REPRODUCTION OF HATCHERY PRODUCED (F1 GENERATION) GREATER AMBERJACK (*Seriola dumerili*) USING GONADOTROPIN-RELEASING HORMONE AGONIST (GnRHa) IMPLANTS

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Introduction

The greater amberjack (*Seriola dumerili*) is a species with high potential for the aquaculture due to its excellent flesh quality, worldwide market availability and high consumer acceptability. However, the industrial production is still negligible as a result of several bottlenecks. One of the most important is the absence of reliable reproduction because of the reproductive dysfunctions that are observed in captivity. Agonists of gonadotropin-releasing hormone (GnRHa) have been used to overcome these problems in several species. The present study shows the results of an effective hormonal spawning induction method using GnRHa delivery systems on the reproduction of hatchery-produced greater amberjack broodstock (F1 generation).

Materials and methods

A group of 15 greater amberjack, which were born in captivity (average weight of 18.5±9.2 kg), was maintained in an outdoor covered raceway tank of 500 m^3 with continuous water supply (6 renewals day^{-1}) under natural photoperiod in the facilities of the Instituto Español de Oceanografía in the Canary Islands, Spain. The broodstock (7 males, 7 females and 1 of undetermined sex PIT (Passive Integrated Transponder) tagged fish) were sampled four times during 2015 spawning season (May, June, July and September) and biometric parameters of length and body weight were measured. Ovarian biopsies for the evaluation of oocyte development were obtained and a wet mount of the biopsy was examined under a compound microscope to evaluate the stage of oogenesis and measure the mean diameter of the largest, most advanced vitellogenic oocytes (n = 10). A portion of the biopsy was fixed in a solution of 4% formaldehyde-1% glutaraldehyde for further histological processing. Maturation of the males was examined by the release of sperm upon application of gentle abdominal pressure. If this was not possible, a sperm sample was obtained by inserting a plastic catheter. The collected sperm was stored (4°C) until quality evaluation. Blood was collected at each sampling from the caudal vessels using heparinized syringes, in order to measure sex steroid hormone concentrations. Blood was centrifuged at 1400 rpm for 20 min and plasma was frozen in liquid nitrogen and stored at –80 °C until hormonal and biochemical analysis.

Table I. Treatment and spawning starting date (Treat. (Sp.), duration (Dur.), number of spawnings (Sp.), number of eggs (Eggs), number of eggs per spawning (Eggs/sp), fertilization (Fertilizat.) and hatching rates in each period after hormonal treatment with GnRHa implants.

Treat. (Sp.) (date)	Dur. (day)	Sp. (n°)	Eggs ($x10^6$)	Eggs/sp ($x10^4$)	Fertilizat. (%)	Hatching (%)
13 (15) May	31	29	7.1	24.3±13.7 b	49.6±37.7 a	5.5±7.5 ab
15 (16) June	16	15	6.6	43.6±25.1 a	55.2±33.8 a	10.3±11.5 a
15 (17) July	9	8	1.4	16.9±20.9 b	6.8±14.3 b	0.1±0.3 b
Total	72	52	14.9	28.7±20.8	41.9±37.1	6.0±8.8

Values are means ± SD. Different letters indicate significant differences ($p<0.05$)

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Fish were treated with an Ethylene–Vinyl acetate (EVAc) GnRHa implant (Mylonas and Zohar, 2001) loaded with Des-Gly10, D-Ala6-Pro-NEth9-mGnRHa (H-4070, Bachem, Switzerland) in May, June and July samplings. Although there were variations in the effective GnRHa dose applied to each fish, due to the fact that implants are loaded with fixed amounts of GnRHa, the fish were treated with a dose ~ 50 μg GnRHa kg^{-1} body weight. At the time of GnRHa implantation, selected females were in advanced vitellogenesis and intratesticular sperm was observed in males.

Results and discussion

Spawning of F1 greater amberjack started 24–48 hours after each hormonal treatment (Table I) and a total of 52 spawnings were obtained during a period of 72 days. The number of spawnings and released eggs after each treatment was decreased from May to September. After the 1st treatment (May to June)., eggs were collected almost daily (29 spawnings in 31 days). Following the 2nd treatment (June to July) a total of 15 spawnings were recorded during the first 16 days and no eggs were collected later on up to the 15th of July. In the third period, a total of 8 spawning were observed during the following 9 days after implantation.

The period following the 1st treatment, a total of $7.1x10^6$ eggs were collected. The decrease in the total number of the collected eggs after the 2nd treatment was related with the reduced number of spawnings. However, the number of obtained eggs per spawning was significantly higher in the 2nd period. Mean fertilization and hatching rate exhibited similar trends during the three spawning periods, reaching their highest values in the second period (June to July).

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Mean sperm motility was 58±21% during the reproductive period and no differences were observed between the samplings. On the contrary, motility duration was significantly higher at the 1st sampling (4.4±1.1min) comparing to the following 3 samplings.

Conclusions

Hatchery produced greater amberjack (F1 generation) were able to finalize vitellogenesis and spermiation, and they underwent repeated spawning for 3 months with a total production of almost 15 million eggs after treatment with GnRHa implants. These results, the first with successful reproduction of F1 greater amberjack broodstock, are a step towards the industrial aquaculture production of this valuable species.

References

Mylonas and Zohar. 2001. Use of GnRHa-delivery systems for the control of reproduction in fish. Rev. Fish Biol. Fish., 10: 463-491.

Acknowledgments

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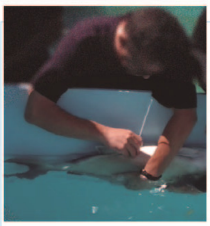
Material and Methods



Broodstock fish maintenance

A group of 15 greater amberjack broodstock born in captivity (average weight of 18.5 ± 9.2 kg) was maintained in an outdoor covered raceway tank of 500 m3 in the facilities of the Instituto Español de Oceanografía in Canary Islands (Spain).
Fish were feed with low commercial value fish (mackerel, *Scomber scombrus*) supplied three days a week to satiation and maintained with continuous water supply (water flow rate of 1000 L/min) under natural conditions of water temperature (17.8 – 24.5°C), salinity (36.5 y 37.2 ‰) and photoperiod.

Fish samplings

The broodstock (7 males, 7 females and 1 undetermined sex PIT (Passive Integrated Transponder) tagg
fish) were sampled four times during 2015 spawning season (May, June, July and September) and biomet
parameters of length and body weight were measured.
Ovarian biopsies were obtained and a wet mount was examined under a binocular microscope to evalua
the stage of oocyte development and measure the mean diameter of the largest, most advanced vitellogen
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pressure. If this was not possible, a sperm sample was obtained by inserting a plastic catheter. The collect
sperm was stored (4°C) until quality evaluation.

Spawning induction therapies

Fish were treated with an Ethylene–Vinyl acetate (EVAc) GnRHa implant (Mylonas and Zohar, 2001) load
with Des-Gly10, D-Ala6-Pro-NEth9-mGnRHa (H-4070, Bachem, Switzerland) in May, June and July (~50
GnRHa kg^{-1} body weight). At the time of GnRHa implantation, selected females were in advanc
vitellogenesis and males were producing intratesticular sperm.
Eggs were collected in a 500 µm net placed at the overflow pipe of the tank (checked daily) and fertilized ra
calculated under binocular microscope.
Date, total eggs released, and the fertilization and hatching rate were registered for each spawning.

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Treat. (Sp.) (date)	Dur. (day)	Sp. (nº)	Eggs (x10^6)	Eggs/sp (x10^4)	Fertilized (%)	Hatching (%)
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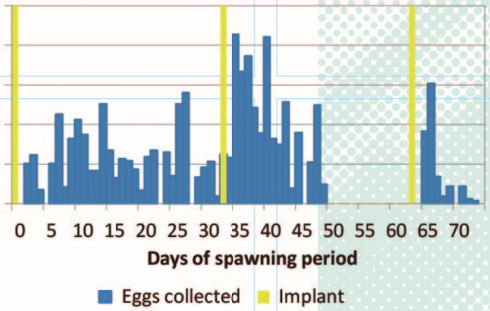
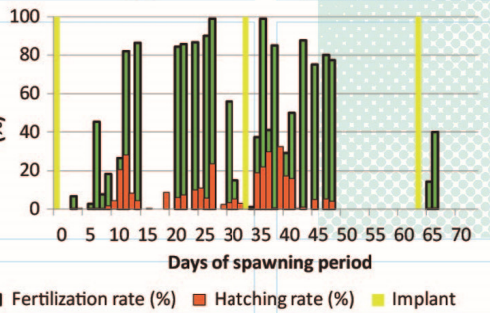
Number of eggs batches and percentage of fertilized eggs and hatching rate per day





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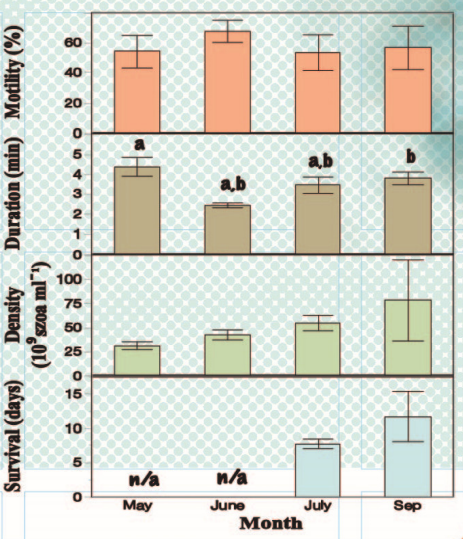

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