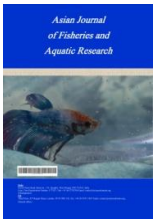

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Induced Spawning of the Fat Snook, *Centropomus parallelus* Poey, 1860 (Perciformes: Centropomidae), via the Application of the Gonadotropin-Releasing Hormone (GnRH)

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Authors' contributions

This work was carried out in collaboration among all authors. Author DCL designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors RCG and EAZM managed the analyses of the study. Author EAZM managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The fat snook (*Centropomus parallelus*), a catadromous species highly valued for human consumption, is widely distributed along the Atlantic coast of the American continent, from the United States to Brazil. In Mexico, it is common in the waters off the coastal states of the Gulf of Mexico, particularly Tamaulipas, Veracruz, Tabasco and Campeche. As it is important to ensure maturation and reproduction during cultivation under controlled conditions, given that this is inhibited in captivity, the spawning of the *Centropomus parallelus* was induced in the present study using a dose of gonadotropin-releasing hormone (GnRH).
Advanced gonad maturity was observed, while two females in a vitellogenic state reached final maturation with the application of a 50 µg/kg dose of the hormone and the male subjects failed to present spermiation under the application of the same dose.

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1. INTRODUCTION

The genus *Centropomus*, whose 12 species are all present in Mexico, is found on both coasts of the American continent, including the islands of West Indies and Galapagos, where this genus is the sole representative of the family [1]. The fat snook (*Centropomus parallelus*) (Poey, 1860) is a catadromous species widely distributed along the Atlantic coast of the American continent, from the south of the United States to southern Brazil [2]. In Mexico, it is common in the coastal states of the Gulf of Mexico, particularly in Tamaulipas, Veracruz, Tabasco, and Campeche [3,4].

The fat snook is one of the fish species most highly valued by both sports and artisanal fishing, due to its flavor and the texture of its meat [5].

It is found in coastal areas, bays, estuaries, brackish lagoons, freshwater environments and occasionally, in hyper-saline lagoons, using saltwater for reproduction, with juveniles then migrating to brackish waters or freshwater. Its prey comprises fish and crustaceans [1].

The induction of reproduction via the use of hormones has guaranteed the mass production of marine fish larvae [6], while the treatments used depend on many factors, such as species, cost, availability, and facilities for reproducers. The following are the main hormonal treatments involved in the induction of fish reproduction: human chorionic gonadotropin (hCG); luteinizing hormone-releasing hormone (LHRH, also known as GnRH); gonadotropin hormone (GtH); pituitary extract; and, gonadal steroids [7].

The use of GnRH factors began in the 1970s with injection techniques and, from the 1980s onwards, continued via the use of implant techniques [8].

Of the little research conducted in Mexico on the family Centropomidae, the most notable have been studies on reproductive and populational dynamics, which have found that the length of females is greater than that of males. Moreover, of the organisms analyzed, males comprised 69% and females 31%, giving a male-to-female ratio of approximately 2.2:1 [9].

Evaluation of the growth of *Centropomus undecimalis* and *C. parallelus* indicates that the speed of growth in terms of length is similar for both species, while, in terms of weight, the fat snook grows more quickly than the robalo [3].

Studies on reproduction in captivity have focused on the induction of maturation and spawning, using hormones such as LHRHa and GCH and obtaining favorable results, such as advanced gonad maturity and, even, spawning [10,7].

Among those studies on *C. parallelus*, some describe embryonic development and the first larval stages, observing that eggs of this species hatch over a 20-hour period [2]. One study described the reproductive variation of *C. parallelus* using oocyte diameter as an indicator for the application of hormonal treatments and the attainment of spawning, reporting that ideal oocyte size is between 300 and 400 microns [4].

At a temperature of 25°C, salinity of 29 to 35 PSU (practical salinity units), and using human chorionic gonadotropin, Godinho et al. [11] obtained a fertilization rate of 70 to 90% [10]. Another study obtained ovulation in 70% of female subjects 40 hours after a 1500 U.I HCG injection for females and a 500-1500 U.I injection for males [12].

2. MATERIALS AND METHODS

Twenty reproducers (Table 1) were captured during the night on the coast of the port of Tuxpan, Veracruz, using a *chinchorro* net and were transported to the aquatic bioassay facility of the Faculty of Biological and Agricultural and Livestock Sciences, Poza Rica-Tuxpan Region, at the University of Veracruz. On arrival, the individuals were then placed in five 1000-liter tanks filled with seawater that had been acclimatized via oxygenation for 20 days.

The temperature, salinity, dissolved oxygen, and pH were measured every four days, while ammonium ion (NH_3) and ammonium (NH_4+) levels were taken every 16 days.

The reproducers were fed fresh shrimp at a proportion of 2% of the total biomass in each tank, once a day.

After 20 days, sexing and biometric measurements were undertaken, with a total of 16 reproducers identified, given that four died during this period.

Table 1. Morphometric data for the *C. parallelus* examples captured

Females		Males	
Length	**Weight**	**Length**	**Weight**
40 cm	473 gr	30 cm	226 gr
		29 cm	224 gr
38 cm	450 gr	27 cm	213 gr
		29 cm	222 gr
36 cm	395 gr	26 cm	182 gr
		29 cm	224 gr
38 cm	450 gr	27 cm	213 gr
		27 cm	204 gr

The separation of males and females was carried out via cannulation, with the individuals then distributed in each tank at a ratio two males per female.

Or,

The males and females were identified via cannulation, with the individuals separated and then distributed in each tank at a ratio two males per female.

In order to ascertain the state of maturity of the reproducers, they were anesthetized with clove oil and cannulated in accordance with Gómez [7] for the females and with Barbieri et al [13] for the males.

The hormone used was GnRH, in the 20 ml presentation known commercially as GONASYL. The dose used was 50 µg/kg of the weight of each reproducer, converted by dividing the weight of each fish by 1000 in order to obtain the quantity of hormone to be used in milliliters. The injections were applied intraperitoneally under the lateral fin of each of the organisms, with four males and two females injected and then placed in the tanks, at a proportion of two males to one female. Four females and two males were selected as a control and were not injected. The state of gonad maturity was ascertained both before and after the injection, via cannulation.

3. RESULTS

While, after 30 hours, null spawning was observed, a change in the coloration of the genital papilla of the females injected was observed, becoming turgid and pink in color. As an increase in the diameter of the oocytes of the females injected with GnRH was also noted, transverse histological sections were taken and the gonads then subjected to the hematoxylin-eosin stain technique.

While the two females injected attained a state of final maturation, they were not able to progress to the ovulation stage (Fig. 1), while those organisms that were not injected did not present any advance in terms of gonad maturity.

Four stages of testicle development were found in the males, corresponding to spermatogonia, spermatids, spermatocytes, and spermatozoids (Fig. 2).

Due to the procedures of capture and manipulation of the organisms I cause them stress and decreased their weight (Fig. 3).

The temperature ranged from 26 to 28°C, salinity from 33 to 34 PSU, and pH from 7 to 9, while ammonium remained at 2 mg/l., which is within the range reported for the reproduction of *Centropomus parallelus*.

4. DISCUSSION

The lack of maturation and spawning in fish in captivity mainly results from a physiological alteration in the hypothalamo-pituitary-ovarian (HPO) axis [10]. Gonadotropin levels increase as the breeding season approaches, although the hormone, which is responsible for stimulating the beginning and normal development of oocyte growth and maturation (a process inhibited by a lack of gonadotropin), is not released into circulation. Said process can, therefore, be corrected and accelerated via the administration of hormones which either stimulate the release of the individual's own gonadotropins or simply replace them [7].

The completion of vitellogenesis triggers the beginning of the final maturation of oocytes and is characterized by the migration of the nucleus to the animal pole and, physiologically, represents the restart of meiosis. Moreover, it is stimulated by the increased concentration of LH gonadotropin circulating in the blood. The

identification of gonads in a vitellogenic state is important for aquaculture, as the maturation, ovulation, and spawning of oocytes are generally induced from this state via the application of hormones such as GCH, GnRH, LHRH, or their analogs.

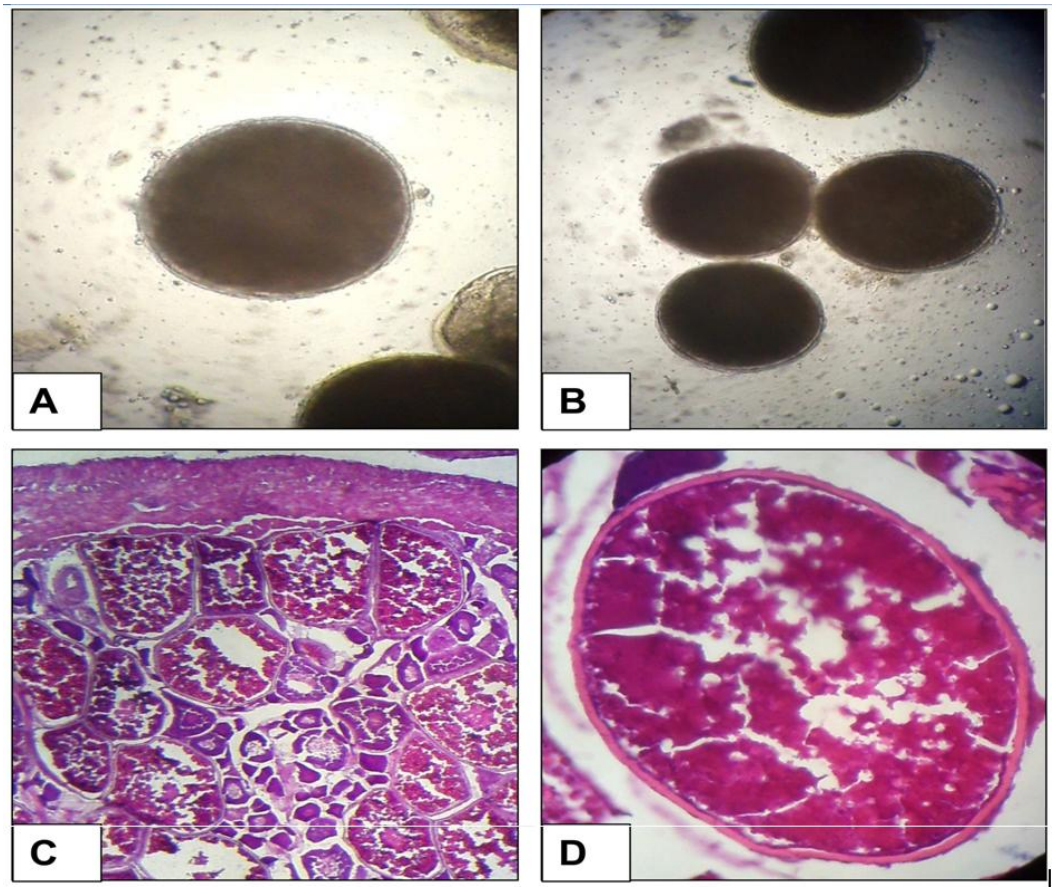

Fig. 1. Vitellogenic oocytes of female 2 before (A) and after (B) the application of the injection, while (C) and (D) show the oocytes in a state of final maturation

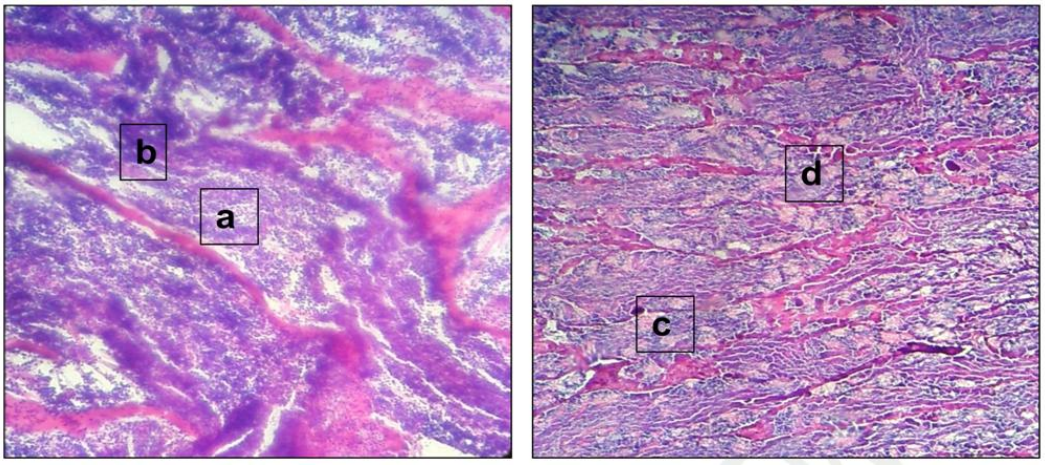

Fig. 2. Histology of *Centropomus parallelus* testicles, where (a) shows the spermatozoids, (b) the spermatocytes, (c) the spermatogonia and (d) the spermatids

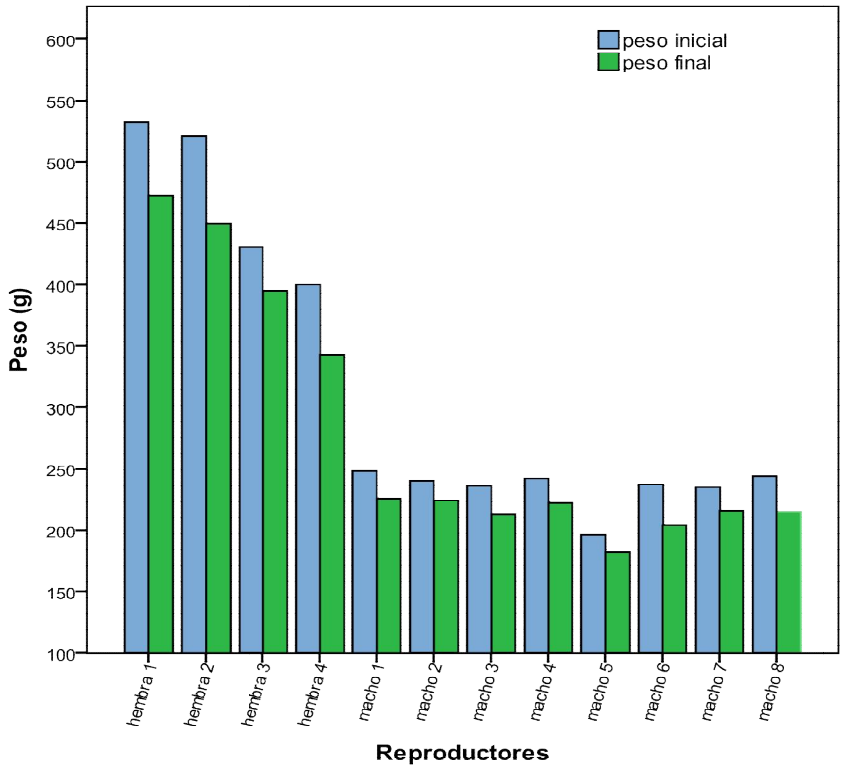

Fig. 3. Initial and final weight of the reproducers on completion of the bioassays

The maturation process of the oocytes, taken from the females and ina vitellogenic state, began after the application of the GnRH hormone, with oocytes in a state of final maturation found 26 hours post-application in the females injected. It was observed that the migration of the germinal vesicle had occurred in the oocytes, as well as the fusion of the lipid droplets and, even, that some oocytes had completed the maturation process. Further to inducing maturation, this hormone stimulates the development of oocytes, which were observed from an initial perinuclear stage or primary vitellogenic phase up to maturity, following the same pattern of development described by Carvajal [10] for *Centropomus nigrescens*. Álvarez and Hernández [8] state that *Centropomus parallelus* is found within the group of species which present asynchronous ovarian development and that spawning is induced in males via the application of a 50-microgram dose. This result is distinct to the present study, in which spawning was not achieved and spermiation was not observed on the application of abdominal pressure [2].

The GnRH or LHRH doses used in the various bioassays are similar to those used successfully in other fish species. For example, Carvajal [10] was able to induce maturation with the hormone LHRHa at doses of 97.6 to 106.2 mg/Kg-1, achieving spawning using the hormone HCG at a dose of 1.000 U.I. HCG.Kg-1 of reproducer weight in *Centropomus nigrescens*. Gómez [7] induced gonad maturity and obtained spawning using the hormone GCH at a dose of 500 UI/kg in *Centropomus undecimalis*, while Álvarez et al. [2] induced spawning in *Centropomus parallelus* with injections at a dose of 50 micrograms per kilogram.

The inefficacy of GnRH in stimulating spawning in this species may be due to insufficient doses of the hormone or that its physiological effects were neutralized due to the stress caused by captivity, as reported by Gómez [7], for *Centropomus undecimalis*, and Cerqueira [14], for *Centropomus parallelus*.

The correct diameter for oocytes to be injected with hormones is 300 microns, at which diameter advances in sexual maturity and, even, spawning, can be obtained, as described by Contreras et al. [4]. For this reason, the female subjects in the present study were injected at lesser diameters, at which an advanced state of

maturity could be observed with the application of the hormone, although spawning was not achieved.

On the application of a simple hormone injection into the reproducers, the half life of GnRH or its duration in the fish's circulatory system is short, for which reason the treatment may not be capable of inducing the maturation and ovulation of those oocytes yet to undergo vitellogenesis. Carvajal [10] states that spawning is best induced when oocytes are in a vitellogenic state.

In terms of reproducer weight, Gómez [7] reports negative effects due to stress in captivity, such as appetite loss and erratic feeding, which cause weight loss, a finding similar to that of the present study, in that, by the end of the bioassays, the final weight of the organisms was lower than that at the start, causing the death of the reproducers. The parameters of temperature and salinity were within the ranges acceptable for the reproduction at 27 and 28°C reported for *Centropomus parallelus* by Álvarez and Hernández [8], as were the oxygen parameters at values between 5 and 7 mg/l. Gómez [7] reports, for *Centropomus undecimalis*, values above 3.5 mg/l, results similar to those obtained in the present study. The pH values were also acceptable, within a range of 7.3 to 8.6. The values for ammonium (NH_4) and ammonia (NH_3) remained acceptable, in that they did not rise above 2 mg/l, with Álvarez and Hernández [8] describing levels over 3.4 mg/l as toxic for the organisms.

5. CONCLUSIONS

The hormone GnRH, at injected doses of 50 µg/kg, had a positive effect in promoting the advance of gonad maturity in *Centropomus parallelus*.

In the present study, those oocytes obtained from *Centropomus parallelus* females injected with the hormone GnRH and in a vitellogenic state did advance to a state of final maturation, while those females not injected did not present any advance in terms of their gonad maturity.

As the fat snook is a species sensitive to handling and confinement in captivity, the correct use of handling techniques should be emphasized during the application of hormones that stimulate reproduction.

As gonad development in females is asynchronous by group, oocytes can be found in different states of maturation.

While maturation and spawning may be induced in both females and males recently captured in the wild, successful results require that they are acclimatized over a longer period of time, above all in terms of diet. As the males did not present spermiation on the application of abdominal pressure, the hormone did not have an effect on the promotion of gonad maturity, making it recommendable to conduct experiments with higher doses.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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