Deliverable Report

Deliverable No:	D3.7	**Delivery Month:**	48
Deliverable Title	Comparative effectiveness of a GnRHa injection vs GnRHa implant treatment for the induction of spawning of greater amberjack in the eastern Atlantic.		
WP No:	3	**WP Lead beneficiary:**	P2. FCPCT
WP Title:	Reproduction & Genetics – greater amberjack		
Task No:	3.3	**Task Lead beneficiary:**	P2. FCPCT
Task Title:	Development of an optimized spawning induction protocol for captive greater amberjack in the eastern Atlantic.		
Other beneficiaries:	P1. HCMR		
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Other Scientists participating: Mylonas, C.C. (HCMR)

Objective: Comparative effectiveness of a GnRHa injection vs GnRHa implant treatment for the induction of spawning of greater amberjack in the eastern Atlantic: The deliverable will be a scientific report that compares effectiveness of a GnRHa injection vs GnRHa implant treatment for the induction of spawning of greater amberjack in the eastern Atlantic. The deliverable will present the results of the two hormonal induction therapies applied in replicated experiments, including egg release frequency, fecundity and egg quality parameters (i.e. morphology, fertilization, hatching and larval survival). Finally, a series of conclusions and protocol recommendations for final users will be provided.

Description: Development of an optimized spawning induction protocol for captive greater amberjack in the eastern Atlantic. Mature wild-caught fish adapted to culture conditions will be divided into 3 groups of 6 males and 6 females. At the onset of the spawning season in the eastern Atlantic (April-May), after evaluation of reproductive status fish will be treated with either single GnRHa injections every 7-10 days or GnRHa implants (that will be produced by HCMR) every 3 weeks, and one group will be left untreated as control. Evaluation of the spawning quality will be carried out for all spawns.

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1. -Introduction

Controlled reproduction of greater amberjack (*Seriola dumerili*), a promising candidate species for Mediterranean aquaculture, still constitutes one of the main bottlenecks for commercial production of this species. However, other species of the *Seriola* genus do mature and reproduce readily in captivity, such as the yellowtail (*Seriola quinqueradiata*), a fish with the highest production worldwide in this genus. This species is cultured in Japan, Korea and Taiwan (Symonds et al., 2014; FAO, 2015). Yellowtail kingfish (*Seriola lalandi*) also reproduces readily in aquaculture conditions and is cultured since the end of the 1990s in Australia, where ~2,000 t was produced in 2010 (Tanner and Fernandes, 2010). At the same time, experimental production was reported for longfin yellowtail (*Seriola rivoliana*) in Ecuador, using wild captured broodstocks and F1 maintained in captivity, with a production of 20,000 juveniles (Cataudella and Mazzola, 2013). Since 2005, the same species has been produced in Hawaii in open seawater cages and in 2008 the production reached 750 t (Cataudella and Mazzola, 2013). Several other countries produce or are in the process of establishing commercial farms of these species including Chile (yellowtail kingfish), New Zealand (yellowtail kingfish), South Africa and Namibia (yellowtail kingfish) (Aguilera et al., 2013; Orellana et al., 2014; Symonds et al., 2014; O'Neill et al., 2015).

For many years, greater amberjack has also been produced in Japan, but this production has been based entirely on wild juveniles, being in the 1990s the second highest among marine fishes in Japan (Nakada, 2000). Experimental production of juveniles has been undertaken in Italy, Greece, Spain and Malta during the last two decades, using wild broodstock captured and kept in captivity and induced hormonally to complete oocyte maturation, ovulation and spawning. In Malta in 2010, 14,000 juveniles were produced (Cataudella and Mazzola, 2013), while small productions have also been reported by commercial operations in Italy (Lampedousa Island) and Spain (Port of Cadiz) (Jerez, 2013).

Of all *Seriola* species, only the greater amberjack has a worldwide distribution, and, particularly, in the EU it can be found in the Mediterranean Sea and the Atlantic Ocean from the United Kingdom (Froese and Pauly, 2016) to the Canary Islands (Moro et al., 2003). This finfish has a great potential for the expansion of the EU aquaculture industry and, therefore was one of the species targeted by the EU Project DIVERSIFY (7FP-KBBE-2013-GA 602131).

The first prerequisite for the culture and industrial production of a new species is the control of its reproduction. To have a sustainable production of high quality eggs, it is necessary to have good knowledge of a species' reproductive physiology and control of reproductive function in captivity. However, in most new aquaculture species, reproduction in captivity is a main bottleneck, as many species exhibit reproductive dysfunctions (Zohar and Mylonas, 2001; Mylonas and Zohar, 2007; Mylonas et al., 2010). Even though spontaneous spawns have been reported in greater amberjack in captivity, in the island of Chichijima (Japan) (Kawabe et al., 1996, 1998) and in Tenerife (Spain) (Jerez et al., 2006), most reproduction studies required hormonal inductions with injections (Kozul et al., 2001; Fernández-Palacios et al., 2013), or implants (Mylonas et al., 2004a; La Barbera, 2014). Hormonal induction is only effective when fish reach an advanced stage of gonadal development (post-vitellogenesis) and is highly temperature-dependent (De la Gándara, 2006). In addition, the quality of eggs obtained with hormonal induction may be often lower than from spontaneously spawning broodstocks in species such as medaka (*Oryzias latipes*) (Shioda and Wakabayashi, 2000), southern flounder (*Paralichthys lethostigma*) (Watanabe et al., 2001), summer flounder (*Paralichthys dentatus*, (Watanabe and Carrol, 2001), European sea bass (*Dicentrarchus labrax*) (Fornies et al., 2001), red snapper (*Lutjanus campechanus*) (Papanikos et al., 2003; Phelps et al., 2009), Atlantic croaker (*Micropogonias undulatus*) (Sink et al., 2010) or yellowtail kingfish (Symonds et al., 2012). Nevertheless,

spawning quality is not different between spontaneous or induced spawns in gilthead seabream (*Sparus aurata*) (Barbaro et al., 1997) and quality of induced spawns is better than of spontaneous spawns in brill (*Scoptlhalmus rhombus*) (Basaran et al., 2008) and cobia (*Rachycentron canadum*) (Nhu et al., 2011).

Therefore, there is a need to improve broodstock management methods for greater amberjack, to optimize the reproductive function of greater amberjack maintained in captivity and promote spontaneous spawning with a potential better quality than hormonal therapies. To develop effective broodstock management methods for greater amberjack, the present study compared the hormonal induction protocols with injections or implants with spontaneous spawns to obtain high quality eggs for a reliable production of greater amberjack juveniles.

2. -Materials and methods

Broodstock maintenance

Twenty-two greater amberjack captured in May 2011 in the Southwestern coast of Gran Canaria (Canary Islands, Spain) were used in the present experiment. Groups of fish with a mean ± SD weight of 3.41 ± 1.12 kg for females and 2.37 ± 1.07 kg for males, were conditioned in tanks of 10 m^3 volume (3 m x 3 m x 1.5 m depth), located in the Planta Piloto de Producion de Alevines (PPPA) of the Grupo de Investigación en Acuicultura (GIA). Fish were kept under spontaneous photoperiod using seawater at a temperature range 20.83 ± 0.32 °C in winter and 23.84 ± 0.18 °C in summer. In January 2013, greater amberjack (females of a body weight of 8.27 ± 1.11 kg and males of a body weight of 8.12 ± 1.82 kg) were transferred to three 40 m^3 circular tanks of (5 m x 2.35 m). There were no mortalities during this acclimation period.

The 40 m^3 tanks were supplied with surface water of 37 ‰ salinity in a flow through system with a water exchange of 600% day^{-1}. Photoperiod was simulated spontaneous following the day extension in concordance with the geographical position (27º 59' 28" N; 15º 22'05" W), having a length day of 10.26 and 13.6 h at the summer and winter solstice, respectively. Temperature and dissolved oxygen was determined continuously through a system of sensors monitored by computer (Miranda, Innovaqua, Sevilla, Spain). Fish were fed twice a week with commercial feeds (13 mm, Vitalis CAL, Skretting, Burgos, Spain) at 1% of their estimated total biomass, and once a week with locally fished *Scomber scombrus* at 2% of their total biomass.

Evaluation of gonad maturation

Before starting the experiment (3 June 2014), in late May 2014, all fish were anesthetized with clove oil (Guinama SL, Valencia, Spain; 50 ppm), weighted and sized (**Table 1**). Gonad maturation state was evaluated by ovarian biopsies obtained with cannulation of the genital pore using a catheter of 1.3 mm outside diameter (Kruuse, Langeslov, Denmark). Serra solution (6:3:1, 60 % ethanol at 96 %, 30 % Formalin at 40 % and 10 % of glacial acetic acid at 96 %) was added to each ovary sample to disperse the oocytes and make them transparent to determine nuclear position. They were then observed in a profile projector (Mitutoyo PJ-3000A, Kanagawa, Japan) to estimate the diameter of 100 oocytes randomly selected. All examined females (♀, n=10) had oocytes of more than 500 µm in diameter, and all males (♂, n=12) emitted sperm upon abdominal massage.

At the end of May 2014, two females from the monitored population had oocytes >800 µm. Since Roo et al. (2009) and Fernandez-Palacios et al. (2013) in Las Palmas, Kawabe et al. (1996, 1998) in Japan, and Jerez et

al. (2006) in Tenerife, obtained spontaneous spawns without hormonal induction with wild-caught females having oocytes of only ~600 µm in diameter, we decided to allow these two females to spawn spontaneously, and therefore were not induced. The other eight females, from which six were selected for the experiment, had a mean oocyte diameter of 672±87 µm. The difference in oocyte size among these females and the two mentioned above could be attributed to the different background of these individuals. The latter eight fish were used in another experiment the previous year, and while they were maintained from January to May 2013 in a 40 m^3 tank, in the experimental period of June-October 2013 they were moved to a 10 m^3 tank, and then were returned to the 40 m^3 tank in November 2013. On the other hand, the two individuals with the higher oocyte diameters in May 2014 were not used for the study of 2013 and remained in the 40 m^3 tank from January 2013 to May 2014, just prior to the start of the present experiment.

Hormonal treatment

The selected fish were distributed in three circular tanks of 40 m^3 in volume, as follows. In tank 1 (2 ♀ and 5 ♂), the fish were not induced hormonally or handled and spawned spontaneously, being considered the control group. In tank 2 (3 ♀ and 3 ♂), the fish were injected intramuscularly with gonadotropin releasing hormone analogue (LHRHa, des-Gly10, [D-Ala6]-; Sigma-Aldrich, St. Louis, MO, USA) at a dose of 20 µg kg^{-1} body weight, based on the reported dosage for greater amberjack (Fernandez-Palacios et al., 2013). These hormonal treatments were applied twice a week alternating the broodstock (1 ♀ and 1 ♂). The three males and three selected females of tank 3 (3 ♀ and 3 ♂), were induced using 500-µg GnRHa implants, (Mylonas and Zohar, 2001). One implant (500 µg) was used for each female (Mylonas et al., 2004a) and half the dose for males (La Barbera, 2014). Implants were given subcutaneously, at about three scale rows down from the posterior end of the dorsal fin. The frequency of GnRHa injection was once every 11.8 ± 3.0 days, while the frequency of GnRHa implantation was once every 26.9 ± 7.6 days.
First spontaneous spawn was obtained in control broodstock on June 1st 2014 and GnRHa injection and implants were given on 3 June 2014 (seawater temperature 20.4 ºC). Last spontaneous spawn was obtained on October 18th, whereas spawns from GnRHa injected and implanted broodstock were respectively obtained on October 21st and 14th (seawater temperature 24.5ºC). Three further hormonal inductions after this period did not result in any spawns.

Evaluation of hormonal induction efficiency

To test the spawning induction efficiency (Fernández-Palacios et al., 2014) the following parameters were determined: number of spawning females that responded to hormone treatment, number of spawns, spawns obtained per induction and latency period (time from the hormonal treatment and the first spawn, based on the presence of eggs in the outflow egg collectors, which were monitored during the day every 15-20 minutes). In the case of spontaneous spawning, the number of spawning females, number of spawns and spawns $hour^{-1}$ were determined. Also, for each treatment the total number of eggs, the number of eggs $female^{-1}$, the number of eggs $spawn^{-1}$ and the number of eggs $spawn^{-1}$ Kg female $weight^{-1}$were determined. For induced spawns the number of eggs Kg female $weight^{-1}$ $induction^{-1}$ was also determined.

Evaluation of egg quality

For each spawn, fertilization rate was determined to evaluate egg quality. Eggs were placed in a 10-l bucket provided with strong aeration, from where a random sample of 10-ml was taken using a plastic pipette, and placed into a 17-ml Falcon tube. From there, all the eggs in the sample were placed in a Bogorov chamber and identified as live (fertilized, L) or dead (D) under a binocular microscope (Lieca- S6E, Wetzlar,

Germany). The fertility of the spawn was calculated as [(L + D) x 1000] and fertilization success was calculated as [L / (L + D)] %. Fertilized eggs were then placed in 96-well microliter plates in replicates (Greiner Bio One, Kremsmunster, Australia) according to the method of Panini et al. (2001) and were incubated in a controlled temperature incubator at 22.1 ± 0.4 ºC, in order to estimate the percentage of viable eggs at 24 hours, hatching percentage and larval survival at 4 and 8 days.

Egg and larvae sizes

Egg diameter was estimated from 150 eggs from 10 different spawns for each treatment. Eggs from the same spawns were also stocked in 500-L incubators (50 eggs l^{-1}) supplied with the same water as the broodstock tanks. From these spawns, 15 newly hatched larvae and 15 larvae at the end of yolk sack absorption were measured for total length (TL), standard length (SL), diameter of oil globule (LGD), yolk sack length (YSL) and width (YSH), using a profile projector as mentioned before for the oocyte diameter (**Fig.1**). All the measurements were made in live larvae anesthetized with clove oil at 1% to avoid deformities produced upon dying. The volume of the yolk sack (YSV) was calculated using the formula proposed by Blaxter and Hempel (1963): $YSV = \pi / 6\ YSL \times YSH^2$.

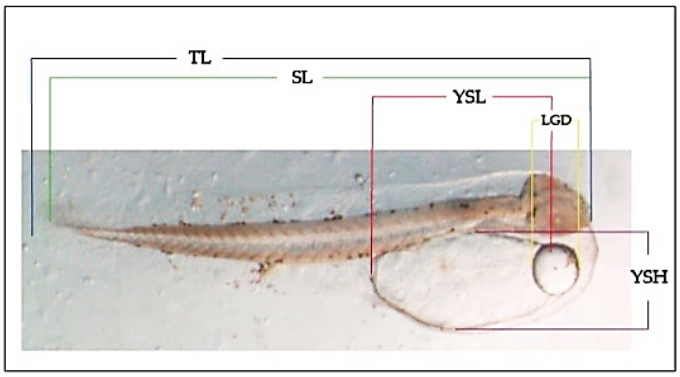


Fig. 1. Morphometric measurement procedures on the larvae of greater amberjack.

Statistical analysis

The data were analyzed using the statistical program SPSS statistics (versión 22.0 for Windows; Inc, IBM, Chicago, IL, USA) and visualized using SigmaPlot 12.0 (Systat software, San José, USA). The data was expressed as a mean ± standard deviation (SD). Normality and homogeneity of the variance of all the variables was evaluated using the Kolmogorov-Smirnov test and Levene tests respectively (Sokal and Rolf, 1996). When the assumptions where correct, one-way ANOVA tests were performed, followed by Duncan's New Multiple Range Test. When an ANOVA was not possible due to the heterogeneity of the variances and or the data was not distributed normally, a Kruskal-Wallis test was applied. The differences between treatments where graphed with a box and whisker plot.

3.- Results

Biometric studies of initial broodstock denoted no significant differences among fish with the different broodstock management protocols (**Table 1**). A low biomass rate was kept in all the tanks (**Table 1**). At the

beginning of the trial, the mean oocyte diameter of non-hormonally treated females (837 ± 167 µm) was statistically (P<0.01) higher than those of hormonally induced (690 ± 100 µm injected females and 648 ± 59 µm implanted females).

Table 1. Biometric data of greater amberjack broodstock with spontaneous spawns (Control) and broddtstocks injected or implanted with GnRHa.

Tank	Treatments	Sex (number)	Weight (kg)	Total length (cm)	Fork length (cm)	Biomass (kg/m^3)
1	Control	Female (2)	9.81±1.09	90.00±2.83	78.50±2.12	1.67*
		Male (5*)	9.48±1.76	87.20±4.81	77.20±4.08	
2	Injected	Female (3)	11.83±1.10	94.50±5.56	83.83±4.72	2.39
		Male (3)	11.78±1.77	95.66±3.78	85.00±3.46	
3	Implanted	Female (3)	10.72±0.98	96.66±2.25	82.33±1.52	1.83
		Male (3)	10.19±1.02	92.17±4.54	82.00±4.77	

* 14 June a male jumped out of the tank and died, biomass changed to 1.44 kg/m^3.

All GnRHa induced females spawned during the study. The number of spawns produced after each GnRHa induction was significantly higher for the implanted broodstock than for the injected one (P<0.01) (**Table 2**). No significant differences were seen between the latency periods of induced spawns (43.06 ± 2.49 h in injected and 44.19 ± 7.44 h in implanted broodstock).

The number of eggs spawn^{-1} and number of eggs Kg female weight^{-1} spawn^{-1} were significantly (P<0.01) higher in spontaneously spawning broodstock, whereas the number of eggs Kg female weight^{-1} induction^{-1} was higher (P<0.05) in implanted fish than in injected fish (**Table 3**).

Table 2. Number of hormonal inductions and spawns in greater amberjack with spontaneous spawns (Control) and broodstocks injected or implanted with GnRHa

Treatments	% Females with spawns	Number of inductions	Number of spawns	Spawns/ Induction*
Control	100	-	23	-
Injected	100	37	29	0.78 ± 0.53^{b}
Implanted	100	17	38	2.23 ± 1.85^{a}

* Mean ± SD. Different superscripts in the same column indicate significant differences (P <0.01).

Table 3. Number of eggs obtained from greater amberjack after treatment with GnRHa injections or implants, in comparison with spontaneously spawning fish (Control)*

Treatments	Number of eggs female^{-1} (x 10^{6})	Number of eggs spawn^{-1} (x10^{6})	Number of eggs Kg female weight^{-1}and spawn^{-1} (x 10^{4})	Number of eggs Kg female weight $^{-1}$ induction^{-1} (x 10^{4})
Control	12.80	1.11 ± 0.32^{a}	5.67 ± 1.66^{a}	-
Injected	4.30	0.44 ± 0.27^{b}	3.72 ± 2.30^{b}	2.89 ± 3.87^{B}
Implanted	3.51	0.28 ± 0.29^{b}	2.52 ± 2.73^{b}	5.37 ± 6.11^{A}

* Mean ± SD. Different superscripts in the same column indicate significant differences (Lower letters P < 0.01; Capital letters P < 0.05).

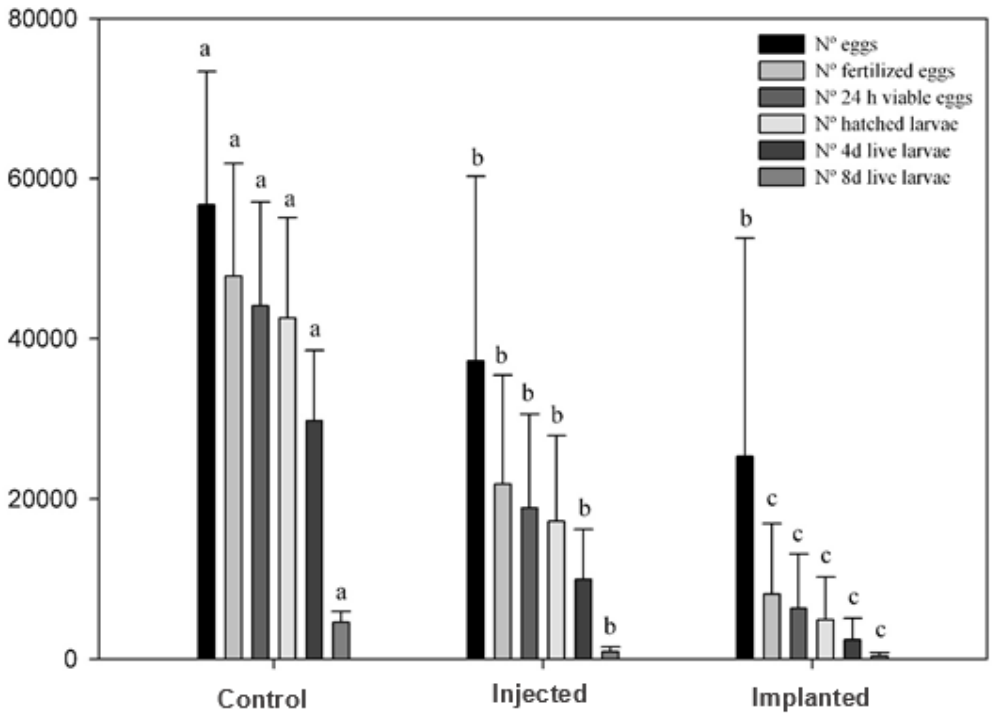
Overall, the spawning quality of the spontaneous spawns was significantly higher than the GnRHa induced spawns (**Table 4**). Thus, the highest (P<0.01) fertilization rates were found in spontaneous spawns, followed by those of injected fish and, then, implanted fish. Percentage of viable eggs at 24 hours (P<0.05) and hatching percentages (P<0.01) were significantly higher in the control fish in comparison to the implanted fish, whereas injected fish showed intermediate values. Highest larval survival (P<0.01) was also found in spontaneous spawns.

Table 4. Quality of egg and larvae obtained from greater amberjack after treatment with GnRHa injections or implants, in comparison with spontaneously spawning fish (Control)*

Treatments	Fertilization rate (%)	Viable eggs percentage (%)	Hatching percentage (%)	Larval survival percentage at 4 days (%)
Control	84.37± 21.57^{a}	92.21 ± 9.43^{A}	96.60 ± 6.56^{a}	69.91 ± 16.51^{a}
Injected	58.82±26.79^{b}	86.37± 25.39^{AB}	91.13± 25.44^{ab}	58.09± 23.66^{b}
Implanted	32.15 ±34.60^{c}	77.60 ± 34.01^{B}	77.96 ± 34.93^{b}	49.45 ± 27.36^{b}

* Meran ± SD. Different superscripts in the same column indicate significant differences (Lower letters P < 0.01; Capital letters P < 0.05).

Total number of eggs produced Kg female weight^{-1} spawn^{-1} in spontaneously spawning broodstock was significantly higher (P<0.01) than in injected or implanted fish (**Fig. 2**). The number of fertilized and viable eggs, as well as the number of larvae produced at the different stages was highest in spontaneous spawns, followed by injected fish, whereas the significantly (P<0.01) lowest values were obtained in implanted fish (**Fig. 2**).



*Bars, of the same shade, with the same letter were not significantly different (P < 0.01).

Fig. 2. Egg production rates (Kg female weight^{-1} spawn^{-1}) in greater amberjack broodstock with spontaneous spawns (Control) and broodstocks injected or implanted with GnRHa.

Eggs from spontaneous spawns were significantly larger than those of induced spawns (**Table 5**). In newly hatched larvae from spontaneous spawns and injected broodstock, total and standard length were similar, and were significantly higher (P <0.01) than larvae from GnRHa implanted fish. Yolk sac volume was significantly greater (P <0.01) in larvae from spontaneously spawning broodstock, compared to yolk sac volume from the other two treatments. The diameter of the oil droplet of larvae obtained in spontaneous spawns was significantly higher (P<0.01) than in the hormonally induced spawns. Total and standard length in larvae with absorbed yolk sac, as well as diameter of oil droplet, were significantly larger (P <0.01) in larvae obtained from spontaneous spawns and GnRHa injected broodstock, compared to the GnRHa implanted fish. A positive correlation was obtained (P<0.05, R=0.99 data not show) between yolk sac volume and survival at 5 days after hatching (dah).

Table 5. Egg and larvae sizes from greater amberjack with spontaneous spawns (Control) and injected or implanted GnRHa*

Treatments	Egg diameter (mm) n=4500	Newly hatched larvae (n=450)			
		Total length (mm)	Standard length (mm)	Yolk-sac volume (mm^3)	Oil droplet diameter (mm)
Control	1.13 ± 0.03 [a]	2.59 ± 0.10 [a]	2.49 ± 0.10 [a]	0.44 ± 0.11 [a]	0.30 ± 0.02 [a]
Injected	1.10 ± 0.03 [b]	2.59 ± 0.13 [a]	2.49 ± 0.13 [a]	0.34 ± 0.09 [c]	0.28 ± 0.02 [b]
Implanted	1.10 ± 0.03 [b]	2.45 ± 0.13 [b]	2.36 ± 0.12 [b]	0.40 ± 0.12 [b]	0.28 ± 0.03 [b]
		Larvae with yolk-sac absorbed (n=450)			
Control	-	3.85 ± 0.13 [a]	3.68 ± 0.13 [a]	-	0.11 ± 0.03 [a]
Injected	-	3.82 ± 0.13 [a]	3.67 ± 0.13 [a]	-	0.11 ± 0.02[a]
Implanted	-	3.53 ± 0.26 [b]	3.42 ± 0.24 [b]	-	0.14 ± 0.03 [b]

* Mean ± SD. Different superscripts in the same column indicate significant differences (P <0.01).

4.- Discussion and conclusions

Discussion

The good growth performance and survival of captured greater amberjack individuals underline that this species may acclimatize well to captivity conditions, in agreement with previous studies (Jerez et al., 2006; Fernández-Palacios et al., 2013; La Barbera, 2014). Despite the fact that vitellogenesis of this species may be inhibited in captivity (Micale et al., 1999), in other stocks only oocyte maturation fails to be completed (Marino et al., 1995; Lazzari et al., 2000; Mandich et al., 2004; Mylonas et al., 2004a) due to unsuitable environmental (Mylonas et al., 2010) or stress inducing conditions (Schreck, 2010). However, in the present study, greater amberjack of only 2-3 kg caught in the Atlantic Ocean and kept in a 40 m^3 tank, acclimatized

very well to captive conditions and completed gonadal development. Thus, at the beginning of the spawning season, oocytes reached a mean diameter of ~670 µm and up to >800 µm, larger than previously described for this species in captivity (Kawabe et al., 1996, 1998; Jerez et al., 2006; Roo et al., 2009, Fernandez-Palacios et al., 2013; La Barbera, 2014). These latter females with larger oocytes proceeded with very successful spontaneous spawning without any hormonal therapy. However, in other females such large oocyte size was not achieved, a fact that could be related to their different background and the fact that, prior to the start of the present trial, they were kept for several months in 10 m^3 tanks instead of the 40 m^3 tank where large oocyte females had been kept. Despite the fact that induced spawns of the *Seriola* genus have been obtained in 10 m^3 tanks (Fernández-Palacios et al., 2015a, 2015b), large volume tanks (50-80 m^3) have been recommended for this species (Benneti, 2008) and spontaneous spawns have been obtained in tank volumes ranging from 56 to 500 m^3 (Kawabe et al., 1996, 1998; Jerez et al., 2006).

Spontaneous spawns were obtained between June and October 2014, with a mean frequency of spawning every 6 days, in agreement with previous studies (Marino et al., 1995; Jerez et al. 2006). Annual changes in photoperiod are responsible for gonadal maturation, activating the endocrine reproductive axis of fish, together with temperature, which is also a synchronizing factor that indicates the appropriate environmental conditions for spawning (Carrillo et al., 1989; Munro, 1990; Huber and Bengtson, 1999; Falcón et al., 2003; Maitra et al., 2006). Spontaneous spawning of greater amberjack in captivity without any exogenous hormonal induction in individuals captured from the wild has been only obtained in the Canary Islands (Spain) or Ogasawara Archipelago (Japan), which are in the same geographical latitude and, therefore, have similar photoperiod (**Fig. 3**). In Ogasawara Archipelago, the spawning season lasts 41-55 days within a range of temperatures of 21.8-25.9ºC (Kawabe et al., 1996, 1998), whereas in the Canary Islands the spawning season lasts 140-156 days, with temperatures between 19.7 and 24.5ºC (Jerez et al., 2006 and present study). Thus, spawning season is 3 times longer in the Canary Islands than in Ogasawara, which could be due to a longer period of water temperature between 19 and 26ºC (**Fig. 4**).

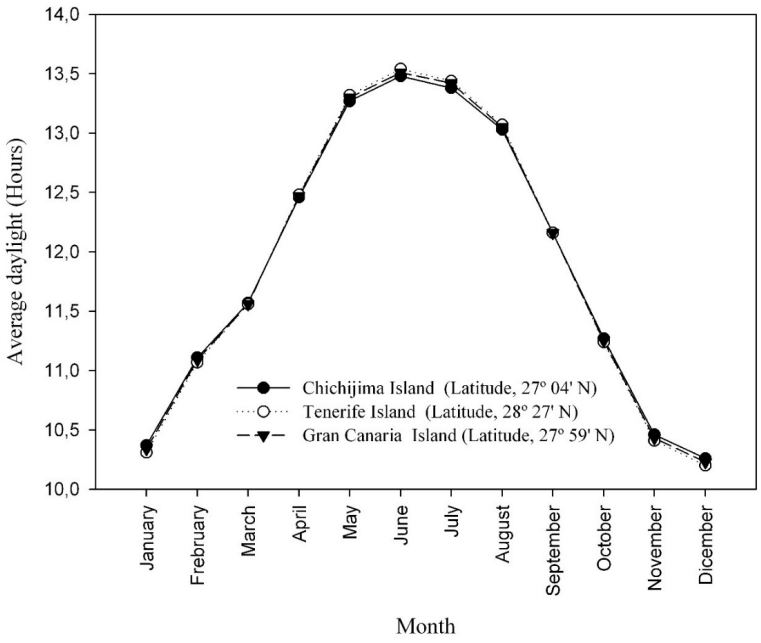


Fig. 3. Mean monthly light hours in the islands of Chichijima (Osagawara, Japan), Tenerife and Gran Canary (Canarias, Spain) (www.climatemps.com).

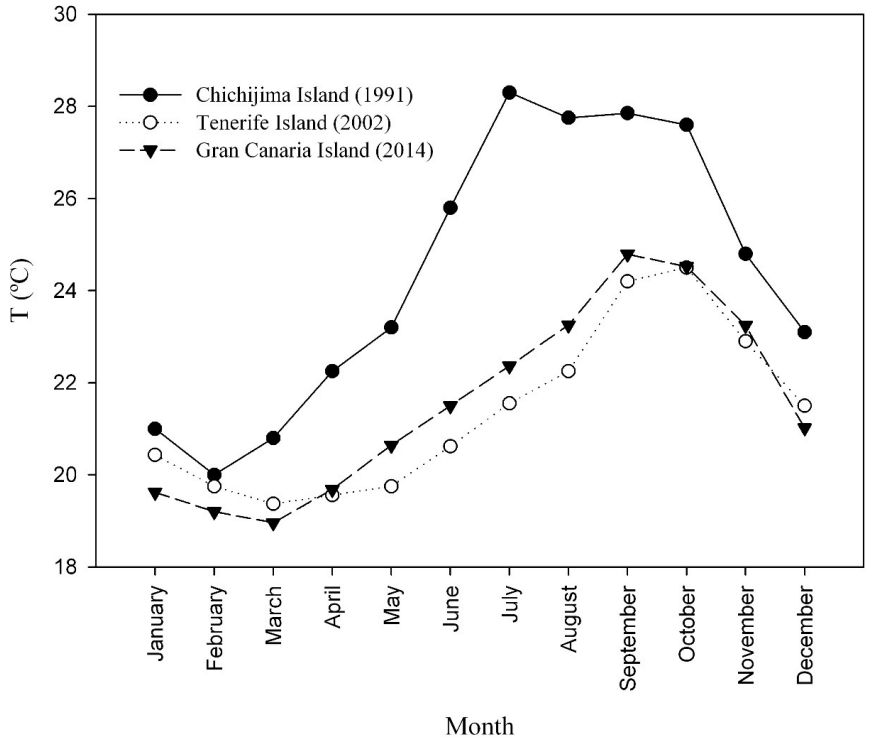


Fig.4. Mean monthly temperature in the islands of Chichijima, Ogasawara, Japan (Kawabe et al., 1996), and Tenerife (Jerez et al., 2006) and Gran Canaria, Spain (This study).

Quality of spontaneous spawns was very high, in terms of total number of eggs Kg female weight^{-1} spawn^{-1}, fertilization and larval survival rates in comparison to previous studies. For instance, total number of eggs female^{-1} (12.8 million eggs female^{-1} spawn^{-1}) was higher than in other studies with amberjack of similar weight (3.1 and 2.86 million eggs female^{-1}/spawn^{-1} in Japan (Kawabe et al., 1996) and Canary Islands (Jerez et al., 2006) assuming a ratio female:male of 1:1). Only if one single female spawned in those previous studies the results would be comparable with the present study. Besides, the number of eggs Kg female weight^{-1} spawn^{-1} in the present study was 5 times higher than in previous studies (Jerez et al., 2006). Moreover, fertilization rates were between 11 and 35% higher than those obtained in previous studies (Kawabe et al., 1996, 1998; Jerez et al., 2006). In addition, hatching rates were also 80% and 17% higher than in those studies in Japan (Kawabe et al., 1996, 1998) and Canary Islands (Jerez et al., 2006), respectively. This improved egg quality in the present study could be related to the larger oocyte size previously discussed, in relation to more favorable temperature, nutrition or fish welfare conditions. Hatched larvae had a similar yolk sac volume to those of other fast-growing species (0.4-0.5 mm^3 for yellowtail amberjack, (Moran, 2007); 0.43 mm^3 for meagre (*Argyrosomus regius*) (Klimogianni et al., 2013)) and higher than other marine species with pelagic eggs of similar size (0.28 mm^3 for red porgy (*Pagrus pagrus*) (Aristizabal, 2006); 0.36 mm^3 for European sea bass (Pope et al., 2014); 0.2-0.3 mm^3 for gilthead seabream (*Sparus aurata*) (Polo et al., 1991)).

Hormonal induction by GnRHa injections or implants successfully induced spawns in females bearing >500 µm oocytes, in agreement with previous studies (Kozul et al., 2001; Mylonas et al., 2004a; De la Gandara et al., 2004; La Barbera, 2014; Fernández-Palacios et al., 2015a, 2015b). Gonadotropin releasing hormone agonist has been used to induce spawning either through injections (Fernández-Palacios et al., 2013; La Barbera, 2014) or through controlled release implants (Mylonas et al., 2004a; La Barbera, 2014). However, none of these studies compares spontaneous spawns with hormonally induced ones. In the present study, hormonal induction with either injections or implants lead to a lower number of eggs produced Kg female

weight^{-1} spawn^{-1} in comparison to the spontaneously spawning fish, which could be related to the potential stress caused by the manipulation during hormonal induction. Indeed, handling stress has been shown to affect fish reproduction in different ways depending on the species, sex, gonad maturation and stress tolerance (Schreck et al., 2001). Besides, hormonal induction also caused a reduction in larval survival rates in comparison to spontaneously spawning greater amberjack, in agreement with studies on other species (Papanikos et al., 2003). During endogenous nutrition, larvae depend on their yolk sac reserves, including the oil droplet (Sanderson and Kupferberg, 1999), which are correlated with egg size (Dabroswki and Luczynski, 1984) and higher larval survival (Brooks et al., 1997). Thus, in the present study, the lower survival rate in larvae from hormonally induced fish could be related to the lower egg and oil drop diameter, frequently used indicators of spawning quality in fish (Faulk and Holt, 2008), suggesting an impaired vitellogenesis in comparison to the spontaneous spawned eggs. Indeed, hormonally induced females produced more spawns than the spontaneously spawning females, suggesting a lower vitellogenetic period in the former. Egg diameter has been also related to the number of spawns during the spawning season in other species (Brooks et al., 1997). Still, in the present study, egg diameters obtained by hormonal induction were higher than in previous studies with GnRHa implanted greater amberjack (1.02 ± 0.01 mm) (Mylonas et al., 2004a) and similar to broodstock injected with human chorionic gonadotropin (hCG) (1.12-1.14 mm) (Kozul et al., 2001) or GnRHa (1.15 mm) (Lazzari et al., 2000).

Hormonal injection with GnRHa was particularly successful, leading to higher fertilization rates than hormonal implants and equal egg viability and hatching rates with spontaneous spawning. Indeed, egg viability in GnRHa injected fish was 30% higher than the 55.5% previously reported for this species (Fernández-Palacios et al., 2013). On the contrary, GnRHa implants lead to the lowest fertilization, egg viability and hatching rates. As a consequence, the lowest number of eggs and larvae were obtained with GnRHa implants in comparison to GnRHa injected or spontaneous spawning greater amberjack. These results are in agreement with the low fertilization rates previously obtained in GnRHa implanted fish that ranged between 22-50% (Mylonas et al., 2004a), whereas in GnRHa injected fish fertilization rates reached up to 80%. Similarly, in previous studies egg quality in terms of hatching rates was higher in eggs from GnRHa injected greater amberjack than from implanted fish (La Barbera, 2014). Moreover, in the present study, length of newly hatched and yolk sac reabsorbed larvae from implanted fish were significantly lower than from injected fish, which showed equal size with larvae from spontaneous spawns. Interestingly, together with smaller length, larvae from implanted fish showed larger yolk sac denoting the lower utilization of nutrient reserves in comparison to injected or spontaneously spawning amberjack.

Considering that in a close related species of the same genus, the Japanese amberjack, there were no differences in fertilization rates between GnRHa implanted or injected fish (Chuda et al., 2002), the protocol for GnRHa implants for greater amberjack must be still improved in order to obtain good quality eggs as with GnRHa injections or spontaneous spawns. For instance, the interval between successive hormonal injections (12 ± 3 days) was closer to the spawning interval of the spontaneous spawns (6 ± 3 days) than the spawning interval of GnRHa implants (27 ± 8 days). In previous studies, hormonal treatment of this species with GnRHa implants produced spawns after the first induction and 3 spawns in the second induction, 15 days after (Mylonas et al., 2004a). In another study, it was observed that an interval of 10 days between hormonal injections was sufficient to have spawns of good quality (Fernández-Palacios et al., 2013). On the contrary, with the shi drum (*Umbrina cirrosa*) when 3 injections of GnRHa were administered within 10 days, females did not respond to the second or third injection, but did respond after a 20 days interval (Mylonas et al., 2004b).

As expected, the mean number of spawns per injection was significantly higher in the implanted broodstock than in the injected ones, due to the long-term release of GnRHa in implants, and therefore a long-term stimulation of reproductive function, resulting in more consecutive spawns (Mylonas y Zohar, 2007). Similar latency periods were obtained between both hormonal treatments and in comparison to other studies (Fernández-Palacios et al., 2013; La Barbera, 2014). Latency periods of 36-52 h have been described depending on water temperature (Tachihara et al., 1993) and a negative correlation between temperature and latency period has been shown in longfin yellowtail (Fernández-Palacios et al., 2014). In greater amberjack, shorter latency periods of 36 h were obtained using implants with a dose of 40 µg kg^{-1} GnRHa (Mylonas et

al., 2004a) or 30 h when injecting broodstock with 50 µg kg^{-1} of GnRHa (García et al., 2001). Other authors have obtained a latency period between 46 and 66 h when inducing wild broodstock with 1000 UI kg^{-1} hCG (Kozul et al., 2001).

Conclusions

This study showed for the first time that it is possible to obtain very high quality spontaneous spawns in greater amberjack, in relation to adequate tank size, environmental conditions, particularly temperature between 19-26ºC, and possibly broodstock management and nutrition. Besides, the GnRHa weekly injections protocol used lead to spawns with higher fertilization rates than GnRHa hormonal implants, equal egg viability and hatching rates with spontaneous spawning and better than in previous studies. Finally, GnRHa implants also produced successful spawns, although the protocol for GnRHa implants for greater amberjack must be still improved in order to improve spawns, egg and larval quality and to reach similar quality with spontaneous spawns and even GnRHa injections.

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Deviations:

This deliverable was prepared according to the work described in Task 3.7.

No deviations exist from the DOW.

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