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Reproduction of hatchery-produced meagre *Argyrosomus regius* in captivity I. Description of the annual reproductive cycle

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ABSTRACT

The aim of the present study was to monitor the reproductive cycle of a hatchery-produced meagre *Argyrosomus regius* broodstock. Four-year-old fish of a mean weight (±SD) of 5.1 ± 1.1 kg for females (n = 7) and 4.6 ± 0.7 kg for males (n = 6), were maintained under simulated natural photo-thermal regime and sampled between November 2010 and July 2011. Vitellogenesis began in March, when the first increase in oocyte diameter was observed. Fully vitellogenic oocytes of mean (±SEM) diameter between 561 ± 23 and 621 ± 9 μm were observed between April and June. Two of the seven females spawned spontaneously in June, producing six major spawns of 390,000–940,000 eggs per day with fertilization success >97%. Vitellogenesis was associated with relatively low levels of testosterone (T) ranging between mean values of 0.036 ± 0.01 ng ml^{-1} and 0.207 ± 0.07 ng ml^{-1}, and of 17β-estradiol (E_2) ranging between mean values of 0.194 ± 0.09 ng ml^{-1} and 0.473 ± 0.12 ng ml^{-1}. Spermiation also began in March, but only 33% of the fish produced significant amounts of milt to allow sperm quality evaluations. In May 100% of the fish were spermiating, and in July spermiation ceased almost completely. No significant changes were observed during the spermiation period (March–June) in sperm concentration (mean ranged between 18.9 and 31.5×10^9 szoa ml^{-1}) or initial sperm motility (mean ranged between 44 and 80%). The mean duration of motility (mean ranged between 0.78 and 1.27 min) was also stable during the reproductive season, with the exception of a significant decrease in May. Mean plasma T and 11-ketotestosterone (11-KT) levels in male meagre increased gradually during the year, reaching their spermiation season peaks in March (0.818 ng 11-KT ml^{-1}) or in May (0.263 ng T ml^{-1}). The study demonstrated that gametogenesis proceeds normally in hatchery-produced meagre and under the influence of relatively low levels of sex steroid hormones. Although spontaneous spawning was observed for the first time in meagre maintained in captivity (2 of 7 females), the study demonstrated also that oocyte maturation is sporadic and inconsistent, and reliable spawning could be obtained using only exogenous hormones. Furthermore, it was shown that females failing to undergo oocyte maturation, maintain their vitellogenic oocytes in a viable condition without significant atresia for a period of at least 2 months (April–June). These oocytes could potentially be induced to undergo maturation, ovulation and spawning at any time during this "post-vitellogenesis" period.

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1. Introduction

The meagre (*Argyrosomus regius*) is a member of the Sciaenidae family and is found mostly near estuaries of the Mediterranean Sea and the Atlantic coast of Europe and North Africa (Gil et al., 2013; Griffiths and Heemstra, 1995; Morales-Nin et al., 2012). The first reproductive maturation in nature occurs at a total length (TL) of 45–62 cm for males and 47–70 cm for females (Abou Shabana et al., 2012; González-Quirós et al., 2011). Earlier maturation has been observed in culture, and it has been reported to occur at a TL of 27 cm (2-years-old) for males and 36 cm (3-years-old) for females (Schiavone et al., 2012). Due to its fast growth of ~1 kg per year (Duncan et al., 2012; El-Shebly et al., 2007; González-Quirós et al., 2011; Monfort, 2010), fast acclimatization to captivity (Duncan et al., 2012), low feed conversion ratio of 0.9–1.2 (Monfort, 2010) and relatively easy larval rearing (Roo et al., 2010; Vallés and Estévez, 2013), meagre is considered as a good candidate species for the diversification of aquaculture production in the Mediterranean region (Duncan et al., 2013; Monfort, 2010).

Although the production of meagre has increased rapidly in the last few years (Duncan et al., 2013), reproduction in captivity still remains a problem, as fish do not mature and spawn spontaneously in captivity (Duncan et al., 2013), and exogenous hormones need to be used to induce spawning (Duncan et al., 2012; Mylonas et al., 2013). Therefore, there is a need to study the reproductive biology of this species in captivity in order to develop more appropriate broodstock management methods to either alleviate the observed reproductive dysfunctions or improve the efficiency of induced spawning protocols. Failure of females to undergo oocyte maturation upon the completion of vitellogenesis is the most common reproductive dysfunction in aquaculture

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(Mylonas and Zohar, 2009; Mylonas et al., 2010), and it might be the result of inappropriate rearing conditions (*e.g.*, tank size or depth), water quality (*e.g.*, temperature or salinity) or stress due to the captive environment (Schreck, 2010). Over time, as new generations of hatchery produced fish become inadvertently better adapted to the captive environment and as aquaculturists gain better understanding of the reproductive requirements of each species, this dysfunction may decline or disappear, as the case of gilthead sea bream *Sparus aurata* underlines (Mylonas et al., 2011; Zohar et al., 1995). Therefore, it is necessary to study the reproductive function of fishes with interest for the aquaculture industry, first using wild-caught captive-reared broodstocks, and then using hatchery-produced broodstocks in order to achieve absolute control of reproduction. Such studies should include (a) identification of the period and progress of gametogenesis in captivity, (b) monitoring of the status of the reproductive endocrine system of fish, (c) the response of the reproductive system to photothermal manipulations, starting with simulation of natural conditions, (d) evaluation of the quality of the produced gametes and eggs, and (e) development of manipulation methods for the induction, synchronization or enhancement of spawning and the production of high quality gametes.

This manuscript is the first of a series of studies in the reproduction of hatchery-produced meagre broodstocks, and it focuses on the description of the endocrine function of the gonads, oocyte development and the variation of sperm quality during the reproductive period.

2. Materials and methods

2.1. Broodstock maintenance

Rearing was undertaken at the facilities of the Institute of Marine Biology, Biotechnology and Aquaculture (previously Institute of Aquaculture) of the Hellenic Centre for Marine Research (HCMR), Iraklion, Crete, Greece. The broodstock came from eggs produced in the hatchery in 2005 and consisted of 13 fish (7 females and 6 males) of a mean weight (±SD) of 5.1 ± 1.1 kg for the females and 4.6 ± 0.7 kg for the males at the peak of the reproductive season in June 2011. Fish were maintained in a 9000-l Recirculation Aquaculture System (ACE, the Netherlands), under controlled, simulated natural water temperature and photoperiod (Fig. 1). Fish were fed daily to apparent satiation with industrial feed (Skretting, EXCEL XL) and frozen squid was offered 3 times a week between February and July 2011. Measurements of temperature and water quality (NH_3 and NO_2) were conducted once per week. A passive egg collector was placed in the outflow of the tank, in order to verify the occurrence of any spawning and collect the spawned eggs. Eggs were collected every morning into a 10-l bucket and their number (fecundity) was estimated by counting the total number of eggs in a sub-sample of 10 ml, after vigorous agitation. Fertilization success was evaluated at the same time by examining each of the eggs in this 10 ml sample for the presence of a viable embryo (usually at the blastula stage) using a stereoscope.

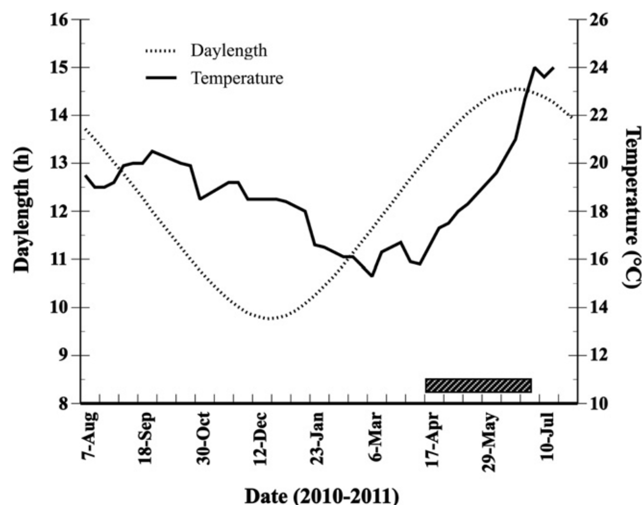

Fig. 1. Annual daylength and water temperature profile for the meagre broodstock monitored in the present study. The horizontal bar at the bottom of the graph indicates the anticipated spawning period in the present study, based on the presence of males in spermiation and females in post-vitellogenesis.

2.2. Fish samplings

The broodstock were sampled between November 2010 and July 2011. No sampling was done between August and November, and the first three samplings (November until March) were conducted only bimonthly in order to reduce unnecessary handling of the fish during the quiescent period and the following 4 samplings were conducted monthly, from April until July. Fish were starved for two days prior to sampling. During sampling, fish were tranquilized initially in their tank with the use of clove oil (0.01 ml l^{-1}) and then transferred to an anesthetic bath for complete sedation with a higher concentration of clove oil (0.03 ml l^{-1}) (Mylonas et al., 2005). Ovarian biopsies for the evaluation of oocyte development were obtained by inserting a glass cannula (Natelson tube) into the ovarian cavity, connected to a TygonR tubing and applying gentle aspiration. A wet mount of the biopsy was examined first 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). A portion of the biopsy was fixed in a solution of 4% formaldehyde-1% glutaraldehyde for further histological processing. Maturation of the males was examined by the release of sperm upon application of gentle abdominal pressure. Sperm was collected after rinsing the fish with clean seawater and blot drying the genital pore. Small volumes of sperm (50–100 μl) were collected in order to avoid influencing the quantity and/or quality of sperm during subsequent collections. The collected sperm was stored on ice and then transferred to a 4 °C refrigerator until evaluation.

Spermiation condition was evaluated based on the presence and ease of milt release upon the application of gentle abdominal pressure (Mylonas et al., 2003). Spermiation condition (Sperm Index) was reported on a subjective scale from 0 to 2, with S0 = no milt released, S1 = only a drop of milt released after multiple stripping attempts and S2 = milt was released easily after the first stripping attempt. Only S2 males released enough milt to be collected for further evaluation. Sperm quality parameters that were evaluated included sperm concentration (number of spermatozoa ml^{-1} of milt), percentage of spermatozoa showing forward motility immediately after activation (initial sperm motility, %) and duration of forward sperm motility of at least 10% of the spermatozoa in the field of view (motility duration, min). Sperm concentration was estimated after a 4221-fold dilution with seawater using a Neubauer haemocytometer under 200× magnification (in duplicate) in a compound light microscope (Nikon, Eclipse 50i). Sperm motility and motility duration were evaluated on a microscope slide (400× magnification) after mixing 1 μl of sperm with a drop of about 50 μl of saltwater (in duplicate). Changes in sperm motility percentage over time after activation were examined with Computer Assisted Sperm Analysis (CASA) using an open source software (Wilson-Leedy and Ingermnn, 2007). Activated sperm samples were observed for the first time 10 s after activation under the compound light microscope at a 400× magnification, connected to a camera, a video monitor and a digital recorder. The number of motile spermatozoa was evaluated for 1 s, every 10 s (in duplicates) until cessation of all forward motility.

Blood was collected from all fish at each sampling, in order to measure sex steroid hormone concentrations. Blood was centrifuged at 6000 rpm for 15 min and plasma was collected and stored at −80 °C until analysis.

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2.3. Histological processing

Before embedding in methacrylate resin (Technovit 7100®, Heraeus Kulzer, Germany) ovarian biopsies were dehydrated in gradually increasing ethanol solutions (70–96%). Serial sections of 3 μm were obtained with a microtome (Reichert Jung, Biocut 2035, Germany). Sections were stained with Methylene Blue (Sigma, Germany)/Azure II (Sigma, Germany)/Basic Fuchsin (Polysciences, USA) according to Bennett et al. (1976). Sections were examined under a light compound microscope (Nikon, Eclipse 50i) and photographed with a digital camera (Jenoptik progress C12 plus).

2.4. Hormone measurements

For the quantification of testosterone (T), 17β-estradiol (E_2) and 11-Ketotestosterone (11-KT) in the plasma, already established and well-described enzyme-linked immunoassays (ELISA) were used (Cuisset et al., 1994; Nash et al., 2000; Rodríguez et al., 2000) with some modifications, and using reagents from SpiBio (France). For steroid extraction, 200 μl of plasma were extracted twice with 2 ml diethyl ether. Extraction was done by vigorous vortexing (Vibramax 110, Heidolph, Germany) for 3 min. After decanting of the organic phase, drying of the supernatant was done under a stream of nitrogen (Reacti-vap III, Pierce, USA). Samples were reconstituted in 250 μl of reaction buffer for running in the ELISA.

2.5. Statistical analysis

Differences in mean oocyte diameter, sperm concentration, initial sperm motility (%) and plasma steroid concentrations during the annual reproductive period were performed using one-way ANOVA, followed by Duncan's New Multiple Range test, at $P \leq 0.05$. Data was examined for normality in the distribution of variances, in order to comply with the prerequisites of ANOVA. Statistical analyses were performed with linear statistics software (SuperAnova and Statview, Abacus Concepts, Berkeley, CA, USA). Results are presented as mean ± SEM, unless otherwise mentioned.

3. Results

The first significant increase in mean oocyte diameter occurred in the March sampling (Fig. 2), when the ovaries contained oocytes in early vitellogenesis (Figs. 3 and 4). The wet mounts of the ovarian biopsies obtained from fish between April and June appeared very similar, having large numbers of oocytes in advanced vitellogenesis, but of various diameters (Fig. 3A and B). One female in June was found to be in early oocyte maturation, having oocytes exhibiting lipid droplet coalescence (Fig. 3C) and another female contained post-ovulated eggs in her ovary (not shown). In July, 4 of the 6 remaining females (one female died in early July) had only atretic and primary oocytes in their ovaries, but 2 females had also a small number of viable vitellogenic oocytes (Figs. 3D and 4D).

Spontaneous spawning was observed for the first time on 13 June when 55,000 eggs were obtained (Fig. 5), but large fecundity spawnings ($n = 6$) began on 16 June and continued intermittently. Relative fecundity is not reported, since it was not possible to know how many females participated in these spawning events. Based on the evaluation of the ovarian biopsies in June (see above paragraph), which was done on 24 June when females were spawning, it was presumed that only 2 females participated in these spawnings.

Mean plasma T and E_2 levels in female meagre did not change significantly between November and April (Fig. 6). In the following sampling in May, both T and E_2 increased significantly and then decreased in the next monthly sampling. Mean plasma T exhibited a second peak in July.

The first spermiating males were observed in March, although only two of the fish (33%) were at stage S2 (Fig. 7), and thus produced enough milt to carry out further sperm evaluations. In April and May, almost all fish were spermiating freely. In June, spermiation index started decreasing and in July only 33% of the males were still in spermiation, albeit in stage S1 (data not shown). As a result, no sperm evaluation could be carried out in July. There were no significant differences during the reproductive period in mean sperm concentration or initial sperm motility percentage. The mean duration of motility was also stable during the reproductive season, but a significant decrease was observed in May (Fig. 7). The percentage of motile spermatozoa decreased gradually after activation, with ~50% of the initially motile spermatozoa still exhibiting forward motility after 30 s from activation, regardless of the time (month) when the evaluation was done (Fig. 8).

Mean plasma T and 11-KT levels in male meagre varied significantly over the reproductive season (Fig. 9). Both androgens increased gradually during the year, but significantly different levels from the quiescent period were achieved between March and April for 11-KT ($P < 0.01$), while two peaks were observed in May and July for T ($P < 0.05$). Similar plasma T levels were observed for almost all months when fish were in spermiation, while 11-KT was significantly higher in March compared to the other months.

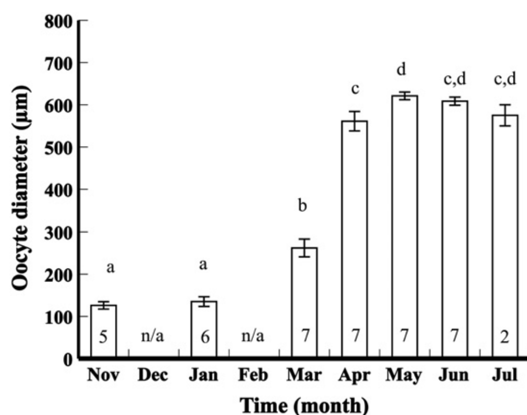

Fig. 2. Mean (±SEM) diameter of the largest vitellogenic oocytes in meagre ($n = 2–7$) during the annual reproductive cycle. Different letter superscripts indicate means that were significantly different (ANOVA, DNMR, $P < 0.05$). The numbers inside the bars indicate the number of fish biopsied. Some females could not be biopsied in November ($n = 2$) and January ($n = 1$). In July, only 2 females had oocytes in vitellogenesis, while all the others ($n = 5$) had atretic and primary oocytes only (see Fig. 3). n/a = Not available, since no samplings were made on these months.

4. Discussion

The timing of vitellogenesis, as well as the reproductive strategy of each species need to be identified for its successful and efficient propagation in aquaculture, in order to be able to anticipate (a) at which time of the year and ambient temperature the fish is expected to spawn and (b) what is the batch and seasonal fecundity, and fertilization success that can be expected. The above information is necessary for proper stocking (number of broodstocks), management of the broodstock (feeding, handling, egg collection, etc.) and planning of larval rearing operations. The first signs of vitellogenesis in meagre were observed in March, while ovaries in full vitellogenesis were observed between April and June, at temperatures between 15.8–22.7 °C. This is in agreement with studies of wild populations (Abou Shabana et al., 2012; González-Quirós et al., 2011), and some captive-reared or hatchery-produced broodstocks (Duncan et al., 2012, 2013; Gil et al., 2013).

Completion of vitellogenesis in meagre in the present study was achieved within 2 months, less than what is required by other Mediterranean species that spawn in early Spring, such as the red porgy *Pagrus pagrus* (December until March) (Kokokiris et al., 2001) and common dentex *Dentex dentex* (February until April–May) (Pavlidis et al., 2000). In our study, meagre began vitellogenesis when the water

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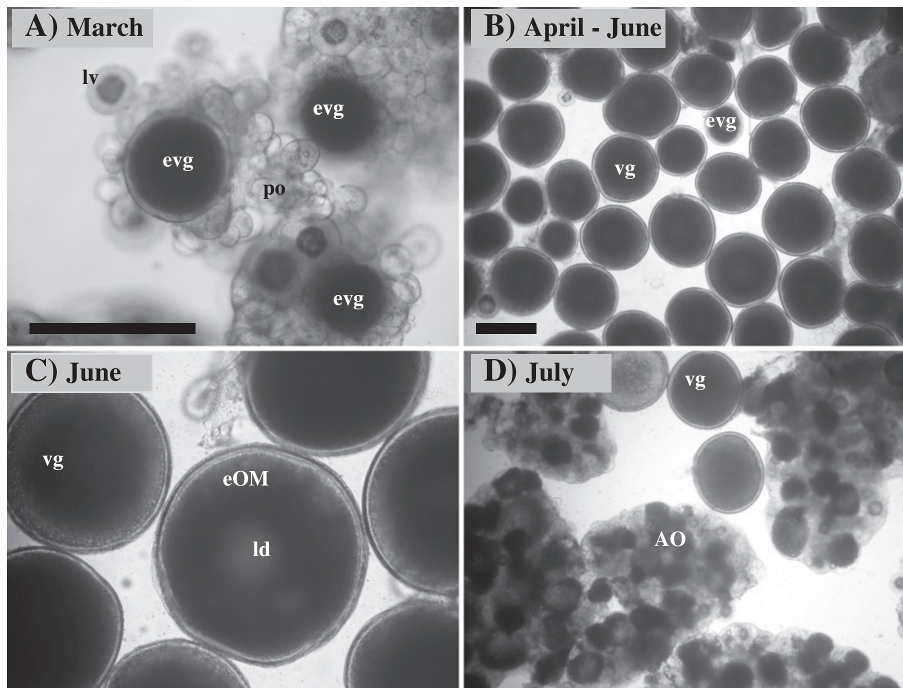

Fig. 3. Photomicrographs of oocytes from meagre ovarian biopsies during the annual reproductive period. In March (A), early vitellogenic oocytes (evg) appeared in the biopsies, though the gonads were mainly full of primary oocytes (po) and some oocytes at the lipid vesicle (lv) stage. Between April and June (B), the ovaries contained mostly oocytes in full vitellogenesis (vg), as well as some in early vitellogenesis (evg). In June (C), one female contained oocytes in early oocyte maturation (eOM), with the lipid droplets having coalesced into larger droplets (ld). In July (D), the ovaries of most fish contained only atretic oocytes (AO) and primary oocytes, but small numbers of remaining vitellogenic oocytes (vg) were present in the ovaries of two of the females examined. The bar at the bottom of each photo represents 500 μm.

temperature reached its minimum in late February and proceeded rapidly when water temperatures began increasing in April. In fact, already in April the largest vitellogenic oocytes were of a diameter that could undergo maturation and ovulation, based on induced spawning experiments (>500 μm, Duncan et al., 2012, 2013). Small increases in oocyte diameter of vitellogenic oocytes were observed thereafter, and the maximum diameter observed in May was similar to what has been reported for vitellogenic oocytes of wild meagre in Egypt (Abou

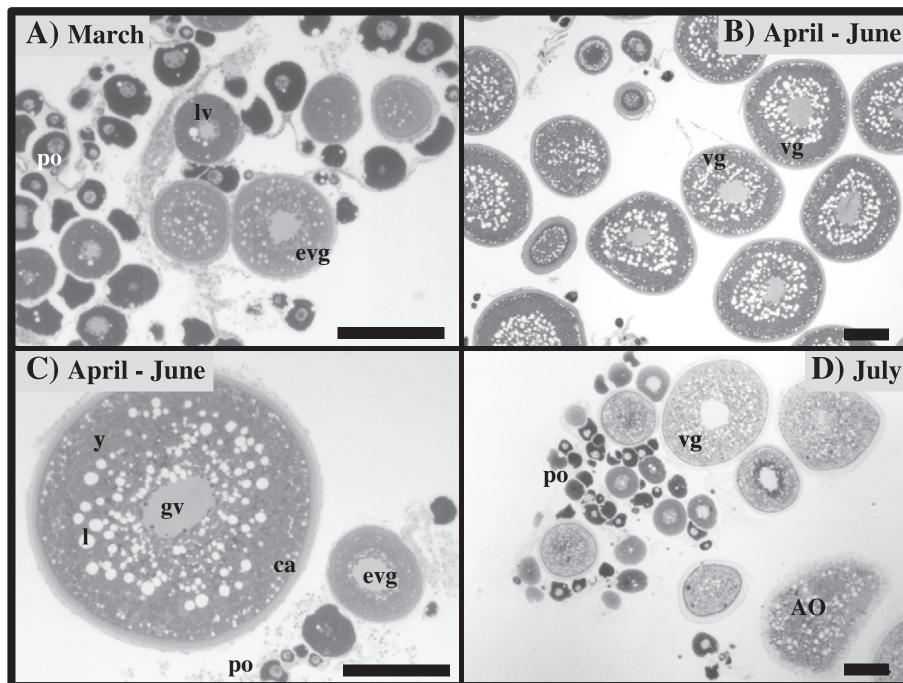

Fig. 4. Photomicrographs of histological sections from ovarian biopsies of meagre during the annual reproductive period. In March (A), in addition to the primary oocytes (po), some oocytes were at the lipid vesicle (lv) or early vitellogenesis stages (evg). Ovaries were in full vitellogenesis between April and June (B and C), containing mainly vitellogenic oocytes (vg), as well as po and evg oocytes. Fully vitellogenic oocytes (C) had a centrally located nucleus (germinal vesicle, gv) and contained a large number of yolk globules (y) and lipid droplets (l) scattered throughout the cytoplasm, as well as cortical alveoli (ca) in the periphery. Regression of the gonads occurred in July (D), with ovaries having mainly atretic oocytes (AO) and po. The bar at the bottom of each photo represents 200 μm.

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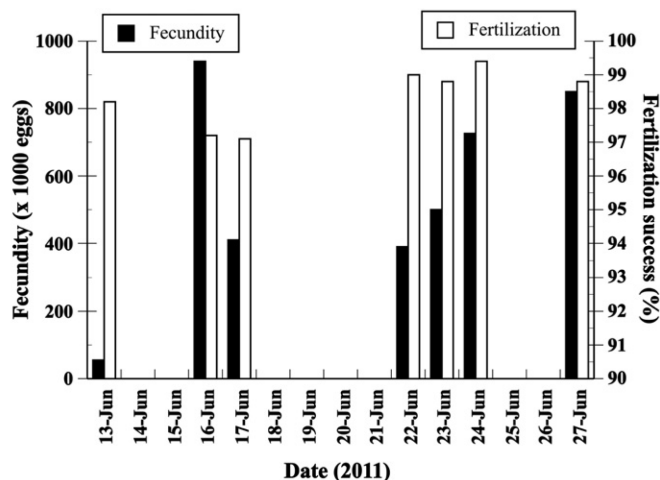

Fig. 5. Daily fecundity (×1000 eggs) and fertilization percentage of spontaneous spawns from the meagre broodstock. Although the stock consisted of 7 females (mean body weight of 5.1 ± 1.1 kg), it was concluded that only two individuals contributed to these spawns (see Discussion).

Shabana et al., 2012) and captive-reared wild meagre in Spain (Duncan et al., 2012). For comparison with other Sciaenids, maximum diameter of vitellogenic oocytes was 515 ± 52 μm in mulloway *Argyrosomus japonicus* (Battaglene and Talbot, 1994), 527 ± 34 μm in shi drum (Mylonas et al., 2004) and up to 600 μm in brown meagre *Sciaena umbra* (Grau et al., 2009).

During the first months of oogenesis in meagre, the levels of both hormones remained unchanged, with a significant (but still small) increase in the middle of the reproductive season in May. The study of Schiavone et al. (2012) reported a similar peak in plasma T and E_2 in May during the first reproductive season of meagre; however, during the second reproductive period in the same study plasma T and E_2 peaked later in June (Schiavone et al., 2012). In a different study, female plasma E_2 levels peaked in April during the first year and in March during the second (Vallés et al., 2011). Nevertheless, in the present study the significant, albeit small, increases in plasma T and E_2 occurred concurrently with the peak in the oocyte diameter of the vitellogenic oocytes, which was observed in May. The second peak of T observed at the end of the spawning period here, has been observed in other fish species as well (Pavlidis et al., 2000; Prat et al., 1990) and has been attributed to the action of androgens on the gonad preparation for the next reproductive cycle (Prat et al., 1990). It is obvious from the present results, that vitellogenesis was initiated and progressed to a large extent under low plasma levels of both T and E_2. These values were similar to another study on meagre during the first monitored vitellogenic cycle (Vallés et al., 2011), but significantly lower than another study on meagre who found peak T and E_2 in vitellogenic females in June of ~5 and 4 ng ml^{-1}, respectively (Schiavone et al., 2012). The use of only a single broodstock in the present study does raise some questions as to the repeatability of these plasma hormone levels in different broodstocks. However, based on the existing (though limited) literature it seems that even though significant variations in the absolute levels of the sex steroid hormones may exist from year to year or from broodstock to broodstock, all reared meagre broodstocks undergo vitellogenesis and spermatogenesis in relatively the same period (April–June), but they all fail to undergo oocyte maturation and do not spawn. Given the failure of most females in the present study to undergo oocyte maturation at the end of vitellogenesis (see discussion below), one is tempted to hypothesize that the low sex steroid plasma levels observed during oogenesis in meagre in the present study underlines another reproductive dysfunction during the process of vitellogenesis. However, the fact that the final diameter of vitellogenic oocytes was similar to other studies with meagre in captivity (Duncan et al., 2012, 2013) or in nature (Abou Shabana et al., 2012; Gil et al., 2013), and some spontaneous spawns were obtained by 2 out of the 7 females in the stock, with >97% fertilization success, leads to the conclusion that vitellogenesis in meagre may occur successfully under seemingly low levels of plasma T and E_2.

The present study provides the first documented report of spontaneous spawning of meagre, since all previous reports on spawning and egg production were the result of administration of exogenous hormones (Duncan et al., 2008, 2012, 2013; Fernández-Palacios et al., 2011; Mylonas et al., 2013). A recent study of hatchery produced meagre reported again this failure of fish to undergo oocyte maturation, ovulation or spawning, even though fish reached advanced vitellogenesis stages with oocytes diameters ~600 μm and gonadosomatic index (GSI) values of 4.5% (Gil et al., 2013). It is not known at this stage, why the broodstock in the present study spawned spontaneously, which is contrary to what has been reported amply in the literature so far, has been observed in our facilities year-after-year in various broodstocks maintained in different sized-tanks (unpublished data) and has been claimed by many commercial facilities (unpublished data). One possibility could be that this stock was better acclimated for reproduction since it has been maintained as broodstock for at least another year before the experiment (4-year-old fish), and in previous years it was used in successful spawning induction trials.

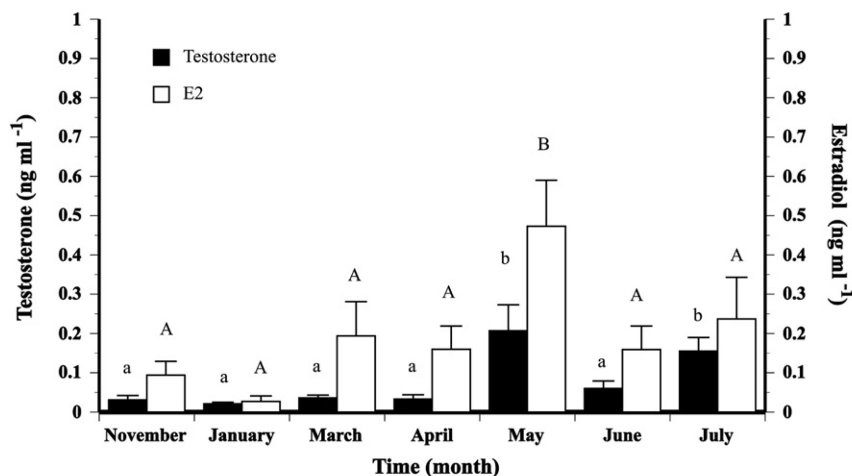

Fig. 6. Mean (±SEM) plasma concentrations of 17β-estradiol and testosterone in female meagre (n = 7, except in July n = 6) during the annual reproductive period. Different small letter superscripts indicate statistically significant differences in testosterone between months, whereas different capital letter superscripts indicate statistically significant differences in 17β-estradiol between months (one-way ANOVAs, DNMR, P ≤ 0.05).

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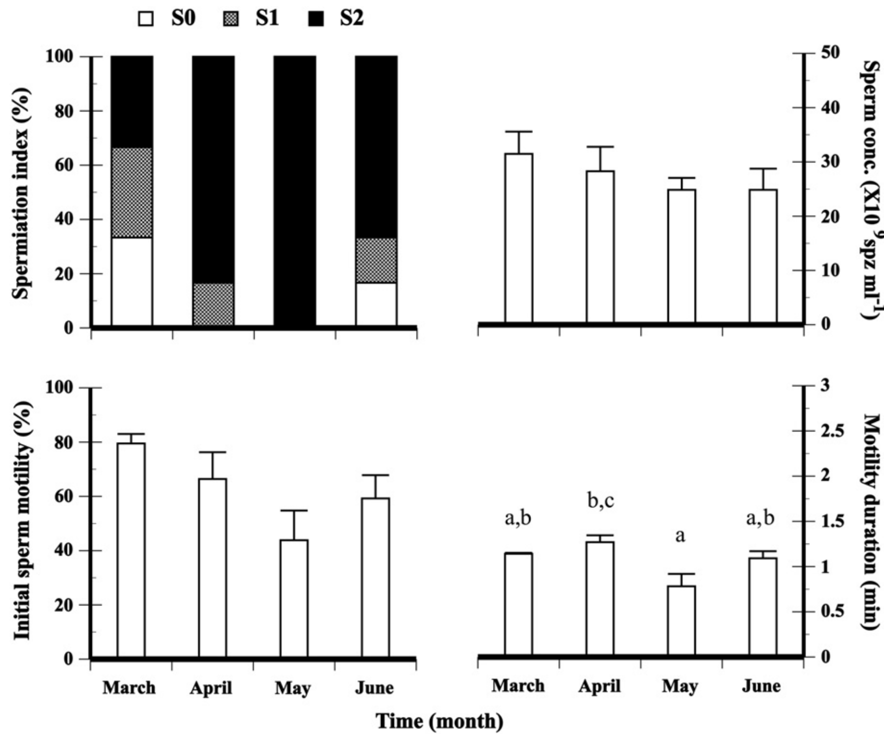

Fig. 7. Spermiation index (% of fish at each category) and mean (±SEM) sperm concentration, initial sperm motility percentage and motility duration of sperm from meagre during the spermiation period (n = 2 in March, n = 5 in April, n = 6 in May and n = 4 in June). Spermiation index was reported on a subjective scale with S0 = no milt released, S1 = only a drop of milt released after multiple stripping attempts and S2 = milt was released easily after the first stripping attempt. Only S2 males released enough milt to be collected for further evaluation. Statistically significant differences existed only in motility duration and are indicated by different letter superscripts (ANOVA, DNMR, $P < 0.05$).

Spawning commenced in mid June even though females contained fully vitellogenic oocytes – with the capacity to mature, if induced with exogenous hormones – since April. Spawning lasted for only 2 weeks and resulted in a total of six major spawns with high fecundity and fertilization success. The number of females participating in these spawns is not certain, but based on the sampling completed in June – which took place on 24 June while fish were spawning – some inferences are warranted. At this sampling time, only one female had ovulated eggs in her ovary, demonstrating that this female produced the eggs obtained on this day (726,000 eggs) with relative fecundity of 137,500 eggs kg^{-1} body weight. Another female had oocytes in early maturation and it is assumed that this latter female produced the spawn that was collected 3 days later (850,000 eggs) with relative fecundity of 170,000 eggs kg^{-1} body weight. All other females had fully vitellogenic oocytes in their ovaries. Given this information, it is assumed that all spawns obtained in the present study came only from the above-mentioned two females, with only one of them spawning at each day and producing a mean (±SD) batch fecundity of 123,750 ± 45,700 eggs kg^{-1} female body weight (without taking into account the first spawn that was of a very small fecundity). Total reproductive season fecundity estimates for wild and captive meagre (Gil et al., 2013) are in supporting of a single female spawning each day in the present study. A histological evaluation of wild fish from the Bay of Cadiz indicated a value of ~900,000 eggs kg^{-1} body weight (Gil et al., 2013). Also, in response to spawning induction of individual females using GnRHa implants, females spawned between 10 and 19 times, producing total reproductive season fecundity values between 522,000 and 699,400 eggs kg^{-1} body weight (Mylonas et al., 2013). In the present study, if one considers the "single female spawning" scenario, total season fecundity for the two females was

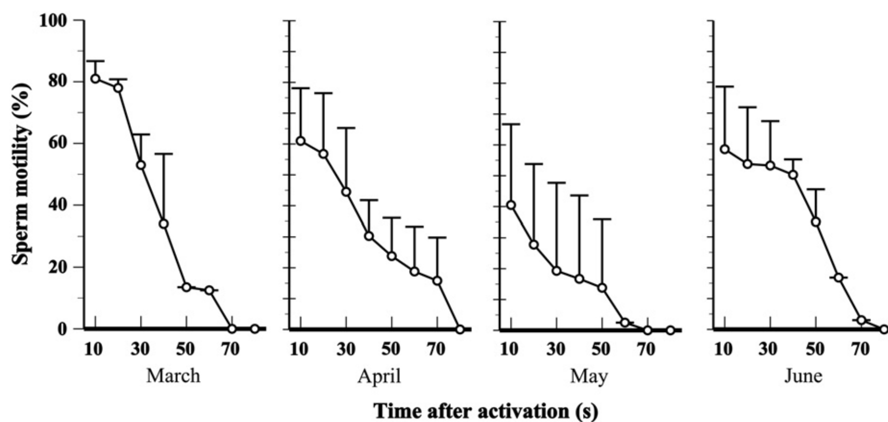

Fig. 8. Mean (±SD) percentage of meagre spermatozoa exhibiting forward motility at different times after activation, examined at different times during the spermiation season (March–June).

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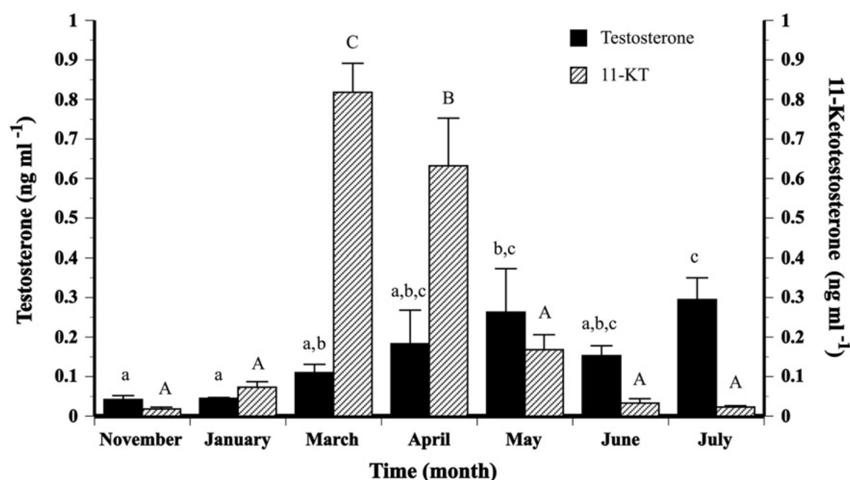

Fig. 9. Mean (±SEM) plasma concentrations of testosterone and 11-ketotestosterone in male meagre (n = 6) during the annual reproductive period. Different small letter superscripts indicate statistically significant differences in testosterone between months (one-way ANOVAs, DNMR, $P \leq 0.05$), whereas different capital letter superscripts indicate statistically significant differences in 11-ketotestosterone between months (one-way ANOVAs, DNMR, $P \leq 0.01$).

376,500 eggs kg^{-1} body weight for 7 spawns, considerably less that the above reported values. Given the presence of large numbers of fully vitellogenic oocytes in the 24 June ovarian biopsies of these two females, it is believed that the 376,500 eggs kg^{-1} body weight produced were only part of the potential of these females for the reproductive season.

Since the present study was limited to a single broodstock, without any tank replication, the results presented here alone could not reliably conclude on the reproductive function of meagre reared in captivity. However, considered together with ample reports in the literature (Duncan et al., 2013), data obtained year-after-year from a number of broodstocks maintained in our facilities (unpublished data) and claims by many commercial facilities throughout the Mediterranean Region, including Cyprus (C. Marangos, Sagro Aquaculture Ltd., personal communication), Turkey (Dr. K. Gamsiz, Ege University, personal communication), Greece (G. Iakovopoulos, Galaxidi Marine Farms S.A., personal communication) and Spain (N. Katribouzas, Andromeda Group S.A., personal communication), the maturation and egg production results obtained here underline the reproductive dysfunction that is common in the aquaculture of meagre throughout the Mediterranean, namely that while gametogenesis is completed, females do not undergo maturation reliably. Since this failure is so widespread throughout broodstock rearing facilities, where other marine fish do reproduce more reliably, it is probably caused by the "lack" of the same physiological/environmental condition. Given the life history of this species, which spawns near the Deltas of large rivers around the Mediterranean Sea and Eastern Atlantic Ocean (Abou Shabana et al., 2012; Gil et al., 2013), it is conceivable that this condition may relate to lower water salinity at the time of spawning. Reducing water salinity is not something marine hatcheries are accustomed or eager to do, given the limited freshwater resources around the Mediterranean coast, but perhaps it is a valid parameter to be examined in future studies.

Furthermore, even if a small percentage of the females do spawn, this may come late in the season in June and the total egg production is significantly less than the female's maximum potential. Interestingly, even if females failed to undergo maturation when their vitellogenic oocytes reached a size at which potentially they could be induced to mature and produce viable eggs and larvae (Duncan et al., 2012), oocyte integrity and viability was maintained for an additional period of between 1 and 2 months (May–June). Examination of ovarian biopsies and histological sections indicated that very little atresia was present in the ovaries during May and June, suggesting that meagre could maintain the viability of their post-vitellogenic oocytes for an extended period of time. These oocytes could, potentially, be induced with exogenous hormones to undergo maturation and produce viable eggs and larvae, as has been shown in an experiment where similarly reared meagre broodstocks were induced to spawn successfully at three different times between 5 May and 9 June (Mylonas et al., 2013). Mean fecundity (365,000 ± 107,000 eggs) and fertilization success (85 ± 9%) were not statistically different between the three different trials of the latter study, indicating that the fish were at a similar stage of development when the hormonal treatment was given, and no improvement or reduction in oocyte condition occurred within a period of 1 month. This finding is important from a broodstock manager's perspective, as it suggests that for production purposes one can expect a meagre broodstock to remain in "post-vitellogenic" condition for quite some time, and induce it to spawn when eggs are required for hatchery production.

Regarding the ovarian mode of development and spawning kinetics of meagre, the present study confirms very recent reports identifying this species as a multiple spawner (Duncan et al., 2012, 2013; Mylonas et al., 2013) with asynchronous or group-synchronous ovarian development (Abou Shabana et al., 2012; Gil et al., 2013; Schiavone et al., 2012) and determinate fecundity (Gil et al., 2013). Multiple spawning is characteristic to all members of the Sciaenidae family, such as the red drum *Sciaenops ocellatus* (Thomas et al., 1995), spotted seatrout *Cynoscion nebulosus* and orangemouth corvina *Cynoscion xanthulus* (Brown-Peterson et al., 1988; Thomas et al., 1994), as well as the Mediterranean shi drum (Barbaro et al., 2002; Mylonas et al., 2004) and brown meagre (Grau et al., 2009).

The spermiation period started 1 month before females were in full vitellogenesis and lasted for 5 months (March–July), although the percentage of spermiating fish in March was low. The majority of males were considered in full spermiation for 3 months between April and June, which coincided with the period when vitellogenic oocytes of the highest diameter were present in the females. In general, the spermiation period encompasses the spawning period in females, with males spermiating before females complete vitellogenesis and enter their spawning season, and continue spermiating after females regress (Carrillo et al., 1995; Kokokiris et al., 2001; Mylonas et al., 2003). In other recent studies with captive-reared meagre, the spermiation period ranged between March–June in Spain (Duncan et al., 2013; Gil et al., 2013), and June–July in Italy (Schiavone et al., 2012). Based on histological or GSI evaluations of meagre in the wild, the spermiation period was estimated to be between March–May in Egypt (Abou Shabana et al., 2012), and between April–August in the Gulf of Cádiz (Atlantic Ocean), Spain (González-Quirós et al., 2011). In the latter study, the water temperature ranged between 16 °C in April and 22 °C in August,

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a range that coincides with the one reported here for spermiating meagre, as well as with other studies (Duncan et al., 2013; Mylonas et al., 2013).

Similar to the females, both T and 11-KT in male meagre exhibited low values during the reproductive season. These values were in accordance with another study on meagre (Vallés et al., 2011), but were significantly lower than the 4–5 ng ml^{-1} reported in the study of Schiavone et al. (2012) for a broodstock in their second reproductive season. Also, plasma androgen levels here were lower than those reported in studies of other fish species (Berlinsky et al., 1995; Kokokiris et al., 2000; Pavlidis et al., 2000; Prat et al., 1990; Sulistyo et al., 2000; Utoh et al., 2004). Nevertheless, the profile of the two androgens during the reproductive season reflected the process of spermiation in the males, with 11-KT increasing significantly in March when the first spermiating fish were observed, and declining gradually thereafter, similar to what has been observed in the European sea bass *Dicentrarchus labrax* (Prat et al., 1990). As expected, plasma 11-KT was much higher than T, in accordance to studies both in meagre and other fishes (Berlinsky et al., 1995; Carragher and Pankhurst, 1993; Koldras et al., 1990; Schiavone et al., 2012). This observation underlines that 11-KT is the main reproductive hormone in males regulating the process of spermatogenesis (Schulz et al., 2010). In a another report on meagre, 11-KT peaked in February during one year and March during another (Vallés et al., 2011), whereas in a study following puberty and subsequent reproductive function, both T and 11-KT reached their peak at the same time in two consecutive years, T in May and 11-KT in June (Schiavone et al., 2012). As with females in the present study, males exhibited a peak in plasma T at the end of the reproductive season in July, which has been also shown in the European sea bass (Prat et al., 1990). Since testicular biopsies were not obtained for histological evaluations during the reproductive season (testicular biopsies would require killing the fish), it is not possible at this stage to correlate the levels of the two androgens with specific stages of spermatogenesis in meagre or to make any inferences on the appropriateness of the levels or profiles of these sex steroid hormones in meagre reproduction in captivity. However, given the accomplishment of spermiation in 100% of the population, the results obtained from the sperm evaluation and the production of viable eggs with >97% fertilization success (see discussion below), it can be concluded that spermatogenesis in meagre, as with vitellogenesis, can take place successfully in cultured meagre under low levels of plasma T and 11-KT.

During the full spermiation period, sperm quality in meagre did not vary significantly, with the exception of a transient reduction in motility duration in May. Regarding sperm density, no published information exists so far for meagre, but the values recorded in the present study were similar to those of other Mediterranean marine fish (see review by Suquet et al., 1994), including the shi drum (13–26 × 10^9 szoa ml^{-1}) (Mylonas et al., 2004), the rabbitfish *Siganus guttatus* (5–20 × 10^9 szoa ml^{-1}) (Garcia, 1993), the red porgy (9–24 × 10^9 szoa ml^{-1}) (Mylonas et al., 2003) and the Atlantic bluefin tuna *Thunnus thynnus* (38 × 10^9 szoa ml^{-1}) (Suquet et al., 2010). The percentage of spermatozoa exhibiting forward motility also remained unchanged, though variable (44–80%), during the spermiation period in meagre, exhibiting values that were similar to another published report of meagre (53–74%) (Schiavone et al., 2012), as well as the shi drum (70–80%) (Mylonas et al., 2004). In the other meagre study (Schiavone et al., 2012), sperm motility percentage was higher in the middle of the spawning season, but in shi drum no change was observed during the three months of monitoring (Mylonas et al., 2004). Regarding the duration of sperm motility, it was brief in meagre in the present study (0.78–1.27 min), but unlike other studies of this species (0.57–3.3 min) (Mylonas et al., 2013; Schiavone et al., 2012), as well as a wide variety of other fishes (see review by Suquet et al., 1994). The percentage of motile spermatozoa after activation started decreasing immediately after activation in meagre, following a gradual decline, and by 0.5 min after activation only ~50% of the initially motile spermatozoa were still exhibiting forward motility. This type of kinetics in percentage of sperm motility has been reported in other species with short duration of sperm motility, such as the European sea bass (Fauvel et al., 1999), the horse mackerel *Trachurus mediterraneus* and the red mullet *Mullus barbatus* (Lahnsteiner and Patzner, 1998). Given the high fertilization success of all spawns obtained by meagre here, which exceeded 97%, it can be safely concluded that sperm quality parameters (*i.e.*, sperm concentration, and percentage and duration of sperm motility) were optimal in the present study and not posing any limitation to the production of large numbers of fertilized eggs.

In conclusion, although a single broodstock was used for the present study, thus limiting the reliability of the plasma sex hormone levels and sperm quality parameters, the results of the present study in terms of meagre gametogenesis in captivity agree with the existing literature and reports from commercial hatcheries that hatchery-produced meagre undergo successful vitellogenesis and spermatogenesis/spermiation in captivity. In addition, the study showed for the first time that there is some capacity of a small percentage of the female population to undergo spontaneous oocyte maturation and spawning, resulting in the production of viable eggs of high fecundity and fertilization success. Females maintained their vitellogenic oocytes without significant atresia for a period of at least 2 months (April–June) and these oocytes could potentially be induced to undergo maturation, ovulation and spawning at any time during this “post-vitellogenesis” period. Sperm of good quality was available throughout this 2-month period when females had fully vitellogenic oocytes in their ovaries, and based on the observed sperm quality parameters, sperm was not limiting for the production of viable eggs. These findings are expected to provide useful information for improving broodstock management and the implementation of spawning induction therapies for the acquisition of spawns of high fecundity and fertilization success. The optimization of hormonal spawning induction therapies is the focus of two articles that will follow, one in the present volume (Mylonas et al., 2013).

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References

Abou Shabana, N.M., Abd El Rahma, S.H., Al Absawy, M.A., Assem, S.S., 2012. Reproductive biology of *Argyrosomus regius* (Asso, 1801) inhabiting the south eastern Mediteraanean Sea, Egypt. Egypt. J. Aquat. Res. 38, 147–156.

Barbaro, A., Franceson, A., Bertotto, D., Bozzato, G., Di Maria, I., Patarnello, P., Furlan, F., Colombo, L., 2002. More effective induction of spawning with long-acting GnRH agonist in the shi drum, *Umbrina cirrosa* L. (Sciaenidae, Teleostei), a valuable candidate for Mediterranean mariculture. J. Appl. Ichthyol. 18, 192–199.

Battaglene, S.C., Talbot, R.B., 1994. Hormone induction and larval rearing of mulloway, *Argyrosomus hololepidotus* (Pisces, Sciaenidae). Aquaculture 126, 73–81.

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, 71–94.

Berlinsky, D.L., Jackson, L.F., Smith, T.I.J., Sullivan, C.V., 1995. The annual reproductive cycle of the white bass *Morone chrysops*. J. World Aquac. Soc. 26, 252–260.

Brown-Peterson, N., Thomas, P., Arnold, C.R., 1988. Reproductive biology of the spotted seatrout, *Cynoscion nebulosus*, in South Texas. Fish. Bull. 86, 373–388.

Carragher, J.F., Pankhurst, N.W., 1993. Plasma levels of sex steroids during sexual maturation of snapper, *Pagrus auratus* (Sparidae), caught from the wild. Aquaculture 109, 375–388.

Carrillo, M., Zanuy, S., Prat, F., Cerda, J., Ramos, J., Mañanos, E., Bromage, N., 1995. Sea bass (*Dicentrarchus labrax*). In: Bromage, N.R., Roberts, R.J. (Eds.), Broodstock Management and Egg and Larval Quality. Blackwell Science, Oxford, pp. 138–168.

Cuisset, B., Pradelles, P., Kime, D.E., Kühn, E.R., Babin, P., Davail, S., Le Menn, F., 1994. Enzyme immunoassay for 11-ketotestosterone using acetylcholinesterase as label:

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application to the measurement of 11-ketotestosterone in plasma of Siberian sturgeon. Comp. Biochem. Physiol. 108C, 229–241.
Duncan, N., Estevez, A., Padros, F., Aguillera, C., Montero, F.E., Norambuena, F., Carazo, I., Carbo, R., Mylonas, C.C., 2008. Acclimation to captivity and GnRHa-induced spawning of meagre (*Argyrosomus regius*). Cybium 32 (2), 332–333 (Suppl.).
Duncan, N.J., Estévez, A., Porta, J., Carazo, I., Norambuena, F., Aguilera, C., Gairin, I., Bucci, F., Vallés, R., Mylonas, C.C., 2012. Reproductive development, GnRHa-induced spawning and egg quality of wild meagre (*Argyrosomus regius*) acclimatised to captivity. Fish Physiol. Biochem. 38, 1273–1286.
Duncan, N.J., Estévez, A., Fernández-Palacios, H., Gairin, I., Hernández-Cruz, C.M., Roo, F.J., Schuchardt, D., Vallés, R., 2013. Aquaculture production of meagre (*Argyrosomus regius*), hatchery techniques, ongrowin and market. In: Allan, G., Burnell, G. (Eds.), Advances in Aquaculture Hatchery Technology. Woodhead Publishing Limited, Cambridge, UK, pp. 519–541.
El-Shebly, A.A., El-Kady, M.A.H., Hussin, A.B., Hossain, M.Y., 2007. Preliminary observations on the pond culture of meagre, *Argyrosomus regius* (Asso, 1801) (Sciaenidae) in Egypt. J. Fish. Aquat. Sci. 2 (5), 345–352.
Fauvel, C., Savoye, O., Dreanno, C., Cosson, J., Suquet, M., 1999. Characteristics of sperm of captive seabass in relation to its fertilization potential. J. Fish Biol. 54, 356–369.
Fernández-Palacios, H., Schuchart, D., Roo, J., Hernández-Cruz, C.M., Sabater, C., Duncan, N., 2011. Efecto de diferentes intervalos de tiempo enter inyecciones con GnRHa, sobre las puestas de reproductores de corvina (*Argyrosomus regius*). Proceedings of the XIII Congreso Nacional de Acuicultura, 21–24 de Noviembre Barcelona, pp. 554–555 (in Spanish, abstract in English).
Garcia, L.M.B., 1993. Sustained production of milt in rabbitfish, *Siganus guttatus* Bloch, by weekly injection of luteinizing hormone-releasing hormone analogue (LHRHa). Aquaculture 113, 261–267.
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. Mar. 77, 105–118.
González-Quirós, R., del Árbol, J., García-Pacheco, M., Silva-García, J., Naranjo, J.M., Morales-Nin, B., 2011. Life-history of the meagre *Argyrosomus regius* in the Gulf of Cadiz (SW Iberian Peninsula). Fish. Res. 109, 140–149.
Grau, A., Linde, M., Grau, A.M., 2009. Reproductive biology of the vulnerable species *Sciaena umbra* Linnaeus, 1758 (Pisces: Sciaenidae). Sci. Mar. 73, 67–81.
Griffiths, M.H., Heemstra, P.C., 1995. A contribution to the taxonomy of the marine fish genus *Argyrosomus* (Perciformes: Sciaenidae), with descriptions of two new species from Southern Africa. Ichthyol. Bull. 65.
Kokokiris, L., Mourot, B., Le Menn, F., Kentouri, M., Fostier, A., 2000. Endocrine changes during the annual reproductive cycle of the red porgy, *Pagrus pagrus* (Teleostei, Sparidae). Fish Physiol. Biochem. 23, 1–11.
Kokokiris, L., Le Menn, F., Kentouri, M., Kagara, M., Fostier, A., 2001. Seasonal cycle of gonadal development and serum levels of vitellogenin of the red porgy, *Pagrus pagrus* (Teleostei, Sparidae). Mar. Biol. 139, 549–559.
Koldras, M., Bieniarz, K., Kime, D.E., 1990. Sperm production and steroidogenesis in testes of the common carp, *Cyprinus carpio* L., at different stages of maturity. J. Fish Biol. 37, 635–645.
Lahnsteiner, F., Patzner, R.A., 1998. Sperm motility of the marine teleosts *Boops boops*, *Diplodus sargus*, *Mullus barbatus* and *Trachurus mediterraneus*. J. Fish Biol. 52, 726–742.
Monfort, M.C., 2010. Present market situation and prospects of meagre (*Argyrosomus regius*), as an emerging species in Mediterranean aquaculture. Studies and Reviews. General Fisheries Commission for the Mediterranean, No. 89. Food and Agriculture Organization of the United Nations (28 pp.).
Morales-Nin, B., Geffen, A.J., Pérez-Mayol, S., Palmer, M., González-Quirós, R., Grau, A., 2012. Seasonal and ontogenic migrations of meagre (*Argyrosomus regius*) determined by otolith geochemical signatures. Fish. Res. 127–128, 154–165.
Mylonas, C.C., Zohar, Y., 2009. Controlling reproduction in aquaculture. In: Burnell, G., Allan, G. (Eds.), New Technologies in Aquaculture: Improving Production Efficiency, Quality and Environmental Management. Woodhead Publishing Ltd., Cambridge, UK, pp. 109–142.
Mylonas, C.C., Papadaki, M., Divanach, P., 2003. Seasonal changes in sperm production and quality in the red porgy *Pagrus pagrus* (L.). Aquac. Res. 34, 1161–1170.
Mylonas, C.C., Kyriakou, G., Sigelaki, I., Georgiou, G., Stephanou, D., Divanach, P., 2004. Reproductive biology of the shi drum (*Umbrina cirrosa*) in captivity and induction of spawning using GnRHa. Isr. J. Aquacult. - Bamidgeh 56, 75–92.
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., Fostier, A., Zanuy, S., 2010. Broodstock management and hormonal manipulations of reproduction. Gen. Comp. Endocrinol. 165, 516–534.
Mylonas, C.C., Zohar, Y., Pankhurst, N.W., Kagawa, H., 2011. Reproduction and broodstock management. In: Pavlidis, M., Mylonas, C.C. (Eds.), Sparidae: Biology and Aquaculture of Gilthead Seabream and Related Species. Blackwell Science Publishers, London, pp. 95–131.
Mylonas, C., Mitrizakis, N., Castaldo, C., Cerviño, C., Papadaki, M., Sigelaki, I., 2013. Reproduction of hatchery-produced meagre *Argyrosomus regius* in captivity II. Hormonal induction of spawning and monitoring of spawning kinetics, egg prodcution and egg quality. Aquaculture 414-415, 309–317.
Nash, J.P., Davail-Cuisset, B., Bhattacharyya, S., Suter, H.C., Le Menn, F., Kime, D.E., 2000. An enzyme linked immunosorbent assay (ELISA) for testosterone, estradiol, and 17,20β-dihydroxy-4-pregnen-3-one using acetylcholinesterase as tracer: application to measurement of diel patterns in rainbow trout (*Oncorhynchus mykiss*). Fish Physiol. Biochem. 22, 355–363.
Pavlidis, M., Greenwood, L., Mourot, B., Kokkari, C., Le Menn, F., Divanach, P., Scott, A.P., 2000. Seasonal variations and maturity stages in relation to differences in serum levels of gonadal steroids, vitellogenin, and thyroid hormones in the common dentex (*Dentex dentex*). Gen. Comp. Endocrinol. 118, 14–25.
Prat, F., Zanuy, S., Carillo, M., De Mones, A., Fostier, A., 1990. Seasonal changes in plasma levels of gonadal steroids of sea bass, *Dicentrarchus labrax* L. Gen. Comp. Endocrinol. 78, 361–373.
Rodríguez, L., Begtashi, I., Zanuy, S., Carrillo, M., 2000. Development and validation of an enzyme immunoassay for testosterone: effects of photoperiod on plasma testosterone levels and gonadal development in male sea bass (*Dicentrarchus labrax*, L.) at puberty. Fish Physiol. Biochem. 23, 141–150.
Roo, J., Hernandez-Cruz, C.M., Borrero, C., Schuchardt, D., Fernandez-Palacios, H., 2010. Effect of larval density and feeding sequence on meagre (*Argyrosomus regius*; Asso, 1801) larval rearing. Aquaculture 302, 82–88.
Schiavone, R., Zilli, L., Storelli, C., Vilella, S., 2012. Changes in hormonal profile, gonads and sperm quality of *Argyrosomus regius* (Pisces, Scianidae) during the first sexual differentiation and maturation. Theriogenology 77, 888–898.
Schreck, C.B., 2010. Stress and fish reproduction, the roles of allostasis and hormesis. Gen. Comp. Endocrinol. 165, 549–556.
Schulz, R.d.W., de FranÁa, L.R., Lareyre, J.-J., LeGac, F., Chiarini-Garcia, H., Nobrega, R.H., Miura, T., 2010. Spermatogenesis in fish. Gen. Comp. Endocrinol. 165, 390–411.
Sulistyo, I., Fontaine, P., Rinchard, J., Gardneur, J.N., Migaud, H., Capdeville, B., Kestemont, P., 2000. Reproductive cycle and plasma levels of sex steroids in male Eurasian perch *Perca fluviatilis*. Aquat. Living Resour. 13, 99–106.
Suquet, M., Billard, R., Cosson, J., Dorange, G., Chauvaud, L., Mugnier, C., Fauvel, C., 1994. Sperm features in turbot (*Scophthalmus maximus*): a comparison with other freshwater and marine fish species. Aquat. Living Resour. 7, 283–294.
Suquet, M., Cosson, J., de la Gándara, F., Mylonas, C.C., Papadaki, M., Lallemant, S., Fauvel, C., 2010. Sperm features of captive Atlantic bluefin tuna (*Thunnus thynnus*). J. Appl. Ichthyol. 26, 775–778.
Thomas, P., Copeland, P.A., Prentice, J.A., 1994. Preliminary observations on the reproductive physiology of female orangemouth corvina in captivity. J. World Aquac. Soc. 25, 214–224.
Thomas, P., Arnold, C.R., Holt, G.J., 1995. Red drum and other sciaenids. In: Bromage, N.R., Roberts, R.J. (Eds.), Broodstock Management and Egg and Larval Quality. Blackwell Science, Oxford, pp. 118–137.
Utoh, T., Okamura, A., Yamada, Y., Tanaka, S., Mikawa, N., Akazawa, A., Horie, N., Oka, H.P., 2004. Reproductive cycle in reared male common Japanese conger, *Conger myriaster*. Aquaculture 240, 589–605.
Vallés, R., Estévez, A., 2013. Light conditions for larval rearing of meagre (*Argyrosomus regius*). Aquaculture 376–379, 15–19.
Vallés, R., Bayarri, M.J., Mañanos, E., Duncan, N., 2011. Plasma sex steroid profiles in meagre (*Argyrosomus regius*). Indian Journal of Science and Technology 9th ISRPF Issue, vol. 4 No S8, p. 108.
Wilson-Leedy, J.G., Ingermnn, R.L., 2007. Development of a novel CASA system based on open source software for characterization of zebrafish sperm motility parameters. Theriogenology 67, 661–672.
Zohar, Y., Harel, M., Hassin, S., Tandler, A., 1995. Gilthead sea bream (*Sparus aurata*). In: Bromage, N.R., Roberts, R.J. (Eds.), Broodstock Management and Egg and Larval Quality. Blackwell Science, Oxford, pp. 94–117.