ECOSISTEMAS Y RECURSOS AGROPECUARIOS

INDUCED SPAWNING OF THE COMMON SNOOK (*Centropomus undecimalis*) IN CAPTIVITY USING GnRH-a IMPLANTS

Inducción de la reproducción del robalo blanco (*Centropomus undecimalis*) en cautiverio usando implantes de GnRH-a

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ABSTRACT. Culture of *Centropomus undecimalis* shows great potential as this species tolerates handling and adapts easily to captivity. However, the difficulty in achieving spawning in captivity is a major obstacle for the development of commercial scale farming. Spawning of common snook was achieved using GnRH-a implants in single 100 and 200 μg doses per fish; control group specimens received no hormone and did not spawn. Both GnRH-a trial doses resulted in spawning with up to 100 % fertilization rates per experimental unit, and a range of 60 - 76 % per treatment, showing no statistical differences ($p > 0.05$). The percentage of hatching rate was between 50 - 100 % and larvae measured between 1.56 $\pm$ 0.08 and 1.98 $\pm$ 0.05 mm total length after yolk sac absorption.
Key words: *Centropomus undecimalis*, snook, induction to spawning, GnRH-a, quality eggs

RESUMEN. El cultivo del robalo blanco (*Centropomus undecimalis*) muestra gran potencial debido a que la especie tolera la manipulación y se adapta fácilmente al cautiverio. La dificultad de obtener desoves en cautiverio es el mayor obstáculo para el desarrollo del cultivo a escala comercial. Se indujo el desove de robalo blanco en cautiverio, usando implantes de 100 y 200 μg de GnRH-a/pez en dosis únicas y un grupo control que no recibió hormona. Con ambas dosis probadas de GnRH-a se obtuvieron desoves con porcentajes de fertilización de hasta 100 % con un rango de 60 a 76 % por tratamiento , sin presentar diferencias estadísticamente significativas ($p > 0.05$). La eclosión obtenida estuvo entre 50 y 100 % ($p < 0.05$), con larvas con tallas entre 1.56 $\pm$ 0.08 y 1.98 $\pm$ 0.05 mm.
Palabras clave: *Centropomus undecimalis*, robalo, inducción al desove, GnRH-a, calidad de huevos

INTRODUCTION

The common snook, *Centropomus undecimalis*, is a tropical euryhaline fish with carnivorous feeding habits greatly appreciated by anglers in the Gulf of Mexico, because of its high quality flesh and high value in local and regional markets (Rivas 1986, Taylor *et al.* 2000). This situation has developed fishing pressure on the wild stocks, to the point of being considered as potentially over-exploited (Perera-García *et al.* 2008). To overcome this, aquacultural practices offer an alternative by means of inland grow-out and juvenile production for re-stocking. The culture of *C. undecimalis* has great potential since this species tolerates handling, adapts easily to captivity and readily accepts artificial feeds (Sánchez-Zamora *et al.* 2003). However, difficulty in achieving spawning in captivity is a major obstacle for the development of commercial scale farming. Álvarez-Lajonchere and Hernández-Molejón (2001), recommended the use of hormone implants that allow the fish to slowly produce and release endogenous hormones thereby inducing ovulation and spawning. More recent studies have significantly improved fertilization and hatching rates, with experiments reporting survival up to juvenile

stages (Ibarra-Castro *et al.* 2011). However, low fertilization, hatching and survival rates are still a constant. More research is needed to validate the methods proposed, and determine their feasibility under low-tech conditions at UJAT. In the present study, we evaluated the effect of hormonal implants that provide a gradual release of the hormone in the intent of determining if GnRH-a dosages can be successfully used at a "per fish" basis, independently of its body weight.

MATERIALS AND METHODS

Capture and maintenance of broodstock

Wild caught common snook adults were adapted to captivity and maintained for three years. Nine females with size ranging from 60 to 91 cm total length and weight from 1.89 to 5.29 kg and 18 males with size ranging from 61 to 83 cm and weight from 1.80 to 3.88 kg were used in our implant trials. Fish were maintained in the Marine Aquaculture Station of the Biological Sciences Academic Division (DACBiol) at the Juarez Autonomous University of Tabasco (UJAT), located in Jalapita, Centla, Tabasco, Mexico. Acclimation of broodstock to captivity was carried out in a circular 25 m^3 polyvinyl chloride (PVC)-lined tanks, where the fish were fed to satiation every other day with live food (Clupeidae) of marine origin and supplemented with Breed M-INVE® (to satiation) three times a week.

Experimental design

A randomized block (replication date) design was used with three treatments (0, 100 and 200 μg GnRH-a $fish^{-1}$). Due to the lack of spawning tanks, only one replicate from each treatment was included in every run. A replicate consisted of one female and two males per tank. A total of three replicates were included for each treatment, allowing one week between replicates.

Induced spawning of *C. undecimalis* using GnRH-a implants

For this experiment, the methodology described by Álvarez-Lajonchere and Hernández-Molejón (2001) was employed. Three circular 12.5 m^3 PVC-lined tanks, connected to a recirculation system were used as experimental units for spawning. Eggs were collected in a 100 L cylinder conical tank containing a collecting bag with a 400 μ m mesh. To assess oocyte maturation, female snook were anesthetized in 50 mg L^{-1} of tricaine methane sulfonate (MS-222®). Oocyte samples were obtained by intra-ovarian cannulation employing an F5 polythene feeding tube (1.65 mm internal diameter). Oocytes were examined under a stereomicroscope (Zeiss®) to measure diameters. Females with oocyte diameters > 300 μm were selected for induction. One female and two males were placed in each experimental tank and a pellet containing GnRH-a (Argent Labs®) was implanted into each female at the corresponding experimental dose (0, 100 or 200 μg $fish^{-1}$) (n=3/treatment). Only males releasing fluid sperm were used; however, all males were implanted with 100 μg of GnRH-a/fish to increase fluency. The implants were inserted into the peritoneal cavity (approximately 1 cm away from the base of the left pectoral fin) using an AVID^{TM} syringe applicator. Gentamicin antibacterial cream was applied at the incision point, to reduce infections (Álvarez-Lajonchere and Hernández-Molejón 2001). Dissolved oxygen, temperature (DO-meter YSI55®) and salinity (Refractometer SR6 Vital Sine Premium®) were monitored and 50 % of water was exchanged daily. The average temperature, dissolved oxygen and salinity were 30.65 $\pm$ 0.45 ^oC; 7.24 $\pm$ 0.53 mg L^{-1}, and 27.5 $\pm$ 4.36 UPS, respectively.

Treatment effectiveness evaluation

Treatments were considered effective if spawning occurred, while the variables used to measure spawning quality were based on the techniques of Álvarez-Lajonchere and Hernández-Molejón (2001). Eggs were collected from overflow using mesh bags of 300 μm, and were placed in 40 L plastic buckets containing water with the same salinity and temperature as the spawning tanks and were gently aerated. The number of eggs from

each spawning event was estimated volumetrically counting three 1 mL sub-samples, taken from three 50 mL samples (n = 9). Fertilization rate was determined by presence or absence of embryonic development in three samples of 100 eggs randomly selected from each experimental tank. Eggs were analyzed and counted under a stereoscopic microscope. The following parameters were measured to determine egg quality, egg diameter, total number of eggs per spawning event, percentage of fertilized eggs, percentage hatched and length of larvae. Egg diameter was measured with a calibrated ocular micrometer under a stereomicroscope from a randomly selected sample of 100 eggs from each spawning. All viable eggs from each spawn were maintained in the spawning tank and percent hatching was determined. Total number of larvae was estimated volumetrically. Hatched larvae were maintained in 200 L white plastic tanks, 53 $\times$ 45 $\times$ 44 cm at 31.10 $\pm$ 0.62 °C; 5.49 $\pm$ 0.87 mg L^{-1}; and 24.1 $\pm$ 3.92 UPS until first feeding. The lengths of 100 larvae per experimental tank were measured when the yolk sac was absorbed.

Statistical analysis

To determine differences between experimental hormone doses, analysis of covariance (ANCOVA) was used, considering diameter of fertilized eggs as the response variable; replication date, weight of females, and mean initial oocyte diameter were included as covariables. A Chi-square analysis was used to determine differences in percent fertilization and percent hatch. For number of larvae and larval size, statistical analyses were not conducted because only one replicate (out of two) per treatment hatched. All analyses were performed using the STATGRAPHICS^{TM} V 5.1 statistical package. For all analyses, statistically significant differences were considered when $p < 0.05$.

RESULTS AND DISCUSSION

Evaluation of treatment effectiveness

The use of GnRH-a implants in *C. undecimalis* resulted in successful spawning and the production of good quality larvae. After implantation, common snook that received GnRH-a performed courtship behaviors including swimming in circles and spiraling to the surface, and returning to the bottom of the tank to repeat this behavior. Four fish implanted with GnRH-a spawned, on average, 27 ($\pm$ 1.63) hours post implantation. Induction was achieved with both experimental doses using females with an average initial oocyte diameter of 333.00 $\pm$ 54.08 μm, at salinities between 21 and 30 UPS. Two females treated with 100 and two with 200 μg GnRH-a $fish^{-1}$ (66 % efficiency) spawned; no spawning was observed in the control group. The use of standardized dosages of GnRH-a per fish (100 and 200 μg $fish^{-1}$) instead of dosages per Kg of female provided spawning with viable eggs. Fish spawned when the dose of GnRH-a was as low as 19.4 μg Kg^{-1}, or as high as 105.5 μg Kg^{-1} of female. Spawning episodes achieved in our study coincide with the reproductive season reported for this species by Taylor *et al.* (1998) on the coasts of Florida, and by Perera-García *et al.* (2008) on the coasts of Tabasco, indicating that captive common snook through hormonal stimulation complete oogenesis in the same period as wild fish. The use of standardized dosages of GnRH-a per fish (100 and 200 μg $fish^{-1}$) instead of dosages per Kg of female provided an optional protocol for inducing spawning in adult snook, resulting advantageous when implants have to be made *in situ*, since availability of commercial implants can be problematic in some places outside of the United States. The results obtained in our study agree with other studies performed using captive common snook, in which viable larvae were produced using implants (Soligo *et al.* 2008, Ibarra-Castro *et al.* 2011, Rodhy *et al.* 2014). In the mentioned studies, the effective dose varied due to the type of application and the weight of the specimen.

The results obtained for reproductive performance are encouraging for snook aquaculture. Fecundity values, egg size, fertilization and hatching rates are high for both dosages of GnRH-a used.

Significant differences were observed for the

Table 1. Data obtained from females induced to spawn using GnRH-a (100 and 200 μg fish^{-1}) and controls (0 μg fish^{-1}).

Table 1. Datos obtenidos de hembras inducidas a desovar usando GnRH-a (100 and 200 μg pez^{-1}) y controles (0 μg pez^{-1}).

Treatment (μg female^{-1})	Female Weight (Kg)	Oocyte Diameter at cannulation (μ)	Dosage (μg Kg^{-1})	Temperature (^{o}C)	Salinity (UPS)
0	6.350	ND	0	31.0	30
0	4.780	375	0	31.0	29
0	2.976	365	0	31.0	27
100	4.720	ND	21.18	30.6	30
100	5.156	310	19.39	30.3	29
100	3.282	360	30.46	30.0	21
200	4.755	ND	42.06	30.7	30
200	1.896	366	105.48	31.0	29
200	5.290	230	37.80	30.9	29

ND= Non-detectable at sampling; NS= No spawning occur; NH= No Hatching; *hatching rate = 50; **hatching rate = 100 %.

Table 1. Continue.

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Number of eggs (millions)	Relative Fecundity (million eggs Kg^{-1})	Egg Diameter at spawning (μ)	Number of larvae (millions)	Size of larvae (mm)
NS	-	-	-	-
NS	-	-	-	-
NS	-	-	-	-
NS	-	-	-	-
2.88	0.55	705	0.54*	1.56
1.08	0.32	658	NH	NH
NS	-	-	-	-
1.82	0.95	743	NH	NH
3.11	0.58	750	1.80**	1.98

ND= Non-detectable at sampling; NS= No spawning occur; NH= No Hatching; *hatching rate = 50; **hatching rate = 100 %.

fertilization rate, which was higher (X2; $p < 0.01$) for the 200 μg GnRH-a fish^{-1} dose (76.84 %) as compared to the 100 μg GnRH-a fish^{-1} dose (60.47 %). Similarly, there were highly significant differences for hatching rates between hormone doses (X2; $p < 0.001$; Table 1); the hatching rate was 100 % for the 200 μg GnRH-a fish^{-1} treatment and was 50 $\pm$ 60.10 % for the 100 μg GnRH-a fish^{-1} treatment. On the other hand, we found no differences (ANCOVA; $p > 0.05$) in the number of eggs produced per female between 100 and 200 μg GnRH-a fish^{-1} treatments (1.98 $\pm$ 1.27 and 2.46 $\pm$ 0.93 million eggs, respectively; Table 1). Fertilized egg diameter was similar between implant doses and the weight of females and initial (pre-implant) oocyte diameter had no effects on the size of the egg (ANCOVA; $p > 0.05$). Although not statistically significant, the 200 μg GnRH-a fish^{-1} dose resulted in slightly larger eggs (746.50 $\pm$ 2.12 μm) than did the 100 μg GnRH-a fish^{-1} dose (681.50 $\pm$ 23.50 μm). Similar fertilization results have been reported for the same species by Ibarra-Castro *et al.* (2011) when using dosages based on the weight of the fish and only females that had oocytes larger than 400 μm during cannulation and for *C. poeyi* by Contreras-Sanchez *et al.* (2014). This suggests that

the use of GnRH-a provides a consistent stimulus and probably more effective integration of spawning with other physiological functions, thereby directly or indirectly affecting release of the necessary hormones for successful final oocyte maturation, spermiation and spawning (Zohar and Mylonas 2001). On top of these reassuring results, other studies report that Centropomids can be induced subsequently in the same spawning season (Almendras *et al*. 1988).

Of all the females that spawned, the larvae of only one female from each hormone treatment survived the first feeding. A total of 540,000 larvae were obtained from the 100 μg GnRH-a fish^{-1} dose and 3.1 million larvae from the 200 μg GnRH-a fish^{-1} dose. The larvae from the 200 μg GnRH-a fish^{-1} dose on average measured 1.56 $\pm$ 0.09 mm TL while those from the 100 μg GnRH-a fish^{-1} dose averaged 1.98 $\pm$ 0.05 mm TL (Table 1). These numbers are much lower than those obtained for the Asian snook, but it is well known that the Asian snook has a high relative fecundity (0.6 - 2.3 x 106 eggs Kg^{-1} of body weight) and larger size (Garrett 1987).

The use of GnRH analogs has become a common practice in marine aquaculture. A variety of species is currently induced to spawn in captivity and doses vary according to the species used. Positive results have been achieved with the Pacific Red Snapper; the shi drum, *Umbrina cirrosa* (Mylonas *et al*. 2004a, Basaran *et al*. 2009); the greater amberjack, *Seriola dumerili* (Mylonas *et al*. 2004b); the dusky grouper, *Epinephelus marginatus* (Marino *et al*. 2003); the spotted rose snapper, *Lutjanus guttatus* (Ibarra-Castro and Álvarez-Lajonchere 2009) and the wreckfish, *Polyprion americanus* (Fauvel *et al*. 2008), among other species. In our laboratory we have observed similar results with other species of snooks (*C. parallelus* and *C. Poeyi*), indicating that fish respond quickly under good water quality conditions and adequate feeding. However, the time of spawning after induction and the reproductive performance varies widely according to the species and the geographic location where the study is conducted (Garret 1986).

Broodstock maintained in captivity for several years with optimal feeding and handling conditions ensure the effect of GnRH-a implants, completing final maturation of oocytes even if the latter's diameters are smaller than those recommended for the Centropomidae family before treatment (Álvarez-Lajonchere and Hernández-Molejón 2001). The use of small doses of GnRH-a can be effective inducing maturation and spawning in snook species in captivity, providing viable larvae.

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