

***EFFECT OF SOME ENVIRONMENTAL AND PHYSIOLOGICAL FACTORS ON THE HISTOLOGY OF THE OVARIES OF MUGIL CAPITO DURING THE BREEDING SEASON.***

By

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***ABSTRACT***

*During the breeding season, experimental changes in some environmental factors were accompanied by histological changes in the ovary of Mugil capito. Prolonged photoperiod (18L + 6D), continuous illumination (24L) and continuous darkness (24D) and high temperature had a negative effect on the ovary of this species. Also, the salinity had no sharp effect. The injection of 500 or 1500 IU HCG were not sufficient for the Mugil capito to spawn in the captivity at 3.4 ‰ salinity.*

***INTRODUCTION***

As one of many target organs, the ovary is affected greatly by the environmental and physiological regulators. In the present investigation, the histological structure of ovaries has been investigated under different experimental conditions in order to demonstrate the relation between external environment and ovarian activity of Mugil capito during the breeding season.

Abraham *et al.* (1967), in their study on Mugil capito stated that the spawning of this species was always achieved only after transfer to sea water and injection of pituitary homogenate or human chorionic gonadotropin.

Devlaming (1972 and 1974), stated that photoperiod is one of the most important factors influencing the reproductive cycle of many subtropical and temperate zone fish. Further more Kuo *et al.* (1974), demonstrated the importance of photoperiod for gonadal activity in the grey mullet, Mugil cephalus. They concluded that retarded

photoperiod, irrespective of preconditioning photoperiod, plays a dominant role in stimulating oocyte growth, while the temperature regulates the vitellogenesis towards functional maturity.

Sundararaj and Vasal (1976), used photoperiod and temperature to regulate the reproduction in the female catfish, Heteropneustes fossilis.

Garg and Jain (1985), reported that in some fish, the development and maturation of gonads are, to a great extent, dependent on day length; in others, temperature appears to be the important factor; while, both day length and temperature are operative for third fish category.

Regarding the hormonal treatment, Pien and Liao (1975) histologically studied the gonad of grey mullet, Mugil cephalus, in relation to hormone treatment. They found that hormone treatment usually have a favorable effect in inducing the maturation of females

The object of the present study was to investigate the effect of photoperiod, temperature, salinity and HCG injection on the ovary of M. capito.

## ***MATERIALS AND METHODS***

### **Fish samples:**

The studied fish in the present work were transported from Barceik fish farm to the laboratory in suitable continually-aerated fiberglass-aquaria of 2 x 1.5 x 1.25 meters dimensions. The fish were left for 7 days before running the experiments to give them time to resume their normal condition. 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 slightly saline water 3.4 ‰, as it was in the natural habitat "Barceik fish farm" 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 + 17.5 D) 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 temperature (17.5 °C) and salinity (3.4 ‰). But these tanks 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, of 100 watt each for each tank. The tank which represent 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 and check for fish or 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 amount of day light (6.5L + 17.5D); neither received any artificial illumination. The salinity of the water in all aquaria was constant at a level of 3.4 ‰, but the temperature was varied in order to study its effect.

In the tank with lower 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 by 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.

The tanks had 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".

#### **Histological procedures**

For studying the histological changes, specimens of the ovaries representing each experimental condition were treated technically by passing it through the processes of fixation, washing, dehydration, clearing, embedding and staining using Heidenhain iron haematoxylin as illustrated by Humason (1979).

## **RESULTS**

The control group which was subjected to the natural conditions, showed that the ovary of *Mugil capito* in the period of study is filled with hyaline oocytes. Also, there are immature oocytes, which will be prepared for the next breeding season and a very small percentage of atretic oocytes (plate: 1)

#### **a- Effect of photoperiod**

According to the group which was subjected to short photoperiod (6L + 18D), it showed a well developed oocytes in hyaline form (plate: 2). At the prolonged photoperiod (18L + 6D), a big vacuole with irregular shape was formed in the middle of the oocytes (plate: 3). At continuous illumination (24L), a complete absorption of the yolk globules was observed, and atresia predominated (plate: 4). The oocytes were spongy in shape and the immature oocytes darkly stained.

Continuous darkness (24D), as shown in plate (5), was characterized by atretic oocytes with large vacuoles and complete resorption of the yolk.

So it can be concluded that, the constant illumination (24L) or constant darkness (24D) as well as long photoperiod (18L + 6D) induced atresia and resorption of the yolk in the breeding season.

#### **b- Effect of temperature**

*Mugil capito* was greatly affected by temperature. At lower (15°C) and high (20°C) temperatures, the yolk was resorbed and a big vacuole was observed in the middle of most oocytes. The immature oocytes were not affected by lower temperatures and appeared darkly stained (plate 6 and 7 respectively).

#### **c- Effect of salinity**

As a result of increasing salinity from 3.4 ‰ in the control group to 15 ‰ salinity, large vacuoles were observed in the middle of oocytes (plate 8). With increasing the salinity to 25 ‰, the oocytes were in a hyaline form (plate 9). At 35 ‰ and 38 ‰ salinities (plate 10 and 11 respectively) the immature oocytes clearly developed.

#### **d- Effect of HCG injection**

The effect of HCG on the ovarian histology of female *Mugil capito* kept in fresh water (3.4 ‰ salinity) was investigated. The injection of fish with 500 IU HCG was tested and the histology of this group was demonstrated after 48 hours (plate 12). The ovaries of this groups contained different developmental stages of oocyte, immature, yolk and fat vesicle stages, also contained primary and secondary vitellogenesis stages. A group of fish dissected after one week of injection with 500 IU HCG was demonstrated in plate (13). Most of oocytes of this group were in hyaline form, few of immature oocytes were present for the next breeding season.

The rest of the fish group, which injected by another two doses to reach a total cumulative dose of 1500 IU HCG and dissected after 10 weeks of the injection, they showed atretic oocytes (plate 14). The yolk in this group was resorbed and the atretic oocytes kept their natural shape. The immature oocytes were not affected by the investigated dose.

### ***DISCUSSION***

Little information is available on the role of photoperiod, temperature and salinity in relation to reproduction of *Mugil capito* (recently: *Liza ramada*). In an attempt to evaluate the role of environment in reproduction of *Mugil capito*, photoperiod, temperature and salinity controlled experiments were conducted during the breeding season between middle October and late December.

Under natural conditions, light and temperature are concomitantly increased so it is not easy to assess the relative importance of either one in inducing gonadal recrudescence. These two factors were dissociated experimentally under laboratory conditions.

The present study revealed that long photoperiod (18L + 6D), continuous illumination (24L) and continuous darkness (24D) induced gonadal resorption in the female Mugil capito. Atretic oocytes were a very common feature of the teleost ovary and may be caused by environmental stress.

The result of the present investigation is in agreement with the results, of Lam and Soh (1975) who studied the effect of long photoperiod (18L + 6D) on the gonads of Siganus canaliculatus (Park). They found that the gonadal maturation of this species is also retarded by long photoperiod.

A clear inhibition of gonadal recrudescence under long photoperiod (16L + 8D) was also found by Kadmon *et al.* (1985) they studied the effect of two photoperiod regimes on the gonads of Sparus aurata (L.). On the other hand, short photoperiod (6L + 18D) initiated vitellogenesis in Mugil cephalus (Kuo *et al.*, 1974).

Yaron *et al.* (1980), reported that the effect of the environmental factors, temperature and photoperiod, on ovarian recrudescence in Mirogrex terrae-sanctae can best be explained by a dual-axis model. According to this model, day-length would regulate the axis photoreceptors, central nervous system, hypothalamus, pituitary gonadotropin, ovarian estrogen secretion, oogonial proliferation and indigenous yolk formation. Temperature may have an effect on the former axis, but also, can probably act directly on the liver, allowing the rate of estrogen-dependent vitellogenesis to be relatively high in a cool environment.

Temperature may act on a variety of loci in the endocrine pathways. It may impinge on the thermo-receptors and through them, affect the central nervous system, the hypothalamus, the pituitary, and finally the gonads (Garg and Jain, 1985).

Previous work has indicated that photoperiod exerts the major factor controlling influence in salmonides (Alison, 1951; Carlson and Hale, 1973; Mc Quarrie *et al.*, 1978; and Peier and Crim, 1979), whereas in cyprinides, temperature seems to be the most important factor (Ahsan, 1966). However, in many of these studies, several environmental factors were allowed to change at the same time and this, together with the wide variety of experimental techniques used, has made it difficult to draw any firm conclusions.

Temperature and photoperiod effects on ovarian maturation and egg laying of the crayfish, Orconectes limosus, were studied by Dube and Portelance (1992). They reported that the ovarian maturation rate peaked from mid-November to mid-December. The combined conditions of warm temperature (10-12°C) and darkness successfully accelerated ovarian maturation from mid-December to mid-January and induced egg laying 5 weeks earlier than in the control group (7°C, darkness). They also reported that long photoperiod (16L) did not accelerate ovarian maturation to the same degree: it did not promote earlier egg laying while the warm temperature successfully accelerated egg laying by 3 months in comparison with natural population.

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*LIST OF PLATES*

- Plate (1): Section in the ovary of control group, subjected to natural environmental conditions [(6.5L + 17.5D), (17.5°C) & (3.4 ‰ salinity)], showing a well developed oocytes. (25 X).
- Plate (2): Section in the ovary of short photoperiod group (6L + 18D), at ambient temperature (17.5°C) and 3.4 ‰ salinity, showing a well developed oocytes. (50 X).
- Plate (3): Section in the ovary of prolonged photoperiod group (18L + 6D) at ambient temperature (17.5°C) and 3.4‰ salinity, showing partially resorbed oocytes with large central vacuole. (50 X).
- Plate (4): Section in the ovary of continuous illumination group (24L) at ambient temperature (17.5°C) and 3.4‰ salinity, showing completely resorbed oocytes. (25 X).
- Plate (5): Section in the ovary of continuous darkness group (24D) at ambient temperature (17.5°C) and 3.4 ‰ salinity, showing highly resorbed oocytes. (25 X).
- Plate (6): Section in the ovary of an experimental temperature (15°C) group at ambient photoperiod (6.5L + 17.5D) and 3.4 ‰ salinity, showing partially resorbed oocytes. (25 X).
- Plate (7): Section in the ovary of an experimental temperature (20°C) group at ambient photoperiod (6.5L + 17D) and 3.4 ‰ salinity, showing highly resorbed and collapsed oocytes. (25 X).
- Plate (8): Section in the ovary of 15 ‰ salinity group at ambient photoperiod (6.5L + 17.5D) and ambient temperature (17.5°C), showing rip oocytes with large amount of vacuoles. (25 X).
- Plate (9): Section in the ovary of 25 ‰ salinity group at ambient photoperiod (6.5L + 17.5D) and ambient temperature (17.5°C), showing a well developed oocytes. (25 X).

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- Plate (10): Section in the ovary of 35 ‰ salinity group at ambient photoperiod (6.5L + 17.5D) and ambient temperature (17.5°C), showing a small development in the immature oocytes. (50 X).
- Plate (11): Section in the ovary of sea water group (38 ‰) salinity at ambient photoperiod (6.5L + 17.5D) and ambient temperature (17.5°C), showing obvious recrudescence in the immature oocytes. (100 X).
- Plate (12): Section in the ovary of injected group after 48 hours of 500 IU HCG, at natural conditions [(6.5L + 17.5D), (17.5°C) & (3.4‰ salinity)], showing developed oocytes at different developmental stages. (50 X).
- Plate (13): Section in the ovary of injected group after one week of 500 IU HCG, at natural conditions [(6.5L + 17.5D), (17.5°C) & (3.4 ‰ salinity)], showing ripe oocytes. (50 X).
- Plate (14): Section in the ovary of injected group after 10 weeks of 1500 IU HCG, at natural conditions [(6.5L + 17.5D), (17.5°C) & (3.4‰ salinity)], showing resorbed oocytes. (25 X).







