EGYPTIAN JOURNAL OF AQUATIC RESEARCH 1687-4285
VOL. 31 NO. 2, 2005: 419-431.

EFFECT OF PHOTOPERIOD, TEMPERATURE AND HCG ON OVARIAN RECRUDESCENCE AND ABILITY OF SPAWNING IN NILE TILAPIA, *OREOCHROMIS NILOTICUS* (TELEOSTEI, CICHLIDAE)

ABD EL-HAKIM E. EL-GAMAL and ZEINAB A. EL-GREISY*

National Institute of Oceanography and Fisheries, Aquaculture Division. Alexandria, Egypt.

Keywords: *Oreochromis niloticus, Nile tilapia, Reproduction, Photoperiod, Temperature, HCG.*

ABSTRACT

Adult fish of Nile tilapia, *Oreochromis niloticus* were netted at the quiescent phase of their reproductive cycle (late of October) from Manzalla Lake, Egypt, in a factorial design combining ambient or elevated temperature with normal or prolonged photoperiod.

Normal winter conditions (9L, 16°C) were applied as a control group. Another group subjected to a higher temperature and a prolonged photoperiod was considered as a simulation of summer (15L, 25°C). Other two different photothermal conditions were applied as follows: (15L, 16°C) as a prolonged photoperiod condition and (9L, 25°C) as an elevated temperature condition.

Gonadsomatic index, hepatosmatic index, ova diameter and histological studies of the ovaries were demonstrated in each group after 60 and 90 days of experimentation. The present study showed that significant differences were recorded in these reproductive parameters from treatment to another. The final maturation in the ovary of *O. niloticus* was accelerated by higher temperature and prolonged photoperiod. The maximum acceleration was recorded at a combination of higher temperature and prolonged photoperiod together at the same time. However, there was a considerable effect (acceleration) when either of these environmental factors was increased alone, but higher in case of temperature than photoperiod.

HCG injection increased the final ovarian maturation, egg diameter, fertilization rate, hatching rate and percent of spawning than the non injected groups, either environmentally controlled or not.

INTRODUCTION

Several environmental and physiological factors are practically involved in fish gonadal maturation and spawning, hence facilitate hatchery production (Bromage *et. al*., 2001). Major environmental factors involved in cueing reproductive activity are temperature and photoperiod (Eyeson 1983; Emit *et al.* 1989; El-Greisy 1993 and El-Naggar *et al.* 2000). Temperature is generally the most variable environmental parameter and also the most controllable hatchery condition. It has been considered as the most thoroughly investigated environmental factor influencing fish reproduction. Photoperiod would regulate the axis photoreceptors, central nervous system, hypothalamus, pituitary gonadotropin, ovarian estrogen secretion, oogonial proliferation and endogenous yolk formation (Yaron *et al.* 1980; Hansen *et. al.*, 2001).

Hormonal therapy is a very important physiological factor that can initiate many vital processes important for fish reproduction. Human Chorionic Gonadotropin (HCG) has an obvious role in this aspect. *Oreochromis niloticus* in El-Manzallah Lake, Egypt, have a normal spawning season extending from April to

*Corresponding author
E-mail: Zeinab_elgreisy@yahoo.com

August with its maximum activity from May to June (El-Ghobashy, 1990; Shalloof, 1991). Accelerating of the final maturation and ovulation (i.e. spawning) can help avoid pre-spawning mortality by reducing the time that fish are held in the hatchery. Acceleration of final maturation also leads to early hatching of offspring which can lead to larger size of release, often a goal of aquaculture facilities (Slater *et al.* 1995).

The purpose of the present study is to investigate the effect of different combinations of photoperiod, temperature and HCG injection on ovarian recrudescence and ability of Nile tilapia, *Oreochromis niloticus*, to spawn out of the time of the spawning season.

MATERIALS AND METHODS

The experiment was carried out at the period ranged from late of October 2004 till February 2005 at El-Mattaria station for fish researches. A period of light in 24 hours is marked by the letter "L". Adults of *Oreochromis niloticus* weighing between 35 and 75 grams in body weight were netted at the quiescent phase of their reproductive cycle (October 2004) from Manzalla Lake. The fish were kept for 10 days at 15°C and natural photoperiod for acclimation. The fish were then divided into four groups and placed in controlled glass aquaria. The temperature in the first two groups was 16°C over 90 days (from end of October till the early of February). While, in the third and fourth glass aquaria, the temperature were increased gradually to become 25°C. the experimental groups were as follows:

1. Winter conditions (9L, 16°C), considered a control group.
2. prolonged photoperiod (15L, 16°C).
3. simulation of summer (15L, 25°C).
4. elevated temperature (9L, 25°C).

Half of fish in each group was examined on the 60th day of the onset of the experiment and the other half of each group was examined by the same manner on the 90th day of the experiment. The fish were weighed to the nearest gram, then the ovaries were excised and weighed to the nearest milligram. Subsamples were taken for histological examination. The gonadosomatic index (GSI) was determined by applying the following equation:

$$GSI = \frac{\text{gonad weight}}{\text{Gutted weight}} \times 100$$

The hepatosomatic index (HSI) was also determined by applying the following equation.

$$HSI = \frac{\text{Liver weight}}{\text{Gutted weight}} \times 100$$

The egg diameters were measured by using a standardized eye-piece micrometer under stereomicroscope. For histological studies, small pieces of ovary were fixed in Bouin's fluid for about 48 hours, thereafter dehydrated through an ascending concentrations of ethanol, cleared in xylene and embedded in paraplast wax (m.p. 56-58°C). Transverse sections were taken at 6 micron thickness and stained with Haematoxylin after Harris (1900) and counterstained with Eosin.

HCG was injected for females at doses of 25 and 50 IU/gm of body weight to show the effect of hormonal treatment at various combinations of temperature and photoperiod on spawning after 90 days of the beginning of the experiment. Male individuals were distinguished from females by appearance of reddish colour in the tip of caudal fin and snout. A single dose of HCG (25 IU/gm of body weight) was used for the stimulation of males.

Statistical analysis: The test described by Fisher (1950) was employed to calculate the statistical significance between these treatments.

RESULTS

I. Effect of photoperiod and temperature on the reproductive parameters

1. Gonadosomatic Index

As shown in Table (1), it is obvious that the GSI values varied at different combinations of photoperiod and temperature. The lowest value of GSI was recorded at short photoperiod and low temperature (9L/16°C) i.e. at winter conditions (control group). A slight increase was recorded when the photoperiod only was raised to 15L hrs (i.e. 15L/16°C). Very highly significant increase was recorded when the temperature only was raised to 25°C (i.e. 9L/25°C). But the highest significant increase was recorded when the photoperiod and temperature were elevated together (i.e. 15L/25°C) which represents the simulated summer conditions.

2. Hepatosomatic Index

The HSI showed the highest value at high temperature combined with prolonged photoperiod (Table 2).

Table (1): Gonadosomatic Index of females *Oreochromis niloticus* at various combinations of temperature and photoperiod after 60 and 90 days of treatment.

Expermental Groups	GSI (After 60 days)				GSI (After 90 days)			
	No. of fish	Min.	Max.	Average ±SD	No. of fish	Min.	Max.	Average ±SD
9L/16°C (control, winter conditions)	18	0.157	0.890	0.558±0.177	17	0.150	2.170	0.860±0.567
15L/16°C (prolonged photoperiod)	16	0.250	1.550	0.876±0.414	19	0.250	2.360	1.229±0.577
15L/25°C (simulation of summer)	16	0.270	2.150	1.283±0.564	13	0.550	3.800	1.731±1.055
9L/25°C (elevated temperature)	17	0.237	1.850	1.0179±0.430	17	0.360	2.750	1.374±0.751

15L/25°C VS 9L/16°C was very highly significant $P < 0.0005$.
15L/25°C VS 9L/25°C was significant $P < 0.10$.
15L/25°C VS 15L/16°C was highly significant $P < 0.025$.
9L/25°C VS 15L/16°C was not significant $P < 0.25$.
9L/25°C VS 9L/16°C was very highly significant $P < 0.005$.

Table (2): Hepatosomatic Index (HSI) of females *Oreochromis niloticus* at various combinations of temperature and photoperiod after 90 days of treatment.

Experimental Groups	No. of fish	HSI		
		Min.	Max.	Average ± SD
9L/16°C (control, winter conditions)	15	2.035	3.250	2.710±0.606
15L/16°C (prolonged photoperiod)	12	1.872	4.336	2.836±0.698
15L/25°C (simulation of summer)	13	1.290	4.492	3.195±1.518
9L/25°C (elevated temperature)	15	1.081	3.980	2.645±1.068

3. Egg diameter

The largest diameter of ova, as shown in Table (3), was recorded when both photoperiod and temperature were increased together i.e. simulation of summer (15L, 25°C). Also, a considerable increase was recorded when either of photoperiod or temperature was increased, but higher and significant in case of temperature rather than photoperiod.

4. Frequency distribution of egg diameters at different combinations of photoperiod and temperature

The frequency distribution of egg diameters in *Oreochromis niloticus* at different photothermal treatments, as shown in figure (1), showed that both prolonged photoperiod and elevated temperature affects positively egg diameter. Elevated temperature showed higher increase in egg diameter more than the prolonged photoperiod. However, the highest increase in egg diameter was recorded in case of the combination of prolonged photoperiod and elevated temperature together.

II. Effect of HCG injection at various combinations of photoperiod and temperature

HCG injection successfully accelerated the final gonadal maturation of Nile tilapia *Oreochromis niloticus*, as shown in Table (4). HCG injection affected positively egg diameter in both control group (winter conditions) and photothermal controlled groups. But successful spawning occurred only in elevated temperature, prolonged photoperiod and the combination of both of them together. The combined effect of HCG injection and photothermal treatments showed an obvious difference in egg diameter, time of spawning, fertilization rate, hatching rate and percent of spawning. However, the maximum increase of hatching rate was recorded when both photoperiod and temperature increased together.

III. Effect of photoperiod and temperature on ovarian histology

The histological study of the ovary of *Oreochromis niloticus* indicated more strict results by the end of three months and after exposure to various combinations of different photoperiod and different temperatures. The ovaries of the control group (i.e. 9L and 16°C) revealed that it was mainly composed of oocytes in early stages of development i.e. early and late perinucleolus stages beside some of small sized eggs (Figure 2a). The yolkly oocytes i.e. vitellogenic oocytes were mainly observed in the ovaries of fish maintained at 15L and 25°C . Few of small eggs in early and late perinucleolus stages were also observed (Figure 2b). In either unnatural combinations 9L and 25°C or 15L and 16°C, the oocytes appeared small in size and some oocytes were observed in abnormal architecture (Figure 2c and d) The abnormal structure of those oocyte reflex the lower significance in gonadosomatic index than that of the fish exposed for simulation of summer conditions.

Table (3): Egg diameter of *Oreochromis niloticus* at various combinations of photoperiod and temperature after 90 days of treatment

Expermental Groups	No. of fish	Egg diameter (mm)		
		Min.	Max.	Average ± SD
9L/16°C (control, winter conditions)	30	0.550	1.770	1.185±0.372
15L/16°C (prolonged photoperiod)	35	0.650	2.180	1.249±0.439
15L/25°C (simulation of summer)	35	0.980	2.650	1.820±0.544
9L/25°C (elevated temperature)	32	0.780	2.210	1.398±0.438

15L, 25°C VS. 9L, 16°C, 15L, 16°C and 9L, 25°C were very highly significant P< 0.0005.
9L, 25°C VS 9L, 16°C was highly significant P<0.025.
15L, 16°C VS 9L was non significant P<0.040.
9L, 25°C VS 15L, 16°C was significant P<0.10.

Table (4): Effect of HCG injection on female *Oreochromis niloticus* at various combinations of temperature and photoperiod after 90 days of treatment.

Groups	No. of fish	Body weight (gm) Av. ± SD	Initial egg diameter (mm) Av. ± SD	Dosage/gm of body weight		Time interval (hrs)	Spawning egg diameter (mm) Av. ± SD	Time of spawning (hrs)	Fertilization %	Hatching %	Spawning %
				1st injection IU/gm body wt.	2nd injection IU/gm body wt.						
9L/16°C (control, winter conditions)	3	24.76±4.05	1.050±0.159	25	50	24	1.340±0.282	-	-	-	-
15L/16°C (prolonged photoperiod)	5	18.1±5.23	1.044±0.142	25	50	24	1.478±1.143	-	-	-	-
15L/25°C (simulation of summer)	4	26.3±2.59	1.247±0.133	25	50	24	1.990±0.517	63	80	67	75
9L/25°C (elevated temperature)	5	23.32±4.154	1.171±0.112	25	50	24	1.637±0.258	75	57	35	40

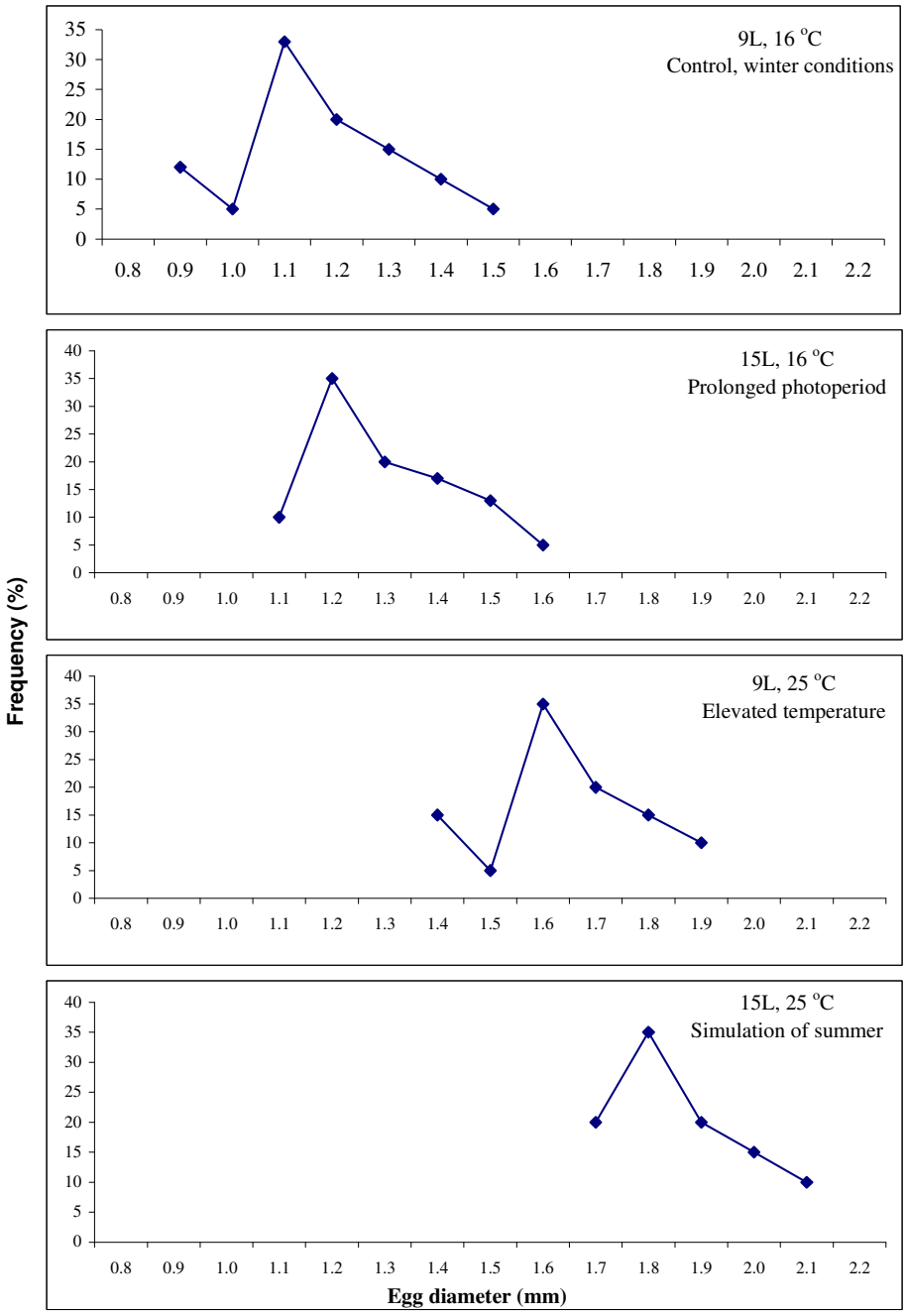

Figure (1): Frequency distribution of egg diameters (mm) of *Oreochromis niloticus* at different combinations of temperature and photoperiod after 3 months of treatments.

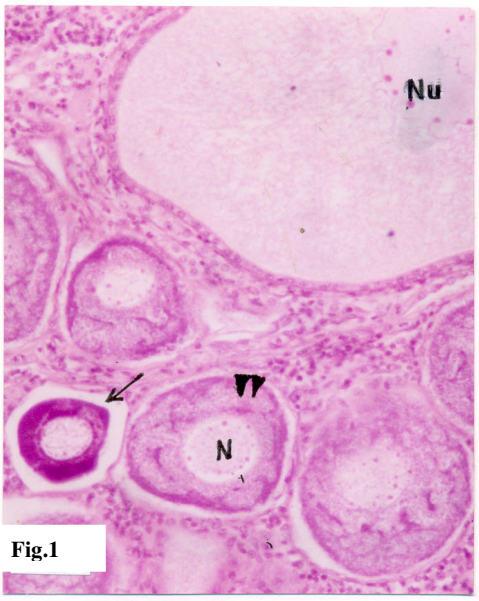

Fig.1

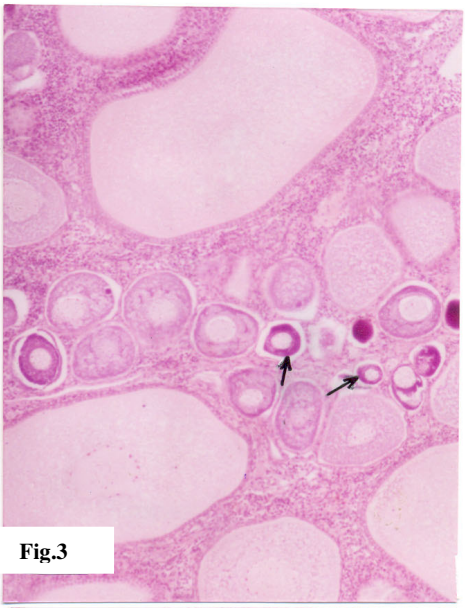

Fig.3

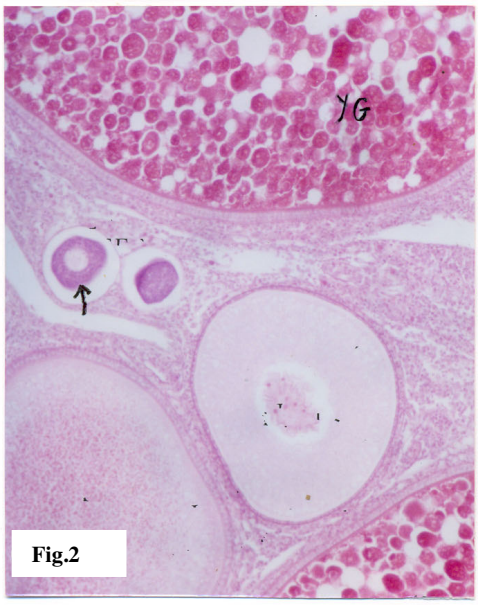

Fig.2

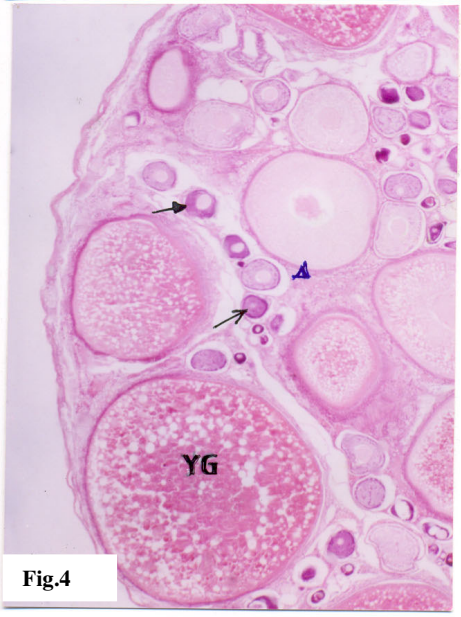

Fig.4

DISCUSSION

Many and great efforts are directed to understand and use either environmental or physiological factors or a combination of both to obtain more and out of season gonadal development and successful spawning. However, the success of induced spawning depends upon several factors which in most of the fishes are not clearly understood (Ram *et al.,* 2001).

In the present study, photoperiod showed a considerable effect on many reproductive parameters of *O. niloticus*. Increases in the values of gonadosomatic index, hepatosomatic index, ova diameter and their frequency distribution were recorded at a prolonged photoperiod, specially that combined with higher temperature.

Photoperiod was recorded as an important cue for the timing of spawning in many fish species, such as Atlantic cod (Norberg *et al.,* 2004), Atlantic salmon (Hansen *et al.,*1992), rainbow trout (Duston and Bromage, 1986 and 1988; Davies *et al.,*1999), sea bream (Devauchelle, 1984), striped bass (Blythe *et al.,*1994), sole (Devauchelle *et al.,* 1987), turbot (Devauchelle *et al.,*1988), Atlantic halibut (Björnsson *et al.,*1994, 1998), sea bass (Devauchelle & Coves 1988, Carillo *et al.,*1989 and Mananos *et al.,*1997), thin lipped grey mullet (El-Greisy, 1993) and Nile tilapia (Ridha & Cruz,2000).

Temperature is considered in many fish species as the crucial cue in gonadal development and out of season induced spawning factor. The present study recorded an obvious effect on all the reproductive parameters under investigation. The values of gonadosomatic index, hepatosomatic index and ova diameters were increased with elevating water temperature. The results of this study indicate the importance of a thermal cue for induction of mature ovaries in *Oreochromis niloticus*.

Generally, the fish in temperate regions and specially summer spawners need a certain range of temperatures to complete the final gonadal maturation and consequently spawning. Huet (1972) recorded that temperature of 21-23°C is the minimum range required for spawning to take place. It is coincide with that recorded by Fryer and Iles (1972), that temperatures above 20°C trigger the development of secondary sexual characteristics and nest building.

The present findings are in agreement with the general view of temperature acting as an important cue in gonadal activity in fish (El-Greisy 1993). El-Naggar *et. al.* (2000) recorded that *Oreochromis niloticus* did not lay eggs when water temperature decreased below 19°C regardless the photoperiod (12L or 24D), but when the temperature increased to 19-21°C, spawning percentage averaged 10 and 34.9% for 24D and 12L. They concluded that the most productive period coincided with a rise in water temperature range from 22 to 27°C. Shimizu (2003) recorded a gonadal change of mummichog (*Fundulus heteroclitus*) under different temperatures regardless of the photoperiod.

Oreochromis niloticus under the present study showed that the time required for hatching tends to be shorter with increasing water temperature. Also, HCG injection of 25 & 50 IU/gm body weight were effective inducers for out of season spawning after 63 and 75 hrs in simulation of summer and elevated temperature respectively. It means that the shorter time was recorded at prolonged photoperiod combined with elevated water temperature and HCG injection. An obvious increase in egg diameter, fertilization rate, hatching rate and percent of spawning were recorded under environmental control with or without HCG injection. The present findings demonstrated that HCG could be used in induced breeding of *O. niloticus* under hatchery conditions for a large scale fry production as a good biotechnological tool. Similar result was recorded by Barray *et al*. (1995), when they used a single intramuscular injection of HCG

to stimulate the final oocyte maturation and ovulation of walleye (*Stizostedion vitreum*), captured from the wild two weeks prior to the normal spawning season.

Histological examination in the ovaries of *Oreochromis niloticus* in winter conditions (9L / 16°C revealed that the ovary were composed of oocytes in small size and devoid from yolk granules. However, in the ovaries maintaind at 15L and 25°C, many of yolky oocytes were prevalent. The absence of yolk in oocytes of *Oreochromis niloticus* maintained a 9L and 16°C indicates that the processes of vitellogenin in the oocytes were impaired.

The effect of temperature and photoperiod on ovarian cyprinids fish, *Mirogrex teyyae-sanctae* was studied by Yaron *et al.* (1980). The authors reported that the impaired oocyte vitellogenesis could be either the synthesis of estrogen by the ovary or, the estrogen dependent vitellogenin synthesis and secretion by the liver or uptake of this protein by the liver. The ovaries of *Oreochromis niloticus* maintained at 15L and 25°C as observed in the present study contained many of yolky oocytes at a period of 90 days indicate that this temperature may be suitable for metabolic clearance in steroid and enhanced the availability of the hormone to the target organ. Yolk in teleost ovaries may be produced by two ways endogenous yolk which is synthesized inside oocyte and exogenous yolk which is synthesized by the liver and released into circulation of blood stream and is sequestered by the oocytes (Korfsmeier, 1966; Plack and Fraser, 1971 and Le Menn, 1979). Autosynthetic yolk formation precedes the accumulation of heterosythetic yolk (TeHeesen and Engels, 1973). The process of incorporation of heterosynthetic yolk into the oocytes could not be induced by purified salmon GTH, but was induced by a whole pituitary extract (Upadhyay *et al.,* 1978). Either unnatural combination 9L and 25°C or 15L and 16°C in which the oocytes in the ovaries of *Oreochromis niloticus* appeared small in size and some of the oocytes appeared in abnormal structure, explain that the decrease of water temperature or short photoperiod may decrease responsibility of hormone to the target organ i.e. ovaries. However, it should be noted that gonadal responsiveness to salmon gonadotropin was independent on temperature (De Vlaming, 1972).

In conclusion, photoperiod and temperature combined with HCG injection are considered as crucial cues in gonadal development and out of season induced spawning. This meaning is obvious in the present study in case of simulation of summer group fish at 15L and 25°C for a period of 90 days, which were likely suitable for ovarian development and induce effect on reproductive performance of one of the most common fish in Egypt, *Oreochromis niloticus.*

REFERENCES

Barray T.P.; Malison J.A.; Lapp A.F. and Procarione L.S. (1995): Effects of selected hormones and male cohorts on final oocyte maturation, ovulation, and steroid production in walleye (*Stizostedion vitreum*). Aquaculture, 138:331-347.

Björnsson B. Th.; Halldorsson, O.; Haux C.; Norberg B. and Brown C.L. (1998): Photoperiod control of sexual maturation of the Atlantic halibut (Hippoglossus): Plasma thyroid hormone and calcium levels. Aquaculture, 116: 117-140.

Björnsson B. Th.; Taranger G.L.; Hansen T.; Stefansson S.O. and Haux C. (1994): The interrelationship between photoperiod, growth hormone and sexual maturation of adult Atlantic salmon. Gen. Comp. Endocrinol. 93: 70-89.

Blythe W.G.; Helfrich L.A. and Sullivan, C.V. (1994): Sex steroid hormone and vitellogenin levels in striped bass (*Morone saxatilis*) maturing under 6-, 9- and 12- month photothermal cycles. Gen. Comp. Endocrinol. 94: 51-62.

Bromage N; Porter M. and Randall C. (2001):

The environmental regulation of maturation in farmed finfish with special reference to the role of potoperiod and melatonin. Aquaculture, 197:63-98.

Carillo M., Bromage N. Zanuy S., Serrano R. and Prat F. (1989): The effect of modifications in photoperiod on spawning time, ovarian development and egg quality in the sea bass (*Dicentrarchus labrax*). Aquaculture, 81: 351-356.

Davies B.; Bromage N. and Swanson P. (1999): The brain-pituitary-gonadal axis of female rainbow trout oncorhynchus mykiss: effects of photoperiod manipulation. Gen. Comp. Endocrinol. 115: 155-166.

De Vlaming V.L. (1972): The role of endocrine system in temperature controlled reproductive cycling in the estuarine gobiid fish, *Gillichthys mirabilis* Comp. Biochem. Physiol. 41(A): 697-713.

Devauchelle N. (1984): Reproduction décalée du bar (*Dichentrarchus labrax*) et de la daurade (*Sparus aurata*). In: Barnabé G., Billard R. (Eds.), L'Aquaculture du Bar et des sparides INRA, Paris pp. 53-61.

Devauchelle N.; Alexandre J.C.; Le Corre N. and Letty Y. (1988): Spawning of turbot (*Scophthalmus maximus*) in captivity. Aquaculture, 69: 159-184.

Devauchelle N., Alexandre J.C., Le Corre N. and Letty Y. (1987): Spawning of sole (*Solea solea*) in captivity. Aquaculture, 66: 125-147.

Devauchelle N. and Coves D. (1988). Sea bass (*Dicentrarchus labrax*) reproduction in captivity: gametogenesis and spawning. Aquatic living Resources. 1: 215-222.

Duston J. and Bromage N. (1986): Photoperiodic mechanisms of reproduction in the female rainbow trout. Fish Physiol. Biochem. 2: 35-51.

Duston J. and Bromage N. (1988): The entrainment and gating of the endogenous circannual rhytem of reproduction in the female rainbow trout (*Salmo gairdneri*). J. Comp. Physiol., 164(A): 259-268.

El-Ghobashy A.E.A. (1990): Biological studies on the western regions of lake Manzallah. Ph.D. Thesis, Zoology Department, Faculty of Science, Mansoura University, Egypt. 259 pp.

El-Greisy Z.A. (1993): Effect of some environmental and physiological characteristics on reproduction of *Mugil capito* in Egypt. M.Sc. Thesis, Institute of Graduate Studies and Research, Alexandria University, Egypt.

El-Naggar G. O.; El-Nady M. A.; Kamar M. G. and Al-Kholy A. I. (2000): Effect of photoperiod, dietary protein and temperature on reproduction in Nile Tilapia (*Oreochromis niloticus*) 5th International symposium for Tilapia aquaculture (ISTA) September 3-7, Barzil.

Emit L.; Etcheri I. and Umoren O. (1989): Aspects of the reproductive biology of *Tilapia guineensi* in Editas Pond, Nigeria. Rev. Zool. Afr. J. Zool., 103: 127-134.

Eyeson K.N. (1983): Stunting and reproduction in pond-reared *Sarotherodon melanotheron*. Aquaculture, 31: 257-267.

Fisher R.A. (1950): Statistical Methods of research workers. 11 th ed., Oliver and Boyd. London.

Fryer G. and Iles T.D. (1972): The chichlid fishes of the Great Lakes of Africa. Their Biology and Evolution. Oliver and Boyd, Edinburgh, Scotland, UK.

Hansen T.; Karlsen Ø.; Taranger G.L.; Hemre G-L; Holm J.C. and Kjesbu O.S. (2001): Growth, gonadal development and spawning time of Atlantic cod (*Gadus morhua*) reared under different photoperiods. Aquaculture, 203: 51-67.

Hansen T.; Stefansson S.O. and Taranger G.L. (1992): Growth and time of ovulation of Atlantic salmon (*Salmo salar*) in sea cages at two different light regimes. Aquac. Fish. Manage., 23: 135-140.

Harris H.F. (1900): On the rapid conversion of hematoxylin into hematin in staining reaction. J. Appl. Microsc., 3:777-780.

Huet M. (1972): Textbook of fish culture: Breeding and cultivation of Fish. Fishing

News Ltd., Farnham. Surry, England, U.K.

Korfsmeier K.H. (1966): Zur Genese des Dottersystems in der oocyte von *Brochydanio rerio. Z. Zellforsch.* 71: 383-396.

Le Menn F. (1979): Some aspects of vitellogenesis in Meleostean fish. *Gobius niger* L. Comp. Biochem. Physiol, 62 (A): 495-500.

Mañanos E.L.; Zanuy S. and Carillo M. (1997): Photoperiodic manipulation of the reproductive cycle of sea bass (*Dicentrarchus labrax*) and their effects on gonadal development, and plasma 17β-estradiol and vitellogenin levels. Fish Physiol. Biochem., 16: 211-222.

Norberg B.; Brown, C.L.; Halldorsson O.; Stensland K. and Björnsson B. Th. (2004): Photoperiod regulates the timing of sexual maturation, spawning, sex steroid and thyroid hormone profiles in the Atlantic cod (*Gadus morhua*). Aquaculture, 229: 451-467.

Plack P.A. and Fraser N.W. (1971): Incorporation of L. (C^{14}) Leucine into egg proteins by liver slices from cod. Biochem. J., 121: 857-862.

Ram R.N., Singh D.M., Singh I.J. and Singh U.P. (2001): Induction of maturational events in gonads of *Labeo rohita* in response to pituitary extract and ovaprium treatment. Indian J. Fish., 48 (3): 259-267.

Ridha M.T. and Cruz E.M. (2000): Effect of light intensity and photoperiod on Nile tilapia *Oreochromis niloticus* L. Seed production. Aquaculture, 31: 609-617.

Shalloof K.A.Sh. (1991): Biological studies on Tilapia species in the Middle regions of Manzallah Lake. M.Sc. Thesis, Zoology Department, Faculty of Science, Mansoura University, Egypt. 244p.

Shimizu A. (2003): Effect of photoperiod and temperature on gonadal activity and plasma steroid levels in a reared strain of the mummichog (*Fundulus heteroclitus*) during different phases of its annual reproductive cycle. Gen. Comp. Endocrinol., 131 (3): 310-324.

Slater C.H.; Schreck C.B. and Amend D.F. (1995): GnRHa injection accelerates final maturation ovulation/spermiation of sockeye salmon (*Oncorhynchus nerka*) in both fresh and salt water. Aquaculture, 130: 279-285.

TeHeesen D. and Engels W.C. (1973): Elekrophoretische untersuchungen zur vitellogenese von Brachyddnio rerio cyprinidae teleostei. Wilhelm Roux Archiv, 173: 46-59.

Upadhyay S.N.; Breton B. and Billard R. (1978): Ultrastructural studies on experimentally induced vitellogenesis in Juvenile rainbow trout (*Salmo-gairdneri* R.). Ann. Biol. Anim. Bioch. Biophys., 18: 1019-1025.

Yaron Z.; Cocos M. and Salzer R. (1980): Effect of temperature and photoperiod on ovarian recrudescence in the cyprinid fish, *Mirogrex terrae*-sanctae. J. Fish. Biol., 16: 371-382.

List of Figures

Figure (2a): Photomicrograph of a histological section in untreated ovary of *Oreochromis niloticus* (control), showing the oocyte in the first growth phase, chromatin-nucleolus stage (arrow), early peri-nucleolus stage (arrow heads). Haematoxylin and Eosin, X 400.

Figure (2b): Photomicrograph of a histological section in treated ovary of *Oreochromis niloticus* (15L and 25 ^{o}C), showing some oocytes reached to secondary yolk granules and few oocytes appeared in the early first growth phase (chromatin-nucleolus stage) (arrow). Haematoxylin and Eosin, X 400.

Figure (2c): Photomicrograph of a histological section in treated ovary of *Oreochromis niloticus* (15L and 16 ^{o}C), showing some oocytes appeared in the early and late peri-nucleolus stage. The others appeared in the first growth phase (chromatin-nucleolus stage) (arrow). Haematoxylin and Eosin, X 250.

Figure (2d): Photomicrograph of a histological section in the treated ovary of *Oreochromis niloticus* (9L and 25 ^{o}C), the oocytes appeared in the secondary yolk granules stage , chromatin-nucleolus stage (arrow), and early and late peri-nucleolus stage. Haematoxylin and Eosin, X 250.