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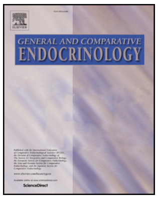

Spawning kinetics and egg/larval quality of greater amberjack (*Seriola dumerili*) in response to multiple GnRHa injections or implants

Ioannis Fakriadis[a,b], Francesca Lisi[a,c], Irini Sigelaki[a], Maria Papadaki[a], Constantinos C. Mylonas[a,*]

[a] *Institute of Marine Biology, Biotechnology and Aquaculture, Hellenic Center for Marine Research, P.O. Box 2214, Heraklion, Crete 71003, Greece*
[b] *University of Crete, Department of Biology, P.O. Box 2208, Heraklion 71409, Crete, Greece*
[c] *University of Barcelona, Gran Via de les Corts Catalanes 585, Barcelona 080007, Spain*

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ABSTRACT

The greater amberjack (*Seriola dumerili*) is one of the most promising finfish species for aquaculture, due to its cosmopolitan distribution and acceptability, high growth rates and large size. However, lack of reproduction control has been one of the main bottlenecks for its commercialization. The study examined two endocrine methods for the induction of oocyte maturation/ovulation and spawning, which are based on the induction of endogenous Luteinizing Hormone (LH) release from the pituitary, through the use of Gonadotropin Releasing Hormone agonist (GnRHa) either in the form of sustained-release delivery systems (implants) or injections (acute release). The stock ($n = 28$) consisted of wild fish captured in 2011 and each GnRHa administration method was conducted in two replicates, with 6 and 8 fish per tank, respectively, at a 1:1 sex ratio. Fish were given a GnRHa injection once a week (three administrations), or a GnRHa implant every 2 weeks (two administrations). Mean daily relative fecundity was significantly higher ($t_{1.3} = -5.24$, $P = 0.012$) in the implanted fish (15,170 ± 2,738 eggs kg^{-1} female day^{-1}) compared to the injected fish (6,119 ± 2,790 eggs kg^{-1} female day^{-1}). Total relative fecundity was also significantly higher ($t_{1.3} = -9.93$, $P = 0.003$) in the implanted fish (102,402 ± 20,337 eggs kg^{-1} female) compared to the injected ones (26,517 ± 9,938 eggs kg^{-1} female), but there were no differences in the quality of eggs in terms of fertilization, 24-h embryo survival, hatching and 5-d larval survival. The number of females with fully vitellogenic oocytes eligible for induction of spawning decreased from the initial to the final sampling, from 7 to 6 females for the GnRHa implant treatment and from 7 to 3 females for the GnRHa injection treatment. In addition to apparently promoting the proper endocrine changes leading to multiple cycles of oocyte maturation, ovulation and spawning, and thus producing larger numbers of eggs; the use of GnRHa implants may be more appropriate in greater amberjack than multiple injections because (a) it also stimulates vitellogenesis and (b) involves less handling of the fish.

1. Introduction

The greater amberjack (*Seriola dumerili*) is one of the most promising species for the diversification of aquaculture production, due to its cosmopolitan distribution (Paxton et al., 1989) and acceptability, high growth rates and large size (Crespo et al., 1994; Grau et al., 1996; Jover et al., 1999; Lazzari, 1991; Lazzari et al., 2000; Mazzola et al., 2000), and late maturation (Micale et al., 1999; Zupa et al., 2017b), which allows for the marketing of the fish before growth is affected by reproductive maturation. As with many other newly cultured species, reproduction control has been one of the main bottlenecks for the commercialization of greater amberjack (Mylonas et al., 2016a). Although some natural-though unpredictable-spawning has been reported in the Canary Islands, Spain (Jerez et al., 2006) and Japan (Kawabe et al., 1996), this has not been the case in the Mediterranean Sea (Grau et al., 1996). Failure of spawning in captivity can result from improper environmental conditions or by captivity-induced stress (Mylonas et al., 2010). However, the main reproductive dysfunction observed in captive fishes is the lack or unreliable oocyte maturation, ovulation and spawning (Mylonas and Zohar, 2001) caused by inadequate pituitary Luteinizing Hormone (LH) synthesis and/or release at the end of vitellogenesis (Zohar and Mylonas, 2001). This reproductive dysfunction has been reported already in cultured greater amberjack (Kozul et al., 2001; Micale et al., 1999; Mylonas et al., 2004) and has so far barred its large-scale production in Europe. Recently, a comparative study of reproductive development in wild and captive-reared greater amberjack has shown the need for minimum handling of greater amberjack during the reproductive season, as this apparently induced significant long-

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[*] Corresponding author.
E-mail address: mylonas@hcmr.gr (C.C. Mylonas).

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term reductions in plasma testosterone (T), 17β-estradiol (E_2) and 17,20β-dihydroxy-4-pregnen-3-one (17,20β-P) (Zupa et al., 2017b) in the females. As a result, the gonadosomatic index (GSI) was significantly reduced at the peak of the reproductive season, and extensive follicular atresia was present in the ovaries. Similarly, significant reductions in plasma T, 11-ketotestosterone and 17,20β-P were observed in the males at the peak of spermatogenesis, again resulting in significant reductions in GSI (Zupa et al., 2017b), concomitant with elevations in plasma E_2, reduction in spermatogonial mitosis and high level of apoptosis at the beginning of the reproductive season (Zupa et al., 2017a). Therefore, more research is needed to understand the endocrine reproductive function of this species in captivity, and to overcome the observed reproductive impairments using the available endocrine therapies employed in a number of other species (Mylonas et al., 2017; Mylonas et al., 2010).

Different hormonal therapies have been used in the past in order to induce oocyte maturation, ovulation and spawning, via the administration of LH (gonadotropin preparations, GtH; or pituitary extracts, PE), human chorionic gonadotropin (hCG) or gonadotropin releasing hormone agonist (GnRHa) (Mañanos et al., 2009; Mylonas et al., 2010; Zohar and Mylonas, 2001). Of these therapies, GnRHa is more widely used due to its advantages in relation to lower species-specificity and targeting a higher level in the brain-pituitary-gonad axis, stimulating the release of the endogenous LH (Mylonas et al., 2010). In addition, GnRHa may be administered in the form of a bolus (liquid injection) or in a sustained-release delivery system (Mylonas and Zohar, 2001), each method having important advantages in different species. For example in meagre (*Argyrosomus regius*), multiple injections of GnRHa were considered more advantageous compared to GnRHa implants due to better egg production control and repeatability of response (Mylonas et al., 2015). In greater amberjack, spawning induction experiments have been done with both GnRHa implants (Mylonas et al., 2004) and multiple GnRHa injections (Fernández-Palacios et al., 2015b), but a proper study comparing the two methods in greater amberjack in the Mediterranean Sea has not been carried out so far.

The aim of the present study was to examine these two endocrine methods for the induction of oocyte maturation/ovulation and spawning in greater amberjack. These methods were based on the induction of endogenous LH release from the pituitary, through the use of GnRHa either in the form of implants (sustained release) or injections (acute release). The efficacy of the two methods was evaluated in terms of oocyte development, spawning kinetics, egg production and quality, with the objective of delivering a sound and efficient protocol to the aquaculture industry. In addition, the study provides some insight into the endocrine control of the process of gametogenesis and oocyte maturation in the species, and the role of hypothalamic GnRH in acute versus sustained administrations.

2. Materials and methods

2.1. Broodstock maintenance

The amberjack broodstock was kept at the sea cage facilities of Argosaronikos Fish Farm S.A. (Salamina Island, Greece). The stock consisted of wild fish captured in 2011 at the juvenile stage with a purse seine from the area of Astakos, Greece. The fish were then moved to Argosaronikos Fish Farm in May 2014 at a body size of 5–7 kg. The broodstock was maintained in a 1000-m^3 sea cage, 300 m off the coast where the on-land facilities of the company were located. At the time of the 2016 reproductive season (late spring-summer), the stock consisted of 14 females (mean ± SD body weight 18.8 ± 2.1 kg) and 14 males (mean ± SD body weight 15.1 ± 3.0 kg). Feed (Vitalis CAL, 22 mm, Skretting, Norway) was given to apparent satiation 6 days a week and fish were starved one day prior to handling.

For the spawning induction trial, fish were selected (see below in “Evaluation of reproductive stage and broodstock selection”) and induced to spawn, just prior to being transferred to the on-land facilities, to four 23-m^3 round tanks. Each GnRHa treatment was conducted in duplicates, with 6 and 8 fish per tank, respectively, at a 1:1 sex ratio, which means that each of the 4 replicates consisted of 3–4 females. The tanks were supplied with a mixture of surface (~16% exchange h^{-1}) and well water (~6% exchange h^{-1}) of ~39 ppt salinity and exposed to ambient photo-thermal conditions, with temperature ranging from 20.1 °C to 24.1 °C in the course of the experiment (Fig. 1). Gaseous oxygen was also provided to the tanks for safety reasons, maintaining the saturation to 110–120%. Measurements of temperature and oxygen saturation were conducted twice a day (a.m. and p.m.). Tank overflows were fitted with 250-L passive egg collectors. Feeding in the tanks was done to apparent satiation between samplings, 5 days a week.

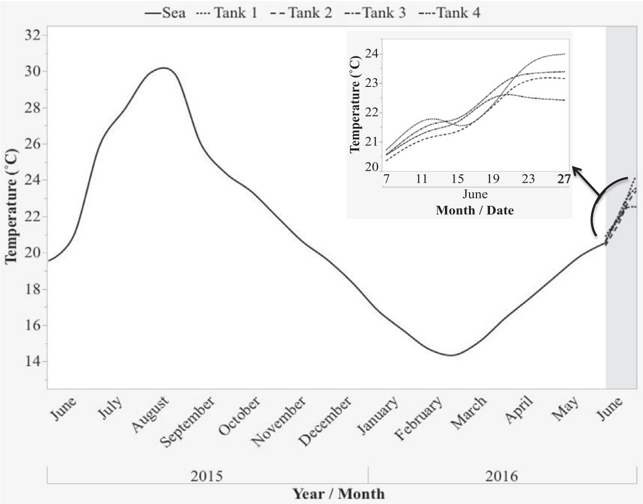

Fig. 1. Water temperature profile for the sea cage maintaining the greater amberjack broodstock during the year (June 2015–June 2016) or for the different tanks during the spawning induction trials (June 2016, inset). The shaded area indicates the experimental period.

For the present study, wild captive-reared greater amberjack were used. Ethical approval was not required by the relevant Greek authorities (National Veterinary Services), because the fish were maintained in a registered aquaculture facility. All procedures involving animals were conducted in accordance to the “Guidelines for the treatment of animals in behavioral research and teaching” (Anon., 1998), the Ethical Justification for the Use and Treatment of Fishes in Research: an Update (Metcalfe and Craig, 2011) and the “Directive 2010/63/EU of the European parliament and the council of 22 September 2010 on the protection of animals used for scientific purposes” (EU, 2010).

2.2. Evaluation of reproductive stage and broodstock selection

Once the fish were tranquilized inside their rearing sea cage with the use of clove oil (0.01 mL L^{-1}), they were moved for complete sedation to an anaesthetic bath at a concentration of 0.03 mL L^{-1} (Mylonas et al., 2005), on a working platform next to the sea cage. Ovarian biopsies were obtained by inserting an endometrial catheter (Pipelle de Cornier) into the ovarian cavity applying gentle aspiration. A wet mount of the biopsy was first examined under a compound microscope (40 and 100 ×) to evaluate the stage of oogenesis and measure the mean diameter of the largest, most advanced vitellogenic oocytes (n = 10), and pictures were taken for further evaluation. A portion of the biopsies was fixed in a solution of 4% formaldehyde-1% glutaraldehyde for histological analysis. Females were considered eligible for spawning induction if they contained fully vitellogenic oocytes with a diameter > 600 µm. Males were selected on the basis of spermiation, which was confirmed with the collection of a sample of milt using a catheter, since gentle abdominal pressure is not effective in releasing milt in greater amberjack, due to the thick muscular nature of the abdominal wall.

2.3. Spawning induction experiments

The spawning induction trial was conducted between 7 June and 28 June 2016. The females were treated either with GnRHa (Des-Gly^{10}, D-Ala^{6}-Pro-NEth^{9}-mGnRHa, H-4070, Bachem, Switzerland) injection (20–25 μg GnRHa kg^{-1}) or with EVAc GnRHa implant (Mylonas and Zohar, 2001), loaded with 750 or 1000 μg of the same GnRHa, for an effective dose of 49–69 μg GnRHa kg^{-1}. The selected doses were those used successfully in previous experiments using GnRHa implants (50 μg GnRHa kg^{-1}) (Mylonas et al., 2004) or GnRHa injections (20 μg GnRHa kg^{-1}) (Fernández-Palacios et al., 2015b). In order to enhance spermiation and ensure adequate milt production, males were treated at the start of the experiment with a GnRHa implant at a dose of 45–70 μg GnRHa kg^{-1} and allocated randomly to the four tanks. Sperm quality (spermatozoa motility percentage, duration of forward motility, spermatozoa density and spermatozoa survival at 4 °C) was also evaluated prior to the GnRHa administration. The dose for the first hormonal administration with GnRHa was given based on an estimated 30% growth from the last weighing time (the previous year, on 15 June 2015). As the fish did not have a uniform growth during this period, there was a variation in the effective GnRHa dose applied to each fish at the first injection. For the calculation of the GnRHa dose for the following hormonal administrations, the actual weight was used. For the GnRHa implant administrations, variations were caused also by the fact that implants are loaded with fixed amounts of GnRHa. Even though implants loaded with different amounts of GnRHa were used, it was still not possible to adjust the dose exactly to the different body weights of the fish.

During the initial GnRHa administration, females were allocated to four tanks in order to have two duplicates per treatment method (3 and 4 fish per replicate). Females were treated weekly in the injected group, and biweekly in the implanted stocks (a total of 3 injections and 2 implants). The subsequent administrations and samplings were implemented with the same procedure used for the first sampling. The water level was reduced in the tanks; the fish were tranquilized and were then moved to an anesthetic bath for complete sedation prior to biopsying and GnRHa administration. Three weeks after the start of the experiment, on 28 June 2016, the final sampling was conducted and the fish were returned to the sea cage.

2.4. Evaluation of egg/larval quality

Egg collectors were examined three times a day (8:00 a.m., 3:00 p.m. and 8:00 p.m.). For each spawn, the date, collection time and developmental stage of the eggs (Tachihara et al., 1993) were recorded, in order to identify different spawns and estimate an approximate spawning time. This allowed calculating the time between hormonal administration and first spawn (latency period). The eggs were collected and transferred into a 10-L bucket. Their number (fecundity) was estimated by counting the total number of eggs in a sub-sample of 10 mL, collected with a pipette after vigorous agitation. Daily relative fecundity was calculated as the number of eggs produced every day in each tank per female biomass, considering the eligible females that had vitellogenic oocytes > 600 μm and were expected to spawn after each GnRHa administration. Total relative fecundity was calculated as the number of eggs produced after each hormonal administration per female biomass in each tank, as above. The fertilization percentage was evaluated at the same time by examining each egg in the subsample. After collecting the sub-sample, the eggs were transferred into a 500 L conical tank-incubator fitted with an overflow filter (250 μm mesh size), and supplied with surface seawater (~90% h^{-1} renewal) and mild aeration. The embryonated eggs were then sent to different hatcheries for larval rearing trials (Mylonas et al., 2016a).

To monitor embryo and larval survival, embryonated eggs from each spawn were collected from the tank incubators and placed individually in 96-well microtiter (mct) plates (in duplicates) according to the procedure of Panini et al. (2001) with some modifications. Briefly, a sample of floating (~100% fertilized) eggs were taken from the tank incubators with a 250 μm mesh sieve, rinsed with seawater and poured in 2 L beakers filled with seawater. Using the sieve, 100–200 floating eggs were scooped from the beaker and placed in a Petri dish. Together with 200 μL of seawater, the fertilized eggs were aspirated with a micropipette one by one and transferred individually to the 96-wells of a mct plate. The plates were checked under a stereoscope and any dead eggs were replaced. Once loaded, the mct plates were covered with a plastic lid, placed in a controlled-temperature incubator and maintained for 7 days at temperatures ranging between 21.0 and 23.5 °C, according to the spawning temperature of each batch. Using a stereoscope, embryonic and early larval development was evaluated daily, recording the number of live embryos 24 h after egg collection (or ~30 h after spawning), hatched larvae (examined ~55 h after spawning) and viable larvae on day 5 after hatching (near the time of yolk sack absorption). At 21.0–23.5 °C, hatching of the amberjack eggs took place in 40–55 h (39.2–48.1°-days). The embryo survival was calculated as the number of eggs having live embryos 1 d after egg collection/number of fertilized eggs initially loaded in the mct plates. The hatching success was calculated as the number of hatched larvae/24-h embryos, and the 5-d larval survival was calculated as the number of live larvae 5 d after hatching. Estimating survival percentage (%) by using as denominator the number of individuals that survived to the previous developmental stage was considered as a more accurate evaluation of survival within specific developmental stages, without the potential of a distortion effect of the previous stage.

2.5. Histological analysis

The samples were fixed in 4% formaldehyde: 1% glutaraldehyde (McDowell and Trump, 1976), dehydrated in a 70–95% ethanol series and embedded in glycol methacrylate resin (Technovit 7100, Heraeus Kulzer, Germany). Serial sections were obtained at a thickness of 3–5 μm on a microtome (Leica RM2245, Germany) using disposable blades. After drying, slides were stained with methylene blue/azure II/basic fuchsin (Bennett et al., 1976), examined under a light microscope (50i Eclipse, Nikon, Japan) and photographed using a digital camera (Progres, Jenoptik AG, Germany).

2.6. Statistical analysis

Differences in mean oocyte diameter, relative and total fecundity and egg/larval performance parameters (fertilization success, 24-h embryo survival, hatching, and 5-d larval survival) between the two GnRHa treatments were examined using a *t*-test at a $P \le 0.05$ significance level. Within GnRHa treatment, differences among data collected in the period between GnRHa administrations were examined using one-way ANOVA followed by the post-hoc test Tukey's HSD at a $P \le 0.05$ significance level (only for total relative fecundity due to unbalanced sample sizes for the other parameters). Data was examined for normality in the distribution of variances, in order to comply with the prerequisites of the ANOVA. Percentages were arcsin transformed before statistical analysis. All analyses were performed with a statistics software (JMP 12, SAS Institute Inc., Cary, NC, USA). Results are presented as mean ± SEM, unless otherwise stated.

3. Results

There were differences between the females from the two GnRHa treatments in the number of females remaining eligible for induction of spawning (*i.e.* having fully vitellogenic oocytes > 600 μm) at the final sampling at the end of the experiment (Fig. 2A). However, there was no

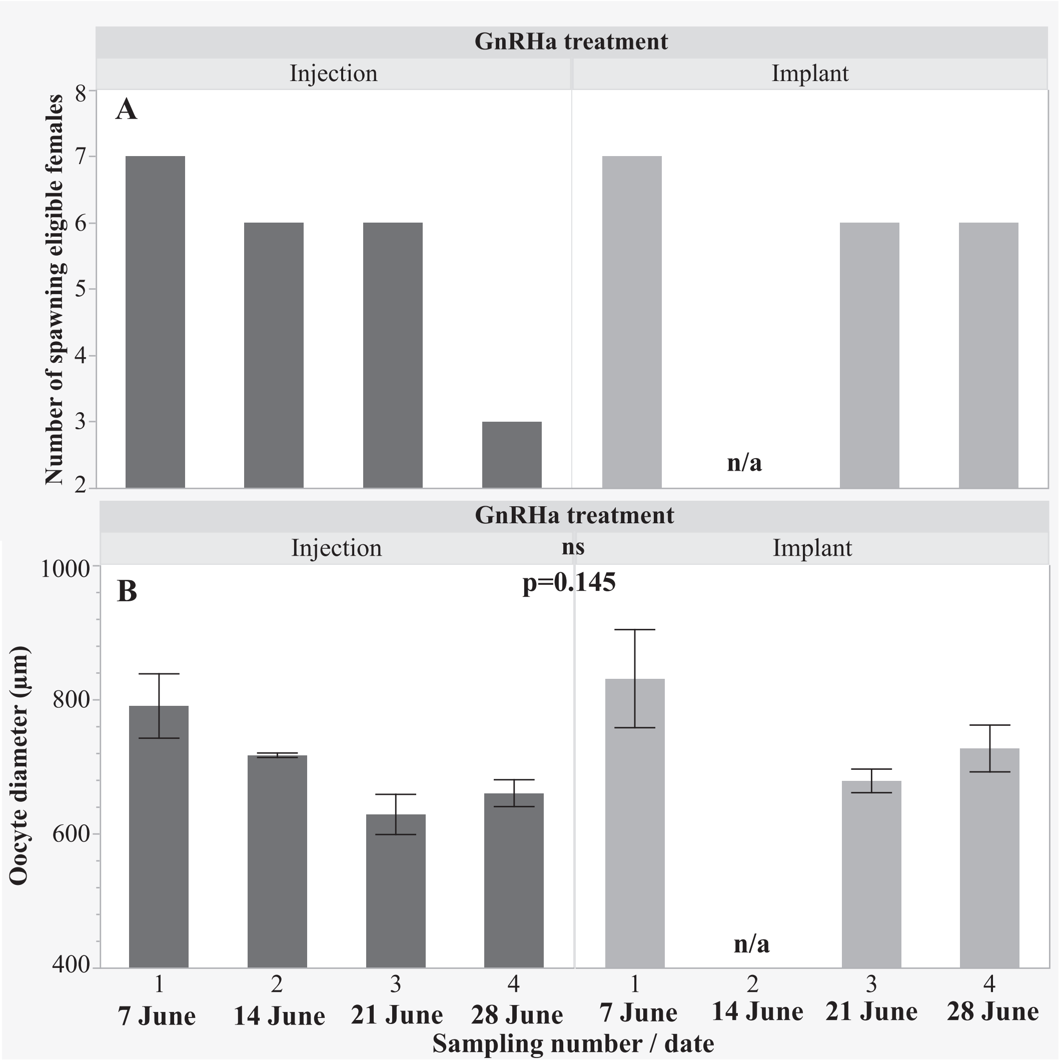

Fig. 2. Number of spawning-eligible females (A) and mean diameter (± SEM) of the largest vitellogenic oocytes (B) from the biopsies of female greater amberjack treated with multiple administrations of a GnRHa injection or GnRHa implant. The P value of the statistical analysis (*t*-test) between the two GnRHa treatments is indicated on the GnRHa treatment legend (ns = no significance). n/a: not applicable.

significant difference in the mean oocyte diameters between the females from the two GnRHa treatments ($t_2 = -0.71$, $P = 0.145$), since the samples represented only females eligible for induction of spawning and the evaluated oocytes were only those in advanced vitellogenesis (Fig. 2B). Specifically, in the GnRHa implant treatment, the number of eligible females decreased from the initial administration from 7 to 6 females at the final sampling, whereas in the GnRHa injection treatment the number of eligible females decreased from 7 to 3 females.

At the time of the 1st GnRHa hormonal administration, females had mainly vitellogenic oocytes in their ovaries, while 4 out of the 14 were in oocyte maturation (Fig. 3, first row) and only a small number of atretic follicles was observed. During the 2nd sampling, only the injected fish were handled and biopsied. Vitellogenic oocytes were still present, except in one female where only ovulated eggs and atretic follicles were observed (Fig. 3, second row). Some ovulated eggs and atretic follicles were observed in almost all the biopsies taken. At the 3rd sampling, vitellogenic oocytes were visible in both treatment groups (Fig. 3, third row), except from one fish from each treatment that had only atretic follicles and ovulated eggs, while the number of primary oocytes was increased. At the 4th sampling, the implanted fish still had vitellogenic oocytes and some of them in oocyte maturation, and the proportion of atresia was minimal (Fig. 3, fourth row). On the other hand, 4 of 7 injected fish had concluded their reproductive period (*i.e.* they were spent), since only primary and early vitellogenic oocytes were present in the ovaries, together with advanced atretic follicles and unreleased, overripe eggs. The other 3 females from the injected treatment still had some vitellogenic oocytes, but with increased number of atretic follicles.

In response to both GnRHa treatments, spawning started 1 day after the 1st application, because of the existence of maturing oocytes, whereas after the following GnRHa administrations spawning commence after 2 days (Fig. 4). Implanted fish spawned for up to ten times after the 1st administration, and only four times after the 2nd administration. Injected fish spawned for seven times after the 1st administration, three to five times after the 2nd administration and one to three times after the 3rd administration.

Mean daily relative fecundity was significantly higher ($t_{1.3} = -5.24$, $P = 0.012$) in the implanted fish (15,170 ± 2,738 eggs kg^{-1} female day^{-1}) compared to the injected fish (6,119 ± 2,790 eggs kg^{-1} female day^{-1}) (Fig. 5). Total relative fecundity was also significantly higher ($t_{1.3} = -9.93$, $P = 0.003$) in the implanted fish (102,402 ± 20,337 eggs kg^{-1} female) compared to the injected ones (26,517 ± 9,938 eggs kg^{-1} female) (Fig. 6). When looking separately at the total egg production in the period between different GnRHa administrations, we observed a significant decrease (ANOVA, Tuckey's HSD, $P \leq 0.05$) over time in fish from the GnRHa

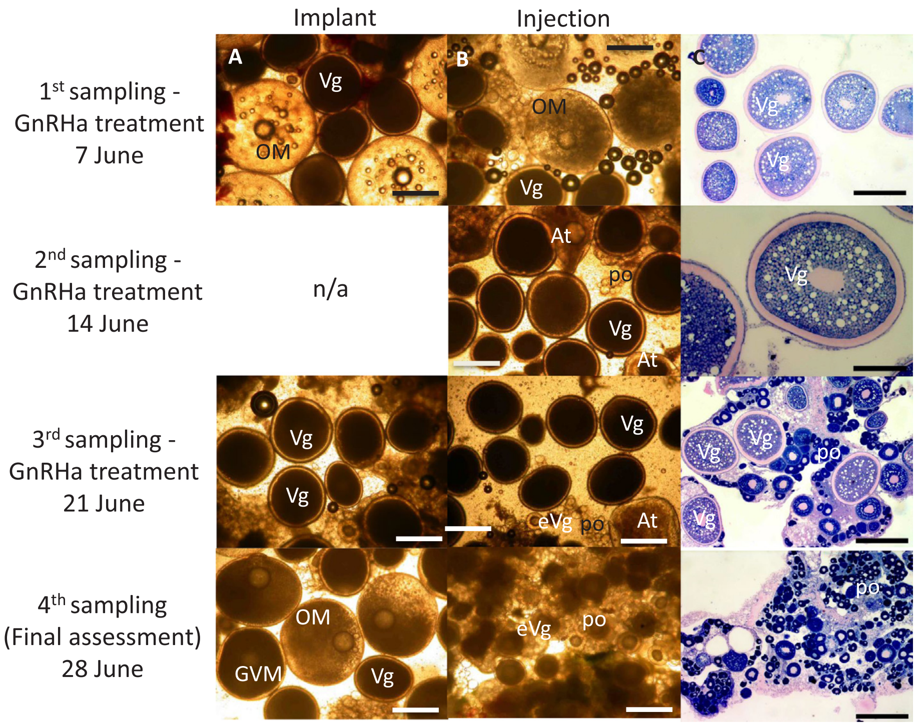

Fig. 3. Microphotographs of representative ovarian biopsies from greater amberjack just prior to the different GnRHa administrations and the final sampling time at the end of the experiment, presented as wet mounts (left and middle columns) or after histological processing (right column). Fish were treated either with GnRHa implants or injections. po = primary oocyte, Vg = vitellogenic oocyte, eVg = early Vg, GVM = Germinal Vesicle Migration, OM = Oocyte Maturation, At = atretic follicle. Bars = 500 μm.

injected treatment, but not in the implanted treatment. The highest daily egg production was observed in implanted fish, with 4,242,000 eggs tank^{-1} two days after the 1st administration, while in injected fish maximum daily egg production was 2,454,000 eggs tank^{-1} at the same time.

Fertilization success, 24-h embryo survival, hatching and 5-d larval survival was similar between eggs obtained with the two GnRHa treatments (Fig. 7). Overall, mean fertilization success was 36 ± 5%, 24-h embryo survival was 53 ± 7%, hatching was 70 ± 4% and 5-d larval survival was 20 ± 4%.

4. Discussion

Greater amberjack in the present study were induced successfully to spawn using either GnRHa implants of ~50 μg kg^{-1} fish or GnRHa injections of 20 μg kg^{-1} fish, spawning many times over the course of 3 weeks, and producing fertilized eggs of adequate quality for large scale larval rearing trials (Mylonas et al., 2016a). These results confirmed previous studies where GnRHa implants (Mylonas et al., 2004) or injections (Fernández-Palacios et al., 2015b) induced multiple spawning of viable eggs, overcoming the observed reproductive dysfunctions of the species in captivity. The GnRHa therapy was effective in all fish treated, and resulted in many more spawns and a longer reproductive season than reported previously (Mylonas et al., 2004). Developing a reliable method for the production of eggs from a large number of breeders is important for the development of a sustainable industry of greater amberjack, because in Europe (and especially in the Mediterranean) spontaneous reproduction of the species is very rare (Jerez et al., 2006; Lazzari et al., 2000; Mylonas et al., 2004; Rodríguez-Barreto et al., 2014). In addition, synchronizing spawning of a large group of breeders could mitigate the problem of disproportionate parental contribution and inbreeding, when eggs are produced from only a limited number of breeders, as it was shown for the congeners yellowtail kingfish (*Seriola lalandi*) (Setiawan et al., 2016) and longfin yellowtail (*Seriola rivoliana*) (Fernández-Palacios et al., 2015a).

Early studies on the induction of spawning of greater amberjack utilizing chum salmon (*Oncorhynchus keta*) PE resulted in the production of 1.4 and 7 million eggs in two different reproductive seasons (Tachihara et al., 1993), whereas the use of hCG injection produced mostly unfertilized eggs (Kozul et al., 2001). The more recent use of GnRHa seems to be the most efficacious method in greater amberjack (Fernández-Palacios et al., 2015b; Jerez et al., 2018; Mylonas et al., 2004), as it was shown in other species as well (Mylonas et al., 2010). However, the differences in response (*e.g.* spawning kinetics, egg production and quality) between the methods of administration of GnRHa have not been systematically evaluated so far. It has been shown long time ago that the *in vitro* degradation rate of even the most potent synthetic GnRHa is very rapid, with a half-life of only minutes (Zohar et al., 1990; Zohar et al., 1989). *In vivo*, most of the GnRHas used stimulated increases in plasma LH for only 48 h (Zohar et al., 1990; Zohar et al., 1989). In other studies, injections of a bolus of GnRHa resulted in measurable levels in the plasma for a period of 2–5 days (Harmin and Crim, 1993; Mylonas et al., 1998), stimulating an increase in plasma LH for a similar period (Mañanos et al., 2002; Mylonas et al., 1998). On the contrary, implants loaded with GnRHa resulted in measurable levels in the plasma for periods > 14 days, resulting in continuously elevated plasma LH for as long as GnRHa could be measured in the plasma, without any indication of downregulation (Mañanos et al., 2002; Mylonas et al., 1997; Mylonas et al., 1998). Due to these differences in the kinetics of plasma LH induced by a bolus versus a controlled release GnRHa administration, it was interesting both from an aquaculture production perspective, but also from a reproductive endocrinology one, to compare the effects of GnRHa injections and GnRHa implants on the reproductive function of greater amberjack, which has an asynchronous ovarian development and multiple spawning frequency during the reproductive season.

In the present study, GnRHa implanted females produced significantly more eggs of the same quality in the same number of spawns compared to the GnRHa injected females, with less handling. In addition, most of the GnRHa implanted females still contained a significant number of vitellogenic oocytes at the end of the experiment, when spawning ceased, and presumably had the potential to produce more eggs if treated with another GnRHa implant. It was shown recently that expression of the β subunit of Follicle Stimulating Hormone (*fshb*) in the pituitary increased, and plasma FSH remained elevated throughout the reproductive season, including the spawning period, at the time that

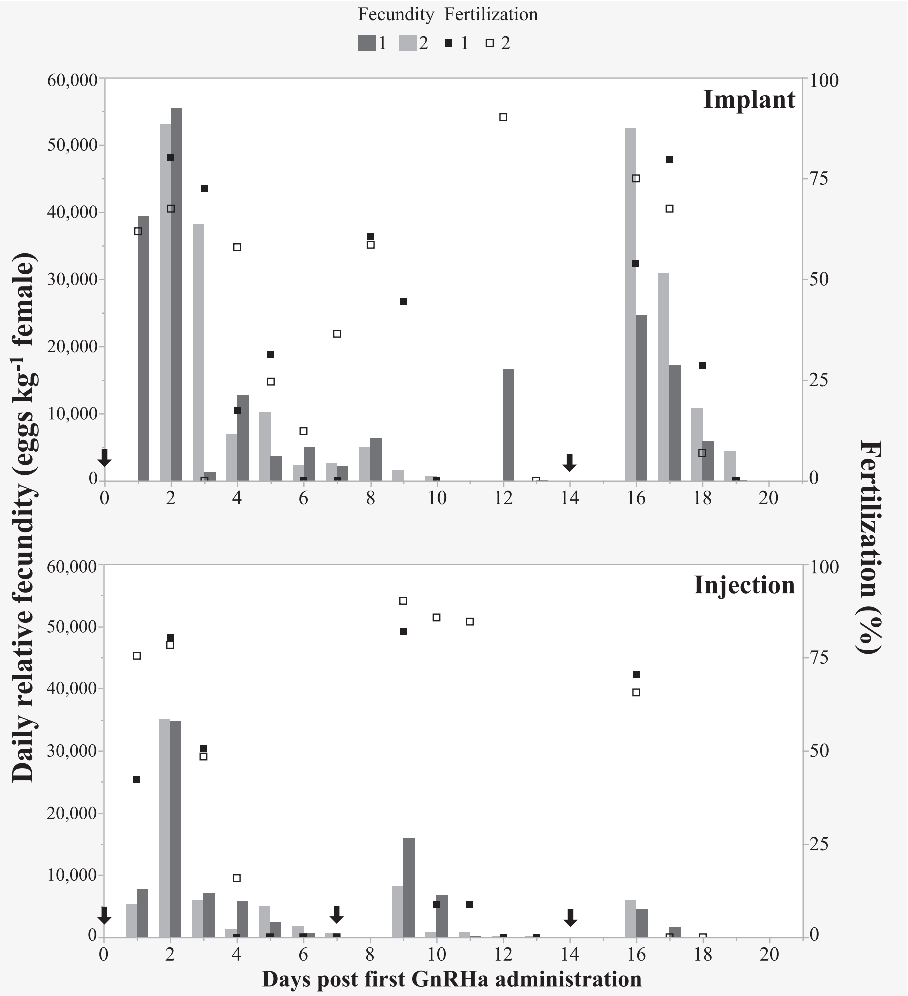

Fig. 4. Daily relative fecundity (bars, x eggs kg^{-1} female) and fertilization success (squares, %) of GnRHa injected or implanted greater amberjack (n = 2 tanks for each treatment group, numbered 1 and 2). Arrows (n = 2 for GnRHa implanted and n = 3 for injected fish) indicate the time of hormonal administration. The first application was done on 7 June 2016.

expression of pituitary *lhb* and plasma LH increases significantly (Nyuji et al., 2016). Therefore, the synthesis and release of FSH from the pituitary during the peak of the reproductive period (*i.e.* during spawning) is quite relevant in this asynchronous species, presumably for the maintenance of high E_2 and vitellogenin levels in the plasma (Mandich et al., 2004) and the support of gametogenesis and the production of multiple batches of oocytes for maturation. The results of the present study suggest that the sustained presence of GnRHa in circulation, resulting from the administration of sustained-release delivery systems was probably more physiologically sound for the continued stimulation of gametogenesis, through FSH release, compared to a multiple GnRHa injection protocol. In a similar comparison with the present study in the Canary Islands (Spain), GnRHa implants were shown to be more reliable in inducing spawning than GnRHa injections, since fish responded consistently after a GnRHa implant administration, spawning an average of 2.2 ± 1.9 spawns per implantation (Sarih et al., 2018). On the contrary, after GnRHa injection fish spawned only 0.8 ± 0.5 spawns per injection, suggesting that only 80% of the injections resulted in spawning. If fish after some injections spawned more than once after the injection (this information is not provided in the article), then the success of that study was even less than 80%. Fecundity was similar in the two methods in that study, even if the number of spawns obtained after GnRHa implantation was more than 2x higher. Based on the higher fertilization success of eggs obtained from the GnRHa injected group, however, it was reported that this was a preferred method of spawning induction (Sarih et al., 2018). Greater amberjack in the Canary Islands exhibit somewhat different reproductive characteristics with a much more prolonged reproductive period – lasting from April to October (Jerez et al., 2006; Sarih et al., 2018) – presumably due to the more stable environmental conditions (photoperiod and temperature) that exist in sub-tropical regions. Perhaps the use of a bolus GnRHa administration once every ~10 days in the study of Sarih et al. (2018) was physiologically a more appropriate method under these conditions, as it was shown in meagre maintained under constant temperatures from the beginning of the spawning season and for the next 4 months (Mylonas et al., 2016b). In the present study, two administrations of GnRHa implants spaced 14 days apart produced significantly more eggs of the same quality compared to three weekly GnRHa administrations with injections, with the GnRHa implantation approach involving less handling.

The greater amberjack is characterized as group synchronous or asynchronous regarding its ovarian development, with a multiple spawning reproductive strategy (Marino et al., 1995). The GnRHa implant is considered as the most appropriate method of administration for this group of fishes, since it can both induce multiple cycles of oocyte maturation and ovulation, and it can support also the final stages of vitellogenesis in fish that are not quite ready to undergo oocyte maturation (Mylonas et al., 2004). In the multiple batch spawner Senegalese sole (*Solea senegalensis*), two different GnRHa sustained-release delivery systems were proven to be the most effective for

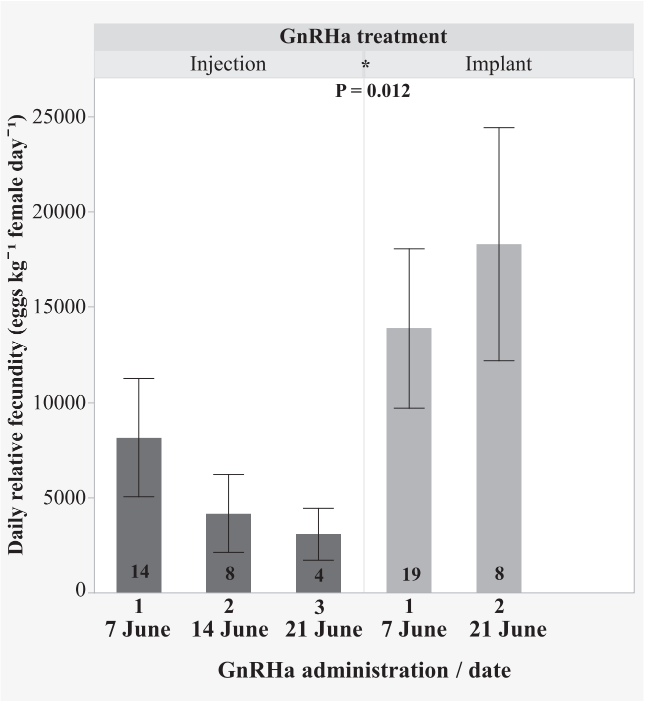

Fig. 5. Mean (± SEM) daily relative fecundity of greater amberjack in the periods between different GnRHa injection or implant administrations. Numbers inside the bars are the spawns constituting each mean. A statistically significant difference between the two GnRHa treatments is indicated by an "*" and the P value of the *t*-test (n = 2 replicate tanks per treatment) on the GnRHa treatment legend.

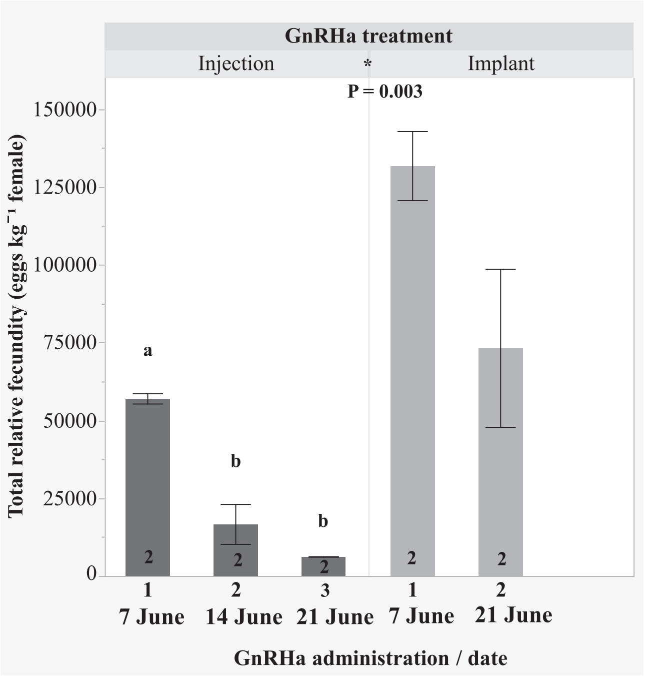

Fig. 6. Mean (± SEM) total relative fecundity of greater amberjack in the periods between different GnRHa injection or implant administrations. Numbers inside the bars are the replicates for each mean. A statistically significant difference between the two GnRHa treatments is indicated by an "*" and the P value of the *t*-test (n = 2 replicate tanks per treatment) on the GnRHa treatment legend. Lowercase letters above the hormonal administration means indicate significant differences within GnRHa administrations (ANOVA, Tukey's HSD, P ≤ 0.05).

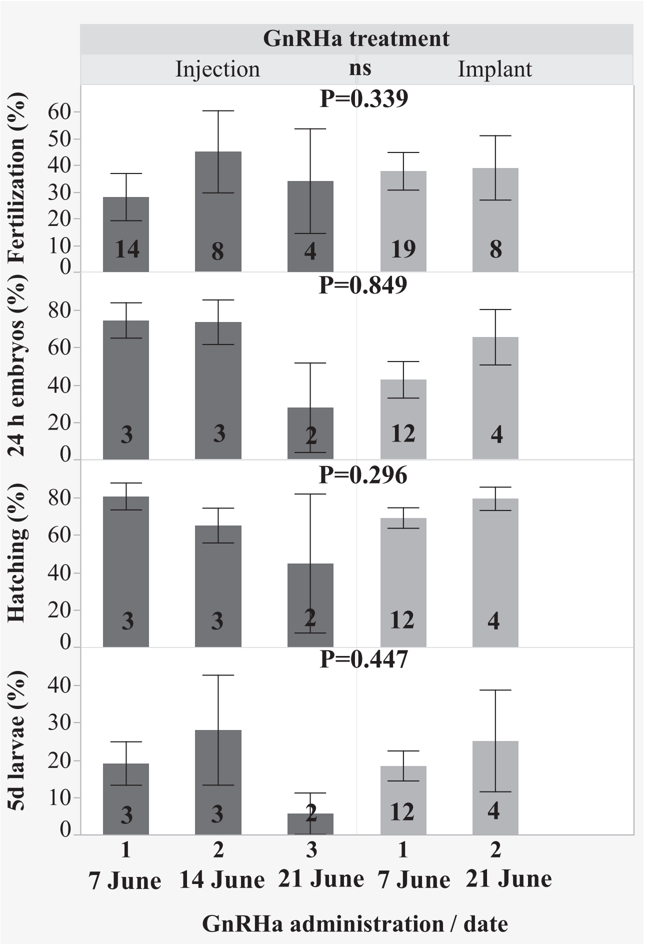

Fig. 7. Mean (± SEM) fertilization, 24-h embryo survival, hatching and 5-d larval survival of greater amberjack eggs collected in the periods between different GnRHa injection or implant administrations. Numbers inside the bars are the number of spawns for each mean. No statistical differences were observed between different GnRHa treatment methods (*t*-test, n = 2 replicate tanks per treatment, P value on each graph).

spawning induction when compared with a single (Guzmán et al., 2009) or multiple injections of GnRHa (Agulleiro et al., 2006). However, in meagre, a species which is also characterized by asynchronous oocyte development (Gil et al., 2013; Mylonas et al., 2013b), multiple GnRHa injections were reported to be a more appropriate method of spawning induction compared to GnRHa implants, even if the overall results of egg production and quality did not differ significantly between the two methods (Mylonas et al., 2015). In meagre, GnRHa implants induce daily spawns for up to 3 weeks, but the majority of the eggs are produced during the first 2–3 spawns after administration, while the following spawns consist of small batchers of eggs that can not be utilized efficiently by commercial hatcheries (Mylonas et al., 2013a), and eventually the gonad is depleted of vitellogenic oocytes and further GnRHa implantation does not produce consistent results (Mylonas et al., 2015). On the contrary, weekly GnRHa injections induced only two high-fecundity spawns after each injection (day 2 and 3 after hormonal administration) – presumably due to the short-lived elevation in plasma LH – and the hormonal administration produced consistently spawns of high fecundity and egg quality for up to 17 weeks (Mylonas et al., 2016b). So, the choice between GnRHa injection(s) or sustained-release delivery systems for the induction of spawning must be examined in each species of interest, as well as in relation to the specific environmental conditions and genetic origin of the broodstock in question. Recent genetic studies suggested that greater amberjack may be separated into an Atlantic and Mediterranean population (Šegvić-Bubić et al., 2016), and this may explain – at least in part – the differences in response to the GnRHa injections and implants of the fish between the present study and the one in the Canary Islands (Sarih et al., 2018).

Group synchronous oocyte development is exhibited also by the congeners of greater amberjack such as the yellowtail kingfish (Poortenaar et al., 2001), the Japanese yellowtail (*Seriola quinqueradiata*) (Kagawa, 1989) and the longfin yellowtail (Fernández-Palacios et al., 2015a), and all have been induced to spawn successfully with various hormonal therapies (Chuda et al., 2002; Fernández-Palacios et al., 2015b; Roo et al., 2014; Setiawan et al., 2016). In the Japanese yellowtail a comparison among three different hormonal therapies was examined, using a single or double hCG injection, or a single cholesterol implant with GnRHa. Even though the latter hormonal administration produced the highest fecundity, a single hCG injection was considered as the most efficient method to induce oocyte maturation and ovulation, based on the production of eggs of better quality (Chuda et al., 2001). However, when hCG administration is used repeatedly in subsequent years, the fish may develop an immune response and the injected preparation is immune-neutralized (Zohar and Mylonas, 2001). This is an important disadvantage of the hCG over the GnRHa preparations for inducing spawning of fishes for aquaculture production. This is especially true in species such as the greater amberjack that are mainly of wild-caught origin, are difficult to acquire and they mature after > 3–4 years.

Eighty six percent of the females from the GnRHa implanted group and 43% of the injected group still had a large number of fully vitellogenic oocytes 3 weeks after the initial hormonal administration and after spawning multiple times. We expect that these fish would produce more eggs if given another hormonal therapy. As discussed earlier, this observation demonstrates the capacity of GnRHa, primarily in a sustained-release delivery system, to not only induce maturation of the available post-vitellogenic oocytes, but also to further support vitellogenesis of the smaller oocytes, as it was shown in the dusky grouper (*Epinephelus marginatus*) (Marino et al., 2003), eventually resulting in multiple cycles of oocyte maturation, ovulation and spawning. Unfortunately, oocyte diameter data at the time of consecutive GnRHa administrations are not available from some other spawning induction studies of greater amberjack (Fernández-Palacios et al., 2015b; Sarih et al., 2018). In a recent study in the Canary Islands, F1 greater amberjack broodstock treated with GnRHa implants every 30–40 days maintained their maximum oocyte diameter above 650 μm for a period of 5 months (May–September) (Jerez et al., 2018). The fact that the fish in these studies spawned for a period of many months after multiple injections every 10 days in the Canary Islands underlines the capacity of GnRHa to induce vitellogenesis, in addition to oocyte maturation, probably through the induction of FSH release, in addition to LH release from the pituitary (Zohar et al., 2010). The same capacity was evident in greater amberjack in the present study, albeit to a lesser extent, since in the Mediterranean (Zupa et al., 2017b) and Japan (Kawabe et al., 1996; Kawabe et al., 1998) the rapid elevation of water temperature in the summer most likely causes a cessation of reproductive function. Perhaps this is the reason that the same species has a much longer spawning season in the Canary Islands (up to October) where the water temperature does not exceed 25 °C (Jerez et al., 2018; Jerez et al., 2006; Sarih et al., 2018), whereas in Japan (Kawabe et al., 1996) and the Mediterranean Sea (Mandich et al., 2004; Marino et al., 1995) the spawning season ends in July. The effectiveness of GnRHa in sustained release delivery systems to promote the process of vitellogenesis has been demonstrated in only limited situations, such as in the ayu (*Plecoglossus altivelis*) (Aida, 1983), bitterling (*Acheilognathus rhombea*) (Shimizu, 1996), winter flounder (*Pleuronectes americanus*) (Harmin et al., 1995) and the congener yellowtail kingfish (Setiawan et al., 2016). In greater amberjack it was suggested recently that an increase in the expression of the receptor of FSH (*fshr*) in the ovarian follicles was more important for regulating vitellogenesis than elevation of FSH content in the plasma (Nyuji et al., 2016). Again, in the present study it appears that the sustained presence of GnRHa in circulation, resulting from the administration of sustained-release delivery systems was probably more appropriate than a multiple GnRHa injection protocol in stimulating the necessary increases in *fshr* expression.

The overall daily and total relative fecundity after the two GnRHa implantations were 2.5 and 3.8 times higher, respectively, than the three GnRHa injections. Considering only the first GnRHa implantation (9.5 ± 0.7 spawns) and injection (7 ± 0 spawns), the overall daily and total relative fecundity were 1.7 and 2.4 times higher, respectively. Multiple GnRHa injections in another study produced less than 45,000 eggs kg^{-1} fish per hormonal administration (Fernández-Palacios et al., 2015b), slightly lower than the respective maximum fecundity in response to GnRHa injections in the present study. In studies using GnRHa implants in the Canary Islands (Jerez et al., 2018) and Greece (Mylonas et al., 2004), the respective daily relative fecundity was less than 6,000 eggs kg^{-1} and 30,000 eggs kg^{-1}, respectively, which is 9 and 2 times lower compared to the maximum observed in the present study after GnRHa implantation. Wild-caught fish spawning spontaneously in the Canary Islands produced 114,490 eggs kg^{-1} (or a total of 14,311,200 eggs) during the extended spawning period (April to October) (Jerez et al., 2006). In the present study, the overall mean (± SD) total fecundity of the GnRHa implanted fish was 204,805 ± 20,214 eggs kg^{-1} (or a total of 13,489,000 ± 1,507,000 eggs), in a period of only 21 days. The mean total fecundity per female was 3,889,000 ± 355,000 eggs $female^{-1}$, which is higher than another study in the Canary Islands using multiple GnRHa injections (2,480,000 eggs $female^{-1}$) (Fernández-Palacios et al., 2015b).

Fertilization success was similar in the eggs obtained using any GnRHa treatment, suggesting that in both treatments the process of oocyte maturation, ovulation and spawning was equally successful, if we assume that the performance of the males was similar in both groups. To ensure the latter, males in the two GnRHa treatment groups were treated in a similar way (GnRHa implants) and the sperm quality was found to be the same at the beginning of the experiment. Similar values of fertilization success were found in a spawning induction experiment of greater amberjack using GnRHa implants in Greece (Mylonas et al., 2004) or in the Canary Islands using F1 broodstock (Jerez et al., 2018). Significantly higher values, however, were reported in a multiple GnRHa injections experiment (96.0 ± 6.5%) with the same species (Fernández-Palacios et al., 2015b). In the latter study, the sex ratio was higher in favor of the males (1:2 ♀:♂) than the present study (which was 1:1 ♀:♂), and this could have resulted in better fertilization success. In a different study where natural (*i.e.* no hormonal administration), GnRHa injected and GnRHa implanted groups were compared, fertilization success was 84.4 ± 21.6%, 58.8 ± 26.8% and 32.5 ± 34.6%, respectively, being significantly different among the groups (Sarih et al., 2018). Again, in the latter study the sex ratio of the most successful group (natural) was higher in favor of the males (2:5 ♀:♂). Based also on reports from greater amberjack in Japan, it seems clear that an increased male to female ratio is preferable for this species, since more than one male appears to fertilize the eggs of one female (Tachihara et al., 1993). In the congener yellowtail kingfish, it has been shown that in a communal tank with 14 breeders, in 50% of the recorded spawning events two males fertilized the eggs of one female, resulting in > 99% fertilization success, suggesting also that a higher male to female ratio is preferable for that congener as well (Moran et al., 2007). In the same species, a high male contribution (60% in all egg batches) in the fertilized eggs was found after parentage analysis of the eggs spawned in a communal tank with 14 females and 10 males (Setiawan et al., 2016).

The quality of the eggs obtained in the present study – in terms of embryonic development, hatching and larval survival until yolk sack absorption—did not exhibit any significant differences between the two

GnRHa treatments, but was lower than in other studies. One day after spawning, only 53 ± 7% embryos were still alive compared to 92.2 ± 9.4%, 86.4 ± 25.4% and 77.6 ± 34.0% for eggs obtained from natural, GnRHa injected or GnRHa implanted females, respectively, in a study in the Canary Islands (Sarih et al., 2018). Hatching was also lower in the present study compared to the above-mentioned study, being 96.6 ± 6.6% for eggs from the natural, 91.1 ± 25.4% from the injected and 78.0 ± 34.9% from the implanted females. However, 5-d larval survival was higher here, compared to the 10.8 ± 14.7% for eggs from the natural, 5.5 ± 7.2% from the injected and 8.0 ± 12.5% from the implanted group, respectively (Sarih et al., 2018). Although there is room for improvement in terms of the quality of the eggs produced in response to the spawning induction therapy, the eggs produced were of adequate fecundity and quality to implement a number of larval rearing trials, for the development of commercial production protocols (Mylonas et al., 2016a). Improvements in egg quality are expected to result from a number of actions, such as better selection of female breeders according to their production characteristics (Symonds et al., 2014), optimized sex ratios, tank size and perhaps above all, broodstock nutrition (Izquierdo et al., 2001; Roo et al., 2015; Valdebenito et al., 2013). The latter is expected to gain more importance once a growing greater amberjack aquaculture industry is established, making it worthwhile for feed companies to invest in both specialized grow out and broodstock feeds for this species.

In conclusion, spawning induction of captive-reared greater amberjack was more effective using GnRHa implants than injections. More eggs were produced using GnRHa implants compared to injections, without altering the quality of eggs in terms of fertilization, 24-h embryo survival, hatching and 5-d larval survival. In addition to apparently promoting the proper endocrine pathways leading to multiple cycles of oocyte maturation, ovulation and spawning, the use of GnRHa implants induced also vitellogenesis. This method may be more effective in greater amberjack than multiple injections, also because of the less handling it involves (*i.e.* one handling every two weeks as opposed to one handling every week). It has recently been shown that handling greater amberjack during the reproductive season may induce significant reductions in spermatogenesis and oogenesis (Pousis et al., 2018; Zupa et al., 2017a,b). Therefore, minimizing the handling involved when implementing spawning induction methods is important for production of good quality gametes in aquaculture. We expect that the spawning induction method presented here would be a valuable tool for the efforts to incorporate this species in the aquaculture industry in Europe, and also that the insights provided into the endocrine control of gametogenesis and oocyte maturation in the species by hypothalamic GnRH will be followed with more detailed endocrine studies.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ygcen.2018.12.007.

References

Agulleiro, M.J., Anguis, V., Cañavate, J.P., Martínez-Rodríguez, G., Mylonas, C.C., Cerdà, J., 2006. Induction of spawning of captive-reared Senegal sole (*Solea senegalensis*) using different administration methods for gonadotropin-releasing hormone agonist. Aquaculture 257, 511–524.

Aida, K., 1983. Effect of LH-releasing hormone on gonadal development in a salmonid fish, the ayu. Bull. Jpn. Soc. Sci. Fish. 49, 711–718.

Anon., 1998. Guidelines for the treatment of animals in behavioural research and teaching. Anim. Behav. 55, 251–257.

Bennett, H.S., Wyrick, A.D., Lee, S.W., McNeil, J.H., 1976. Science and art in preparing tissues embedded in plastic for light microscopy, with special reference to glycol methacrylate, glass knives and simple stains. Stain Technol. 51, 71–97.

Chuda, H., Imayoshi, T., Arakawa, T., Matsuyama, M., 2001. Hormonal treatment for induction of oocyte maturation and ovulation in cultured yellowtail, *Seriola quinqueradiata*. Sci. Bull. Faculty Agric., Kyushu University 55, 169–177.

Chuda, H., Nakao, T., Arakawa, T., Matsuyama, M., 2002. Effects of hCG injection on oocyte maturation and ovulation in cultured two-year-old yellowtail, *Seriola quinqueradiata*. Suisanzoshoku 50, 235–236.

Crespo, S., Grau, A., Padrós, F., 1994. The intensive culture of 0-group amberjack in the western Mediterranean is compromised by disease problems. Aquacult. Int. 2, 262–265.

EU, 2010. Directive 2010/63/EU of the European parliament and the council of 22 September 2010 on the protection of animals used for scientific purposes. Off. J. Eur. Union L 276/33, Animal Protection.

Fernández-Palacios, H., Schuchardt, D., Roo, J., Hernández-Cruz, C., Izquierdo, M., 2015a. Spawn quality and GnRHa induction efficiency in longfin yellowtail (*Seriola rivoliana*) broodstock kept in captivity. Aquaculture 435, 167–172.

Fernández-Palacios, H., Schuchardt, D., Roo, J., Hernández-Cruz, C.M., Izquierdo, M., 2015b. Multiple GnRHa injections to induce successful spawning of wild caught greater amberjack (*Seriola dumerili*) matured in captivity. Aquac. Res. 46, 1748–1759.

Gil, M.D.M., Grau, A., Basilone, G., Ferreri, R., Palmer, M., 2013. Reproductive strategies and fecundity of meagre *Argyrosomus regius* Asso, 1801 (Pisces: Sciaenidae): implications for restocking programs. Sci. Marina 77, 105–118.

Grau, A., Crespo, S., Riera, F., Pou, S., Sarasquete, M.C., 1996. Oogenesis in the amberjack *Seriola dumerili* Risso, 1810. A histological, histochemical and ultrastructural study of oocyte development. Sci. Marina 60, 391–406.

Guzmán, J.M., Ramos, J., Mylonas, C.C., Manaños, E., 2009. Spawning performance and plasma levels of GnRHa and sex steroids in cultured female Senegalese sole (*Solea senegalensis*) treated with different GnRHa-delivery systems. Aquaculture 291, 200–209.

Harmin, S.A., Crim, L.W., 1993. Influence of gonadotropin hormone-releasing hormone analog (GnRH-A) on plasma sex steroid profiles and milt production in male winter flounder, *Pseudopleuronectes americanus* (Walbaum). Fish Physiol. Biochem. 10, 399–407.

Harmin, S.A., Crim, L.W., Wiegand, M.D., 1995. Manipulation of the seasonal reproductive cycle in winter flounder, *Pleuronectes americanus*, using a gonadotropic hormone releasing hormone. Mar. Biol. 121, 611–619.

Izquierdo, M.S., Fernández-Palacios, H., Tacon, A.G.J., 2001. Effect of broodstock nutrition on reproductive performance of fish. Aquaculture 197, 25–42.

Jerez, S., Fakriadis, I., Papadaki, M., Martín, M., Cejas, J., Mylonas, C.C., 2018. Spawning induction of first-generation (F1) greater amberjack *Seriola dumerili* in the Canary Islands, Spain using GnRHa delivery systems. Fishes 3, 1–22.

Jerez, S., Samper, M., Santamaría, F.J., Villamados, J.E., Cejas, J.R., Felipe, B.C., 2006. Natural spawning of greater amberjack (*Seriola dumerili*) kept in captivity in the Canary Islands. Aquaculture 252, 199–207.

Jover, M., García-Gómez, A., Tomás, A., De la Gándara, F., Pérez, L., 1999. Growth of Mediterranean yellowtail (*Seriola dumerili*) fed extruded diets containing different levels of protein and lipid. Aquaculture 179, 25–33.

Kagawa, H., 1989. Reproductive physiology and induced spawning in yellowtail (*Seriola quinqueradiata*). Marine Ranching 18, 15–18.

Kawabe, K., Kato, K., Kimura, J., Okamura, Y., Ando, K., Saito, M., Yoshida, K., 1996. Rearing of broodstock fish and egg-taking from amberjack *Seriola dumerili* in Chichijima, Ogasawara Islands, Southern Japan. Aquac. Sci. 44, 151–157.

Kawabe, K., Kimura, J., Ando, K., Kakiuchi, K., 1998. Natural spawning from 2-year-old reared amberjack, *Seriola dumerili* in Chichijima Ogasawara Islands, Southern Japan. Aquac. Sci. 46, 31–36.

Kozul, V., Skaramuca, B., Glamuzina, B., Glavic, N., Tutman, P., 2001. Comparative gonadogenesis and hormonal induction of spawning of cultured and wild mediterranean amberjack (*Seriola dumerili*, Risso 1810). Sci. Marina 65, 215–220.

Lazzari, A., 1991. Some notes to the aquaculture development of the new Mediterranean species: the yellowtail (*Seriola dumerilii*) case and strategy to come. In: De Pauw, N., Joyce, J. (Eds.), Aquaculture Europe 91. European Aquaculture Society, Dublin, Ireland, pp. 183–184 Special Publication No. 14.

Lazzari, A., Fusari, A., Boglione, A., Marino, G., Di Francesco, M., 2000. Recent advances in reproductional and rearing aspects of *Seriola dumerilii*. In: Basurco, B. (Ed.), Cahiers Options Méditerranéennes, vol. 47: Mediterranean Marine Aquaculture Finfish Species Diversification. C.I.H.E.A.M., Zaragoza, Spain, pp. 241–247.

Mañanos, E., Carrillo, M., Sorbera, L.S., Mylonas, C.C., Asturiano, J.F., Bayarri, M.J., Zohar, Y., Zanuy, S., 2002. Luteinizing hormone and sexual steroid plasma levels after treatment of European sea bass with sustained-release delivery systems for gonadotropin-releasing hormone analogue. J. Fish Biol. 60, 328–339.

Mañanos, E., Duncan, N., Mylonas, C.C., 2009. Reproduction and control of ovulation, spermiation and spawning in cultured fish. In: Cabrita, E., Robles, V., Herraez, P. (Eds.), Methods in Reproductive Aquaculture. CRC Press Taylor and Francis Group, Boca Raton, pp. 3–80.

Mandich, A., Massari, A., Bottero, S., Pizzicori, P., Goos, H., Marino, G., 2004. Plasma sex steroid and vitellogenin profiles during gonad development in wild Mediterranean amberjack (*Seriola dumerilii*, Risso). Mar. Biol. 144, 127–138.

Marino, G., Mandich, A., Massari, A., Andaloro, F., Porrello, S., 1995. Aspects of reproductive biology of the Mediterranean amberjack (*Seriola dumerilii* Risso) during the spawning period. J. Appl. Ichthyol. 11, 9–24.

Marino, G., Panini, E., Longobardi, A., Mandich, A., Finoia, M.G., Zohar, Y., Mylonas, C.C., 2003. Induction of ovulation in captive-reared dusky grouper, *Epinephelus marginatus* (Lowe, 1834) with a sustained-release GnRHa implant. Aquaculture 219, 841–858.

Mazzola, A., Favaloro, E., Sará, G., 2000. Cultivation of the Mediterranean amberjack, *Seriola dumerili* (Risso, 1810), in submerged cages in the Western Mediterranean Sea. Aquaculture 181, 257–268.

McDowell, E.M., Trump, B.F., 1976. Histologic fixatives suitable for diagnostic light and electron microscopy. Arch. Pathol. Lab. Med. 100, 405–414.

Metcalfe, J.D., Craig, J.F., 2011. Ethical justification for the use and treatment of fishes in research: an update. J. Fish Biol. 78, 393–394.

Micale, V., Maricchiolo, G., Genovese, L., 1999. The reproductive biology of the amberjack, *Seriola dumerilii* (Risso 1810). I. Oocyte development in captivity. Aquac. Res. 30, 349–355.

Moran, D., Smith, C.K., Gara, B., Poortenaar, C.W., 2007. Reproductive behaviour and early development in yellowtail kingfish (*Seriola lalandi* Valenciennes 1833). Aquaculture 262, 95–104.

Mylonas, C., Katharios, P., Grigorakis, K., Papandroulakis, N., Robles, R., Corriero, A., Pousis, C., Zupa, P., Fernandez-Palacios, H., Montero, D., Izquierdo, M., Roo, F.J., Hernandez-Cruz, C.M., Rodriguez, C., Peres, J.A., Jerez, S., Secombes, C.J., Guerrero, L., 2016a. Advances in greater amberjack (*Seriola dumerili*) research: the DIVERSIFY project, Aquaculture Europe. European Aquaculture Society, Belgium, pp. 12–19.

Mylonas, C.C., Cardinaletti, G., Sigelaki, I., Polzonetti-Magni, A., 2005. Comparative efficacy of clove oil and 2-phenoxyethanol as anesthetics in the aquaculture of European sea bass (*Dicentrarchus labrax*) and gilthead sea bream (*Sparus aurata*) at different temperatures. Aquaculture 246, 467–481.

Mylonas, C.C., Duncan, N.J., Asturiano, J.F., 2017. Hormonal manipulations for the enhancement of sperm production in cultured fish and evaluation of sperm quality. Aquaculture 472, 21–44.

Mylonas, C.C., Fatira, E., Karkut, P., Sigelaki, I., Papadaki, M., Duncan, N., 2015. Reproduction of hatchery-produced meagre *Argyrosomus regius* in captivity III. Comparison between GnRHa implants and injections on spawning kinetics and egg/larval performance parameters. Aquaculture 448, 44–53.

Mylonas, C.C., Fostier, A., Zanuy, S., 2010. Broodstock management and hormonal manipulations of fish reproduction. Gen. Comp. Endocrinol. 165, 516–534.

Mylonas, C.C., Mitrizakis, N., Castaldo, C.A., Cerviño, C.P., Papadaki, M., Sigelaki, I., 2013a. Reproduction of hatchery-produced meagre *Argyrosomus regius* in captivity II. Hormonal induction of spawning and monitoring of spawning kinetics, egg production and egg quality. Aquaculture 414–415, 318–327.

Mylonas, C.C., Mitrizakis, N., Papadaki, M., Sigelaki, I., 2013b. Reproduction of hatchery-produced meagre *Argyrosomus regius* in captivity I. Description of the annual reproductive cycle. Aquaculture 414–415, 309–317.

Mylonas, C.C., Papandroulakis, N., Smboukis, A., Papadaki, M., Divanach, P., 2004. Induction of spawning of cultured greater amberjack (*Seriola dumerili*) using GnRHa implants. Aquaculture 237, 141–154.

Mylonas, C.C., Salone, S., Biglino, T., de Mello, P.H., Fakriadis, I., Sigelaki, I., Duncan, N., 2016b. Enhancement of oogenesis/spermatogenesis in meagre *Argyrosomus regius* using a combination of temperature control and GnRHa treatments. Aquaculture 464, 323–330.

Mylonas, C.C., Scott, A.P., Vermeirssen, E.L., Zohar, Y., 1997. Changes in plasma gonadotropin II and sex steroid hormones, and sperm production of striped bass after treatment with controlled-release gonadotropin-releasing hormone agonist-delivery systems. Biol. Reprod. 57, 669–675.

Mylonas, C.C., Woods III, L.C., Thomas, P., Zohar, Y., 1998. Endocrine profiles of female striped bass (*Morone saxatilis*) in captivity, during post-vitellogenesis and induction of final oocyte maturation via controlled-release GnRHa-delivery systems. Gen. Comp. Endocrinol. 110, 276–289.

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

Nyuji, M., Kazeto, Y., Izumida, D., Tani, K., Suzuki, H., Hamada, K., Mekuchi, M., Gen, K., Soyano, K., Okuzawa, K., 2016. Greater amberjack Fsh, Lh, and their receptors: plasma and mRNA profiles during ovarian development. Gen. Comp. Endocrinol. 225, 224–234.

Panini, E., Mylonas, C.C., Zanuy, S., Carrillo, M., Ramos, J., Bruce, M., 2001. Incubation of embryos and larvae of marine fish using microtiter plates. Aquacult. Int. 9, 189–196.

Paxton, J.R., Hoese, D.F., Allen, G.R., Hanley, J.E., 1989. Pisces. Petromyzontidae to Carangidae. Australian Government Publishing Service, Canberra.

Poortenaar, C.W., Hooker, S.H., Sharp, N., 2001. Assessment of yellowtail kingfish (*Seriola lalandi lalandi*) reproductive physiology, as a basis for aquaculture development. Aquaculture 201, 271–286.

Pousis, C., Mylonas, C.C., De Virgilio, C., Gadaleta, G., Santamaria, N., Passantino, L., Zupa, R., Papadaki, M., Fakriadis, I., Ferreri, R., Corriero, A., 2018. The observed oogenesis impairment in greater amberjack *Seriola dumerili* (Risso, 1810) reared in captivity is not related to an insufficient liver transcription or oocyte uptake of vitellogenin. Aquac. Res. 49, 243–252.

Rodríguez-Barreto, D., Jerez, S., Cejas, J.R., Martin, M.V., Acosta, N.G., Bolaños, A., Lorenzo, A., 2014. Ovary and egg fatty acid composition of greater amberjack broodstock (*Seriola dumerili*) fed different dietary fatty acids profiles. Eur. J. Lipid Sci. Technol. 116, 584–595.

Roo, J., Fernández-Palacios, H., Hernández-Cruz, C., Mesa-Rodriguez, A., Schuchardt, D., Izquierdo, M., 2014. First results of spawning and larval rearing of longfin yelowtail *Seriola rivoliana* as a fast-growing candidate for European marine finfish aquaculture diversification. Aquac. Res. 45, 689–700.

Roo, J., Fernández-Palacios, H., Schuchardt, D., Hernández-Cruz, C.M., Izquierdo, M.S., 2015. Influence of hormonal induction and broodstock feeding on longfin yellowtail *Seriola rivoliana* maturation, spawning quality and egg biochemical composition. Aquac. Nutr. 21, 614–624.

Sarih, S., Djellata, A., La Barbera, A., Fernández-Palacios Vallejo, H., Roo, J., Izquierdo, M., Fernández-Palacios, H., 2018. High-quality spontaneous spawning in greater amberjack (*Seriola dumerili*, Risso 1810) and its comparison with GnRHa implants or injections. Aquac. Res. 49, 3442–3450.

Šegvić-Bubić, T., Marrone, F., Grubišić, L., Izquierdo-Gomez, D., Katavić, I., Arculeo, M., Lo Brutto, S., 2016. Two seas, two lineages: how genetic diversity is structured in Atlantic and Mediterranean greater amberjack *Seriola dumerili* Risso, 1810 (Perciformes, Carangidae). Fish. Res. 179, 271–279.

Setiawan, A.N., Muncaster, S., Pether, S., King, A., Irvine, G.W., Lokman, P.M., Symonds, J.E., 2016. The effects of gonadotropin-releasing hormone analog on yellowtail kingfish *Seriola lalandi* (Valenciennes, 1833) spawning and egg quality. Aquacult. Rep. 4, 1–9.

Shimizu, A., 1996. Long-term efects of a luteinizing hormone-releasing hormone analogue and/or a dopamine antagonist, pimozide, on gonadal activity in an autumn-spawning bitterling, *Acheilognathus rhombea*, during various phases of the annual reproductive cycle. J. Exp. Zool. 276, 279–286.

Symonds, J.E., Walker, S.P., Pether, S., Gublin, Y., McQueen, D., King, A., Irvine, G.W., Setiawan, A.N., Forsythe, J.A., Bruce, M., 2014. Developing yellowtail kingfish (*Seriola lalandi*) and hāpuku (*Polyprion oxygeneios*) for New Zealand aquaculture. N. Z. J. Mar. Freshwater Res. 48, 371–384.

Tachihara, K., Ebisu, R., Tukashima, Y., 1993. Spawning, eggs, larvae and juveniles of the purplish amberjack *Seriola dumerili*. Nippon Suisan Gakkaishi 59, 1479–1488.

Valdebenito, I.I., Gallegos, P.C., Effer, B.R., 2013. Gamete quality in fish: evaluation parameters and determining factors. Zygote 23, 177–197.

Zohar, Y., Goren, A., Fridkin, M., Elhanati, E., Koch, Y., 1990. Degradation of gonadotropin-releasing hormones in the gilthead seabream *Sparus aurata* II. Cleavage of native salmon GnRH, mammalian LHRH and their analogs in the pituitary, kidney and liver. Gen. Comp. Endocrinol. 79, 306–319.

Zohar, Y., Goren, A., Tosky, M., Pagelson, G., Leibovitz, D., Koch, Y., 1989. The bioactivity of gonadotropin-releasing hormones and its regulation in the gilthead seabream, *Sparus aurata: in vivo* and *in vitro* studies. Fish Physiol. Biochem. 7, 59–67.

Zohar, Y., Muñoz-Cueto, J.A., Elizur, A., Kah, O., 2010. Neuroendocrinology of reproduction in teleost fish. Gen. Comp. Endocrinol. 165, 438–455.

Zohar, Y., Mylonas, C.C., 2001. Endocrine manipulations of spawning in cultured fish: from hormones to genes. Aquaculture 197, 99–136.

Zupa, R., Fauvel, C., Mylonas, C.C., Pousis, C., Santamaría, C.A., Papadaki, M., Fakriadis, I., Cicirelli, V., Mangano, S., Passantino, L., Lacalandra, G.M., Corriero, A., 2017a. Rearing in captivity affects spermatogenesis and sperm quality in greater amberjack, *Seriola dumerili* (Risso, 1810). J. Anim. Sci. 95, 4085–4100.

Zupa, R., Rodríguez, C., Mylonas, C.C., Rosenfeld, H., Fakriadis, I., Papadaki, M., Pérez, J.A., Pousis, C., Basilone, G., Corriero, A., 2017b. Comparative study of reproductive development in wild and captive-reared greater amberjack *Seriola dumerili* (Risso, 1810). PLoS One 12, e0169645.