Pakistan J. Wildl., vol. 16(1), pp 23-32, 2025

Gonadal Development, Fecundity Rate and Hatchability of Giant Snakehead (Channa marulius) by using different synthetic hormones in Captivity.

Sadia Nazir[1]*, Noor Khan[1], Simon J. Davies[2], Sheeza Bano[1], Muhammad Asghar[1]
[1]Department of Fisheries & Aquaculture, University of Veterinary and Animal Sciences, Lahore-Pakistan.
[2]Aquaculture and Nutrition Research Unit (ANRU), Carna Research Station, Ryan Institute and School of Natural Sciences, College of Science and Engineering, University of Galway, Galway city, Ireland
*Corresponding author: sadianazir824@gmail.com

Running Title: Hormonal Effects on Channa marulius Reproduction

ABSTRACT

This research was conducted to evaluate the induced breeding using the application of synthetic hormones Conceptal (T1), Suprefact (T2), and Ovaprim (T3), as well as to understand the reproductive biology and gonadal maturation of the bullseye snakehead, Channa marulius. There were nine ponds with a dimension of [2 (W) 4 (L) 1.5 m (D)] representing three treatments (in triplicate) with a pair of fish in each pond. The hormones were injected into the test fish at the following proportions (0.3ml, 0.4ml, and 0.5ml to males and 0.8ml, 0.9ml, and 1.0ml to females' body weight) and then released into the pond for large-scale production of seed. Over the study period, estimates of the gonado-somatic index (GSI) and absolute fecundity were estimated. In addition to these, gonadal histology was carried out for a better assessment of ovarian maturity. The results showed no successful spawning on Conceptal. The highest average GSI values were found for both females and males in Suprefact (3.32±1.62 % and 1.67±0.18 %, respectively), then in Ovaprim (1.13±0.56 and 1.22±0.68 %, respectively). The result showed that fish stimulated with suprefact (T2) obtained the highest average fecundity (3079.3±100.7) and survival rate (95.75±1.51%), respectively. Gonads were observed microscopically, including staining and histology. The four stages of ovarian development were identified by ovarian histology: (1) primary growth, (2) yolk globules, (3) vitellogenesis (4) maturity. In conclusion, the use of Suprefact® and Ovaprim® allows for to maximization of the effects of breeding induction of C. marulius in small experimental breeding ponds.

Keywords: Channa marulius, induced breeding, gonadosomatic index, reproductive biology, gonadal histology.

INTRODUCTION

The bullseye snakehead (Channa marulius), belonging to the family Channidae, commonly referred to as the snakehead murrel, is a significant freshwater fish of Africa, East Asia, and Southeast Asia. Due to the high market demand for it as food and its delicious flavor, lower quantity of intramuscular spines than most fish, and high nutritional content, it is a fish with significant economic value (Dayal et al., 2013). Since overfishing, habitat destruction, and a lack of fish culture technology, the snakehead population is now in decline and becoming harder to find. This fish should be domesticated and artificially bred using synthetic hormones to preserve its existence and maintain production. The commercially available synthetic inducing hormones in readymade form (Ovaprim®, Suprefact®, Ovopel®, and Aquaspawn®) are becoming more popular and are proven to be effective in fish spawning. A few studies have been reported on testing the effectiveness of using different doses of these synthetic hormones for the induced spawning of catfish and snakehead (Marimuthu et al., 2015; Nazir et al., 2022).

An essential factor in assessing fish reproductive performance is the gonadosomatic index and egg size (Biswas et al., 2005). Fish larval size, viability, and hatchability are influenced by egg size (Sarmento et al., 2018). The consensus is that larger eggs can result in more viable larvae than smaller eggs, probably because the relatively larger eggs have a higher yolk content (Clarke, 1993). Due to their seasonal breeding habits, freshwater murrels change their gonads during the reproductive cycle (Marimuthu et al., 2001). Studies on the cyclic gonadal changes of some

Channa species, including C. punctatus (Srivastav and Srivastav, 1998) and C. striatus (Al Mahmud et al., 2016), have been conducted, and the reproductive performance of snakehead murrel, Channa striatus (Ghaedi et al., 2014).

Although Channa marulius is a significantly commercially important fish, little is known about the reproductive biology and histologic alterations of the gonads when kept in captivity. Gonadosomatic index (GSI) and gonadal histological data are essential for selecting the inducing agents, brood fish, and timing for induced breeding. However, data obtained from GSI and the histological examination of the gonads of C. marulius in reproductive biology can be used as the basis for further research on C. marulius to develop the package of induced spawning and to manage and conserve the fish from the threatening condition.

The purpose of this study is to ascertain how hormones affect induced breeding, as well as to examine the bullseye snakehead's reproductive biology using GSI and histological analysis of the gonads (testis and ovaries) for a more accurate assessment of gonadal ripening status.

MATERIALS AND METHODS

The experiment was carried out at the Department of Fisheries & Aquaculture, UVAS, Ravi Campus, Pattoki, in spring 2020.

Collection and rearing of broodstock

The study was performed with the ethical approval of the Animal Care Committee Review Board at the University of Veterinary and Animal Sciences, Lahore, Pakistan. Fish were acclimatized in a brooder pond and fed twice a day with a commercial fish feed (6mm floating pellet) containing 40% crude protein at @3% of their body weight (BW) daily for three months, until reaching sexual maturity. Poultry viscera was also given to all the broodstock fishes at regular intervals as a supplementary feed. The mature brood stock of Channa marulius was collected from the brooders' pond and stocked in small experimental breeding ponds (4 m x 2 m x 1.5 m) at the Department of Fisheries and Aquaculture, UVAS, Ravi Campus, Pattoki. There were a total of nine small ponds, each containing one pair of brooders having an average weight of 1.5-2.18 kg and a length of 66-70 cm. They were again acclimatized for one week. Aquatic macrophytes (Hydrilla verticillata and Eichhornia crassipes) were introduced into the breeding ponds for hiding purposes. Water quality parameters such as temperature, dissolved oxygen, and pH were monitored during the period of the experiment. The water temperature, pH, and Dissolved Oxygen (DO) were measured by using digital meters such as YSI Model 55 Dissolved Oxygen and Temperature System, Ohio, 4387, USA.

Sex determination of matured brood fish:

The selected brood stock could be identified sexually. The genital opening is located behind the genital papilla on the slender male body. The genital opening is located above the genital papilla, and the female body is chubby. The mature males were selected on the basis of pressing on the male's belly, a white color milt oozes out from the genital papilla. While a mature females were identified by a soft, swollen, yellow belly that was protruding. One male and one female make up each pair (ratio: 1:1).

Preparation of superfact-20 hormonal solution for 10 kg of biomass of fish:

Ten tablets of Motilium-V have been taken, thoroughly ground, and added to a small petri dish with 10 ml of distilled water to form a solution. With the help of a 1ml syringe, 0.3ml suprefact® hormone was then added, and the motilium solution was thoroughly mixed.

Brood stock selection, induced breeding, conditioning, spawning, and hatching of eggs

A total of 18 pairs of sexually mature, healthy broodstock of males and females (1.5-2.5 kg bw) of *C. marulius* were collected from the small breeding ponds. For the induced breeding trial, three different hormones, Ovaprim (Syndel Laboratories, Vancouver, BC, Canada) and Suprefact-20 Hormone (Sanofi Aventis, Germany), Conceptal injection 5 ml (Star Laboratories (Pvt.) Ltd. were used in triplicate. The suprefact was mixed with Motilium-V tablets and diluted by adding distilled water to make a solution and then injected at different concentrations into males and females (0.3ml, 0.4ml, and 0.5ml for males and 0.8, 0.9ml, and 1.0ml for females/kg body weight) respectively. The hormonal doses were injected into the recipient fish intramuscularly. After the hormonal injections of three stimulatory hormones each pair of brooder was transferred back into

their small experimental ponds. After 24 hours of injection of synthetic hormones, the response of brooders was observed in experimental ponds.

Table 1. Synthetic hormones used as stimulators, their dosage concentrations for *Channa marulius.*

Groups/ Treatments	Hormones used	Ponds	Dosage concentrations (ml/kg BW)		Time Interval of second dose (Days)
			Male	Female	
T1	Conceptal	P1	0.3	0.8	15 Days
		P2	0.4	0.9	
		P3	0.5	1.0	
T2	Suprefact	P4	0.3	0.8	15 Days
		P5	0.4	0.9	
		P6	0.5	1.0	
T3	Ovaprim	P7	0.3	0.8	15 Days
		P8	0.4	0.9	
		P9	0.5	1.0	

Body Measurements:

The specimens (3-5) were brought to the laboratory in fresh condition, measured for their total length (cm) in centimeter scale, and total body wet weight (w/w). After anaesthetizing with 10 ppm clove oil. Subsequently, a longitudinal ventral incision was made on each fish, and the gonad was dissected for microscopic classifications. The fish was wiped with a dry napkin before weighing, and body weight and ovary weight were measured using a digital weighing balance (Model NBL 254e (250 g × 0.0001 g). Gonad sections were collected from the mid-part of the ovary. To sketch out the peak breeding season and gonadal cycle, *C. marulius* were sacrificed and gonads collected for a gonadosomatic index study.

The Gonadosomatic index was calculated according to Strum (1978) as follows:

$$\text{Gonado-Somatic Index (GSI)} = \frac{\text{Weight of gonad}}{\text{Weight of fish}} \times 100$$

$$\text{Fecundity (F)} = \frac{\text{N x Gonad weight}}{\text{Sample weight}}$$

$$\text{Fertilization rate (\%)} = \frac{\text{Number of fertilized eggs}}{\text{Total number of eggs collected}} \times 100$$

$$\text{Hatching rate (\%)} = \frac{\text{Number of hatched eggs.}}{\text{Number of fertilized eggs}} \times 100$$

$$\text{Larval survival rate (\%)} = \frac{\text{No. of actual fish survived}}{\text{Number of actual fish stocked}} \times 100$$

Histological study of the gonads

Gonads belonging to a variety of developmental stages were kept at room temperature and preserved in 10% formalin solution for histological study. Samples of preserved gonads were taken from the fixative. Then, the 0.5 cm-thick samples from the central portion of the gonads were separately placed for histological examination into the cassettes. Then, dehydrated in a series of graded alcohol, embedded in paraffin, sectioned with a microtome (AEM 450) for 5-7 µm thickness, and then mounted in DPX mount after being stained with haematoxyline and eosin, photographs of different developmental stages of gonads were estimated to be observed under the microscope (LABOMED America, Inc., U.S.A.) by (Navarro et al., 1989; Morton, 1990).

Statistical analysis

Two-way ANOVA (Analysis of variance) was used to statistically analyse the collected data using SPSS-22 version software. Tukey's test was used to compare the means.

RESULTS

Reproductive performance / Induced spawning

Synthetic hormones and their dosage concentrations for male and female *C. marulius* are presented in Table 1. The result of the induced breeding performance of *C. marulius* was presented in Table 2. After 10 hours of post-injection, the fish that had been exposed to hormones exhibited

aggressive behavior. The courtship behaviour of the breeding pairs, which began 1-2 days before spawning, was observed as well as their mutual roaming, nudging, and splashing in the water. Fish breeding behaviour was closely monitored for the entire 48-hour spawning process. *Channa marulius* formed a floating nest of weeds for the deposition of eggs. After 48 hours, eggs hatch and fry can be seen, and parents guard the fry for about a month. *Channa marulius* did not spawn in treatment T1 (Conceptal®). Gonado-Somatic Index did not appear to be significantly ($P \geq 0.05$) affected by hormone doses. Overall, the average higher values for female and male GSI (3.32±1.62 and 1.67±0.18, respectively) appeared in treatment T2 (Suprefact®). The highest ($p<0.05$) average fecundity (3079.3±100.7) was observed in treatment T2 (Suprefact®), while treatment T3 (Ovaprim®) had comparatively low fecundity (1669.3±836.5). In snakehead, hormone doses had a significant ($P < 0.05$) impact on the fertilization rate, hatchability, and survival rate, with higher average values (96.33±1.20, 94.67±2.40, and 95.75±1.51) appearing in treatment T2 (Suprefact®).

Table 2. Reproductive performance of *Channa marulius* treated with Conceptal® (GnRH Analogue), Suprefact® (LHRH), and Ovaprim® (GnRH + dopamine inhibitor). (Means±SE, N = 3).

Parameters	T1 (Conceptual)	T2 (Superfact)	T3 (Ovaprim)	P-value
GSI% % (Female)	0.00±0.00^{a}	3.32±1.62^{a}	1.13±0.56^{a}	0.134
GSI% % (Male)	0.00±0.00^{a}	1.67±0.18^{a}	1.22±0.68^{a}	0.127
Fecundity rate %	0.00±0.00^{b}	3079.3±100.7^{a}	1669.3±836.5^{ab}	0.026
Fertilization rate %	0.00±0.00^{b}	96.33±1.20^{a}	58.00±29.02A^{b}	0.038
Hatching rate %	0.00±0.00^{b}	94.67±2.40^{a}	61.27±30.65^{ab}	0.041
Survival rate %	0.00±0.00^{b}	95.75±1.51^{a}	64.67±32.34^{ab}	0.049
Time interval (response after 2nd dose)	0.00±0.00^{b}	15.00±0.00^{a}	10.00±5.00^{ab}	0.049
Mortality of broodstock	0.00±0.00^{a}	0.00±0.00^{a}	0.67±0.67^{a}	0.444

Means sharing different letters in a row or a column are statistically significant ($p < 0.05$). Comparison of means ± SD (n = 3).

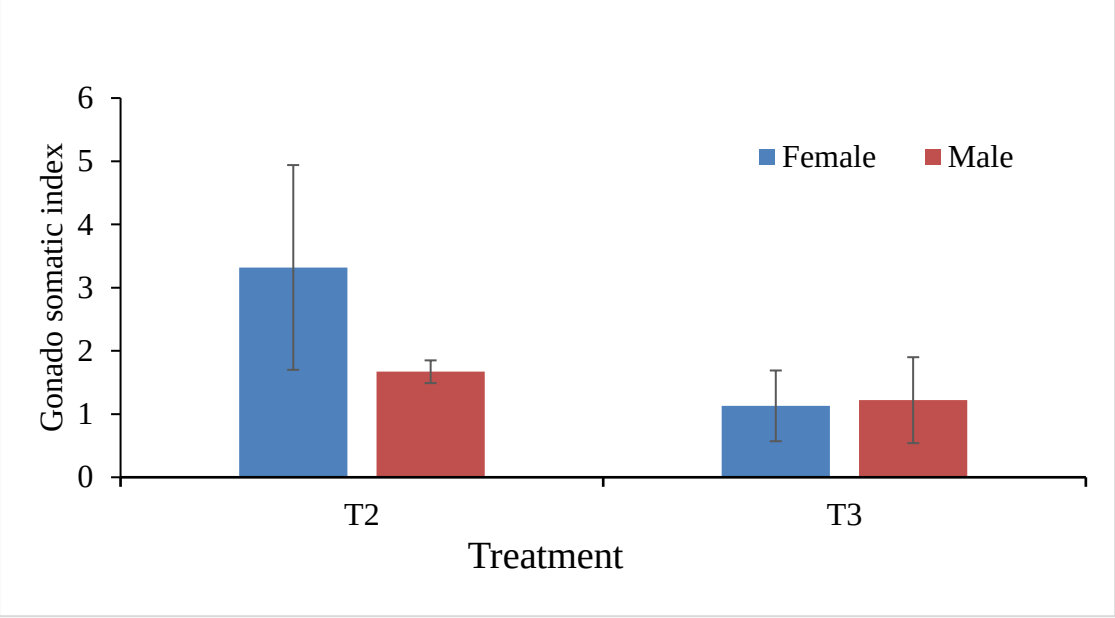


Figure 1. GSI% % of Male & Female in different Gonadal developmental stages.

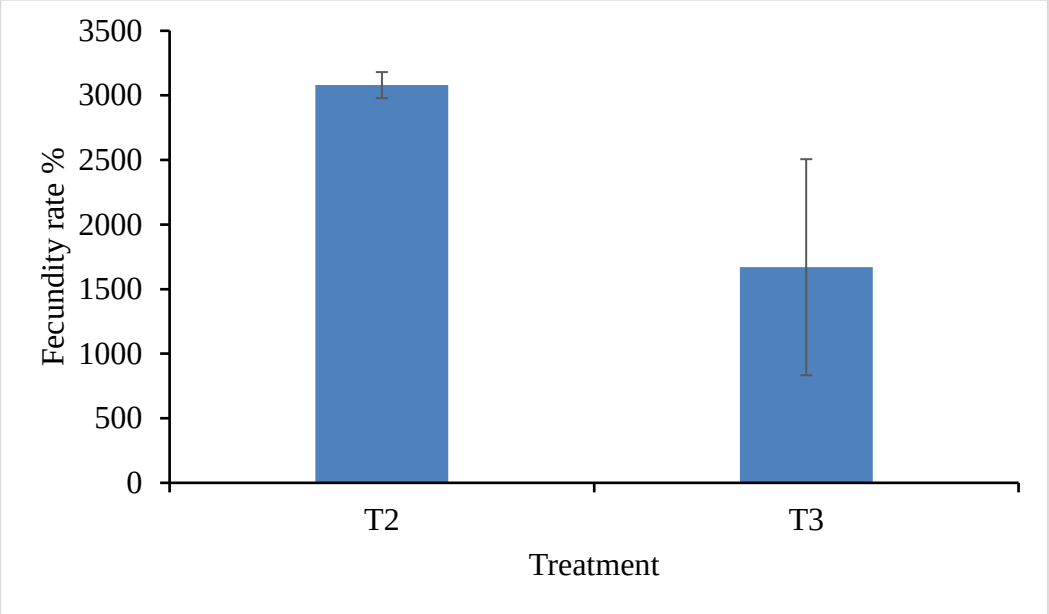


Figure 2. Absolute fecundity of *C. marulius*

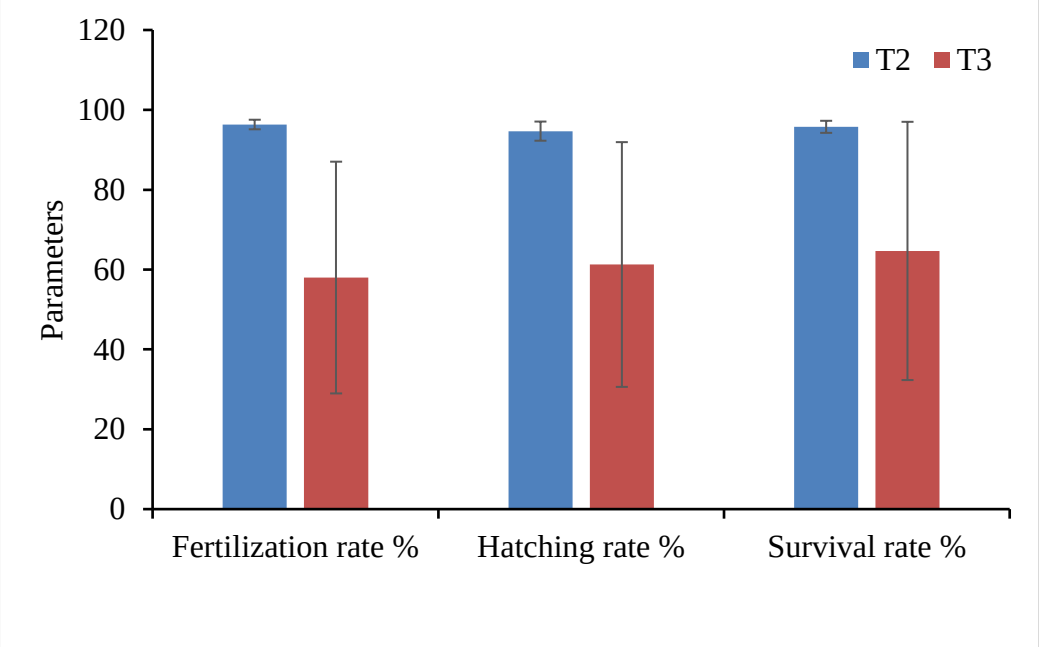


Figure 3. Induced breeding parameters of *C. marulius*

Histological description of ovaries and stages of oocytes in female *C. marulius*

Four major phases of oocyte development in *C. marulius* throughout the study period have been confirmed (Table 3). Based on histological criteria, the stages of oocyte growth were divided into primary growth phase (Fig. A), yolk globules (Fig. B), vitellogenesis (Fig. C), and mature (Fig. D).

Primary growth / Chromatin nucleolar stage

Primary growth involves the period from the stage of meiotic chromatin nucleus to the stage of oocyte development of early cortical alveoli formation. Small cells with a thin, unclear peripheral zone were chromatin nucleolar stage oocytes. In further information, there was more than one nucleolus commonly present.

Yolk globules stage

This phase was characterized by the formation in the cytoplasm of oval, rounded yolk globules that were shown to be empty, unstained vacuoles. At the periphery of the oocyte, these yolk globules first appear and gradually spread to the center of the nucleus.

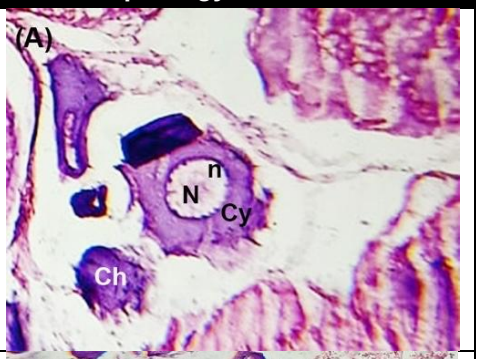
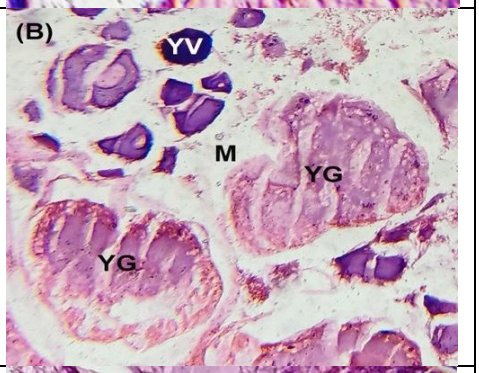
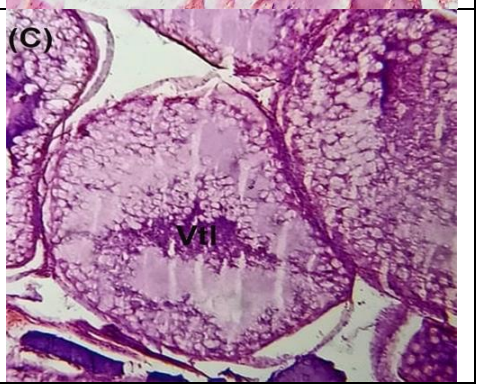
Vitellogenesis stage

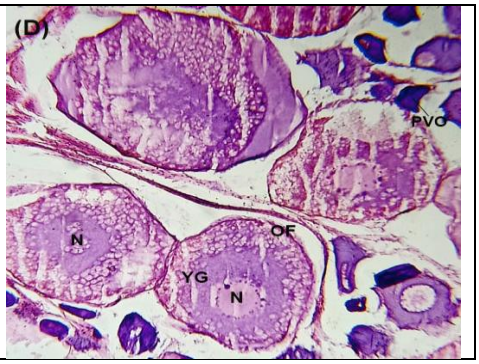
Vitellogenesis, which begins with the oocyte's incorporation of vitellogenin (VTG) proteins and their processing into yolk proteins, is the third stage of oocyte maturation, ending with passage of the nucleus to the animal pole.

Mature stage

Oocytes undergone the stage of maturation at the end of vitellogenic stage. Wall of the ovary is thinner. Oocytes larger and more mature. Some oogonia were still present.

Table 3. Histological assessment showing dominant stages of development of oocytes of *C. marulius* during a study period of 3 months.

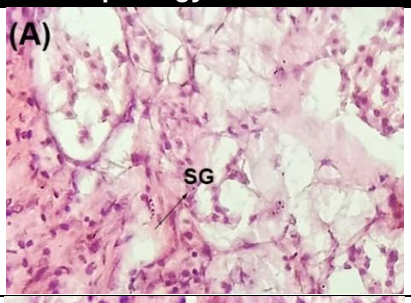
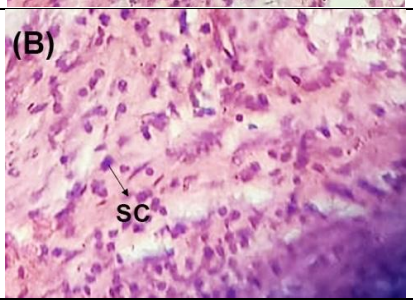
Stages	Description of ovaries	Histomorphology of ovaries
Stage I (Primary growth stage)	Oogonia, chromatin nucleolus (ch), Nucleus (N), nucleoli (n), and cytoplasm (cy) at 40× magnification.	
Stage II (Yolk globules)	Yolk globules (Yg) and oocytes of the well-developed yolk vesicle, Oocyte membrane (M) in the ovary of *C. marulius* at 40× magnification.	
Stage III (Vitellogenesis)	Vitellogenin (Vtl) stages of oocytes in the *C. marulius* ovary, 40× magnification.	

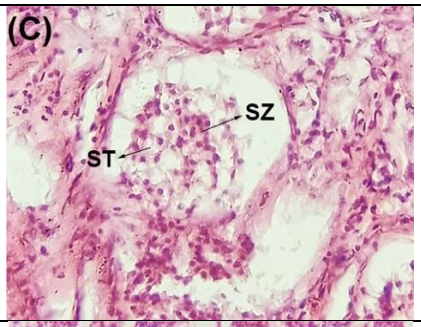
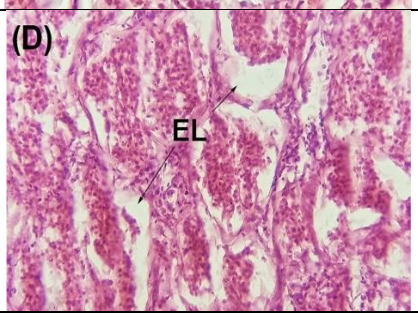
Stage IV (Mature)	Ovarian follicle (OF), yolk globule (YG), primary vitellogenic oocyte (PVO, migratory nucleus (N) showing a mature egg in the ovary of *C. marulius* at 40× magnification.	

Testicular maturity stages in snakehead

There are four stages of testis growth and development in *C. marulius,* illustrated in Table 4. Four stages of sperm development, namely, were revealed by a histological study of the testis. Spermatogonia (Fig. A), spermatocytes (Fig. B), spermatids and spermatozoa (Fig. C), empty lumen (Fig. D), and these are described as: Spermatogonia stage, being the primary spermatogenesis phase, is the largest germ cell in the testicles. It was spherical and prominently displayed the cell membrane. Spermatocytes stage, where spermatogonia were transformed through meiotic division into spermatocytes that were also spherical, with a central nucleus. Spermatocytes were smaller than spermatogonia in size. Spermatid stage, spermatids were produced by the spermatocytes. Spherical shapes, in which, because of their dark appearance, the nucleus was not seen under the microscope. Spermatozoa stage, under the microscope, spermatozoa appear as small black spots, the smallest cells of the spermatogenic lineage. These were the functional male gametes derived through the spermiogenesis process from spermatids. Empty lumen tubules are where few spermatozoa in sperm ducts and more spermatocytes may be observed.

Table 4. Testes of *C. marulius* at various stages of maturation over the 3-month study period.

Stages	Testes description	Histomorphology
Stage I (Immature)	There were very small testes, many spermatogonia (SG). (Fig. A) at 10× magnification.	
Stage II (Maturing)	Thinly scattered were also a few spermatocytes (SC) (Fig. B) at 10× magnification.	

Stage III (Mature)	In this stage, the testes were larger than in the immature phase. More abundant were Spermatids (ST). Testes showed a white, pinkish color. A large number of (SZ) spermatozoa were observed (Fig. C) at 10 × magnification.	
Stage IV (Spent)	There were darker and more opaque testes. Empty lumen of tubules (EL). (Fig. D) at 10 × magnification.	

DISCUSSION

In the current study, *Channa marulius* is an air-breathing fish, successfully spawned and experienced changes in its gonadal development in small experimental breeding ponds after receiving a single intramuscular injection of the synthetic hormones Suprefact® (LHRH) and Ovaprim® (GnRH + dopamine inhibitor). A very similar observation has been made by Nazir et al. (2022), who used a special combination of synthetic hormones Suprefact® (LHRH) and Ovaprim® (GnRH + dopamine inhibitor) in the case of *C. marulius.* Each pair of brooders was then transferred to blue-colored fiberglass drums after receiving the first hormonal dosages of Motilium-V mixed Suprefact (0.3, 0.4, and 0.5 ml for males and 0.8, 0.9, and 1.0 ml for females per kilogram body weight). The experimental fish were given a second dose of the second hormone (Ovaprim®) at the same concentration after 24 hours. *C. marulius* was spawned successfully after 48 hours, and fertilized eggs were moved with the help of plastic bowls into a circular cemented tank that had a gentle flow of aerated water, where eggs were incubated. Three different hormones, Conceptal®, Suprefact®, and Ovaprim®, were also used in the current study, without combination but with the same hormonal dosage concentrations (0.3, 0.4, and 0.5 ml for males and 0.8, 0.9, and 1.0 ml for females per kilogram body weight). From these three hormones, no induced spawning was observed with Conceptal®. However, these two separate studies showed that suprefact® and ovaprim® hormones were effective for the induced breeding of *Channa marulius*.

In our study, both the male and female gonadosomatic index (GSI), which indicates gonadal development and maturation, were higher and peaked in April. Gupta et al. (2013) observed normal fish ovarian development in *L. dyocheilus* kept in captivity and observed a similar trend in GSI. In addition to predicting the breeding season, GSI can also show a fish's maturity level and frequency of spawning (Khanna, 2003). In an additional study, Rinku et al. (2013) observed that *C. bleheri's* highest GSI value occurred between April and July. Similarly, Sunita et al. (2011) confirmed the maximum GSI value in the rainy season during May and August.

In the present study, fecundity rate, fertilization, and hatchability were observed to be higher in treatment T2 (Suprefact®) than in treatment T3 (Ovaprim®). Our finding is comparable to previous findings by Maradun et al. (2018), who observed a 72 to 88% fertilization rate in *C. gariepinus*. Rivastava et al. (2012) used Ovaprim® 1.0-2.0 ml kg^{-1} BW, where the doses were effective in Asian Catfish, *Clarias batrachus*. Another research observed that 2.5 $ml.kg^{-1}$ BW of females in *Mystus gulio* showed maximum ovulation and a hatching rate of 80% injected with Ovaprim® Mijkherjee et al., 2002). The spawning occurred in *Clarias batrachus* within 21-22 h after pituitary extract administration (Rahman et al., 2011) and 24 hours after ovaprim injection (Abdulraheem et al., 2012). While Mosha (2018) observed the highest fertilization rate of 87.34% in African

Catfish, *C. gariepinus,* with Ovaprim® induction.

The accuracy of a hormone's ability to induce ovulation and the production of spermatozoa is evaluated using an important parameter known as fertilization. The oocyte stages of *C. marulius* were reported according to the largest quantity of advanced oocytes in the ovary sections. Developmental stages of spermatogenic phases are spermatogonia, spermatocytes, spermatids, and spermatozoa. The evidence of spawning season and gonadal maturation can be seen through histological observation. The number of gonadal stages and sub-stages can vary depending on the gonadal development of each species and the different criteria each author uses. In the current study, the ovary and testes of *C. marulius* were studied under microscopes, and four stages of gonadal development were noted. Briefly, primary germ cells called oogonia and spermatogonia multiply through mitotic division to become previtellogenic oocytes and spermatocytes, which are unique to the immature ovary and testis, respectively. The development of the yolk in the oocyte signals the start of vitellogenesis, during which the cell reaches its maximum size, goes through maturation/ovulation, and then the egg is extruded to the outside. Proliferation, primary and secondary growth, and the maturation phase made up the process of egg and gonad maturation. Primary oocytes were still seen without yolk granules before the injection, indicating that the gonad was still in the previtellogenesis phase (Nurhidayat et al., 2017).

Conclusion

The hormones Suprefact® and Ovaprim® were both effective synthetic hormones that could be used as an appropriate spawning agent for *C. marulius,* for successful induced breeding and seed production. Thorough observation of histological analysis of the gonads, the current study, for the first time, documented gonadal development and sexual maturity of *C. marulius* raised in captivity. According to this study of the reproductive biology of *C. marulius,* the gamete maturation and the highest GSI value were seen in April with treatment T2 (Suprefact®) than treatment T3 (Ovaprim®). It is clear from the study's findings that a better understanding of the gonadal histological observation of *C. marulius* will aid in the development of conservation strategies and the maturation and rearing of this priceless species in captivity, and be appropriate for managing the fisheries sustainability and selectively breeding *C. marulius* in Pakistan's natural habitats.

REFERENCS

Dayal, R., Srivastava, P. P., Bhatnagar, A., Raizada, S., Chowdhary, S., Yadav, A. K., & Lakra, W. S. (2013). Captive spawning of the striped murrel, *Channa striatus* (Bloch) using sGnRH, in the Gangetic plains of India. *Proceedings of the National Academy of Sciences, India Section B: Biological Sciences*, *83*, 65-70. https://doi.org/10.1007/s40011-012-0089-y.

Marimuthu, K., Satthiyasilan, N., Rahman, M. A., Arshad, A., Raj, M. G., & Arockiaraj, J. (2015). Induced ovulation and spawning of African catfish *Clarias gariepinus* (Bloch) using Ovaprim. *Journal of Environment and Biotechnology Research*, *1*(1), 2-9.

Nazir, S., Khan, N., Azmat, H., Naveed, S., Ramzan, M. M., & Davies, S. J. (2023). Efficacy of various concentrations of synthetic hormones on the induced breeding of *Channa marulius* (Sole). *Journal of the World Aquaculture Society*, *54*(1), 143-155.

Biswas, A. K., Morita, T., Yoshizaki, G., Maita, M., & Takeuchi, T. (2005). Control of reproduction in Nile tilapia *Oreochromis niloticus* (L.) by photoperiod manipulation. *Aquaculture*, *243*(1-4), 229-239. https://doi.org/10.1016/j.aquaculture.2004.10.008.

Sarmento, N. L., Martins, E. F., Costa, D. C., Mattioli, C. C., da Costa Julio, G. S., Figueiredo, L. G., ... & Luz, R. K. (2018). Reproductive efficiency and egg and larvae quality of Nile tilapia fed different levels of vitamin C. *Aquaculture*, *482*, 96-102. https://doi.org/10.1016/j.aquaculture.2017.08.035.

Clarke, A. (1993). Egg size and egg composition in polar shrimps (Caridea; Decapoda). *Journal of Experimental Marine Biology and Ecology*, *168*(2), 189-203. https://doi.org/10.1016/0022-0981(93)90259-Q.

Marimuthu, K., Haniffa, M. A., Muruganandam, M., & Raj, A. A. (2001). Low-cost murrel seed production technique for fish farmers. *Naga*, *24*(1), 21-22.

Srivastav, S. K., & Srivastav, A. K. (1998). Annual changes in serum calcium and inorganic

phosphate levels and correlation with gonadal status of a freshwater murrel, *Channa punctatus* (Bloch). *Brazilian*

Al Mahmud, N., Rahman, H. M. H., Mostakim, G. M., Khan, M. G. Q., Shahjahan, M., Lucky, N. S., & Islam, M. S. (2016). Cyclic variations of gonad development of an air-breathing fish, *Channa striata,* in the lentic and lotic environments. *Fisheries and Aquatic Sciences*, *19*, 1-7.

Ghaedi, A., Kabir, M. A., & Hashim, R. (2014). Effect of lipid levels on the reproductive performance of Snakehead Murrel, *Channa striatus*. *Aquaculture Research,* p 1-9.

Sturm, M. D. L. (1978). Aspects of the biology of *Scomberomorus maculatus* (Mitchill) in Trinidad. *Journal of Fish Biology*, *13*(2), 155-172.

Navarro, E., Iglesias, J. I. P., & Larranaga, A. (1989). Interannual variation in the reproductive cycle and biochemical composition of the cockle Cerastoderma edule from Mundaca Estuary (Biscay, North Spain). *Marine Biology*, *101*, 503-511.

Morton, B. (1990). The life cycle and sexual strategy of *Gafrarium pectinatum* (Bivalvia: Veneridae) in a Hong Kong mangrove. *Malacological review*, *23*(1-2), 53-62.

Gupta, M., Pandey, N. N., Tiwari, V. K., Singh, R., Prakash, C., & Chaturvedi, C. S. (2013). Study of Gonadosomatic Index and fecundity of pond raised Labeo dyocheilus in cold water conditions. *International Journal of Advanced Research*, *1*(7), 137-140.

Khanna SS. (2003) Reproduction and development. In: An introduction to fishes. Central Book Depot, Allahabad, India. p. 280-283.

Rinku, G., Behera, S., Bibha, C. B., & Sonmoina, B. (2013). Sexual dimorphism and gonadal development of a rare murrel species, *Channa bleheri* (Bleher), in Assam. *The Bioscan*, *8*(4), 1265-9.

Sunita, K., Kulkarni, K. M., Gijare, S. S., & Tantarpale, V. T. (2011). Seasonal changes of the gonadosomatic index observed in the freshwater fish *Channa punctatus*. *Bios*, *6*(4), 571-3.

Maradun, H. F., Umar, F., Ibrahim, A., Mubarak, A., Zarau, I. J., & Muhammad, S. A. (2018). Effect of different doses of Ovulin hormone on the induced breeding performance of Clarias. *J Anim Vet Sci*, *5*(1), 1-5.

Srivastava, P. P., Raizada, S., Dayal, R., Chowdhary, S., Lakra, W. S., Yadav, A. K., ... & Gupta, J. (2012). Breeding and larval rearing of Asian catfish, Clarias batrachus (Linnaeus, 1758) on live and artificial feed. *Journal of Aquaculture Research and Development*, *3*(4), 1000134.

Mijkherjee, M., Praharaj, A., & Das, S. (2002). Conservation of endangered fish stocks through artificial propagation and larval rearing techniques in West Bengal, India. *Aquaculture Asia*, *7*(2), 8-11.

Rahman, S. M., Habib, M. A., Hossain, Q. Z., Siddiqui, M. N., Rahman, M. M., & Ahsan, M. N. (2011). Embryonic development of Clarias batrachus under the influence of aeration and water flow. *Ecoprint: An International Journal of Ecology*, *18*, 25-31.

Abdulraheem, I., Otubusin, S. O., Agbebi, O. T., Olowofeso, O., Adeyemi, K. A., & Ashley-Dejo, S. S. (2012). Induced breeding of African catfish (*Clarias gariepinus*) under varying broodstock ratios. *Global Journal of Science Frontier Research Agriculture and Veterinary Sciences*, *12*(8), 53â.

Mosha, S. S. (2018). Recent comparative studies on the performance and survival rate of African catfish (*Clarias gariepinus*) larval produced under natural and synthetics hormones: A review. *Journal of Aquaculture Research and Development*, *9*(3), 1-6.

Nurhidayat, L., Arfiani, F.N., & Retnoaji, B. (2017). Gonadosomatic index and histological structure of uceng fish gonads (*Nemacheilus fasciatus*, Valenciennes in Cuvier and Valenciennes, 1846). *Biosfera*, 34(2), 67-74. doi.org/10.20884/ 1.mib.2017.34.2.456.