EFFECT OF SOME ENVIRONMENTAL AND PHYSIOLOGICAL FACTORS ON SOME REPRODUCTIVE PARAMETERS OF MUGIL CAPITO DURING THE BREEDING SEASON.

By

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ABSTRACT

*The effect of some environmental and physiological factors on reproductive parameters of **Mugil capito** in Egyptian fish farm has been studied. Long photoperiodicity and high temperature had a negative effect on the gonads of **Mugil capito** during the breeding season (winter). The change in salinity did not shift the frequency distribution curve of the egg diameter to a certain direction. Human chorionic gonadotropin (HCG) had a stimulating effect on the gonads of **Mugil capito** within the first week of the injection. The spermatozoa concentration and the volume was affected by the dose of this hormone.*

INTRODUCTION

Many environmental and internal factors are thought to act as cues for the initiation of complex behavioral, physiological, biochemical and neuroendocrine changes controlling the reproduction of teleosts. Among these factors, photoperiod, temperature and salinity are the most important ones to initiate pituitary activity in fish in temperate and sub-temperate regions. However, the relative importance of each factor varies with different species of teleosts.

Hormonal treatment was studied in many fish species as an important physiological factor controlling the reproduction of teleost. Protracted spawning season and multiple spawning are characteristic of tropical and sub-tropical teleost, Nikolsky (1963). Photoperiod, water temperatures and food availability have been shown to affect synchronous reproduction in teleost, Devlaming (1972), Johannes (1978) and Bond (1979). Human chorionic gonadotropin (HCG), is one of the most important hormones used in this aspect.

Retarded photoperiod, irrespective of preconditioning photoperiod, plays a dominant role in stimulating oocyte growth in the grey mullet, Mugil cephalus, while the temperature regulates the vitellogenesis towards functional maturity (Kuo ***et al.***, 1974).

Zaki ***et al.*** (1993), studied the spermatogenesis process and production of sperms in ***Liza ramada (Mugil capito)***. They reported that the reproduction of this species occur in January in Lake Edku, Egypt, at 30.34‰ salinity. Males which are reared in fresh water attained sexual maturity and sperms can be obtained by hormonal injection for artificial fertilization.

The present study aims to investigate the effect of photoperiod, temperature, salinity and HCG injection on ***Mugil capito*** during the breeding season.

MATERIAL AND METHODS

Fish samples

The fish samples utilized in the present work were obtained from Barceik fresh water fish farm, Beheira governorate, Egypt. The ***Mugil capito*** in Barceik has a prolonged spawning season extending from October to February. The peak percentage value of the ripe stage in Barceik fish farm is attained in November, December' and January for males and females (Zaki & El-Gharabawy 1991).

The fish studied in the present work were transported from the fish farm to the laboratory in suitable continually-aerated aquaria.

The fish were left for 7 days before running the experiments to give them time to resume their normal condition.

The aquaria (2 x 1.50 x 1.25 meters) were of fiber glass. All the experiments have been done in the same time (from mid October to late December), i.e. within the breeding season, before the natural resorption of the gonads, which starts at January and February.

Experiments

a- Effect of photoperiod

Five aquaria were used in this experiment to determine the effect of photoperiod on the reproduction of ***Mugil capito***. All these aquaria were provided with 3.4‰ saline water, as it was in the natural habitat "Barceik fish farm" to keep the same salinity, and allowed to be aerated several days before the onset of the experiment.

The average of the actual sunshine duration over Alexandria during this period was (6.5L + 17.5D) as reported by Mosalam (1991). So, the control group received (6.5L + 17.5D) during the experiment.

All the tanks of the photoperiodicity experiment had the same ambient temperature (17.5°C) and salinity (3.4‰). But these tanks which received different light conditions arranged as follows:

(1) control (6.5L + 17.5D), (2) short photoperiod (6L + 18D), (3) long photoperiod (18L + 6D), (4) continuous illumination (24L) and (5) continuous darkness (24D).

The illumination in this experiment was done by 3 lamps for each tank, the lamp was 100 watt. The tank which represented continuous darkness had a heavy black plastic glued to its sides and top so that absolute darkness was obtained. The food was added from a small opening in the top of the aquarium. This opening was covered with a piece of the black plastic cover, it was removed only for few minutes daily during the feeding, observing salinity, checking fish or to check the aeration.

b- Effect of temperature

The aquaria used in this experiment were placed directly in the front of windows of the laboratory and thus received identical amounts of day light; neither one of these receive any artificial illumination. All of them received the same amount of light (6.5L + 17.5D), and salinity (3.4‰). But these tanks received different temperature conditions.

In the tank with temperature (15°C), the temperature had been lowered by using ice bags which were filled with frozen oil able to lower the temperature for 24 hours. These bags were changed daily to maintain the low temperature.

In the tank with higher temperature (20°C), the water temperature was thermostatically controlled using electric heaters.

c- Effect of salinity change

Four aquaria beside the control were used in this experiment to determine the effect of salinity on the reproduction of ***Mugil capito***.

Fish used were severely selected as healthy and more or less robust. Their body length ranged between 29.1 cm and 36 cm, their body weight ranged between 280 and 390g. The age of such population lies within the end of the second year class.

All the tanks have been subjected to the same conditions of light (6.5L + 17.5D) and temperature (17.5°C). While salinities were: 3.4‰ (control), 15‰, 25‰, 35‰, and 38‰ (sea water).

d- Effect of Human chorionic gonadotrophic hormone (HCG)

A group of males and females ***Mugil capito*** was injected three times with human chorionic gonadotrophic hormone (HCG) intramuscularly, each was 500 IU weekly. The hormone is produced by the Nile company for Pharmaceutical and Chemical Industries, Egypt, as a drug named "Pregnyl".

The fish were dissected, gonads were weighed and the gonadosomatic index (GSI) was computed as the percentage of gonads to the gutted weight of the fish.

A piece of 0.05 gm of the ovaries "Free from ovarian wall" was preserved in 4% formalin for the determination of egg diameter. The sperms were striped from the fish in the morning at 10 A.M. The method, which used for the determination of volume and number of sperm was according to Belova (1981,a).

RESULTS

1. Gonadosomatic index (GSI) was calculated by the formula:

$$\frac{\text{weight of ovary} \times 100}{\text{gutted body weight}}$$

a- Effect of photoperiod

Through photoperiodic manipulation, the acceleration or delaying of gonadal maturation can be achieved Donaldson, (1973).

In the present work, the effect of photoperiod on the GSI has been studied. From Figure 1, it was observed that the GSI in both sexes decreased with the increase of photoperiod. At the same time, the GSI in complete darkness also decreased greatly. So, continuous illumination (24L) as well as continuous darkness (24D) are not suitable for the gonads of ***Mugil capito*** to achieve gonadal maturation.

b- Effect of temperature

The temperature is one of the most important factors that affect the GSI. From Figure 2, it is clear that the GSI decreased by increasing the temperature. At high temperature (20°C), the GSI of ***Mugil capito*** greatly decreased from 12.733 in thecontrol female and 4.229 in the control male to 2.232 in female and 2.915 in male. The female seemed to be slightly more affected by high temperature than male.

At 15°C, the GSI of the male was 4.239, but the GSI of the female decreased to 3.792. These results indicated that the female is affected negatively by increasing or decreasing the temperature, while the male is affected negatively by increasing temperature and there is no sharp difference between the GSI in the control group (17.5°C) and the lower temperature group (15°C).

c- Effect of HCG injection

The effect of HCG injection on the ***Mugil capito*** without any change in the environmental factors is demonstrated in Figure 3. (The experiment began on the sixteenth of October, 1991).

The GSI of females increased from 12.733 in the control to 14.330 within one week after injection with 1500 IU HCG. After that, the GSI decreased with time due to the resorption of the gonad. After 10 weeks, the gonads were resorbed completely and the GSI decreased to 3.089.

Regarding the males, the injection stimulated spermiation while the GSI slightly decreased to 4.00 within one week of the injection. After 10 weeks, the GSI decreased to 0.427.

2. Egg diameter

Egg diameter is one of the most important criteria that reflect the physiological responses of the fish due to internal or external stresses. In the present investigation, the egg diameter profile is demonstrated under the following effects:

a- Effect of photoperiod

Egg diameter of ***Mugil capito*** is affected greatly by photoperiod. The relation between egg diameter and photoperiod is demonstrated in Figure 4. It is obvious that the egg diameter decreased with the increase of photoperiod. The egg diameter of the control (6.5L + 17.5D) ranges from 0.09 mm to 0.81 mm. As the photoperiod increased to (18L + 6D), the egg diameter ranged from 0.09 mm to 0.63 mm. At continuous light (24L), the maximum egg diameter decreased to 0.54 mm with the majority of 0.27 mm. At continuous darkness (24D), the maximum egg diameter decreased to 0.45 mm with the majority of 0.27 mm.

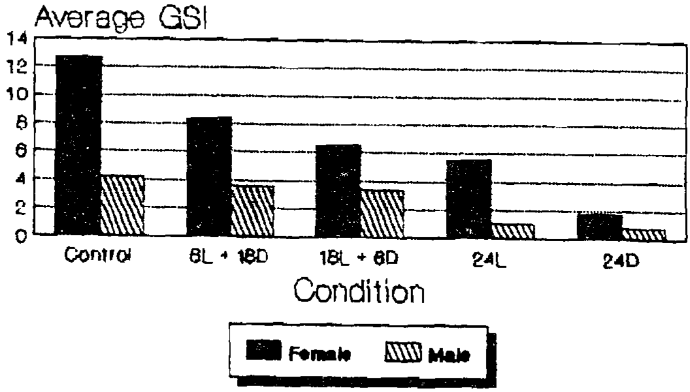

Figure 1: Effect of Photoperiod on the GSI of *Mugil capito*.

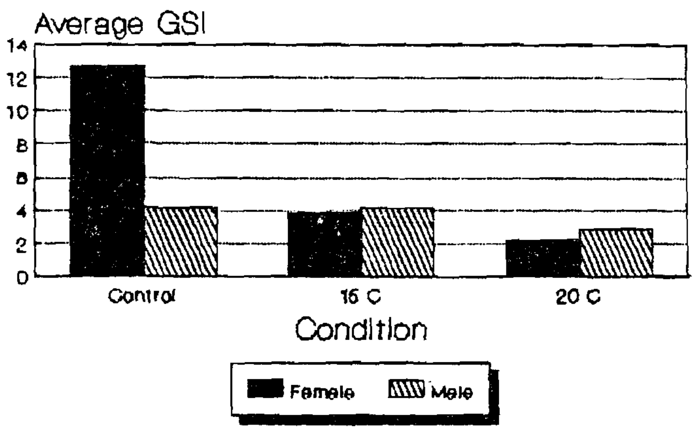

Figure 2: Effect of temperature on the GSI of *Mugil capito*.

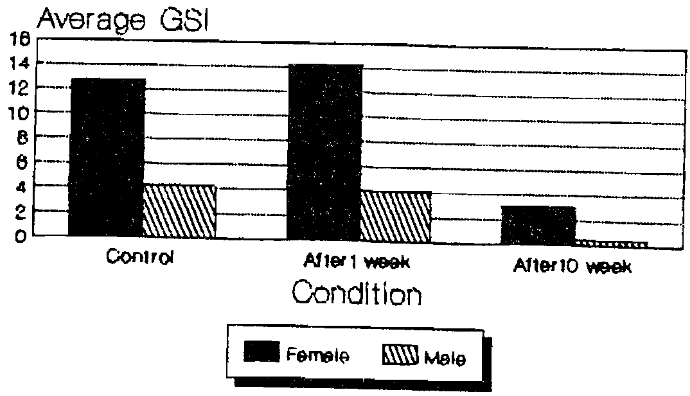

Figure 3: Effect of HCG injection on the GSI of *Mugil capito* Dose = 1500 I.U HCG.

b- Effect of temperature

The frequency distribution curve of egg diameter of ***Mugil capito*** is demonstrated in Figure 5. It is observed that the high temperature of the water was accompanied by a decrease in the egg diameter. The maximum egg diameter at 20°C is 0.36mm compared to 0.81mm in the control. The majority of egg diameter at 20°C was found at 0.18 mm compared to 0.63 mm in the control group. At the temperature of 15°C, the maximum egg diameter decreased to 0.54 mm. The majority at this condition was found at 0.36 mm.

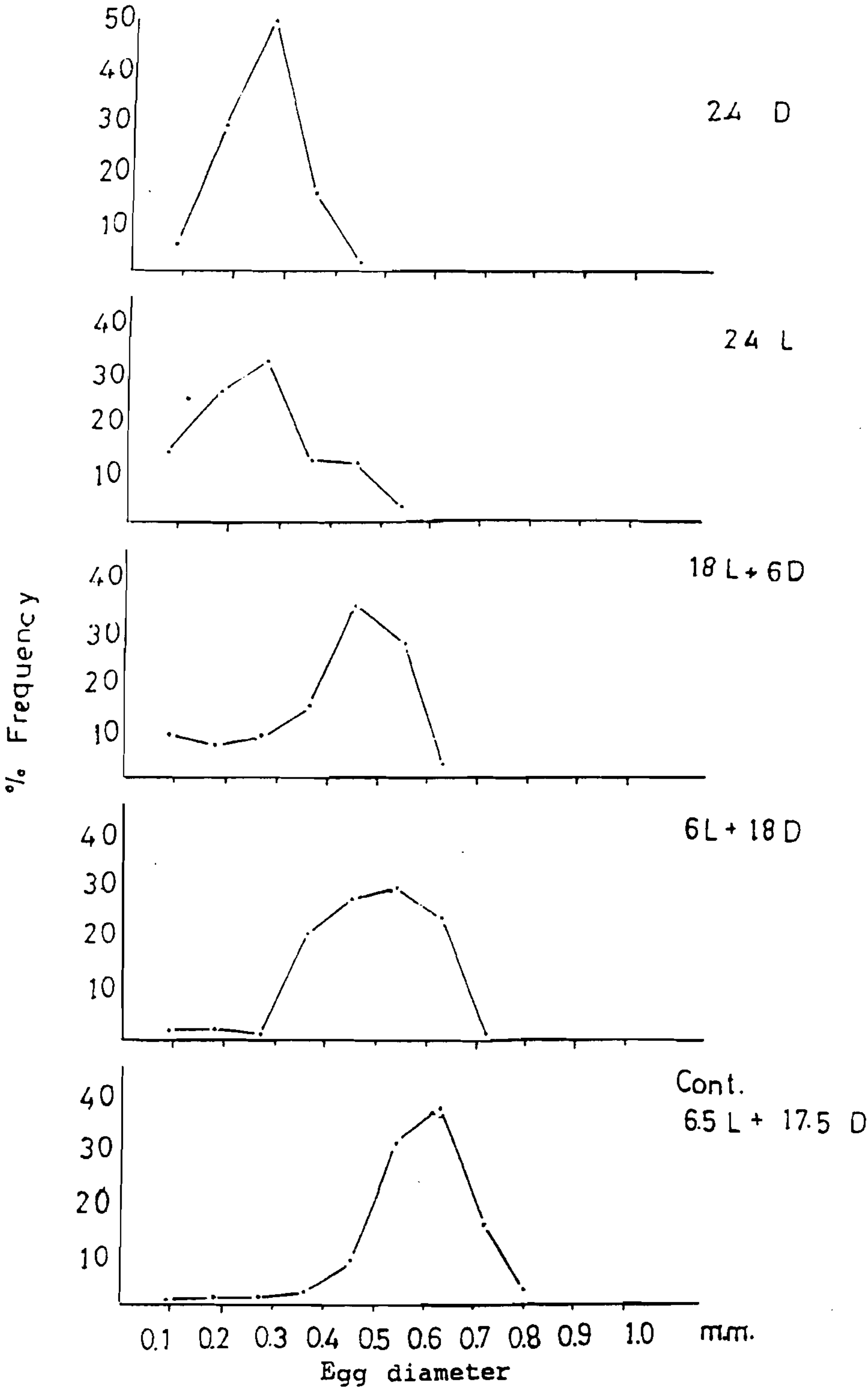

Figure 4: Frequency distribution of egg diameter in *Mugil capito* at different photoperiods during the breeding season.

c- Effect of salinity

The egg diameter is not affected greatly by the salinity. The maximum egg diameter of the group at 15‰ and 25‰ salinities were slightly shifted to the longer egg diameter to reach 0.9 mm compared to 0.81 mm in the control (Figure 6). Whereas, the egg diameter decreased again at 35‰ salinity to confirm that the salinity importance is second to photoperiod and temperature in inducing gonadal maturation. However, the importance of salinity for the ***Mugil capito*** appears in the spawning process (Abraham ***et al***., 1967).

d- Effect of HCG injection

The effect of HCG injection on the egg diameter of ***Mugil capito*** is demonstrated in Figure 7. (This experiment started on the sixteenth of October, 1991). After one week of the injection (1500 IU HCG), the egg diameter frequency profile is slightly shifted to the larger egg diameter. This shift is clear at 0.72 mm and 0.81 mm which increased from 15.7 % and 3 % respectively to 22 % and 5 %, respectively.

After 10 weeks of 1500 IU HCG injection, the frequency distribution profile is shifted to the lower egg diameter to a degree that the majority of the egg diameters are observed at 0.27 mm with a percentage of 44.8.

3. The effect of HCG injection on the milt volume and spermatozoa concentration at different times and different doses of hormone

Human chorionic gonadotropin (HCG) is one of the gonadotropins which are responsible for the gametogenesis and steroidogenesis in the gonads (Murray ***et al***., 1988).

In the present investigation, it was observed that the male of ***Mugil capito*** gave positive response to the human chorionic gonadotropin injection (Table 1). Part (A) of this table represents the relation between the milt volume, spermatozoa concentration and the time passed after 1500 IU HCG.

It was obvious that the milt volume, taken from the same fish, decreased with time. The second part of this table, Part (B), represents the relation between the milt volume, spermatozoa concentration and the time passed after 2000 IU HCG.

It was obvious that the milt volume for the group of fish that received 2000 IU HCG was much more than that in case of the group of fish that received 1500 IU HCG. However, the milt volume and the sperm concentration decreased with time in the case of the two doses of hormone.

Table (1): Effect of HCG injection on the milt volume and spermatozoa concentration at different times and different doses of hormone. Part (A): at 1500 IU HCG & Part (B): at 2000 IU HCG

Dose	Time passed after injection (day)	volume of milt (ml)	Count of sperms/ml	Observations
(A) 1500	1 day	0.15	3,075,200	1st time to be striped milt
1500 IU	3 days	0.10	2,505,600	2nd time to be striped milt
1500	13 days	< 0.10	2,555,200	3rd time to be striped milt
(B) 1500 + 500 IU	9 days after the 1st injection and 1 day after the 2nd injection	3.00	4,392,000	1st time to be striped milt
1500 + 500 IU	10 days after the 1st injection and 2 days after the 2nd injection	0.10	1,390,400	2st time to be striped milt

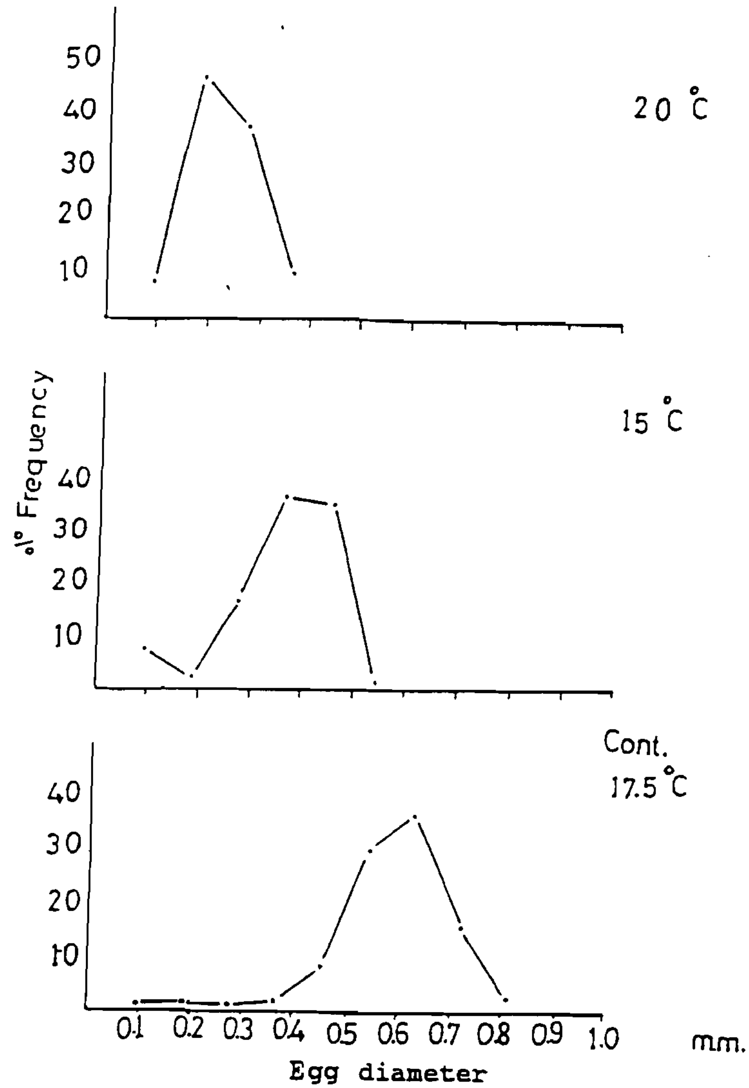

Figure 5: Frequency distribution of egg diameter in *Mugil capito* at different temperatures during the breeding season.

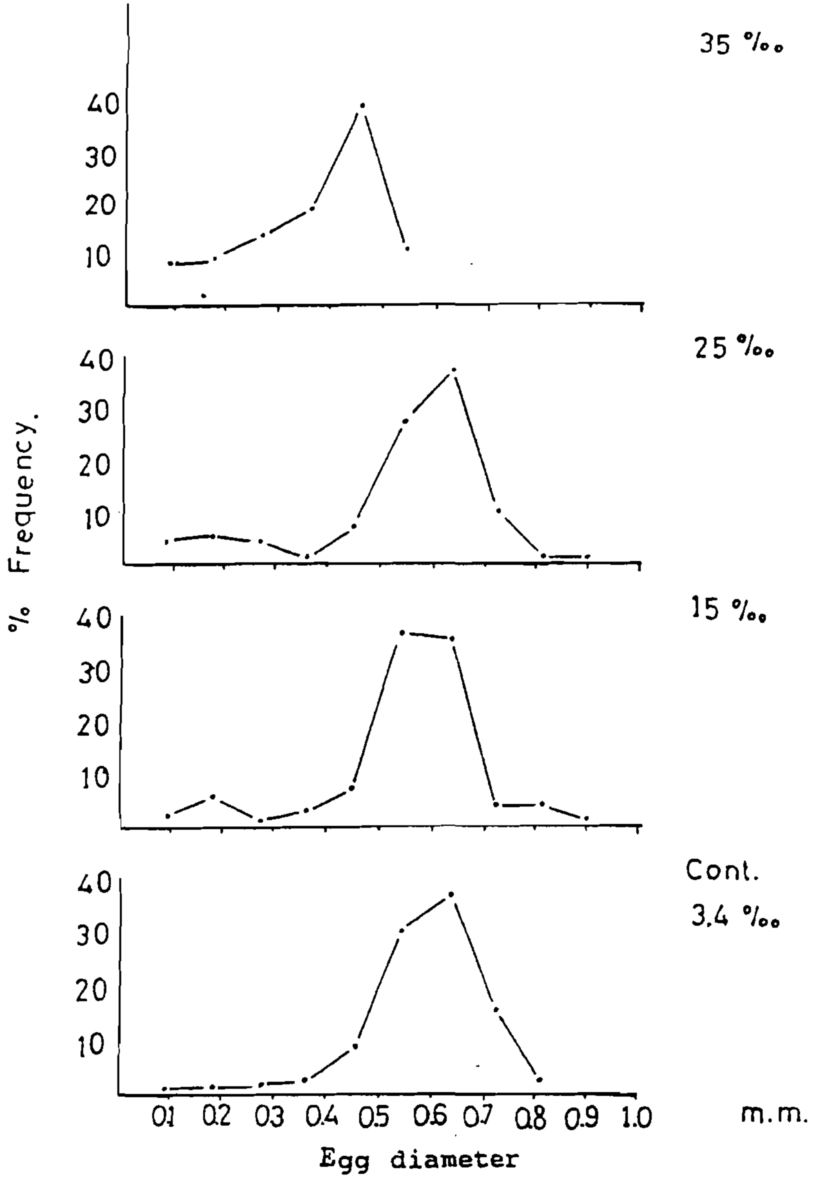

Figure 6: Frequency distribution of egg diameter in *Mugil capito* at different salinities during the breeding season.

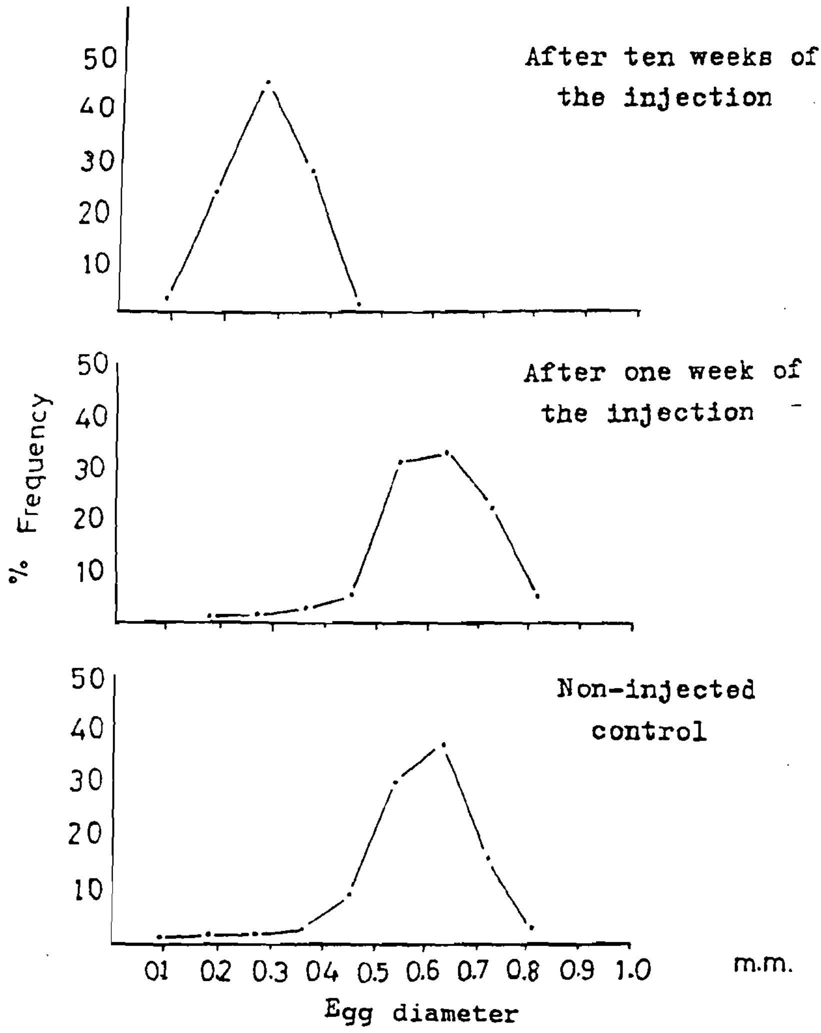

Figure 7: Frequency distribution of egg diameter in *Mugil capito* in injected and non-injected females.

DISCUSSION

The GSI is an important parameter that can be used to reflect the effect of environmental factors on the gonadal activity of the fish. The present investigation revealed that the increase in photoperiod (18L + 6D) as well as continuous illumination (24L) and continuous darkness (24D), showed an obvious decrease in the GSI compared to that of the control (6.5L + 17.5D).

This result is in affirmative agreement with that given by Lam and Soh (1975) who studied the effect of long photoperiod (18L + 6D) on the GSI of ***Siganus canaliculatus***. They observed that the GSI of this fish decreased at this condition compared to that of the control of their experiment (12L + 12D).

The limitation of the breeding season in ***Mugil capito*** to December or mid January is presumably due to the light conditions. Short photoperiodicity is apparently necessary to stimulate oogenic or spermatogenic activity.

The present study also revealed that the increase in temperature showed an obvious decrease in the GSI of both male and female ***Mugil capito*** while the decrease in the temperature showed a decrease in the GSI of the female and a slight increase in the GSI of the male. These results indicate that the female is affected negatively by increasing or decreasing the temperature,while the male is affected positively by decreasing the temperature & negatively by increasing it.

The present results are in agreement with that recorded by Yaron ***et al.***, (1980) in their study on cyprinid fish ***Mirogrex terrae-sanctae.*** They observed that the higher GSI was found in the fish which had been exposed to the stimulated winter regime (16°C, 9L) than that at (16°C, 14L). The lowest GSI was found in those exposed to the stimulated summer regime (27°C, 14L) than those exposed to (27°C, 9L).

In contrast, the increase in temperature led to an increase in the GSI of green sunfish, ***Lepomis cyanellus***, (Clavin and Hasler, 1972).

The egg diameter of ***Mugil capito*** is affected greatly by the change in the environmental factors. From Figure 4, it is obvious that the maximum egg diameter in the control (6.5L + 17.5D) is 0.81 mm. This value decreased in long photoperiod (18L + 6D), continuous illumination (24L) and continuous darkness (24D) recording 0.63 mm, 0.5 mm and 0.45 mm respectively. It is also observed that the frequency distribution curve is shifted to the lower values in the same direction. This indicates that the egg diameter is inversely proportional to the photoperiod.

The relation between egg diameter and temperature is demonstrated in Figure 5. It is observed that the increase or decrease in temperature is accompanied by a decrease in the egg diameter. In both cases, the frequency distribution curve is shifted to the lower values compared to that of the control.

The change in salinity does not shift the frequency distribution curve to a certain direction.

As a conclusion, the present study showed that the egg diameter of ***Mugil capito*** decreased with the increase in photoperiod and temperature. However, the egg diameter is not affected greatly by the change in salinity.

The increase in photoperiod also led to a decrease in the oocyte diameter in cyprinid fish, ***Mirogrex terrae-sanctae*** (Yaron ***et al***., 1980). They found that at 27°C, the oocyte diameter at 9L is larger than that at 14L. At the same time, at 16°C, the oocyte diameter at 9L is also larger than that at 14L. The highest oocyte diameter was found at (16°C 9L), i.e. winter conditions, while the lowest was found at (27°C, 14L), i.e. summer conditions.

The present investigation is also in affirmative agreement with Tamaru ***et al.***, (1991) who studied the egg diameter of ***Mugil cephalus.*** They reported that the average egg diameters in mature females exhibited a high but negative correlation with the average water temperature; the average egg diameters were only moderately positively correlated with day length. They also reported that no correlation could be detected between egg growth and salinity.

It is generally accepted that photoperiod and temperature are the two major environmental cues that mediate reproductive activities in fishes (Crim, 1982 and Lam, 1983). This means that light and temperature have a direct effect on vitellogenic activity in the ovary of ***Mugil capito***.

In contrast to our results, the egg diameter of sunfish, ***Lepomis cyanellus*** increased with the increase in temperature and light (Clavin and Hasler, 1972). This is perhaps due to periodicity of spawning season i.e. summer spawners show an increase in vitellogenesis with light and temperature while winter spawners show a decrease in vitellogenesis.

Hormonal intervention is one of the most important aspects for the study of fish reproduction. Many environmental factors when used alone, however, do not always trigger the gonadal maturation process. Also, manipulating of these factors is not possible in all locations. Thus, alternative methods, such as hormone therapies, need to be found to overcome the physiological constraints to gonadal recrudescence.

In the present study, HCG injection had an obvious effect on many reproductive items. Gonadosomatic index (GSI), egg diameter, milt volume, were found to be affected by HCG injection.

The GSI of females increased to 14.33 within one week of injection with 1500 IU HCG. After 10 weeks, the gonads were resorbed, the GSI decreased to 3.089. While in the control, the GSI was 12.733.

In males, the GSI slightly decreased after one week of the injection but the spermiation process was increased. The GSI was 4.00 and 0.427 after one and 10 weeks respectively compared to 4.22 in the non-injected controls.The increase in GSI due to hormonal injection is observed in Mugil cephalus after the injection directly (Pien and Liao, 1975).

Regarding the egg diameter in the present work, Figure 7 showed that the frequency distribution curve of egg diameter was shifted to the longer diameter after one week of the injection. At the end of the experiment (after 10 weeks), it was shifted to the shorter ones.

An increase in oocyte diameter was observed in the stimulated females of black porgy, ***Acanthopagrus schlegeli*** due to injection of HCG (Change ***et al.***, 1991).

Milt volume and sperm concentration were also studied in the present work in the injected group.

Since the present experiments were done during the breeding season, the male fish used had mature testes fully packed with mature sperms, the hormone treatment made it easier to strip the males specially at the first time to give milt.

Striped milt volume in the group that received the highest dose (2000 IU HCG) was greater than that in the other group that received the lower dose (1500 IU HCG). The striped milt volume was 3 ml and 0.15 ml in the higher and lower doses respectively for the first time to give milt for both groups. It indicated that the increase in striped milt volume was due to enhancing gametogenesis including spermiation stimulated by the secretion of gonadotropin or androgens (Yamazaki and Donaldson, 1969; Billard ***et al.***, 1982; Change ***et al.***, 1991).

The present study also revealed that the sperm concentration in the seminal fluid decreased with time and with the number of times for the same fish to give milt (Table 1).

REFERENCES

Abraham, M., Yashouv, A. and Blanc, N., 1967. Induction experimentale de la ponte chez ***Mugil capito*** confine en eau douce. CR Acad. Sci. Paris, 265: 818-821.

Billard, R., Fostier, A., Weil, C. and Breton, B., 1982. Endocrine control of spermatogenesis in teleost fish. Can. J. Fish. Aquat. Sci., 39: 65-79.

Belova, N.V., 1981a. Some methods used for the determination of pre-spawning and spawning periods of male fish. Methods of reproduction and development in fishes. M. Science, p: 58-66.

Bond, C.E., 1979. Biology of fishes. Philadelphia: W.B. Saunders Co.

Chang, C.-F., Yueh, W.-S. and Lee, M.F., 1991. Effects of LHRH-A and HCG on the steroid profiles of bisexual and mature male and female protandrous black porgy, ***Acanthopagrus schlegeli.*** Aquaculture, 92: 83-92.

Clavin, M.K. and Hasler, A.D., 1972. Photoperiod and temperature effects on the gonads of green sunfish, ***Lepomis cyanellus*** (Rafinesque), during the Quiescent, winter phase of its annual sexual cycle. Trans. Amer. Fish Soc., 101: 270-275.

Crim, L.W., 1982. Environmental modulation of annual and daily rhythms associated with reproduction in teleost fishes. Can. J. Fish. Aquat. Sci., 39: 17-21.

De-Vlaming, V.L., 1972. Environmental control of teleost reproductive cycles: a brief review. J. Fish. Biol., 4, 131-140.

Donaldson, E.M., 1973. Physiological and physicochemical factors associated. with maturation and spawning. EIFAC Workshop on controlled Reprod. Cultiv. Fishes, May 21st-25th, 1973, Hamburg. FAO Publication.

Johannes, R.E., 1978. Reproductive strategies of coastal marine fishes in the tropics. Environ. Biol. Fish, 3, 65-84.

Kuo, C.M., Nash, C.E. and Shehadeh, Z.H., 1974b. The effects of temperature and photoperiod on ovarian development in captive grey mullet (***Mugil cephalus*** L.). Aquaculture, 3: 25-43.

Lam, T.J. and Soh, C.L., 1975. Effect of photoperiod on gonadal maturation in the rabbit fish, ***Siganus canaliculatus***, Park 1797. Aquaculture, 5: 407-410.

Lam, T.J., 1983. Environmental influences on gonadal activity in fish. In. W.S. Hoar and D.J. Randall (Editors), Fish Physiology, Vol. 9B. Academic Press, New York, Ny, pp 65-116.