

Full Length Research Paper

Spawning induction in the Mediterranean grey mullet *Mugil cephalus* and larval developmental stages

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Accepted 20 June, 2006

Spawning induction in the present study was carried out in two steps. The first treatment consisted of a priming dose of carp pituitary extract with 20 to 70 mg, or 10000 IU of human chorionic gonadotropin (HCG) per fish, while the second dose given 24 h later was the resolving dose of 200 µg/Kg lutenizing and releasing hormone (LHRH-a). After the resolving dose the females were maintained together with males and spawning occurred after 17 to 24 h on average. Running ripe males rated (+2) were used in 3:1 male to female sex ratio, in all spawning trials to optimize fertilization rates. The fecundity varied between 1 - 2.70 million eggs/spawn which represents 1395.3 ± 190 eggs/g bw. The ripe unfertile eggs of *M.cephalus* were rounded, colorless and transparent with one oil globule. The surface of the fertilized egg shell is smooth, however; the yolk was unsegmented. After 20 min from fertilization the egg membrane swells up and separated from the previtellic space. The embryo began to be noticed after 13 ± 1 h post fertilization. The egg diameter was 870 ± 30 µm and oil globule was 350 µm. After about 26 ± 4 h post fertilization hatching was started at 25 ± 1 °C and water salinity of 34 ppt. The percent of fertilization varied between 75 to 80% and hatching rate was about 90%. The newly hatched *M. cephalus* larvae was about 1.97 ± 0.23 mm in total length; larvae began feeding 3–5 days after hatching. The time of eye completion is nearly that of mouth opening and body axis becomes straight, which helps the larvae to identify their food as internal-external feeding starts.

Key words: *Mugil cephalus*, carp pituitary extract (CPE), human chorionic gonadotropin (HCG), lutenizing and releasing hormone (LHRH-a), embryonic and larval stages.

INTRODUCTION

The striped mullet, *Mugil cephalus*, is a catadromous fish with world – wide distribution (between latitudes 40° North and 40° South). It spawns in the sea but thrives and grows rapidly in a wide range of salinities. In the wild, the spawning season of the Mediterranean *M. cephalus* ranges from July to December (Brusle, 1982) depending on the salinities and geographical location. Grey mullet females undergo vitellogenesis irrespective of the salinity. However; the rate of oocyte growth is slower in fresh water and a lower proportion of the females successfully complete vitellogenesis (Tamaru et al., 1994).

The grey mullet does not spawn naturally in captivity; females may complete vitellogenesis but will not proceed

with final oocyte maturation (shehadeh et al., 1973). The lack of spawning of the fish in captivity has been attributed to a failure at one or more sites along the hypothalamo-hypophyseal gonadal axis (Zohar, 1989).

Monbrison et al. (2003) indicated that the presumed lack of gonadotropin in the circulation of captive fish could result from an insufficient amount of gonadotropin in the pituitary, inadequate secretion of gonadotropin-releasing hormone (GnRh) from the hypothalamus, or a combination of these reasons. To overcome possible failure along the endocrine axis controlling gonadal function, various hormonal treatments have been adopted to induce maturation and spawning. Reports on induced spawning and larval rearing in grey mullet are primarily based on experiments carried out in Taiwan (Kuo, 1995; Liao, 1997) and Hawaii (Weber and Lee, 1985; Lee et al., 1987, 1988; Tamaru et al., 1989) where

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Table 1. The dosage, number of injections and egg diameters of six unsuccessful spawns utilization.

| Fish no. | Body weight (g) | No. of injections | Initial egg diameter (im) | CPE (mg/Kg) | Dose HCG. I.U./fish | Egg diameter after 1 st injection (im) | 2 nd injection LHRH-a dose (µg/Kg) | Egg diameter after 2 nd injection (im) | 3 rd injection LHRH-a (µg/Kg) |
|----------|-----------------|-------------------|---------------------------|-------------|---------------------|---|---|---|--|
| 1 | 1420 | 2 | 580 | - | 10.000 | 600 | 300 | 620 | - |
| 2 | 1130 | 2 | 550 | - | 10.000 | 570 | 200 | 590 | - |
| 3 | 980 | 2 | 560 | 20 | - | 580 | 200 | 610 | - |
| 4 | 800 | 2 | 550 | - | 10.000 | 565 | 200 | 580 | - |
| 5 | 700 | 2 | 480 | - | 10.000 | 510 | 200 | 520 | - |
| 6 | 580 | 3 | 560 | 20 | - | 610 | 120 | 630 | 100 |

fertilized mullet eggs have been obtained consistently. However, no commercial production of mullet eggs has been reported to date in the Mediterranean basin. Grey mullet commands high price and the ability of juvenile and adult to tolerate large fluctuation of salinity qualifies them as an attractive species for farming (Monbrison et al., 2003).

The present paper reports studies on the acceleration of gonadal development in local grey mullet using hormonal injections and investigation of the embryonic and larval development in aquaria conditions and the first descriptions of the early life history stages of *M. cephalus*. This study will improve the production of fingerlings in the hatchery to increase production of fish in aquaculture farms.

MATERIAL AND METHODS

Broodstock

Grey mullet broodstock (1.42 to 0.58 Kg body weight), used in the spawning trials were obtained from Mallahat Port-Fouad (Port-Said Governorate) in north of Egypt. They were obtained during the end of September to October 2003 – 2004 and maintained at National Institute of Oceanography and Fisheries Marine Hatchery in (3 m³) fiberglass tanks with stocking density of 2 fish/m³, manipulated light exposure at the shortest day (14 D/10 L) during September and October. Females at a body weight of 1.3 ± 0.25 Kg (mean ± SD), and males, at a body weight 0.8±0.28 Kg, were placed in a 3 m³ tank. The state of ovarian maturity for each female was assessed prior to each spawning attempt by using polyethylene canula. The 500 µm size of oocyte diameter has been established as minimum egg diameter for successful induction of spawning in mullet.

System preparation

A flow through sea water 34 ppt system supplied salinity. The water temperature was about 25 ± 1°C throughout the experiment. Fish were fed diet containing 30% crude protein and 6% lipid, with daily rate of 1.5% of the body weight.

Induction of spawning (females)

Two different strategies for spawning mullet were attempted, the first strategy called "a priming" dose of carp pituitary homogenate

(CPH; purchased from Argent chemical laboratories Lot # CP, 1408R) 2 mg/Kg of fish, or human chorionic gonadotropin (HCG; purchased from Argent chemical laboratories Lot # CG, 2304 R) 10000 IU/Kg. Twenty four hours later a second "resolving" injection was giving consisting of LHRH-a (des Gly¹⁰[D-Ala⁶] LHRH ethylamide), (purchased from Argent chemical laboratories Lot # LH2508 R, Redmond, WA 98052). The appropriate dosage was dissolved in 0.9% saline and injected into the dorsal musculature. For each injection, the females were anaesthetized using 2-phenoxyethanol at concentration 0.3 ml/L sea water. The individual was cannulated and then weighed to the nearest gram.

Males

Three males were placed with each treated female after receiving the second (resolving) injection; milting males were identified by anesthetizing as described previous, followed by applying pressure to their abdomen, and scoring the relative amount of milt extruded from the sperm duct. Rating of zero for no sperm, to +3 denoting sperm being extended on the first squeeze was given to each male. Spawning eggs were fertilized naturally, collected and incubated in 1000 L open system fiberglass tanks. Fertilization rates, average spawned egg diameters, hatching rates and larval developmental stages were recorded.

Feeding of larvae

From the 3rd day after hatching the larvae were fed on a mixture of *Chlorella* species and *Brachionus* species; then newly hatched *artemia* were added to tanks at the eight day after hatching.

RESULTS

The mullet was acclimatized for 5 days in the spawning tank accompanied by running ripe males. During 2003 some of the trials were negative with no response but in other trials the fish were spawned with infertile ova due to presence of negative males. In 2004 about 40% spawning success rate (i.e. 4 of 10 attempts) was achieved. Table 1 indicate six unsuccessful spawning attempts occurred, the first five attempts using CPH or HCG as a priming dose followed by LHRH-a in the resolving dose. In the sixth unsuccessful attempt, one extra LHRH-a dose was used for female. The four successful spawning attempts occurred when the females received

Table 2. The dosage, number of injections and egg diameters of four successful spawning utilizations.

| Fish no. | Body weight (g) | Initial egg diameter (im) | CPE (mg/Kg) | Dose HCG. I.U./fish | Egg diameter after 1 st injection (im) | 2 nd injection LHRH-a (µg/Kg) | Egg diameter after 2 nd injection (im) | 3 rd injection LHRH-a (µg/Kg) | Egg diameter after 3 rd injection (im) | Total No. of spawned ovae |
|----------|-----------------|---------------------------|-------------|---------------------|---|--|---|--|---|---------------------------|
| 1 | 1150 | 580 | 70 | - | 610 | 200 | 720±30 | - | - | 2 million |
| 2 | 1300 | 570 | 20 | - | 600 | 200 | 720±30 | - | - | 2 million |
| 3 | 1500 | 590 | - | 10.000 | 620 | 200 | 645 | 100 | 710±10 | 2.170000 |
| 4 | 940 | 570 | - | 10.000 | 610 | 200 | 640 | 100 | 710±10 | 1 million |

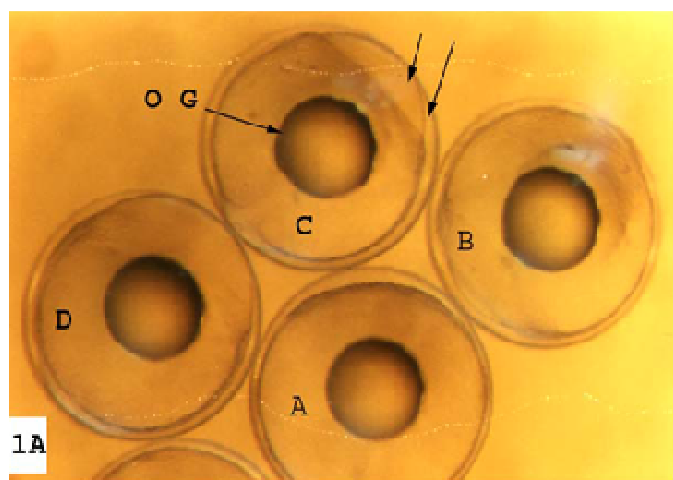


Figure 1a. Photomicrograph of unfertilized egg of *M. Cephalus* (A), fertilized egg at germinal disc stage (B), morula stage after 3.5 h. from fertilization (C), the blastoderm consisted of multiple layered cellular plate (arrows), and blastula after about 4.25 h from fertilization (D). Note, large oil globule (OG) X31.

the two doses, while in the third and fourth attempts the females received an extra dose of LHRH-a as indicated in Table 2.

In the first and second successful attempt, after 24 h from the resolving injection the female spawned the number of spawned egg were about two millions per female. In the third and fourth attempts, after 17 ± 1 h from the third injection the females were spawned, the number of spawned ova was about 2.7 and 1 million for the third and fourth females, respectively. The spawned egg diameter varied between 650 to 720 µm. the percent of fertilization varied between 80 and 75% at water temperature of $25 \pm 1^\circ\text{C}$ and salinity of 34 ± 1 ppt.

In both spawning strategies, a common qualitative change in the oocyte could be observed. Approximately 24 h after the initial injection, a clearing in central portion of the oocytes could be often seen. The percentage of eggs exhibiting this change varied significantly from fish to another. After 24 h from second injection the appearance of oocytes exhibiting central clear type of

morphology was used as an indicator of the final stage of maturation.

Embryonic developmental stages

The ripe unfertilized eggs of *M. cephalus* appeared rounded, colorless and transparent, with a diameter of about 620 ± 30 µm. The egg membrane is smooth, not separated from the yolk and the cytoplasm is reduced to a thin layer covering the yolk; one oil globule was noticed in ripe ova (Figure 1a). After about 20 min from fertilization the egg membrane swells up and separate from perivitelline space; the diameter of the eggs increases to 700 ± 30 µm, then the stages takes place as follows:

Germinal disc stage: After about 30 min from fertilization, the protoplasm was gradually differentiated from yolk to form germinal disc (blastodisc) at the animal pole. Blastdisc was in the form of circular-shaped cap with diameter of 500 µm and height of 112 µm, the perivitelline space was visible only above the cytoplasm (Figure 1a).

Cleavage and morula stage: Serial of cleavage takes place for the blastodisc (two, four, eight and sixty four) and then irregular cleavage. Thereafter, the number of blastomeres become a multicellular cape (morula stage after about 3.5 h from fertilization). The mean total diameter of egg was 715 ± 20 µm, the height of blastodermal cap was about 176 µm and the oil globule was 300 µm. (Figure 1a).

Blastula stage: After about 4.25 h from fertilization, the blastoderm was flattened out over the yolk to be come circular; the height of the cellular cap was 224 µm. The mass of cytoplasm remains in the form of syncytial layer adjacent to the yolk called periblast (Figure 1a).

Gastrula stage: After about 5 h from fertilization, the perimeter of the blastodisc formed a thickened blastoderm and movement of the cells over the whole surface of the yolk in the epiboly process. The embryonic shield enlarg-

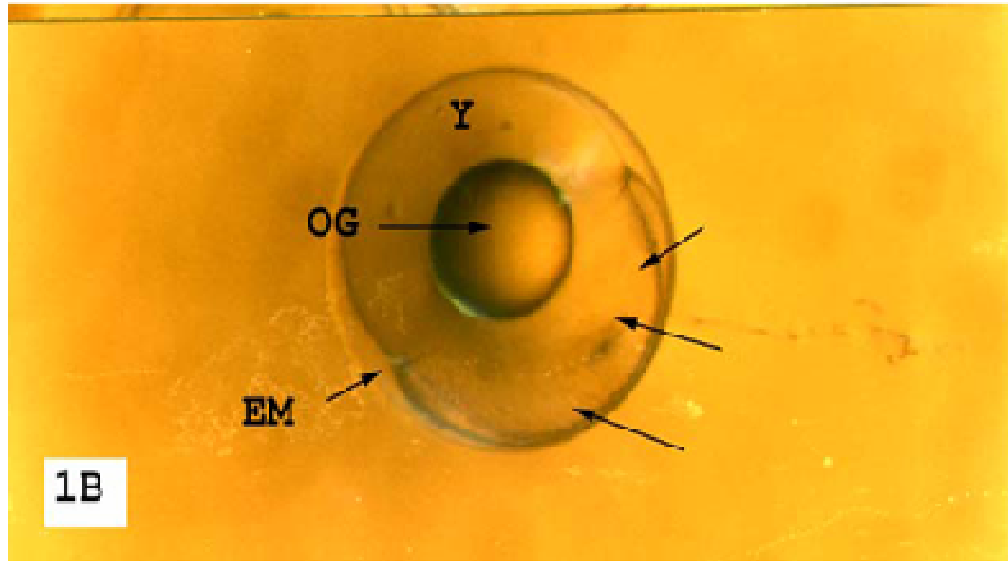


Figure 1b. Gastrula stage, after about 5 h. from fertilization, egg membrane (EM), oil globule (OG) and the blastoderm layer (arrows) which spread over the yolk material (Y), X31.

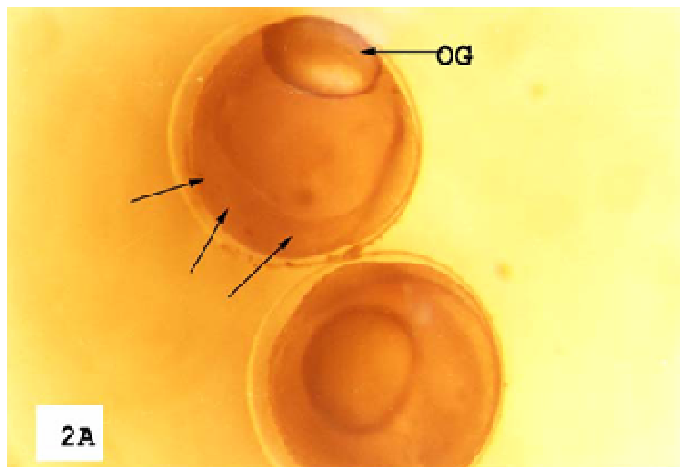


Figure 2a. Embryonic fold stage (EF), 7 hours post fertilization, Note, beginning of organogenesis process as slight folding has not any clear boundaries (arrows), oil globule (OG), X31.

ges by addition of cells. The egg diameter was about $820 \pm 19 \mu\text{m}$ (Figure 1b). The gastrula stage was characterized by cell movement and recognition within the embryo.

Organogenesis stage: After about 7 h of post fertilization, the embryonic fold was observed as a thickening along the dorso-lateral margins of the yolk, consisted of aggregation of cells but did not form any clear cut division or boundaries (Figure 2a). At the age of about 13.5 ± 1 h, the embryo began to be noticed. The head region started to be shaped and optic cup and tail region were formed. Dark pigments cover almost all of



Figure 2b. Embryo at age 13.5 hours, head region (HR) started to be shaped, dark pigments cover almost all of the embryo (arrows) and on the oil globule (OG), X31.

the embryo, yolk sac and the oil globule. The melanophores appeared as stars in shape. The egg diameter was $870 \pm 30 \mu\text{m}$ and oil globule was $350 \mu\text{m}$ in diameter (Figures 2b, c). The jerky striated muscle movement of the tail somites increase by time and the tail start to bend to help and accelerate the hatching process, leading to the loosening of the egg membrane.

Hatching stage: After about 26 ± 4 h post fertilization, hatching process started; the hatching rate was about 90%. As a result of tail movement the egg membrane ruptured as indicated in Figure 3a. The total length of the newly hatched embryo was about 1.97 ± 0.23 mm and the yolk has an oval shape ($0.7 \text{ mm} \times 0.34 \text{ mm}$) Oil globule in the central part of the yolk sac with a diameter of 0.3 mm and head region bent on the yolk sac with hei-



Figure 2c. Embryo at age 13.5 hours, head region (HR) started to be shaped, dark pigments cover almost all of the embryo (arrows) and on the oil globule (OG), X31.

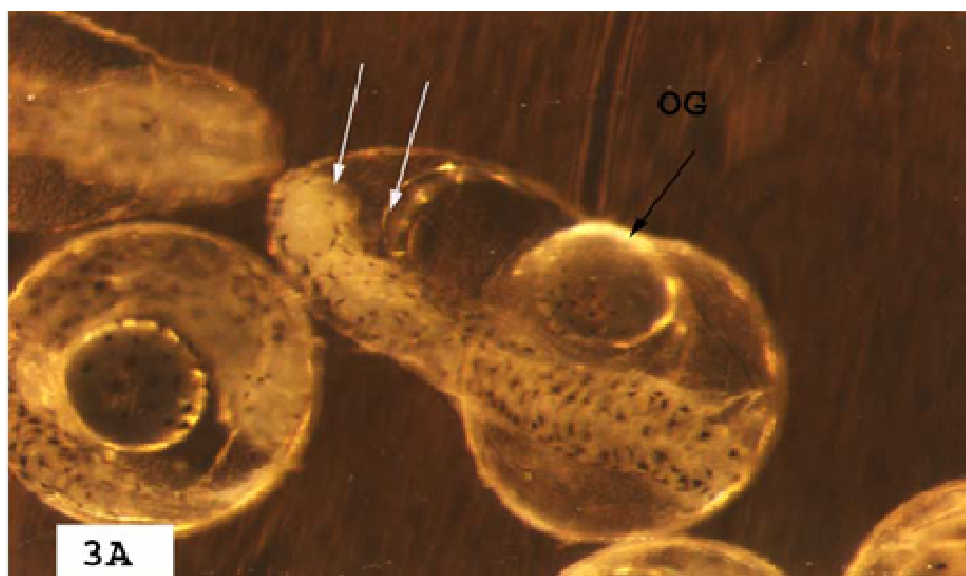


Figure 3a. Hatching of *M. cephalus* embryo (arrows) at age about 26±4 hour's post-fertilization, dark field X31.

ght of 0.39 mm and head length of 0.2 mm was noticed. Tail region bent on the dorsal direction was observed.

Larval developmental stages

After about 12 h from hatching, the yolk sac was still large; the dorsal, ventral and caudal fin were connected together to form the embryonic fin fold. The intestine

appeared as thin tube extended upper to the end of yolk sac (Figure 3b). The blood circulation was clear as a strong flow occurred in the heart.

After about 24 h from hatching a sharp decrease was observed in the yolk sac diameter (0.26 mm). The body axis was straightened and the total length increased to 2.42 ± 0.24 mm. Eye lens was formed and retina were darkly pigmented; eye diameter was about 0.15 mm (Figure 3c).



Figure 3b. Larva after about 12 hours from hatching, the yolk sac (YS) was still large, the dorsal, ventral and caudal fins were connected together to form the embryonic fin fold (EFF). The intestine (I) appeared as thin tube extended upper to the end of yolk sac, X31.



Figure 3c. *M. cephalus* larva after about 24 hours from hatching, a sharp decrease was observed in the yolk sac (YS), eye lens (EL) was formed and eye retinas (E) were darkly pigmented, X25.

After about 36 h from hatching, head region was elevated and increased in width (0.37 mm) with more rounded configuration, eye became more darker with increasing pigmentation; also the mouth parts began to be formed and the mouth was slightly opened. The intestine extended downwards through the ventral fin fold with increasing in both thickness and diameter, while oil globule still appeared at the abdominal region (Figure 4a).

Internal external feeding stage: After about 48 ± 12 h of post hatching the mouth parts were completely formed, upper and lower jaws could be easily noticed. The length

of the lower jaw slightly exceeds that of the upper jaw. Separation of the dorsal and anal fin folds from the caudal fin clearly appeared with mesenchyme deposition. The notochord was clearly seen extending through the whole body. The swimming activity of the larvae increased as the enlargement of the swimming bladder proceeds; several individual swam inefficiently around the bottom of the aquarium or in water column, but others were still clustered at the bottom. At this stage the larvae can eat the external food material reaching it in addition to internal food yolk material. The elimination of dark, mariculated faces and presence of large particles in the intestinal tract were indicated that mixed feeding had begun as shown in Figure 4b.

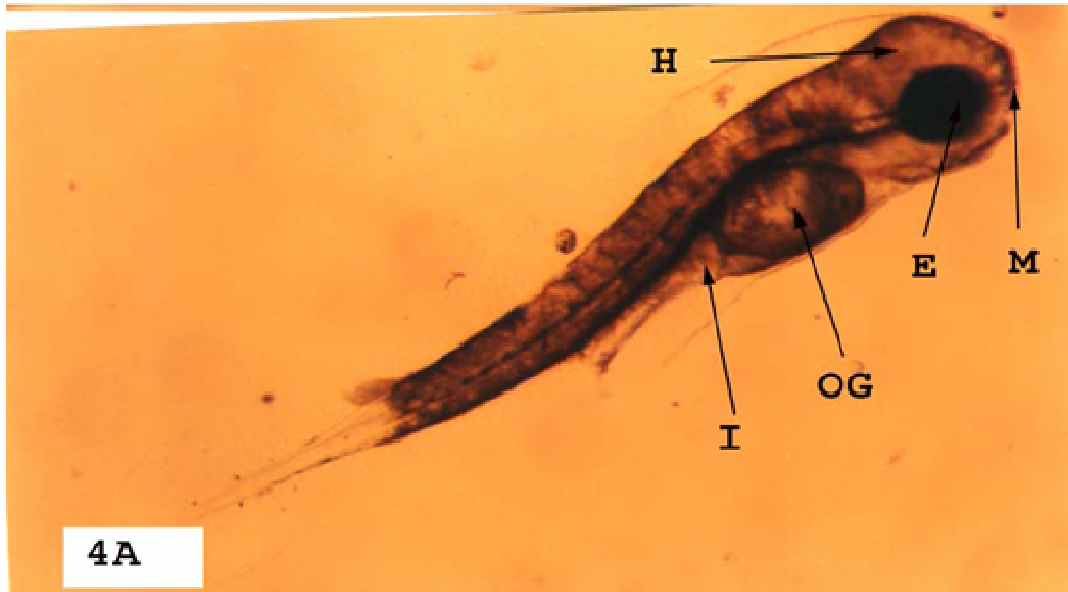


Figure 4a. Larva at 36 hours after hatching, head (H) was elevated, eye (E) became more darker and the mouth (M) was slightly opened, the intestine (I) extended downwards through the ventral fin fold, oil globule (OG) was still appear, X25.

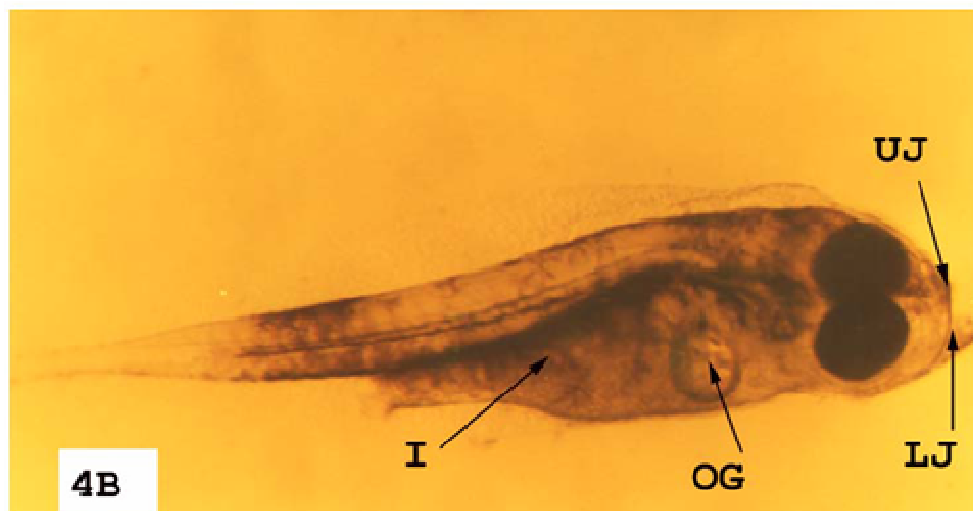


Figure 4b. Larva at about 48 hours after hatching, the mouth parts were completely formed, upper jaw (UJ) and the lower jaw (LJ) could be easily seen, and the intestine (I) was function, while the oil globule (OG) was still present, X25.

External feeding stage: At fourth day after hatching, the mouth was completely opened, become and functional (Figure 5a), and the maxillaries were distinct. The foregut and hindgut were visible, the anus was opened and the eye was completely pigmented. The body and the notochord were straight; the yolk sac was decreased in diameter to 0.1 mm. Greatest body depth ranged from 0.37 to 0.38 mm, total length ranged from 2.55 to 2.75 mm, and preanal length ranged from 1.27 to 1.3 mm. Percentage of the head length increased from 13.26 to 17.05% of the total body length. At the end of sixth day after hatching the total length of larvae ranged between

2.68 and 2.83 mm, preanal length was about 1.35 ± 0.3 mm, head length varied between 0.53 to 0.58 mm and the height of the head was 0.37 mm (Figure 5a). After eight days from hatching larvae averaged body length 2.8 ± 0.4 mm, with pigmented and functional swimming bladder present. The oil globule decreased in size to reach 0.07 mm (Figure 5b). At eleventh day after hatching the larvae average total body length were 3.1 ± 0.4 mm; the body depth was 0.5 mm, the head length was 0.59 mm, the eye diameter was 0.2 mm and the oil droplet was completely absorbed (Figure 5c).

At the fifteenth day after hatching larvae average total

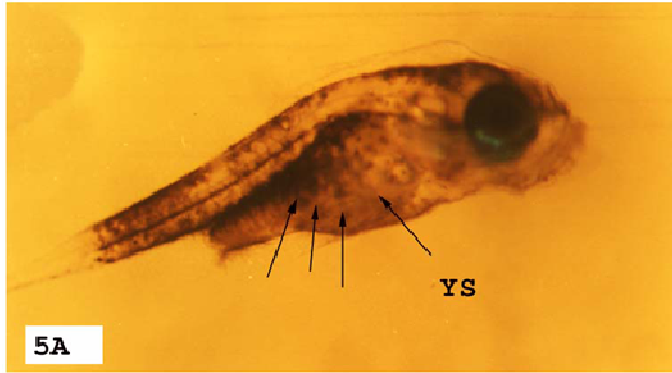


Figure 5a. Larva at fourth day after hatching (4DAH), yolk sac (YS) diameter decrease, the body pigmentation increased especially at the abdomen region (arrows), X25.

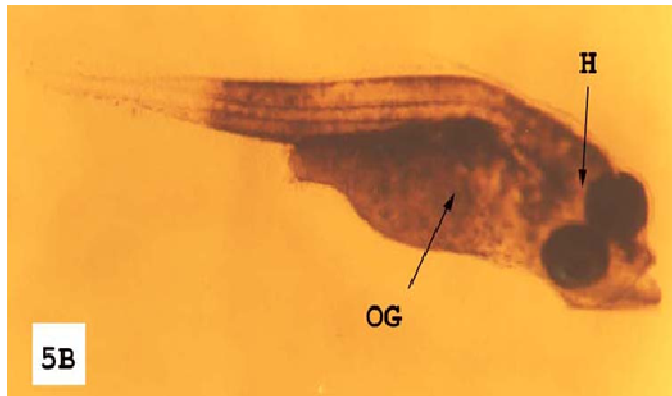


Figure 5b. Larva at the end of 8DAH, the total length and the head length (H) increased, Note rudiment of oil globule (OG), X25.

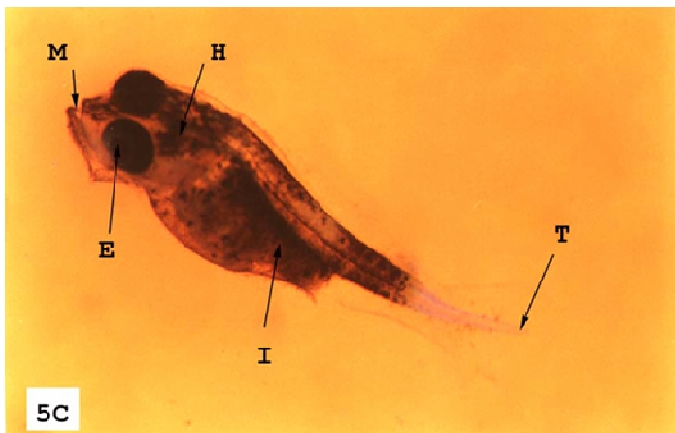


Figure 5c. Larva at 11 DAH, the oil droplet was completely absorbed, Note well developed head (H), eye (E), mouth (M), tail (T) and intestine (I) X 30.

body length were 3.39 ± 0.41 mm, body depth 0.73 mm, head length was 0.75 mm, head width 0.69 mm and the eye diameter increased to 0.25 mm. Slow transformation

of *M. cephalus* post larval stage is shown in Figure 6 to resembled the young fish.

DISCUSSION

In this work, preliminary results on the acceleration of gonadal recrudescence and induction of spawning in a local population of the grey mullet, *M. cephalus*, were presented. In the wild, grey mullets are reproductively active at different periods of the year, depending on their geographical location (reviewed by Tamaru et al., 1989). In captivity, grey mullets do not spawn spontaneously. However, successful spawning induction was reported in Hawaii and Taiwan where, by manipulation of temperature, photoperiod and hormone administration, ovarian maturation could be induced in and out the season (Kuo et al., 1974). The presence of females with oocytes of ≤ 500 μm in diameter and spermated males indicated spawning season, which corresponds to the months from August to October. In agreement with our observations, Monbrison et al. (2003) indicated that once oocytes reached the diameter of 500 μm females were induced to spawn with hormonal treatment. Similar results were recorded in Hawaii by Shehadeh et al. (1973) and Taiwan Kuo et al. (1974) who concluded that grey mullet males were able to complete spermatogenesis without hormonal treatment.

Spawning induction in the present study was carried out in two steps as commonly used in various fish species (Lee et al., 1987 reviewed by Marte 1989). The first treatment consisted of a priming dose of carp pituitary extract from 20 to 70 mg, or 10000 IU of HCG per fish, while the second given dose 24 h later, was the resolving dose of 200 $\mu\text{g}/\text{Kg}$ LHRH-a. After the resolving dose, the females were maintained together with males and spawning occurred after 17 to 24 h on average. In two attempts, two injection of LHRH-a (for a total three injections) were required to induce spawning. In the pacific population of the grey mullet, spawning occurred after a shorter latency of 38 h (Lee et al., 1988; Marte, 1989). These differences could be attributed to the type and dose of hormonal treatment, environmental conditions or differences in physical response of the local and the pacific mullet populations.

In the present investigation running ripe males were used, as 3:1 male to female sex ratio in all our spawning trials to optimize fertilization rates according to the sex ratio recommended by Lee et al. (1988). However; Monbrison et al. (2003) used two spermating male with only one treated female. The highest fecundity of *M. cephalus* in the wild was reported in Cuban waters (14 million eggs), however, most reports present fecundities of 0.25-2.8 million eggs/fish (reviewed by Greeley et al., 1987). In Hawaii and Taiwan captive conditions, the fecundity was 1-2 million eggs/fish with an average of 900 eggs/g bw. The fecundity in the present work was va-

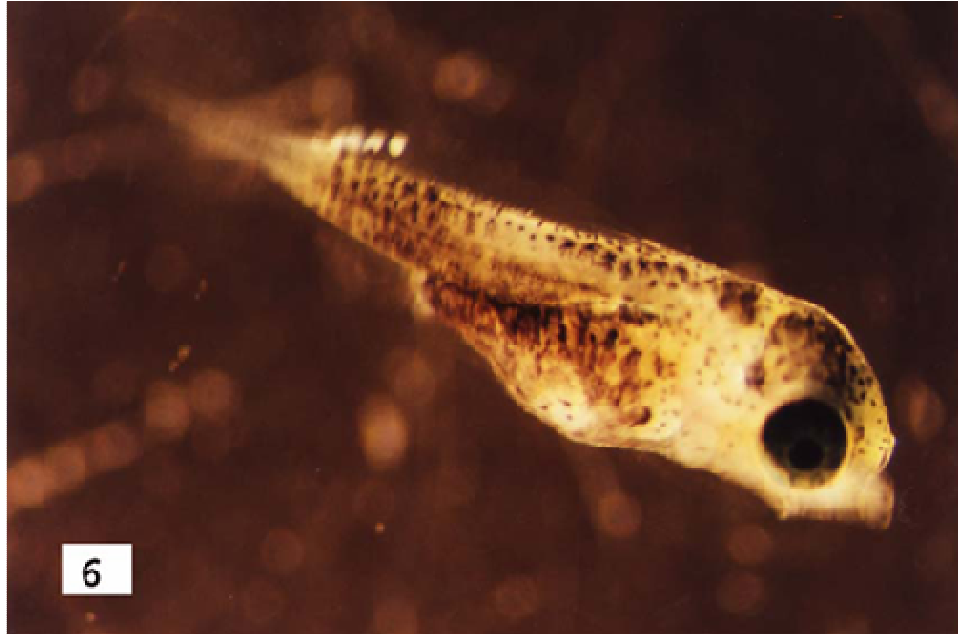


Figure 6. *M. cephalus* larva at 15 DAH, slow transformation of post larval stage to be resembled the young fish, X20.

ried between 1-2.70 million eggs/spawn fish which represents 1395.3 ± 190 eggs/g bw.

The grey mullet has a synchronous ovarian maturation and usually spawns only once a year. Similar results were reported by (Greeley et al., 1987) Brusle (1981) raises the possibility of more than one spawn a season of Mediterranean grey mullet under warm water conditions. Monbrison et al. (2003) indicated that 35% of the treated females which spawned underwent a second round of gonadal recrudescence and spawning after about a month.

The ripe unfertile eggs of *M. cephalus* were rounded, colorless, transparent with one oil globule. The morphology of egg before fertilization was described for *M. cephalus* by Kuo et al. (1973) and Tung (1973) in which the surface of the fertilized egg shell is smooth and the yolk unsegmented. After 20 min from fertilization the egg membrane swells up and separated from the previtelline space. Present data from *M. cephalus* eggs indicate more than one oil globule. On hatching time the larvae had one oil globule (rarely two) located in the yolk sac. Kuo et al. (1973) stated that the frequency of multiple oil droplets in eggs of *M. cephalus* increased with the manual pressure of artificial stripping. Nash et al. (1974) considered that spontaneous release of the eggs by the females produced eggs with a single oil globule to be normal and desirable. The survival of eggs which initially contained multiple oil droplets was always low.

In present study, 13 ± 1 h after post fertilization the embryo began to be noticed; the egg diameter was 870 ± 30 μ m and oil globule 350 μ m. Nash and shehadeh (1980) concluded that the comprehensive data revealed

a wide range of diameters reported for the same species in different location. Kuo et al. (1973) and Tucker (1998) reported the main egg diameter of fertilized egg of *M. cephalus* as 930 μ m, with a range of 880 - 980 μ m. The single large oil globule had a uniform diameter of 330 μ m. Tung (1973) quoted a mean egg diameter of 0.89 mm for the same species, and globule diameter of 0.39 mm. Nash et al. (1974) specified a mean egg diameter of 0.93 mm. In present work after 5 h from fertilization the diameter of egg increased from 620 ± 30 μ m to 820 ± 19 μ m. This increase in the diameter of ovulated egg was due to the rapid intake of external water during hydration as concluded by Nash and shehadeh (1980). In the four hours post ovulatory period, a remarkable increase in the osmolarity adjusted the osmotic pressure from hypotonic to isotonic to sea water at the time of spawning. During this process, there is a net increase in egg electrolytes (Na, K, Ca, Mg, and Cl) although concentration decreases due to increase in egg water content.

After about 26 ± 4 h post fertilization, hatching was started at $25 \pm 1^\circ\text{C}$ and water salinity of 34 ppt. The fertilization percentage was varied between 75 to 80% and hatching rate was about 90%. Similar observations by Yashouv and Berner-Samsonov (1970) showed that under laboratory conditions, the eggs of *M. cephalus* and *M. capito* developed and hatched within 36-44 h at 22-32°C. Kuo et al. (1973) and Tucker (1998) stated that hatching of *M. cephalus* egg was evident 34 - 38 h after fertilization at 24°C and salinity of 32 ppt.

Liao (1974) stated that hatching of *M. cephalus* eggs took place in 34 - 38 h at 23 - 24.5°C and at 49 - 54 h in 22.5 - 23.7°C with salinity between 30.1 and 33.8 ppt. For

the other marine species hatching was recorded 35 h after fertilization for *Dentex gibbosus* at water temperature 20°C (Fernandez Palacios et al., 1994), 104 h for *Labrus merula* at water temperature 14.3°C (Dulcic et al., 1999) and 115 h for *Trigla lucerna* at water temperature 13.5°C (Dulcic et al., 2001).

The newly hatched *M.cephalus* larvae was about 1.97 ± 0.23 mm in total length which in agreement with Kuo et al. (1973) who stated that the total length of the newly hatched *M. cephalus* larvae was 2.65 ± 0.23 mm. In the present study, larvae began feeding 3–5 days after hatching. Such result are in agreement with study of Tung (1973) and Liao (1997) whom concluded that in *M. cephalus* mouth opening and the well development of its upper and lower jaws allow the larvae to take food at three to four days. The time of eye completion is nearly that of mouth opening and body axis becomes straight, which helps the larvae to identify their food as internal-external feeding starts. Kvenseth et al. (1996) stated that the development of functional eye at the time when the larvae halibut have been observed to capture prey and when the digestive system appeared histologically functional.

In the present study, the newly hatched larvae were inactive and usually remained upside down suspended in water column. Thereafter, larval activity increased after the second day as also mentioned by Liao (1997) who described the larvae of *M. cephalus* as having weak swimming activity with the posture of belly up and head down, sometimes moving with jerky motion up and down. The larva gained sustained swimming powers between the tenth and twelfth day after hatching. Nash and shehadeh (1980) reported that the mixed regime for *M.cephalus* larvae is economic in decreasing the numbers of individual organisms which have to be produced on a daily basis. Same results were observed in our study which recommended that the wide choice of organisms probably provides a better qualitative as well as quantitative diet for the larvae.

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