

INDUCED SPAWNING OF THE MULLET *Mugil platanus* GÜNTHER, 1880, IN CANANÉIA, SÃO PAULO, BRAZIL\*

[Reprodução induzida da tainha *Mugil platanus* Günther, 1880, em Cananéia, São Paulo, Brasil]

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ABSTRACT

Studies concerning the induced spawning of the mullet *Mugil platanus* were undertaken in June-September, 1989 and July-October, 1990 at the "Laboratório de Maricultura" in Cananéia, SP, Brazil. Females were chosen observing initial oocyte diameter, graphs of oocyte diameter frequency distribution, location of germinal vesicle, and number of oil droplets present in the oocyte. A total of 49 females were injected; 34 females received two doses of HCG: 20 and 40 IU per gram of total weight applied at 24-h intervals; 8 females received 10 mg/kg of mullet pituitary extract (MPE), and 30 IU/g of HCG; and 7 females received 20 mg/kg of MPE and 30 IU/g of HCG. The initial mean oocyte diameter of the selected females was between 532 µm and 621 µm and the percent frequency distribution of oocyte diameter was unimodal with the mode in 586 µm. High rates of fertilization and hatching were obtained with the either one of the above three treatments.

KEY WORDS: mullet, *Mugil platanus*, induced spawning, oocyte diameters, HCG, mullet pituitary extract

RESUMO

Estudos relativos à reprodução induzida de tainhas *Mugil platanus* foram realizados no período de junho-setembro de 1989 e julho-outubro de 1990, no "Laboratório de Maricultura" do Instituto de Pesca em Cananéia, SP, Brasil. Fêmeas foram escolhidas observando-se o diâmetro médio inicial dos ovócitos, o gráfico de distribuição de frequência, a localização da vesícula germinativa e o número de gotas de óleo presentes no ovócito. No total, 49 fêmeas foram injetadas com hormônio, sendo que 34 receberam 2 doses de HCG (Human Chorionic Gonadotropin) na concentração de 20 e 40 IU por grama de peso total, aplicadas em intervalos de 24 h; 8 receberam 10 mg/kg de extrato de hipófise de tainha (MPE) e 30 IU/g de HCG e 7 receberam 20 mg/kg de MPE e 30 IU/g de HCG. O diâmetro médio inicial dos ovócitos das fêmeas selecionadas para indução variou entre 532 e 621 µm, e a distribuição percentual da frequência dos diâmetros dos ovócitos foi unimodal, com maior frequência em 586 µm. Altas taxas de fertilização e de eclosão de larvas foram obtidas com os três tratamentos propostos.

PALAVRAS-CHAVE: tainha, *Mugil platanus*, reprodução induzida, diâmetro de ovócitos, HCG, extrato de hipófise de tainha

1. INTRODUCTION

The grey mullets do not breed in captivity. In order to be independent of nature, and to ensure a regular supply of good quality mullet seed at the desired time, place, and quanti-

ty, works on induced spawning of grey mullets were started in Brazil by BENETTI & FAGUNDES NETTO (1980), ANDREATTA et alii (1982), and GODINHO et alii (1984a). Due to

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the lack of governmental support, the works were interrupted, but just more recently, with the collaboration of CIRM (Interministerial Commission for Marine Resources), it was possible to return to the experiments.

In Brazil, there are seven marine species belonging to the Mugilidae family, and among them *Mugil liza* and *Mugil platanus* are the best for cultivation (GODINHO; SERRALHEIRO; SCORVO FILHO, 1988).

Spawning of grey mullets has been induced by several hormones like salmon and carp pituitary homogenates preserved in ethyl

alcohol (ethanol), purified salmon gonadotropin, HCG (human chorionic gonadotropin), and LHRH-a (SHEHADEH & ELLIS, 1970; SHEHADEH; KUO; MILISEN, 1973b; KUO; SHEHADEH; NASH, 1973; SEBASTIAN & NAIR, 1975; LIAO, 1975; LEE; TAMARU; KELLEY, 1988; ALVAREZ-LAJONCHERE et alii, 1988).

This report presents the results of utilizing HCG alone and in combination with high doses of acetone-dried *Mugil platanus* pituitary extract (MPE) in an attempt to decrease the costs related to the hormonal therapy.

## 2. MATERIAL AND METHODS

In June-September, 1989 and July-October, 1990 that represent peak periods of the natural spawning season of mullet in Brazil, females and males of mullet *Mugil platanus*, captured from estuarine region of Cananéia, São Paulo, Brazil, Lat. 25° 01' 00" S, were analysed.

A total of 183 females were examined in relation to the development stages of the intra-ovarian oocytes sampled with a plastic catheter. Approximately 200 oocytes were removed; some of them were preserved in Serra's solution (LEMANOVA & SAKUN, 1975) with the aim of verifying the location of the germinal vesicle (BRUZSKA, 1979), and the remainder were preserved in Gilson's solution (SIMPSON, 1951), for 30 min., to measure the diameter with stereomicroscope ( Wild M5, objective lens 25 x, eye-piece 10 x ). These measures were used to calculate the mean oocyte diameter and to construct the graphs of oocyte frequency distribution.

For these experiments, females with initial mean oocyte diameter of approximately 600 µm were selected according to SHEHADEH; KUO; MILISEN (1973a). They were weighed and placed individually in a 1000-L

tank with running sea water (30-34‰) and continuously aerated; the water temperature was maintained between 19 and 21°C.

The induced spawning treatments for females were: two intramuscular injections of either 20 and 40 IU/g of HCG (human chorionic gonadotropin), or 10 mg/kg of MPE (mullet pituitary extract) and 30 IU/g of HCG, or 20 mg/kg of MPE and 30 IU/g of HCG at 24-h intervals.

The pituitary glands were collected from mature females and then preserved in acetone with three changes before final storage at 5 °C.

After resolving injection each female was placed with two or three males.

Males did not receive hormonal injections; they were selected according to the amount and viscosity of milt released after gentle pressure on the abdomen.

Females identified as controls received two injections of 0,9% saline solution.

At the time of ovulation, eggs were again sampled in order to calculate the mean egg diameter and to observe the shift of the mode, the location of the germinal vesicle, and the number of oil droplets present.

Fertilization was performed using the "dry" method (von IHERING & AZEVEDO, 1934). For estimating the rate of fertilization, 2-L samples of eggs were removed from a 60-L container, and the value was calculated as follows: rate of fertilization = (number of developing eggs/ total number of eggs) x 100 as described by GODINHO et alii (1984b) and

ROMAGOSA; PAIVA; GODINHO (1990).

The rate of hatching was calculated, and the larvae were transferred to other tanks.

The larval rearing methods were used according to YAMANAKA et alii (1990).

Thrice a day, temperature and salinity data of sea water were recorded.

### 3. RESULTS

Data of length, weight, mean egg diameter, rate of fertilization, and time of spawning are shown in TABLES 1, 2 and 3 for the three treatments. It was verified that the time of the first hormone injection did not interfere in the results.

A total of 54 females were utilized in this experiment, being five females in control group. None of that showed any progress in oocyte maturation (FIGURE 1-A).

From the 34 females treated with HCG alone, 11 died during the treatment, 20 responded positively, and 3 did not respond. The treatment with HCG presented the best results when the females showed the initial percent frequency distribution of oocyte diameter with modes in 586 and 625  $\mu\text{m}$  (FIGURE 1-B). Twelve females showed this distribution; they spawned around 54-h after the first injection, with mean egg diameter reaching values of  $831 \pm 31 \mu\text{m}$ , and showing high rate of fertilization varying between 60 and 90% (TABLE 1). The treatment MPE combined with HCG was effective for the females with initial mean oocyte diameters over 530  $\mu\text{m}$  and with a high frequency of oocyte diameters in 586  $\mu\text{m}$  (FIGURE 1-C). Among the 8 injected females with 10 mg / kg of MPE and 30 IU/g of HCG, 3 died during the treatment, one did not ovulate, and 4 responded positively (TABLE 2). Three of these females showed at spawning mean oocyte di-

ameters over 800  $\mu\text{m}$  and high rate of fertilization (65-99%). With the doses of 20 mg/kg of MPE and 30 IU/g of HCG, among the 7 injected females, 2 died, one did not respond, and 4 responded positively. These presented in the ovulation small eggs with mean diameter around  $773 \pm 23 \mu\text{m}$ , and the rate of fertilization was lower than that of the others (4-60%). They spawned 49-h after the first injection, but exceptionally only one spawned 25-h after (TABLE 3).

At the first sampling, all the females examined presented oocytes with central nucleous and with many visible oil droplets. In the females that showed high rate of fertilization, at the second sampling, 24-h after the first hormonal injection, it was verified a high percentage of oocytes with eccentric germinal vesicles, and some of them with coalescent oil droplets. At the third sampling, mostly of these oocytes had breakdown of germinal vesicle and the complete aggregation of oil droplets. The oocytes of females that showed low rates of fertilization presented, at the second sampling, central nucleous and various oil droplets. At the third sampling, a high percentage of oocytes showed shift of the nucleous towards the periphery, and in some of them followed by breakdown. In those females that did not reproduce, no change was observed in the location of germinal vesicle and in the number of oil droplets.

Approximately 30-h after the first injection, females eliminated a white substance with pH 9-10, and 20-h after this time they initiated the release of eggs. Eggs extrusion and male spermiation were performed. Ferti-

lization was observed followed by the embryonic development.

The sea water salinity was 32‰ ± 2 and the temperature, 20 °C ± 1 during the experiments.

TABLE 1  
Data of females injected with 20-40 IU/g body weight of HCG

| Total length (cm) | Body weight (g) | Mean initial egg diameter (µm) | First hormonal injection |       | Time of spawning (h) | Mean spawned egg diameter (µm) | Fertilization rate (%) |
|-------------------|-----------------|--------------------------------|--------------------------|-------|----------------------|--------------------------------|------------------------|
|                   |                 |                                | Date                     | Time  |                      |                                |                        |
| 49.6              | 1100            | 573                            | 30/06/89                 | 12:00 | 50                   | 753                            | 10                     |
| 49.4              | 1100            | 572                            | 30/06/89                 | 22:00 | 61                   | 555                            | -                      |
| 47.6              | 1150            | 601                            | 28/07/89                 | 11:00 | 54                   | 844                            | 80                     |
| 55.3              | 1950            | 615                            | 29/07/89                 | 11:00 | 58                   | 862                            | 95                     |
| 45.2              | 950             | 605                            | 03/08/89                 | 12:00 | 59                   | 827                            | 90                     |
| 45.0              | 1420            | 601                            | 08/08/89                 | 12:00 | 48                   | 882                            | 80                     |
| 51.0              | 1300            | 621                            | 16/08/89                 | 17:00 | 68                   | 834                            | 80                     |
| 52.0              | 1310            | 596                            | 28/08/89                 | 16:00 | 56                   | 808                            | 20                     |
| 52.2              | 1480            | 616                            | 29/08/89                 | 12:00 | 53                   | 837                            | 99                     |
| 51.0              | 1440            | 607                            | 30/08/89                 | 12:00 | 52                   | 813                            | 30                     |
| 51.5              | 1460            | 577                            | 30/08/90                 | 11:00 | 54                   | 810                            | 2                      |
| 41.5              | 790             | 588                            | 30/08/90                 | 18:00 | 63                   | 584                            | -                      |
| 46.6              | 1120            | 611                            | 20/09/90                 | 11:00 | 46                   | 735                            | -                      |
| 59.2              | 2080            | 563                            | 25/09/90                 | 13:00 | 55                   | 807                            | 60                     |
| 58.0              | 1980            | 585                            | 26/09/90                 | 19:00 | 53                   | 799                            | 60                     |
| 43.5              | 960             | 591                            | 26/09/90                 | 19:00 | 71                   | 736                            | 1                      |
| 43.0              | 870             | 584                            | 10/10/90                 | 14:00 | 54                   | 878                            | 97                     |
| 40.3              | 1120            | 557                            | 13/10/90                 | 14:00 | 54                   | 812                            | 82                     |
| 51.6              | 1380            | 564                            | 13/10/90                 | 11:00 | 43                   | 798                            | 10                     |
| 45.5              | 900             | 581                            | 22/10/90                 | 15:00 | 55                   | 822                            | 2                      |
| 56.4              | 1850            | 579                            | 22/10/90                 | 15:00 | 52                   | 786                            | 83                     |
| 38.5              | 915             | 590                            | 23/10/90                 | 14:00 | 52                   | 805                            | 95                     |
| 44.4              | 995             | 559                            | 23/10/90                 | 14:00 | 53                   | 796                            | 2                      |

TABLE 2  
Data of females injected with 10 mg/kg body weight of mullet crude pituitary extract as a priming dose followed by 30 IU/g HCG

| Total length (cm) | Body weight (g) | Mean initial egg diameter (µm) | First hormonal injection |       | Time of spawning (h) | Mean spawned egg diameter (µm) | Fertilization rate (%) |
|-------------------|-----------------|--------------------------------|--------------------------|-------|----------------------|--------------------------------|------------------------|
|                   |                 |                                | Date                     | Time  |                      |                                |                        |
| 42.5              | 1140            | 586                            | 02/08/89                 | 12:00 | -                    | 652                            | -                      |
| 53.5              | 1650            | 583                            | 20/09/89                 | 16:00 | 57                   | 871                            | 99                     |
| 51.6              | 1290            | 567                            | 23/07/90                 | 17:00 | 65                   | 753                            | 5                      |
| 55.1              | 1650            | 555                            | 26/09/90                 | 20:00 | 51                   | 805                            | 80                     |
| 51.3              | 2350            | 581                            | 10/10/90                 | 14:00 | 42                   | 844                            | 65                     |

TABLE 3  
Data of females injected with 20 mg/kg body weight of mullet crude pituitary extract as a priming dose followed by 30 IU/g of HCG

| Total length (cm) | Body weight (g) | Mean initial egg diameter (µm) | First hormonal injection |       | Time of spawning (h) | Mean spawned egg diameter (µm) | Fertilization rate (%) |
|-------------------|-----------------|--------------------------------|--------------------------|-------|----------------------|--------------------------------|------------------------|
|                   |                 |                                | Date                     | Time  |                      |                                |                        |
| 49.0              | 1180            | 583                            | 29/08/90                 | 18:00 | 47                   | 789                            | 20                     |
| 58.6              | 1170            | 578                            | 18/09/90                 | 12:00 | 25                   | 774                            | 50                     |
| 47.2              | 1130            | 571                            | 18/09/90                 | 15:00 | 55                   | 780                            | -                      |
| 51.5              | 1150            | 532                            | 25/09/90                 | 14:00 | 48                   | 783                            | 60                     |
| 49.0              | 1150            | 556                            | 26/09/90                 | 12:00 | 52                   | 741                            | 4                      |

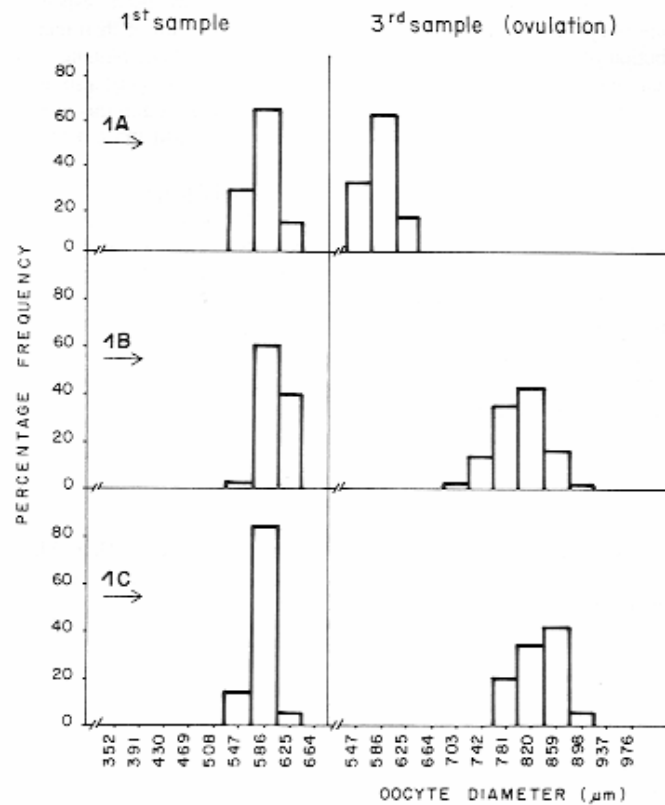


FIGURE 1- Pattern of oocyte diameter frequency distribution in females of *Mugil platanus*. 1A - control; 1B - injected with HCG; 1C - injected with MPE combined with HCG

#### 4. DISCUSSION

From the results obtained for *Mugil platanus*, we suggest that selection of recipient females can be based on the pattern of frequency distribution of the initial oocyte diameter, the mean oocyte diameter, the number of oil droplets, and on the observation of the shift of germinal vesicle.

The results are in accordance with GODINHO et alii (1984a), regarding the oocyte diameter distribution, the mean egg diameter, and response to treatment.

We observed that in species with synchronous groups oocyte development, the frequency distribution of oocyte diameter is the most appropriate measure for routine assessment of gonad maturation degree and the most important factor to be considered in standardizing the dosage of spawn inducing agents. Similar results were also obtained by FENERICH-VERANI; GODINHO; NARAHARA (1984), GODINHO et alii (1984a and b), GREELEY; CALDER; WALLACE (1987), ROMAGOSA; PAIVA; GODINHO (1990), and WEST (1990).

One of the problems associated to the use of HCG and other hormones is determining the effective dosages for each species and for the respective stage of gonadal devel-

opment. We agree with SHEHADEH; KUO; MILISEN (1973b), LAM (1982), DONALDSON & HUNTER (1983), LEE et alii (1986), and LEE et alii (1987), in that the dosages of HCG required to bring about the spawning in mullets are higher than those reported for other species. In our researches with "pacu", *Colossooma mitrei* (GODINHO et alii, 1977), *Piaractus mesopotamicus* (ROMAGOSA; PAIVA; GODINHO, 1990), and "curimbatá", *Prochilodus scrofa* (FENERICH-VERANI; GODINHO; NARAHARA, 1984) we used respectively dosages 50% and 75% lower than that used for "tainha", mullet *Mugil platanus*. ALVAREZ-LAJONCHERE et alii (1988) also used higher dosages than that used in this work and that one reported by BENETTI & FAGUNDES NETTO (1980).

With the purpose of decreasing the costs related to hormonal therapy, according to LEE; TAMARU; KELLEY (1988), we replaced the priming dose of HCG by MPE. The fish was induced to spawn using 10 mg/kg and 20 mg/kg MPE associated with 30 IU/g HCG. The best results were obtained with MPE (10 mg/kg) and HCG and using HCG alone in two applications.

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