Egyptian Journal for Aquaculture
P-ISSN: 2090-7877
E-ISSN: 2636-3984
www.eja.journals.ekb.eg/
Naiel *et al.,* 2023; 13 (2):21-39
DOI: 10.21608/eja.2024.319020.1095

Induced spawning of thin-lipped grey mullet (*Liza ramada*) under captive conditions using synthetic and natural hormonal resources.

Mohammed A.E. Naiel [1], Eman M. Zaki [2] and Usama M. Abdel-Monem [1]

[1]Department of Animal Production, Faculty of Agriculture, Zagazig University, Zagazig 44519, Egypt.

[2]Reproduction and Fish Physiology Department, Central Laboratory for Aquaculture Research (CLAR), Abbassa 44662, Agriculture Research Center, Giza, Egypt.

Received: May 02, 2023; Accepted: June. 10, 2023 published: 2023 Vol.13 (2):21-39

Abstract

The thin-lipped grey mullet (*Liza ramada*) belongs to the Mugilidae family. *Liza ramada* mature females do not spawn without hormonal injection treatments in captivity. This study describes the application of human chronic gonadotrophic hormone (hcg), carp pituitary glands (cpg), Melatonin (MEL) and Mullet Brain extract (MBE) as an effective single-dose inducer of spawning in this species. A total of 40 wild mature female brooders (n = 10; 33.5 ± 3 cm total length, TL and 511 ± 20 g body weight, BW). The mature females were subjected into equal four groups as follow; the first group served as control (no hormonal treatment), while, the second and the third group received hormonal treatment of 4500 IU hcg in combined with 3 mg cpg per (hcg+cpg), and 3 mg melatonin (hcg+MEL). The last fish group received single hormonal treatment of 3 mg mullet brain (MB). The experiment results indicated significant increments ($P > 0.01$ or 0.001) on plasma estradiol (E2) and progesterone levels of adult female *L. ramada* fish received single injection dose from hcg in combined with cpg and MEL. Finally, the trial results suggest that mature *L. ramada* has recognized reproductive traits, adapts well to captive conditions, and can be easily induced to spawn by administering a single dose of human chronic gonadotrophic hormone (hcg) in

combination with carp pituitary glands (cpg) and melatonin.

Keywords.

The Mugilidae family, (*Liza ramada*)**,** human chronic gonadotrophic hormone (hcg), carp pituitary glands (cpg), the relative and absolute fecundity.

Introduction

As the global population continues to grow, finding alternatives to meat-based seafood is crucial to meet the increasing demand for sustainable products **(Naiel *et al.,* 2024).** The Mugilidae family offers a promising option due to their fast growth, low trophic feeding habits, and adaptability to different ecological and culture systems **(El-kady *et al.,* 2024).** However, the large-scale commercial production of these species faces huge challenges due to the absence of a cost-effective and reliable method for producing juvenile **(Lee *et al.,* 1987).** Currently, only three species of Mugilidae are farmed at a basic level of domestication, and several challenges arise when developing farming protocols for key stages of their life cycle under captive conditions **(Mousa and Khalil 2013).** Not only does this limit their cultivation potential, but it also poses challenges in producing high-quality seeds for the mullet farming industry's sustainability **(Sukumaran *et al.,* 2021).**

As a consequence, the shortage of Mugilids juveniles for on-growing creates a gap between seed supplies and farmers' demand **(Ramos-Judéz *et al.,* 2021).** Currently, this gap is filled by collecting mullet juveniles during their seasonal migrations from the natural habitats and coastal areas **(Magnotti *et al.,* 2020).** However, the intensive collection of juveniles from the wild can have negative impacts on wild population stocks **(Ramos-Júdez *et al.,* 2022a).** Moreover, concerns about animal welfare and the overall sustainability of this practice arise due to the high mortality rates during transportation of wild-caught fries to on-growing sites and the lack of specific treatments to prevent disease outbreaks **(Mousa 2010).** Therefore, research efforts are focused on optimizing juvenile production techniques, ranging from broodstock management to larval and juvenile rearing **(Tamaru *et al.,* 1989).** Recent advances in understanding sex determination mechanisms, the development of hormonal therapies to induce sexual maturation, and the identification

of suitable treatments for spawning induction and synchronization are paving the way for a more knowledge-based approach to managing Mugilids broodstock **(Yousif *et al.*, 2010; Vallainc *et al.*, 2021; Kumar *et al.*, 2023).** Implementing these new protocols at a commercial scale could help overcome the current challenges in scaling up and intensifying mullet aquaculture **(Kuo 1982).**

Most Mugilidae, including the thin-lipped grey mullet (*Liza ramada*), are low-trophic, euryhaline species that can be widely farmed in various marine and freshwater regions **(Haniffa *et al.*, 2000).** This versatility allows for the production of grey mullet in different environments, reducing the environmental impact compared to traditional carnivorous species **(Carvalho *et al.*, 2019).** Like many commercially important Mugilidae fish species, the thin-lipped grey mullet does not undergo final oocyte maturation, ovulation, or spawning under captivity without hormonal treatments **(Lee *et al.*, 1988).** Previous research has shown that using pregnyl and human chorionic gonadotropin (hCG) as a priming injection followed by a second injection 24 hours later effectively induces final oocyte maturation, ovulation, and spawning **(Mousa 2010; Mousa and Khalil 2013; Brzuska 2021).**

The main hypothesis of this experiment is to identify suitable, non-expensive hormonal treatments that could be employed as effective single-dose inducers of final oocyte maturation and spawning in *L. ramada* brooders under captive conditions and outside of the seasonal breeding season. The description of the induction protocols, broodstock management, and reproductive performances will be evaluated, alongside the examination of histological development, to support our main objective of the current trial.

Material and Methods

Applied chemical and extracts

Pregnyl (hcg) were obtained from the Nile Company for Pharmaceuticals, Cairo, Egypt. While, Melatonin (concentration 3 mg per tablet) was obtained from NATROL, Inc. Chatsworth, CA 91311 USA. The formulation of acetone dried mullet brain was done following **Pham *et al.*, (2020)** approach to obtain the full brain hormone content. The dried brain powder mixtures, after stored in a dark area and cooled, remain stable for uses afterwards.

Adult brooders collection and spawning induction

The adult female brooders of *L. ramada* were gathered from natural habitats (Bardaweel Lagoon, Egypt) during their natural reproductive season (November). The conventional fishing method based on the presence of lagoon artificial enclosures which trap breeders during their natural reproductive migration journey toward the sea. All collected adult fish were phenotypically identified according to **Vallainc *et al.*, (2021)** protocol. The total of 40 mature individuals (10 females per each treated group) were carefully allocated into equal four groups. Each experimental group were subjected to contain 10 female fish. The adult female fish weighing was approximately 511 ± 20 g body weight, with 33.5 ± 3 cm total length. All females were tested for their maturation phase via examining protruded papilla and bulging abdomen, presenting vitellogenic oocytes with diameters higher than 657 µm. Each group (n=10) was kept at net hapa (5x5x1 meter (m) per each, = 25 m^3) allocated into outdoor earthen pond at Sahl El-Hussinia aquaculture farm (30°57'26"N, 32°4'3"E). Water quality criteria were preserved to be under acceptable ranges that would be profitable for *L. ramada* ovulation. The ambient water temperature was ranged from 22 to 32◦C, dissolved oxygen content was 5-6 mg O_2/L, total ammonia level as a nitrogen level was ranged between 3.4 to 5.1 mg/l, total alkalinity 690-700 mg/L, total hardness 30-37 mg/L, total dissolved solids 6.20-6.59 ppt, salinity 33 ppt and pH value was 8.07, whereas, the light regime was performed under natural photoperiod (12L/12D). Fish were anesthetized by transferring them to a new 200 L white rectangular tank filled with a solution of 0.08 % clove oil **(Vallainc *et al.*, 2022)** in groups of 3 / 4 individuals to avoid any stress during handling and injecting processes. The adult brooders were reserved in the anesthetic bath until loss of equilibrium and then quickly subjected to gentle pressure of the abdomen for sex determination. The head and back of each fish were shielded with a wet hand towel and they were injected with a single intramuscular treatment of 4500 IU hcg in combined with 3 mg cpg per (hcg+cpg group), 4500 IU hcg in combined with 3 mg melatonin (hcg+MEL group), 3 mg mullet brain (MB), while the remaining fish group was served as control without any hormonal treatment.

Steroid hormone determination

After 24 hours from injection process, the blood samples of injected brooders were collected from caudal vein using heparinized

syringes and transferred immediately into tubes containing 0.1 mM Complete (Roche, Mannheim, Germany) to prevent proteolysis. The blood was centrifuged (3000 x*g* for 30 min at 4 °C) and plasma was recovered. Steroids were extracted with ethyl ether. The progesterone and 17β-estradiol (E2) were assessed employing specific enzyme-linked immunosorbent assay (ELISA), according to **Nash *et al.*, (2000)** procedure, using acetylcholinesterase as a label.

Reproductive performance characteristics

The mean egg weight per adult fish (EW/F) was weighed closet gram. The total egg count per gram (NE/G) was computed via collecting 1 g and counting their eggs. The relation between eggs weight and adult fish weight was estimated through dividing the egg volume released each brooder on their total biomass.

The relative and absolute fecundity measurements were computed employing **Bhujel (2000)** formulas as follows:

$$Absolute\ fecundity\ (ABS) = total\ released\ egg\ biomass\ per\ mature\ female\ fish\ (g) x\ total\ egg\ number\ per\ 1\ g$$

$$\text{Relative fecundity (REL)} = \frac{ABS}{female\ biomass\ (FBW, g)}$$

Ovarian histological examination

Ovarian specimens were collected after 24 hrs from the single injection dose and then fixed into Bouin's solution and preserved at 70% ethanol solution, then were dehydrated in ascending ethanol levels, embedded in paraffin, then sectioned at 5 µm thickness and stained with hematoxylin and eosin (Casa Álvarez, Spain). The histological slides were examined under a light microscope (Leica DMLB, Houston, United States). The different stages of oocyte development were identified and the maturation stage of brooders was defined according to the most developed and abundant stage of oocytes present as ascribed by **Ramos-Júdez *et al.*, (2022b)** characterization.

Statistical procedure

The trial results were testing for normality and homogeneity employing the Kolmogorov–Smirnov test. Prior to ANOVA analysis, the covariance statical method was employed to avoid the effect of the linear correlation between adult fish age and determines

reproductive characteristics in order to determine adjusted means and avoid statical errors. Using SAS software, one-way ANOVA analysis was subjected to estimate the variances between the hormonal treatments on reproductive features at 95% confidence values. The Dunnett's multiple group comparisons test was subjected to compare the hormonal treatment groups with the control group via GraphPad prism v9.

Results

Serum steroids hormonal contents

Figure 1 illustrates the effects of different hormonal treatments on the levels of steroid hormones (progesterone and estradiol E2) in adult *L. ramada* female fish kept in captivity during the non-spawning season. Compared to the untreated group, the adult fish treated with hcg+MEL exhibited significantly higher levels of serum progesterone and estradiol ($P < 0.001$). Conversely, the injection of hcg+cpe in adult fish did not significantly affect serum progesterone levels but did result in a marked increase in serum estradiol levels compared to the control group ($P < 0.001$). On the other hand, the fish group that received MB treatments showed a significant decrease in both serum progesterone and estradiol levels compared to the control treatment ($P < 0.01$).

Egg estimated parameters

The effects of different hormonal treatments on egg parameters (egg diameter in µm and egg weight per female in grams) of adult female L. ramada fish in captivity during the non-spawning season are shown in Figure 2. The results indicate that adult female fish injected with hCG along with CPE or MEL displayed significantly larger egg diameter and higher egg weight per female compared to the control group ($P < 0.001$). Specifically, female fish treated with hCG+CPE had the highest egg diameter and weight per female, followed by the group of fish that received hCG+MEL treatment. On the other hand, female brooders injected with MB exhibited a significant increase in egg weight per female, but a noteworthy decrease in mean egg diameter ($P < 0.01$).

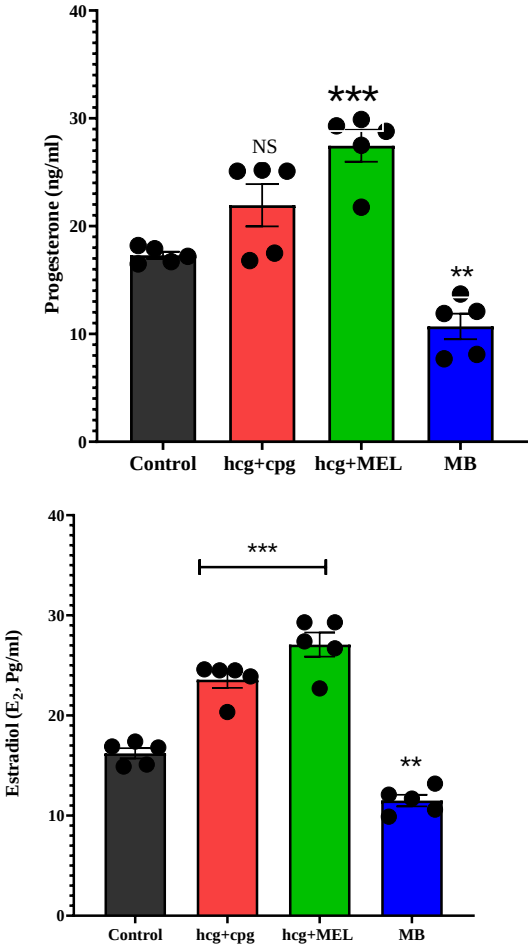

Figure 1. Effects of various hormonal treatments on steroid hormone level (progesterone and estradiol E_2) of adult *L. ramada* female fish under captive conditions out of spawning season. The Dunnett test was employed to estimate the significant variances ($p < 0.05$) between the hormonal treated groups and the control group. N.S, nonsignificant; *, significant at 0.05; **, significant at 0.01; ***, significant at 0.001.

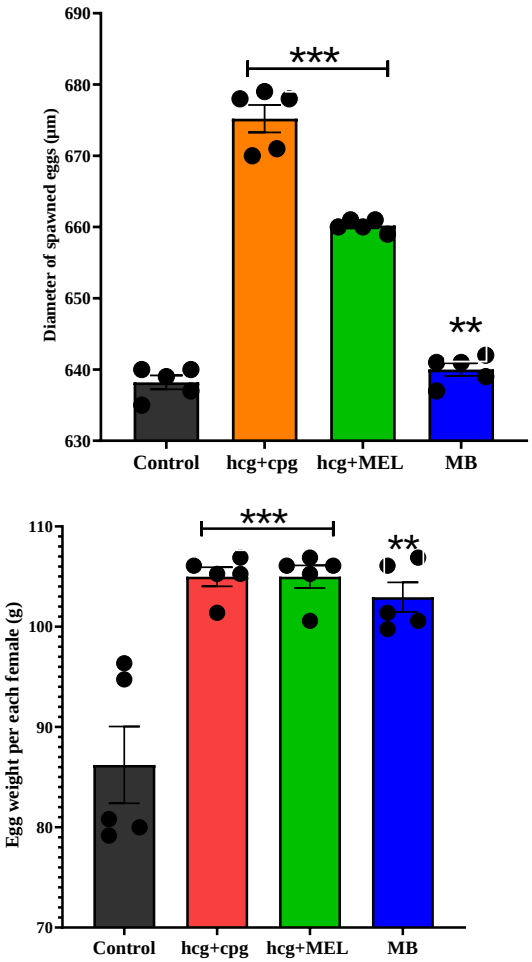

Figure 2. Effects of various hormonal treatments on egg parameters (egg diameter, µm and egg weight per female, g) of adult *L. ramada* female fish under captive conditions out of spawning season. The Dunnett test was employed to estimate the significant variances ($p < 0.05$) between the hormonal treated groups and the control group. N.S, nonsignificant; *, significant at 0.05; **, significant at 0.01; ***, significant at 0.001.

Absolute and relative fecundity

Figure 3 displays the results of a current trial that examined the effects of various hormonal injection protocols on the fecundity (both absolute and relative) of adult female *L. ramada* fish under captive conditions during the non-spawning season. The results showed significant increases in both absolute and relative fecundity for all

fish groups that received hormonal treatments, when compared to the untreated group ($P < 0.001$). Among the groups that received hormonal injections, the adult fish group that received hcg+cpe showed the highest levels of both absolute and relative fecundity, followed by the hcg+MEL group.

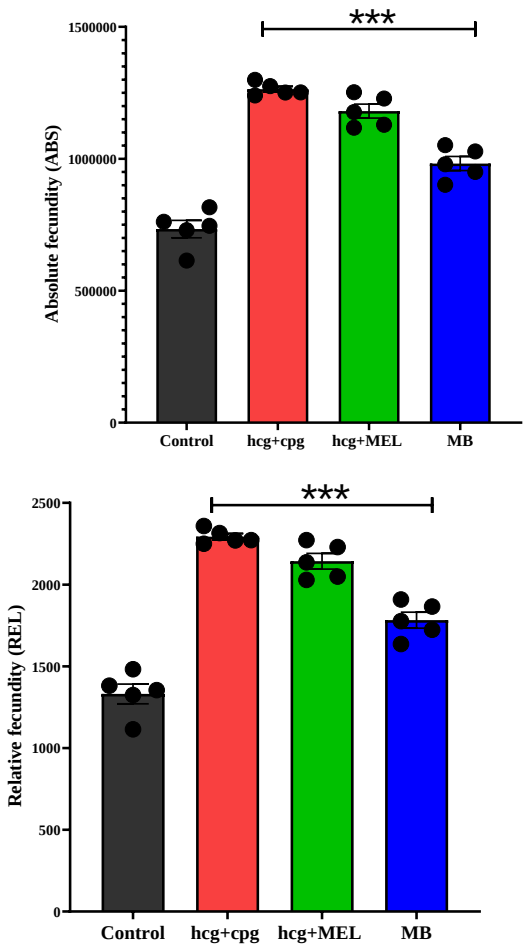

Figure 3. Effects of various hormonal treatments on fecundity (absolute and relative fecundity) of adult *L. ramada* female fish under captive conditions out of spawning season. The Dunnett test was employed to estimate the significant variances ($p< 0.05$) between the hormonal treated groups and the control group. N.S, nonsignificant; *, significant at 0.05; **, significant at 0.01; ***, significant at 0.001.

Ovary histological architecture

The group of fish that did not receive any hormonal treatments exhibited a high count of oogonia (o), a high rate of degenerative chromatin nucleolus stage (Ch), few early perinuclear oocytes (EPO), numerous late perinuclear oocytes (LPO) with few vacuoles (v), and few atretic oocytes (AO) (Figure 4a). Meanwhile, the fish group treated with the hcg+cpg combined injection protocol showed a low content of oogonia cells (O), few chromatins nucleolus stage (Ch), a high rate of degenerative early perinuclear oocytes (EPO), numerous late perinuclear oocytes (LPO) with few vacuoles (v), and a significant presence of ripe oocytes with yolk granules and fat vacuoles in the cytoplasm (R) (Figure 4b). Additionally, adult female fish injected with hcg+MEL demonstrated a moderate level of oogonia (O), a high rate of degenerative chromatin nucleolus stage (Ch), few early perinuclear oocytes (EPO), numerous late perinuclear oocytes (LPO) with few vacuoles (V), and many previtellogenic oocytes (PVO) (Figure 4c). Finally, female fish brooders subjected to the MB injection procedure exhibited a high content of oogonia cells (O), a medium level of chromatin nucleolus stage (Ch), few early perinuclear oocytes (EPO), numerous late perinuclear oocytes (LPO) with few vacuoles (V), and few atretic oocytes (AO) (Figure 4d).

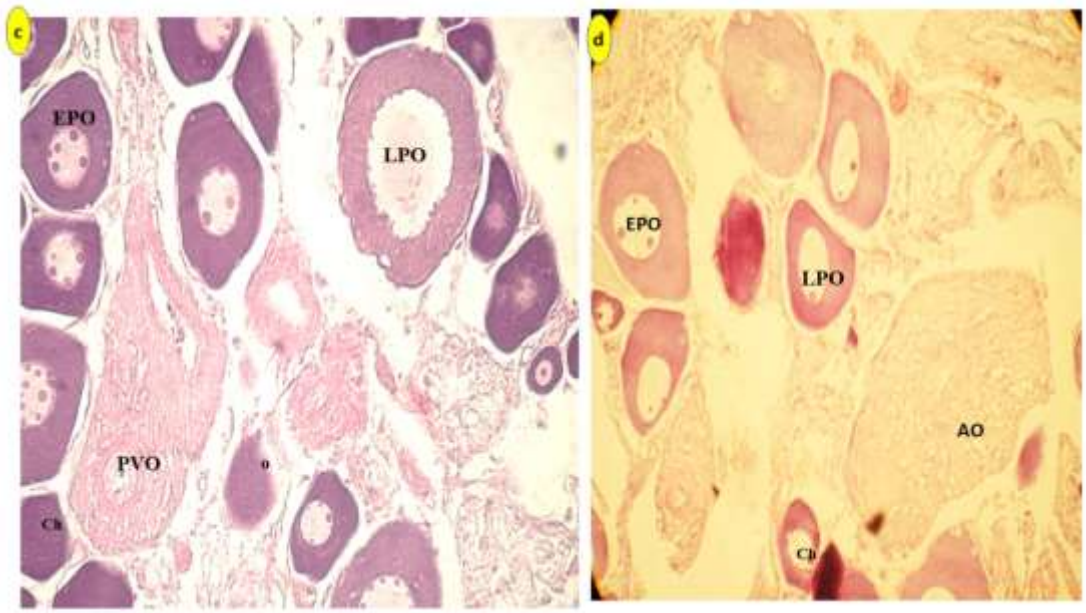

Figure 4. Representative photomicrograph of (H&E) ovary-stained sections (100X) of adult *L. ramada* female fish injected with single

hormonal treatment (a) control group received no hormonal treatment (b) hcg+cpg group, injected with 4500 IU hcg + 3 mg carp pituitary gland, cpg (c) the hcg+MEL group, injected with 4500 IU hcg + 3 mg melatonin, MEL (group), d) the MB group, injected with 3 mg mullet brain, MB. a) control group showed high count of oogonia (o), high rate of degenerative chromatin nucleolus stage (Ch), few early perinuclear oocytes (EPO) and numerous late perinuclear oocyte (LPO) with few vacuoles (v), and few atretic oocytes (AO). b) the hcg+cpg group representing low content of oogonia cells (O), few chromatins nucleolus stage (Ch), high degenerative of early perinuclear oocyte (EPO) and numerous late perinuclear oocyte (LPO) with few vacuoles (v), and many of ripe oocyte's appearance of yolk granules and fat vacuoles in cytoplasm (R). c) the hcg+MEL group exhibited mediate level of oogonia (O), high degenerative of chromatin nucleolus stage (Ch), few early perinuclear oocytes (EPO) and numerous late perinuclear oocyte (LPO) with few vacuoles (V), and incidence of many previtellogenic oocytes (PVO). d) the MB group showed high content from Oogonia cells (O), medium level of chromatin nucleolus stage (Ch), few early perinuclear oocytes (EPO) and numerous late perinuclear oocyte (LPO) with few vacuoles (V), and few atretic oocytes (AO).

Discussion

Our experimental trial confirms that it is easy to adapt mature *L. ramada* adults to captive conditions. Additionally, this study demonstrates the effect of different hormonal protocols on the spawning of adult female *L. ramada* brooders. The study designed to examine the impact of hormonal injection applicable methods to stimulate ovulation and spawning in *L. ramada* fish on gonad histological structure, fecundity, serum steroid hormone levels, and egg measurements under captive conditions. For this purpose, the fish group administered a single injection dose of human chorionic gonadotropin hormone (hcg) combined with either melatonin (MEL), Carp pituitary extract (cpe), or mullet brain extract (MB). These combinations were tested as spawning agents to increase final oocyte maturation (FOM) and ovulation in captivity, as reported by **Vallainc *et al.*, (2021).** The doses of hcg and melatonin used in this study were based on the results of previous investigations, as indicated by **Naiel *et al.*, (2016).**

The present study achieved a high rate of final oocyte maturation (FOM), ovulation (incidence of high rate of ripe oocytes), and spawning in thin-lipped grey mullet females. This success was observed when a single dose of hcg was injected in combined with cpe. Multiple previous reports have indicated successful use of pregnyl (hcg; 15,000 IU/kg weight) as a priming injection. This

initial injection should be followed by a resolving injection of 30,000 IU hcg combined with 200 µg LHRH-a/ kg weight, administered 24 hours later **(Mousa 1999).** This treatment is applied to achieve final oocyte maturation and ovulation in thin-lipped grey mullet. In addition, various studies have been conducted to induce final maturation and spawning in female *Mugil cephalus* with vitelline oocytes (tertiary yolk stage) using higher doses of hormonal treatments. These include 50,000–80,000 IU hcg/kg weight **(Kuo *et al.*, 1972),** 28–48 mg of fresh mullet pituitaries plus 10,000–80,000 IU hcg/kg weight (Kulikova and Gnatchenko 1987), and 20 mg carp pituitary homogenate/kg weight of fish, followed by 200 µg LHRH-a/kg of fish **(Suzuki *et al.*, 1991)**.

Estradiol and progesterone are steroid hormones that play an important role in fish reproduction **(Ramos-Júdez *et al.*, 2022a).** Owing to our study results, the completion of vitellogenesis in females treated with hcg+ cpg was accompanied by an increase in plasma E_2 levels. This increase was not observed in the control group, demonstrating the gonadotropic stimulation of the ovary by the combination of hcg and cpe. At the same tend, **Ramos-Judéz *et al.*, (2021)** obtained a nearly identical result using other types of gonadotropins, with an increase in plasma E_2 levels and nearly a 89% of the treated females completing oogenesis in *Mugil cephalus*. Naturally during the spawning season, there is a noticeable increase in hormone levels, which triggers the release of fish eggs in a sequential manner **(Mousa 1999).** However, mature farmed fish face difficulties in undergoing oocyte maturation, ovulation, and spawning due to a lack of LH secretion during spawning and out spawning season **(Mousa 2010).** Thus, the use of exogenous hormone treatments has proven to be effective in managing the reproductive processes of captive fish.

Comparisons of spawning success in other studies with flathead grey mullet are limited due to differences in methodology and initial gonadal development stage of individuals. Some studies have attempted to enhance vitellogenesis by administering Domperidone at a dose of 5 mg/kg weight or in combination with implants of Gonadotropin-Releasing Hormone agonist (GnRHa) at a dose of 10 µg/kg weight **(Ramos-Júdez *et al.*, 2022a).** These studies obtained lower rates of fully mature females (50–85%). Many other studies directly worked with fully mature females and applied different treatments to induce oocyte maturation and spawning. For example, **Aizen *et al.*, (2005)** applied GnRHa at a dose of 10 µg/kg weight for

priming and 20 μg/kg weight for resolving, combined with Metoclopramide at a dose of 15 mg/kg weight for both priming and resolving. **Meseda and Samira (2006)** injected carp pituitary extract at a dose of 20 to 70 mg/kg weight or 10,000 IU of hcg per fish for priming, followed by one or two injections of 100–200 μg of GnRHa per kg for resolving. **Besbes *et al.*, (2020)** treated the fish with a priming dose of 10,000 IU of hcg per kg weight and a resolving dose of 10,000 IU of hcg per kg weight, along with 200 μg of GnRHa per kg weight. **Vallainc *et al.*, (2021)** injected 200 μg of GnRHa per kg weight. These treatments resulted in high fecundities ranging from 418,945 to 1,649,000 eggs per kg of body weight. In general, these studies showed highly variable spawning success and/or fecundity percentages. In contrast, our study presents reliable higher absolute and relative fecundity levels obtained from females successfully induced from previtellogenesis after receiving hcg in combination with cpe or MEL.

In the same context, **Fahmy and El-Greisy (2014)** found that a minimum egg diameter of 600 μm was necessary to successfully induce spawning in *Liza ramada*. They observed that female mullet first showed early vitellogenic oocytes in November, and after several weeks of progression, spawning could be induced when the mean oocyte diameter reached at least 600 μm. In contrast, **Monbrison *et al.*, (1997)** reported that hormonal treatment could induce spawning in female grey mullet (*Mugil cephalus*) once the oocyte diameter reached 500 μm. In our study, the injection of hormones, particularly hcg combined with cpe, had an effect on increasing the oocyte diameter. These results suggest that a single dose of hormone injection influences the efficiency of nutrient transfer from the liver to the gonads **(Naz 2009).** The nutrient reserves in fish eggs are used by developing larvae for energy metabolism and as a structural component in membrane biogenesis **(Sargent 1995).**

Finally, in the current experiment, we have successfully demonstrated the effectiveness of a single-dose injection procedure in stimulating ovulation in *Liza ramada*, which is a commercially important and valuable fish species in the fisheries industry. However, the scientific community is currently making significant efforts in managing reproduction in Mugilids. It is anticipated that achieving complete artificial control over sexual maturation and reproduction in Mugilids will be possible in the near future. However, until this becomes a reality, it seems that capturing a

limited number of sexually mature individuals from the wild and later releasing them following spawning in captivity is a far more sustainable option than harvesting millions of juveniles from the ecosystem.

References

Aizen, J.; Meiri, I.; Tzchori, I.; Levavi-Sivan, B. and Rosenfeld, H. (2005): Enhancing spawning in the grey mullet (Mugil cephalus) by removal of dopaminergic inhibition. General and comparative endocrinology. 142: 212-221.

Besbes, R.; Benseddik, AB.; Kokokiris, L.; Changeux, T.; Hamza, A.; Kammoun, F. and Missaoui, H. (2020): Thicklip (Chelon labrosus) and flathead (Mugil cephalus) grey mullets fry production in Tunisian aquaculture. Aquaculture Reports. 17: 100380.

Bhujel, R.C. (2000): A review of strategies for the management of Nile tilapia (Oreochromis niloticus) broodfish in seed production systems, especially hapa-based systems. Aquaculture. 181: 37-59.

Brzuska, E. (2021): Reproduction effectiveness of carp (Cyprinus carpio L.) from the Hungarian W breeding line after stimulating ovulation with spawning inducing agents of natural (CPH, hCG, PMSG) and/or synthetic origin (Ovopel, Dagin, Ovaprim, mGnRH-a). Aquaculture. 532: 736023.

Carvalho, C.; Passini, G.; Sterzelecki, F.C.; Baloi, M.F. and Cerqueira, V.R. (2019): Maturation, spawning and larviculture of the mullet Mugil liza under laboratory conditions. Revista Brasileira de Reprodução Animal. 43: 31-36.

El-kady, M.A.; Mansour, H.A.; Elmor, M.E. and Ali, A.A. (2024): Comparative Gene Expression Level of Reproductive Genes Along the Brain-Pituitary-Gonad Axis in the Ripe Female of the Wild and Captive Grey Mullets; Mugil cephalus. Egyptian Journal of Aquatic Biology & Fisheries. 28.

Fahmy, A.F. and El-Greisy, Z.A-B. (2014): Induced spawning of Liza ramada using three different protocols of hormones with respect to their effects on egg quality. African Journal of biotechnology. 13.

Haniffa, M.; Merlin. T. and Mohamed, J.S. (2000): Induced spawning of the striped murrel Channa striatus using pituitary extracts, human chorionic gonadotropin, luteinizing hormone releasing hormone analogue, and ovaprim (r). Acta Ichthyologica et Piscatoria. 30: 53-60.

Kulikova, N. and Gnatchenko, L. (1987): Response of prespawning female Black Sea striped mullet, Mugil cephalus, to chorionic gonadotropin. J. Ichthyol. 27.

Kumar, P.; Behera, P.; Biswas, G.; Ghoshal, T.; Das, S.; Kailasam, M. and Jithendran, K. (2023): Gonad recrudescence and annual sex steroid patterns in female goldspot mullet, Liza parsia reared in brackishwater pond. Animal Reproduction Science. 248: 107161.

Kuo, C-M.; Shehadeh, Z.H. and Nash, C.E. (1972): Induced spawning of captive grey mullet (Mugil cephalus L.) females by injection of human chorionic gonadotropin (HCG). Aquaculture. 1: 429-432.

Kuo, C. (1982): Induced breeding of grey mullet, Mugil cephalus L, Proceeding of the International Symposium on Reproductive Physiology of Fish, pp. 181-184.

Lee, C-S.; Tamaru, C.S. and Kelley, C.D. (1988): The cost and effectiveness of CPH, HCG and LHRH-a on the induced spawning of grey mullet, Mugil cephalus. Aquaculture. 73: 341-347.

Lee, C.; Tamaru, C.; Miyamoto, G. and Kelley, C. (1987): Induced spawning of grey mullet (Mugil cephalus) by LHRH-a. Aquaculture. 62: 327-336.

Magnotti, C.C.; dos Santos CIPRIANO F.; Pedrotti, F.S. and Cerqueira, V.R. (2020): Advances in reproduction of the lebranche mullet Mugil liza: maturation and spawning of f1 breeders in captivity. Boletim do Instituto de Pesca. 46.

Meseda, M. and Samira, S.A. (2006): Spawning induction in the Mediterranean grey mullet Mugil cephalus and larval developmental stages. African Journal of Biotechnology. 5: 1836-1845.

Monbrison, D.d.; Tzchori0, I.; Holland, M.C.; Zohar, Y.; Yaron, Z. and Elizur, A. (1997): Acceleration of gonadal development and

spawning induction in the Mediterranean grey mullet, Mugil cephalus: Preliminary studies.

Mousa, M. (1999): Hormonal induction of oocyte final maturation and ovulation in thin-lipped grey mullet, Liza ramada (Risso). Bull. Nat. Inst. Oceanogr. Fish., ARE. 25: 331-355.

Mousa, M.A. (2010): Induced spawning and embryonic development of Liza ramada reared in freshwater ponds. Animal Reproduction Science. 119: 115-122.

Mousa, M.A. and Khalil, N.A. (2013): Environmental and hormonal control of induced spawning in thin-lipped grey mullet, Liza ramada (Risso). Egyptian Journal of Histology. 36: 449-458.

Naiel, M.A.; Abd El-Naby, A.S.; Samir, F. and Negm, S.S. (2024): Effects of dietary Thalassodendron Ciliatum supplementation on biochemical-immunological, antioxidant and growth indices of Oreochromis niloticus exposed to ammonia toxicity. Aquaculture. 585: 740702.

Naiel, M.A.; El-Darawany, A.; Nasr, A. and Al-Marakby, K.M. (2016): INDUCTION OF OVULATION IN Liza ramada (RISSO, 1826) OUT OF SEASON. Zagazig Journal of Agricultural Research. 43: 1245-1252.

Nash, J.; Cuisset, B.D.; Bhattacharyya, S.; Suter, H.; Le Menn, F. and Kime, D. (2000): An enzyme linked immunosorbant assay (ELISA) for testosterone, estradiol, and 17, 20β-dihydroxy-4-pregenen-3-one using acetylcholinesterase as tracer: application to measurement of diel patterns in rainbow trout (Oncorhynchus mykiss). Fish Physiology and Biochemistry. 22: 355-363.

Naz, M. (2009): Ontogeny of biochemical phases of fertilized eggs and yolk sac larvae of Gilthead Seabream (Sparus aurata L.). Turkish Journal of Fisheries and Aquatic Sciences. 9.

Pham, X.K.; Dao, V.H. and Amano, M. (2020): Changes in brain and pituitary GnRH levels during a reproductive cycle in wild female Asian sea bass, Lates calcarifer (Bloch, 1790). Iranian Journal of Fisheries Sciences. 19: 3255-3268.

Ramos-Judéz, S.; Chauvigné, F.; González-López, W.Á.; Rosenfeld, H.; Cerdà, J.; Giménez, I. and Duncan N (2021): Providing recombinant gonadotropin-based therapies that induce oogenesis from previtellogenic oocytes to produce viable larvae in a teleost,

the flathead grey mullet (Mugil cephalus). Aquaculture. 536: 736418.

Ramos-Júdez, S.; Giménez, I.; Gumbau-Pous, J.; Arnold-Cruañes, L.S.; Estévez, A. and Duncan, N. (2022a): Recombinant Fsh and Lh therapy for spawning induction of previtellogenic and early spermatogenic arrested teleost, the flathead grey mullet (Mugil cephalus). Scientific Reports. 12: 6563.

Ramos-Júdez, S.; Danis, T.; Angelova, N.; Tsakogiannis, A.; Giménez, I.; Tsigenopoulos, C.S.; Duncan, N. and Manousaki, T. (2022b): Transcriptome analysis of flathead grey mullet (Mugil cephalus) ovarian development induced by recombinant gonadotropin hormones. Frontiers in Physiology: 2294.

Sargent, J. (1995): Origins and functions of egg lipids: nutritional implications. . In: Bromage NR, Roberts RJ (eds) Broodstock Management and Egg and Larval Quality. Blackwell Science, Oxford.: 353-372.

Sukumaran, K.; Thomas, D.; Rekha, M.; Angel, J.R.J.; Bera, A.; Mandal, B.; Subburaj, R.; Thiagarajan, G.; Makesh, M. and Ambasankar, K. (2021): Reproductive maturation and induced breeding of two geographical groups of grey mullet, Mugil cephalus Linnaeus, 1758. Aquaculture. 536: 736423.

Suzuki, K.; Asahina, K.; Tamaru, C.S.; Lee, C-S. and Inano, H. (1991): Biosynthesis of 17α, 20β-dihydroxy-4-pregnen-3-one in the ovaries of grey mullet (Mugil cephalus) during induced ovulation by carp pituitary homogenates and an LHRH analogue. General and comparative endocrinology. 84: 215-221.

Tamaru, C.S.; Kelley, C.D.; Lee, C-S.; Aida, K. and Hanyu, I. (1989): Effects of chronic LHRH-a+ 17-methyltestosterone or LHRH-a+ testosterone therapy on oocyte growth in the striped mullet (Mugil cephalus). General and comparative endocrinology. 76: 114-127.

Vallainc, D.; Concu, D.; Loi, B.; Pitzalis, A.; Frongia, C.; Chindris, A. and Carboni, S. (2022): Spawning induction and larval rearing in the thinlip gray mullet (Chelon ramada): The use of the slow release gonadotropin releasing hormone analog (GnRHa) preparation, leuprorelin acetate. Animal Reproduction Science. 247: 107145.

Vallainc, D.; Concu, D.; Papiol, G.G.; Loi, B.; Leggieri, F.; Brundu, G.; Chindris, A.; Sanna, G.; Fois, N. and Antognarelli, F. (2021): Producing flat-head grey mullet Mugil cephalus (Linnaeus, 1758) fries in captivity from sexually mature adults collected in Sardinian lagoons. Aquaculture Reports. 21: 100844.

Yousif, O.M.; Fatah, A.; Krishna Kumar, K.; Minh, D. and Hung, B. (2010): Induced spawning and larviculture of grey mullet, Mugil cephalus (Linnaeus 1758) in the Emirate of Abu Dhabi. Aquaculture Asia. 15: 41-43.

دراسة تأثير الهرمونات على التبويض في أسماك الطوبار تحت ظروف الأسر باستخدام محفزات هرمونية طبيعية وصناعية.

محمد عبد الهادي نايل [1]، إيمان محمود محمد زكي [2]، أسامه محمد عبدالمنعم [1]

قسم الانتاج الحيواني- كلية الزراعة- جامعة الزقازيق- مصر. [1]

[2] قسم التفريخ وفسيولوجيا الأسماك – المعمل المركزي لبحوث الثروة السمكية بالعباسة - أبوحماد شرقية - مركز البحوث الزراعية – مصر.

تنتمي أسماك الطوبار إلى العائلة البورية. إناث أسماك الطوبار الناضجة لا يحدث بها تبويض بدون الحقن هرمونيا وذلك تحت ظروف الأسر, لذا فان هذه التجربة تحقق استخدام هرمون الغدد التناسلية المزمن والغدد النخامية لأسماك المبروك والميلاتونين ومستخلص دماغ البوري كمحفز فعال بجرعة واحدة للتفريخ في هذا النوع، بمجموع 40 أنثى حاضنة برية ناضجة ن= 10 ، 33.5 ± 3 سم الطول الاجمالي، 511 ± 20 جم من وزن الجسم..

تم تقسيم الإناث الناضجة الي أربع مجموعات متساوية على النحو التالي: كانت المجموعة الأولى بمثابة مجموعة تحكم (بدون علاج هرموني) بينما تلقت المجموعة التانية والثالثة علاجا هرمونيا قدره 4500 وحدة دولية من هرمون الغدة التناسلية مع 3 ملجم من الغدد النخامية لأسماك المبروك و 3 ملجم من الميلاتونين. المجموعة الأخيرة من الأسماك تلقت علاجا هرمونيا منفردا بجرعة 3 ملجم من مستخلص مخ البوري.

أشارت نتائج التجربة إلى زيادة كبيرة معنوية في مستويات بلازما الإستراديول والبروجسترون في الإناث الناضجة التي تم حقنها من هرمون الغدة التناسلية بالإضافة إلى الغدد النخامية لأسماك المبروك و الميلاتونين.

وأخيرا تشير نتائج التجربة إلى أنه سمكة الطوبار الناضجة يمكن رفع قدرتها التناسلية، وتتكيف جيدا مع ظروف الأسر، ويمكن حسها بسهولة على التبويض خارج موسم التناسل عن طريق إعطاء جرعة واحدة من هرمون الغدة التناسلية مع الغدد النخامية لأسماك المبروك و الميلاتونين.