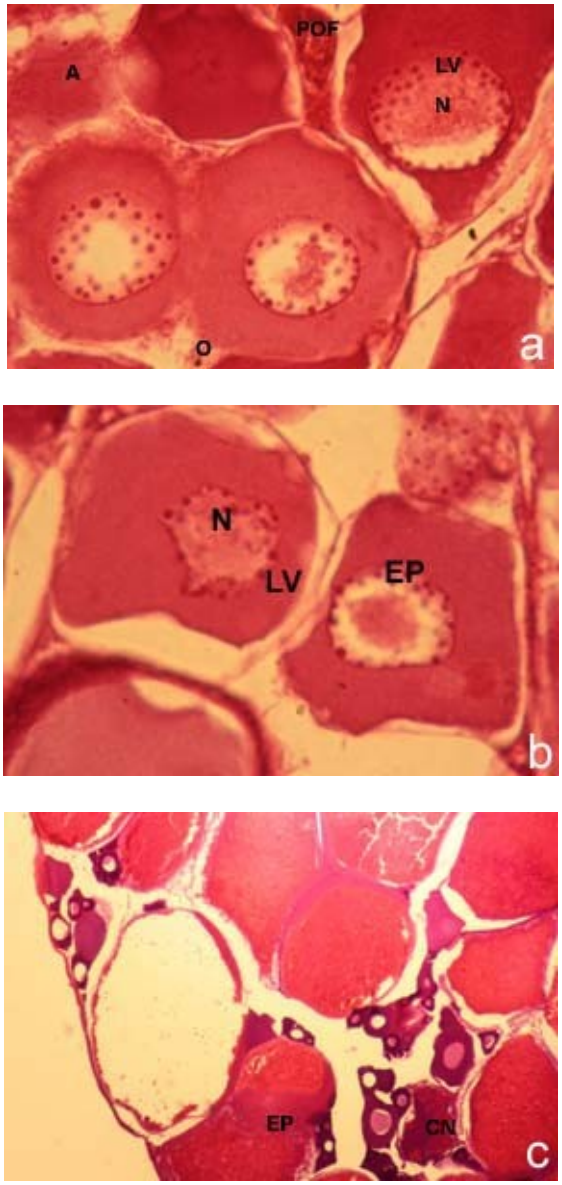

Fig.-II Histological appearance of ovary maturation in *C. auratus* under different experimental regimes (a) 19L/5D at 20^{0}C (b) 12L/12D at 20^{0}C (c) 8L/16D at 20^{0}C
A-Artresia, LV- Lipid vesicle N- Nucleus, EP – Early peri-nucleus, POF – Post ovulatory Follicle, O – Oogonia, CN - Chromatin nucleus

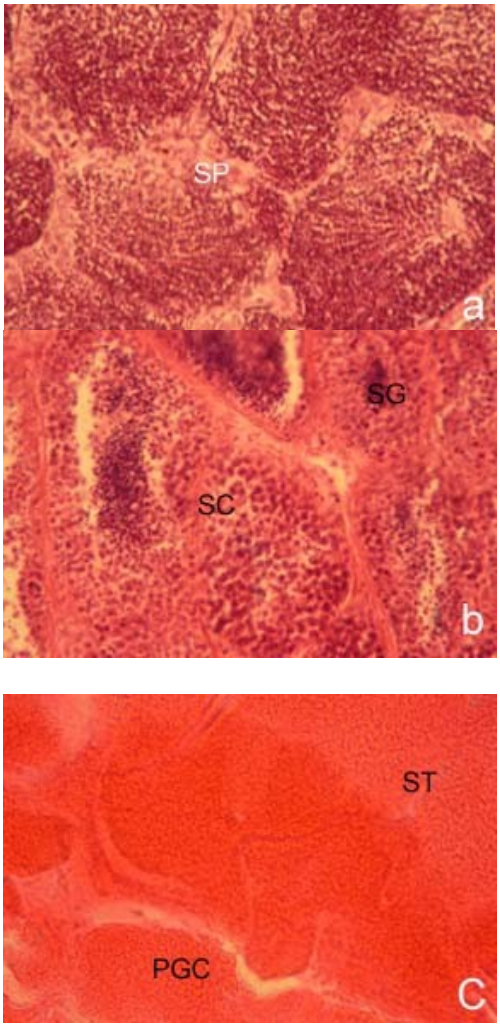

Fig.-III Histological appearance of testis maturation in *C. auratus* under different experimental regimes (a) 19L/5D at 20^0C (b) 12L/12D at 20^0C (c) 8L/16D at 20^0C
PGC – Primary germ cells, SG – Spermatogonia, SC – Spermatocytes, SP – Spermatozoa
ST - Spermatid

Fishes showed gonadosomatic index males (3.2±0.4) females (3.6±0.2) (Fig -I) Testis was thin and colourless, nucleolus was located centrally. In females ovaries were creamy to whitish, cytoplasm contained few primary oocytes.(Fig-II c and III c).In control group 6 out of 10 fishes laid eggs and male and female showed GSI of 5.3±0.7 and 5.7±1.5.

The date presented hare indicate that both photoperiod and temperature are important factors in regulating sexual development in goldfish, *Carassius auratus*. Further more, the effects of various photoperiod temperature regimes on gonadal activity in goldfish appear to vary with different photoperiods. Long photoperiod with warm temperature regimes stimulates testicular and ovarian development. Therefore, a long photoperiod in combination with warm temperature is apparently required for final gonadal maturation in this species.

Long day stimulates body fattening and ovarian growth white short day length are inhibiting in stimulation of this response (Sarkar and Arora, 2001). Several authors have studied the effect of environmental factors on gonadal maturation of goldfish. Goldfish could be induced to fully mature in winter by raising temperature (Yamazaki, 1965). Day length changes in combination with various temperature appears to be important in controlling reproduction in many species (De Vlaming, 1974).Photoperiod also affects the GSI as reported by (Hansen *et. al.* 2001).

GSI values showed a variations throughout all the experimental regimes. The 19L/5D experimental regime stimulates spawning in goldfish. Apparently then, spawning in goldfish depends primarily on photoperiod and temperature conditions. In addition, these data suggests that a combination of a long photoperiod and warm temperature are required for spawning in goldfish.

In fact short photoperiod and warm temperature treatment did result in gonadal regression. In goldfish during short photoperiods, 6L/18D short photoperiod warm temperature regime did not stimulate advancement in gonadal development, but did promote spermatocyte proliferation and the initiation of vitellogenesis. Long photoperiod, in the absence of warm temperature ,do not promote final gonadal maturation in *Carassius*. A short photoperiod in combination with a warm temperature maintains vitellogenesis in ovary but does not promote final ovarian maturation or spawning the long photoperiod and warm temperature brought about sexual maturity in goldfish (Kawamura and Otsuka, 1950).

A short photoperiod regime is not effective in stimulating spermiogenesis in goldfish this regime maintains gametogenesis but does not promote final maturation. These data indicated that the effects of a short photoperiod warm temperature regime on gonadal development in goldfish will never stimulate final gonadal maturation or spawning. A short photoperiod warm temperature regime is effective in maintaining vitellogenesis, final ovarian maturation however, will not occur in these conditions, a short photoperiod regime promotes spermatogonial and spermatocyte proliferation but not spermiogenesis.

Combined the results of these experiments indicate that spermiation depends on a combination of long photoperiod with warm temperatures. The long photoperiod condition is also effective in stimulating spawning, so long photoperiod warm temperature regime is a powerful tool to modify gonadal development in goldfish.

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