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Evolution of sex ratio and egg production of gilthead seabream (*Sparus aurata*) over the course of five reproductive seasons

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A B S T R A C T

Spawning performance -relative fecundity and fertilization success- was studied in two hatchery-reared broodstocks of gilthead seabream (*Sparus aurata*) held under relatively constant well-water temperature (18–20 °C) and simulated natural photoperiod, for five consecutive spawning seasons, between 3 and 7 years of age. The spawning season lasted between 4 and 6 months each year, and the total number of eggs produced annually ranged between 1 480 000 and 3 100 000 eggs/kg female body weight, being the highest during the first and second spawning season. No difference was observed in monthly relative fecundity between years -although high variation existed within years, whereas fertilization success was the highest during the second and third reproductive season, and dropped significantly during the last year of the study. The male percentage of the broodstocks was 35% during the first spawning season of the females, and thereafter it decreased further and remained stable at around 15%–20% for the second and third spawning season. Substitution of older females with smaller males to readjust male percentage to 50% in the fourth spawning season, was followed by sex change of larger males to females and a drop of the male percentage to 18% in the following spawning season. The present study demonstrated the long spawning season of gilthead seabream -especially under constant water temperature, the high fecundity and fertilization success, and the stabilization of male percentage to ~20% after the first spawning season or when a broodstock is modified to increase male percentage. These results are useful to the aquaculture industry, demonstrating that there is no need to add males to gilthead seabream broodstocks over the years, as the sex ratio is stabilized and egg production and fertilization success remain high with a relatively low male:female sex ratio.

1. Introduction

The gilthead seabream *Sparus aurata* is one of the two important species for the Mediterranean aquaculture industry. In nature, it reproduces from December until April at water temperatures between 13 and 17 °C (Zohar, Harel, Hassin, & Tandler, 1995). After many years of farming and many efforts by fish reproduction experts (Zohar, 2020), the gilthead seabream is now fully adapted to captivity conditions, releasing more than 2 000 000 eggs/kg female body weight for at least 4–5 months every year (Ibarra-Zatarain & Duncan, 2015; Mylonas, Zohar, Pankhurst, & Kagawa, 2011). Although it has been shown that both temperature and photoperiod are important environmental parameters involved in the control of reproduction in fish, in gilthead seabream the environmental cue that induces gametogenesis and spawning is undoubtedly photoperiod, as fish kept under a constant temperature of 19–21 °C and the appropriate seasonal photoperiod are able to spawn for a long period of time during the year (Mylonas et al., 2011). Spawning may occur in the morning or afternoon, and the spawning behaviour has been described in captivity, with the participation of one female and one to three males (Ibarra-Zatarain & Duncan, 2015). Gilthead seabream has been shown to continue to spawn even when starved during the spawning season, with no effect of starving on fecundity, fertilization success and egg quality (Chatzifotis et al., 2021). A number of studies have been conducted to monitor the effect of different parameters, such as photoperiod (Kissil, Lupatsch, Elizur, & Zohar, 2001), broodstock nutrition (Chatzifotis et al., 2021; Ferosekhan et al., 2021; Scabini, Fernández-Palacios, Robaina, Kalinowski, & Izquierdo, 2011), female age (Jerez et al., 2012) and endocrine

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disrupting chemicals (Forner-Piquer, Fakriadis, et al., 2019; Forner-Piquer, Mylonas, et al., 2019; Garcia Hernandez et al., 2020) on gilthead seabream spawning performance. However, limited information is available on the sex ratio shifts and egg quality under captivity for a number of consecutive years.

The gilthead seabream is a protandrous species, exhibiting high male percentages at small sizes in nature (Chaoui, Kara, Faure, & Quignard, 2006; Fateh, Lyamine, & Mostefa, 2018; Hadj-Taieb, Ghorbel, Hadj-Hamida, & Jarboui, 2013). Hermaphroditism is very common in the Sparidae family, with different types shown depending on the species. It includes protogyny in the red porgy *Pagrus pagrus* (Pajuelo & Lorenzo, 1996) and the common pandora *Pagellus erythrinus* (Pajuelo & Lorenzo, 1998) and rudimentary hermaphroditism in the sharpsnout seabream *Diplodus puntazzo* (Papadaki et al., 2018). Gonochorism is also found in the Sparidae family, as in the common dentex *Dentex dentex* (Loir, Le Gac, Somarakis, & Pavlidis, 2001). In terms of broodstock management, when broodstocks of protandric species, such as gilthead seabream, remain unmanaged for many years, there is concern that they could end up with a high female percentage, as sex conversion from males to females may happen every year. This may reduce sperm availability in the broodstock, and potentially decrease fertilization success. In order to avoid this problem, the common practice in fish farms is to remove larger, older fish (females) and substitute them with smaller individuals (males) every few years (Mylonas et al., 2011).

The aim of the present study was to monitor sex ratio shifts and spawning performance of gilthead seabream broodstocks under captivity, over many consecutive reproductive seasons. We studied two populations of gilthead seabream produced in our facilities, for seven consecutive years and five reproductive seasons and we monitored egg fecundity and fertilization, as well as shifts in sex ratios over these years. We also tested the effect of removing older females and substituting them with younger males in the following year's sex ratio and spawning performance. The obtained results could provide useful information for commercial broodstock management of gilthead seabream and could be relevant also for other species exhibiting protandrous hermaphroditism in aquaculture.

2. Materials and methods

2.1. Ethical issues

Experiments were conducted at the AQUALABS facilities of the Hellenic Center for Marine Research, Crete, Greece, a registered facility for the maintenance of farmed fish (HCMR, Registration No EL91-BIObr-03 and EL91-BIOexp-04 for animal experimentation and fish production), under the approved protocol No 255356 (Regional Veterinary Services). All procedures involving animals were conducted in accordance to the "Guidelines for the treatment of animals in behavioral research and teaching" (Anonymous, 1998), the Ethical justification for the use and treatment of fishes in research: an update (Metcalfe & Craig, 2011) and the "Directive 2010/63/EU of the European parliament and the council of September 22, 2010 on the protection of animals used for scientific purposes" (EU, 2010).

2.2. Broodstock maintenance

A hundred and seventeen fish of an initial weight of around 50 g produced in our facilities in 2009 were maintained in two 2000-L tanks for two years. During these two years, four different samplings were conducted (January 2010, September 2010, January 2011 and February 2012) and gonads were dissected (n = 5–10) in order to monitor the sex reversal process. After dissection, gonads were preserved in a fixative solution of formaldehyde: glutaraldehyde (4:1) until histological analysis.

In 2012, two broodstocks were formed and moved to two 5000-l tanks, where they were kept under simulated natural photoperiod and allowed to spawn. The first broodstock consisted of 8 males (mean body weight ± S.D. of 1.13 ± 0.15 kg) and 17 females (mean body weight ± S.D. of 1.28 ± 0.25kg) and the second broodstock consisted of 10 males (mean body weight ± S.D. of 1.17 ± 0.26 kg) and 16 females (mean body weight ± S.D. of 1.26 ± 0.21 kg), respectively. Well water was supplied to the tanks, with no heating or cooling, with temperatures ranging from 18 to 20 °C. Fish were fed with industrial feed (Style, IRIDA S.A., Greece) throughout the year, whereas during the spawning period they were offered frozen squid and industrial feed (Vitalis, Skretting, Norway) three and four times per week, respectively. Temperature, oxygen and pH were measured on a weekly basis. From 2012 until 2016, during the reproductive season, an overflow egg collector was placed in the outflow of the tanks for egg collection and estimation of egg production and quality.

2.3. Evolution of sex ratio and egg quality evaluation

At the beginning of each year (January or February), from 2012 until 2016, samplings for broodstock sex ratio, wet weight (WW) and maturity stage were conducted for each of the two broodstocks. During the samplings, fish were initially tranquilized in their tank with the use of clove oil at a concentration of 0.01 mL/L and later transferred to an anesthetic tank, where they were completely sedated with a higher clove oil concentration (0.03 mL/L). In males, gentle abdominal pressure was applied, in order to check for the presence of sperm. After that, all the fish were weighed. In 2015, after the sex ratio sampling, 4 large females (mean weight ± S.D. of 2.20 ± 0.52 kg) were removed and 4 younger males (3 years old, mean weight ± S.D. of 0.68 ± 0.14 kg) were added in each of the two tanks. All the males of the two populations were marked with passive integrated transponder (P.I.T.) tags (AVID, UK), and their sex was checked again in January and November 2016, to monitor the sex reversal incidence in the stocks.

From 2012 until 2016, daily egg quality evaluation was conducted, in terms of fecundity and fertilization success (%). A net was used to

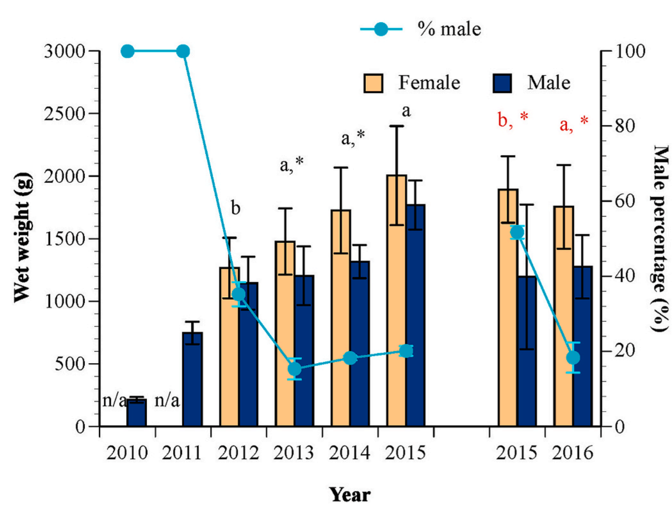

Fig. 1. Annual evolution of mean (±S.E.M.) wet weight (WW) and male percentage of two gilthead seabream (*Sparus aurata*) broodstocks during the seven years of the study, starting with juveniles produced in 2009 (See also Table 1). In 2015, at the start of the spawning season, the two broodstocks were modified by removing some of the larger females, and adding younger (smaller) males, in order to readjust the male percentage to 50%. Letter superscripts indicate significant differences between years in the male percentage (ANOVA, Tukey's HSD, $P < 0.05$), whereas asterisks indicate differences in mean WW between females and males (ANOVA, Tukey's HSD, $P < 0.05$), beginning in 2013. Two different statistical analyses were performed: the first one for reproductive years 2012–2015 (black Latin superscripts and asterisks); and the second one for the years 2015 and -2016 after the modification of the broodstocks (red Latin superscripts and asterisks).

transfer the eggs that were collected in a passive collector fitted on the surface outflow of the tank, into a 10-L bucket. After that, a 10-mL sample was obtained and all the eggs in this sample were observed and counted under a stereoscope to estimate fecundity and fertilization success. Fecundity was estimated as the total number of eggs collected, and fertilization success was calculated as the number of fertilized eggs/total number of eggs present in the 10 ml sample × 100. Relative fecundity was expressed as fecundity/kg female WW.

2.4. Histological analysis

For histological processing, gonadal samples obtained from representative fish at 1, 2 and 3 years of age, were embedded in methylmethacrylate resin (Technovit 7100, Heraeus Kulzer, Germany). Sections were obtained at a thickness of 3–5 μm in a semi-automatic microtome (Leica RM 2245, Germany). Subsequently, slides were stained with methylene blue/basic fuchsin (Bennett et al., 1976). Samples were observed under an optical microscope (Nikon Eclipse 50i) and photographed with the use of a camera (Jenoptik progress C12 plus).

2.5. Statistical analysis

Data on number of spawning days per year, sex ratios and WW of female and male fish between reproductive seasons were analyzed with the use of analysis of variance (ANOVA) followed by Tukey's HSD test with minimum significance value of $P < 0.05$. For WW and sex ratio, two different statistical analyses were performed: the first one for reproductive years 2012–2015, and the second one for the years 2015–2016 after the modification of the removal and addition of fish. Data on % fertilization, monthly relative fecundity, and daily relative fecundity (year × month interaction) during five successive reproductive years were analyzed with the use of two-way ANOVA, Tukey's HSD, $P < 0.05$. All statistical analysis was conducted using JMP statistical package (Cary, USA).

3. Results

Mean female wet weight (Fig. 1 and Table 1) increased significantly each year of the study (ANOVA, Tukey's HSD, $P < 0.05$). On the contrary, the first significant weight increase in males was observed in 2015 (ANOVA, Tukey's HSD, $P < 0.05$). There was a significant difference in body size between males and females (ANOVA, $P < 0.05$) beginning in 2013 (Fig. 1), with females being larger than males. Mean male percentage of the two broodstocks decreased significantly (one-way ANOVA, $P < 0.05$) from 100% until 2011 to 35% ± 3% in 2012 and then 15% ± 3% in 2013 (ANOVA, Tukey's HSD, $P < 0.05$), but then remained unchanged until 2015, when we changed the male percentage to 52% ± 2% after the addition (at the beginning of the spawning season) of younger males and the removal of older females from the broodstocks (Fig. 1 and Table 1).

Sex reversal occurred after the removal of large females and the addition of young males in the broodstocks in 2015 and male percentage decreased again (ANOVA, Tukey's HSD, $P < 0.05$) from 52% ± 2% to 18% ± 4% in 2016 (Fig. 1 and Table 1). More specifically, 5 males from one tank and 4 males from the other tank were found to be females the following reproductive season (January 2016). One more fish was found to have changed sex from male to female in November 2016. A total number of 5 males (the largest ones) from each tank were found to have changed sex between 2015 and 2016, whereas the 2 smallest males from the original stock continued to reproduce as males (data not shown).

At 1 year of age (January 2010), all fish were males with spermatogonia, primary and secondary spermatocytes and spermatozoa in their testes (Fig. 2A). In September 2010, bisexual gonads could be found, in which ovaries contained primary oocytes and testes contained primary and secondary spermatocytes and spermatozoa (Fig. 2B). Only male fish were found again at 2 years of age (January 2011), with testes exhibiting all types of germ cells (Fig. 2C). At 3 years of age (January 2012), in addition to males with well-developed testes, the first females were found in the broodstocks, exhibiting oocytes at the primary, cortical alveoli, vitellogenic and early maturation stage (Fig. 2D).

Spawning begun between December and February, and the end of the reproductive season was observed from the end of May until mid-August during the five spawning seasons (Fig. 3). Within spawning months, daily and mean monthly relative fecundity exhibited variations among years (Fig. 4A and B), but overall the trend was for lower values at the beginning and the end of the reproductive season (January and

Table 1
Mean ± standard error mean (S.E.M.) of various spawning parameters of two gilthead seabream populations during 5 consecutive spawning seasons (2012–2016). The measured parameters included. Daily relative fecundity (eggs/kg female WW, n = 6 from Fig. 4A), Monthly relative fecundity (eggs/kg female WW, n = 6 from Fig. 4B), Total annual relative fecundity (eggs/kg female WW, n = 2 from Fig. 3), Fertilization success (%, n = 6 from Fig. 4C), Number of spawning days (n = 2), Female and Male body weight, and Percentage of males (%, n = 2). Different Latin letters refer to statistically significant differences among the 5 years (one-way ANOVA, *P* values in the last column), whereas lack of significance is shown by "ns" (not significant). In 2015, just before the onset of the spawning season, some females were removed and younger males were added to the two populations, to adjust the sex ratio to 50% males. The resulting new Male and Female weight, and Sex ratio (% males) are shown in separate rows below.

Mean ± S.E.M.	2012	2013	2014	2015	2016	P<
Daily relative fecundity ($\times 10^3$ eggs/kg)	18 ± 0.7^c	17 ± 0.5^c	12 ± 0.4^a	11 ± 0.5^a	15 ± 8.9^b	**0.05**
Monthly relative fecundity ($\times 10^3$ eggs/kg)	433 ± 98	510 ± 59.8	363 ± 33.7	296 ± 52.1	391 ± 83	**ns**
Total annual fecundity ($\times 10^3$ eggs/kg)	$2587 \pm 205^{b,c}$	3100 ± 202^c	$2239 \pm 7^{a,b}$	$2074 \pm 173^{a,b}$	1482 ± 15^a	**0.05**
Fertilization (%)	87 ± 1^b	92 ± 1^c	91 ± 1^c	$89 \pm 1^{b,c}$	83 ± 1^a	**0.05**
Number of spawning days	141 ± 3	180 ± 19	191 ± 19	156 ± 6	130 ± 4	**ns**
Female weight (kg)[1]	1.27 ± 0.04^a	1.48 ± 0.04^b	1.72 ± 0.05^c	2.00 ± 0.08^d	–	**0.05**
			w/new fish	1.89 ± 1.22	1.75 ± 0.07	**ns**
Male weight (kg)[1]	1.14 ± 0.05^a	1.20 ± 0.09^a	$1.32 \pm 0.05^{a,b}$	1.77 ± 0.07^b	–	**0.05**
			w/new fish	1.19 ± 0.12	1.28 ± 0.16	**ns**
Sex ratio (% males)[1]	35 ± 3^b	15 ± 3^a	18 ± 1^a	20 ± 1^a	-	**0.05**
			w/new fish	52 ± 2^b	18 ± 4^a	**0.05**

[1] Measured just prior to the onset of the spawning period.

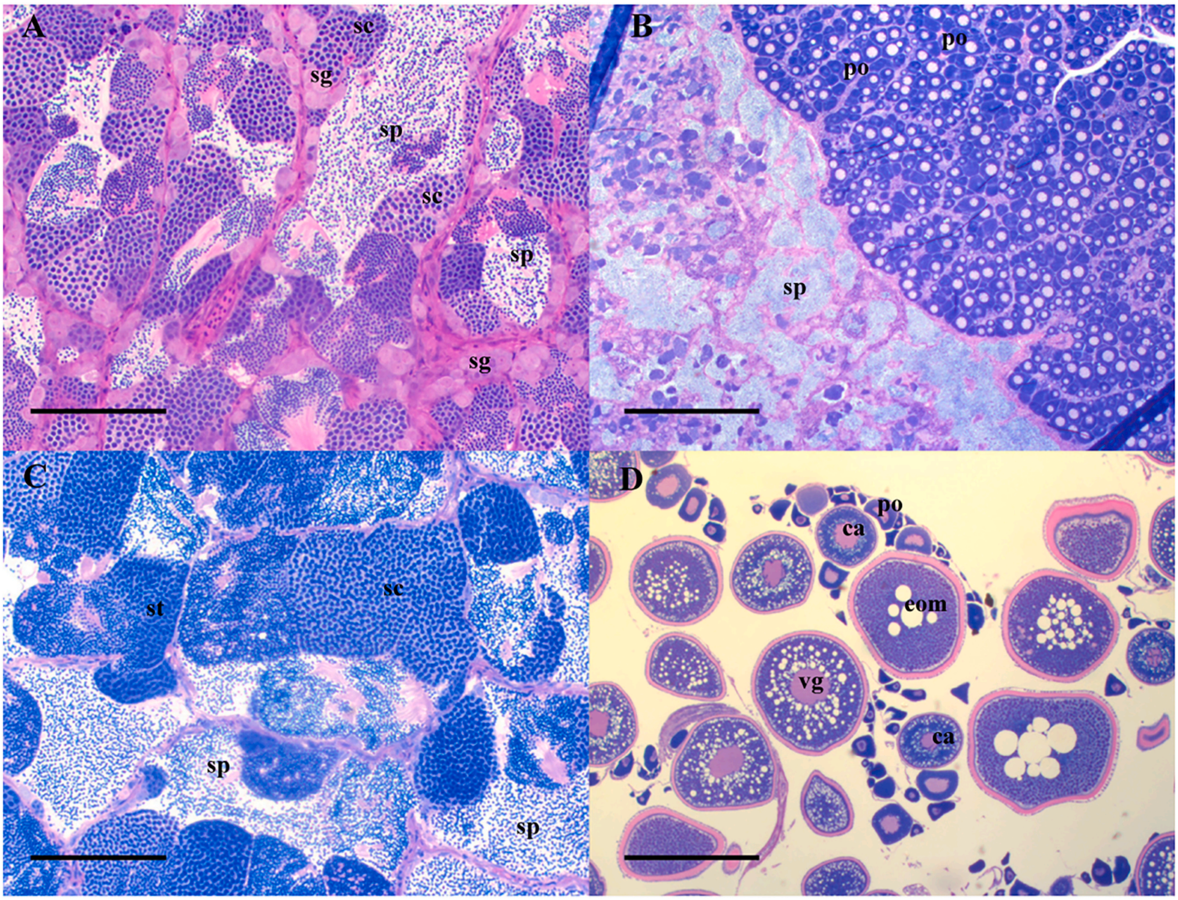

Fig. 2. Histological sections of gilthead seabream (*Sparus aurata*) gonads during the first three years of life. A. Testis of 1-year-old fish (January 2010) with spermatogonia (sg), spermatocytes (sc), and spermatozoa (sz). B. Bisexual gonad of 1-year-old fish (September 2010), with a testis (left) mostly filled with sz and an ovary (right) with primary oocytes (po). C. Testis of 2-year-old fish (January 2011) with sc, st and sz. D. Ovary of 3-year-old fish (January 2012) with po, cortical alveoli (ca), vitellogenic (vg) and early maturing (eom) oocytes.

June). Overall, small (daily) or no significant differences (monthly) in relative fecundity were observed among years (Table 1). Fertilization success was very high and with little variation among the five years of the study and commonly was >80% (Table 1 and Fig. 4C). As with fecundity, lower values were observed at the beginning or the end of the reproductive season with the first and last month being the ones with the lowest fertilization success (Fig. 4C). There was no statistically significant difference in mean number of spawning days among years, however caution should be taken in interpreting these results, as the number of broodstock available for the study was only n = 2 and there was a large variation in the data (Table 1).

Evaluation of the egg production results among the five reproductive seasons suggests a trend towards lower annual relative fecundity and fertilization success during the last two or three monitored reproductive seasons (Table 1).

4. Discussion

Well-developed testes with spermatozoa were found in gilthead seabream already at 1 year of age. The fish increased significantly in size, and became reproductively mature as males at 2 years of age, as expected (Mylonas et al., 2011). When sex reversal took place at 3 years of age, males had already slightly smaller body weight than females. The differences in body mass between sexes increased over the following years, when females continued to grow significantly every year, while the growth of males was considerably less. The failure to observe a significant increase in body size in males between 2012 and 2013 has probably resulted from the sex reversal of the largest males into females. However, this could not be verified, since the fish were not tagged individually at this time. In nature, sex reversal has been shown to occur at a size of 43–45 cm (Chaoui et al., 2006), or at the completion of the second reproductive season in fish held in captivity (Liarte et al., 2007; Zohar, Abraham, & Gordin, 1978; Zohar et al., 1995). At this time, a degenerative process begins in the testes, and up to 40% of the fish change sex to females before their third reproductive season (Liarte et al., 2007), in accordance with the results of the present study. Sex change is also controlled by the dynamics and the social interactions of the population (Zohar et al., 1995). For example, when younger males are added in the broodstock at the end of the reproductive season, the older (and larger) ones change sex before the next reproductive season. In the present study we removed older, large females and we substituted them with smaller males at the beginning of the 4th reproductive season, re-adjusting the sex ratio to 50% males in the two studied populations. In the following reproductive season, after larger males changed sex to females, the male percentage of the populations was found to be similar to the one before the addition of younger males (~18%). These results suggest that a male percentage of around 15%–20% males is "preferred" by the spawning broodstocks of gilthead seabream. According to the size-advantage model (Ghiselin, 1969), protandry is favored in species in which: a) large females have more reproductive success than smaller females and similar-sized males and b) spawning occurs in pairs or small groups, and sperm competition is low. However, in contrast to this model, males of protandric species have been shown to exhibit very high gonadosomatic index values (Erisman, Petersen, Hastings, & Warner, 2013), which can be attributed either to their participation in group spawnings, increasing sperm competition, or to their effort to invest in

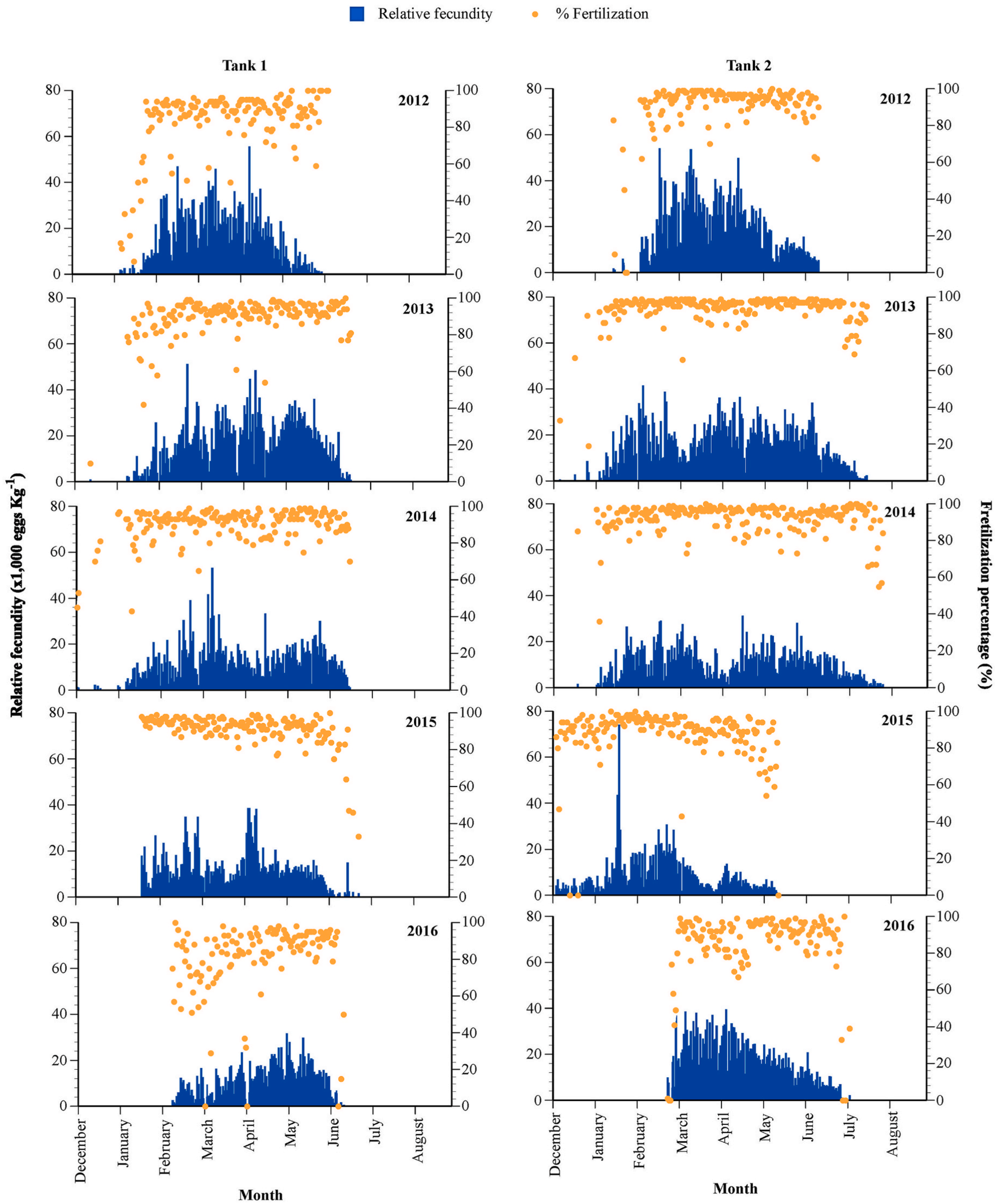

Fig. 3. Daily relative fecundity (eggs/kg female wet weight, blue bars) and fertilization success (%, orange dots) of two gilthead seabream (*Sparus aurata*) broodstocks from 2012 until 2016.

gonad development, in order to be able to fertilize the large number of eggs produced by the large females (Pla, Benvenuto, Capellini, & Piferrer, 2020, 2022). In the present study, when young males were added to the populations, all larger males changed sex to female for the following reproductive season and in 2016 only two small-sized males were found in each broodstock. This led to a small, but significant, reduction in the annual fertilization percentage, but with the fecundity remaining similar as the previous years, indicating that even in the presence of two small males, gilthead seabream broodstocks are able to produce large quantities of high-quality fertilized eggs in aquaculture conditions without the need for hatchery managers to modify the population and the sex ratio of the broodstocks.

The spawning period of gilthead seabream in the present study begun in December of most years and was completed between mid-June and mid-August. Initiation of the spawning period in December has been also shown in previous studies (Zohar et al., 1995; Zohar & Gordin, 1979). However, the duration of the spawning period was rather long in the present study, presumably because of the relatively constant temperature, which was within the spawning temperature range for the species. Mean annual number of spawning days ranged between 131 and 191 days and was somewhat higher than other studies on gilthead seabream, where spawning lasted for 100–150 days per reproductive

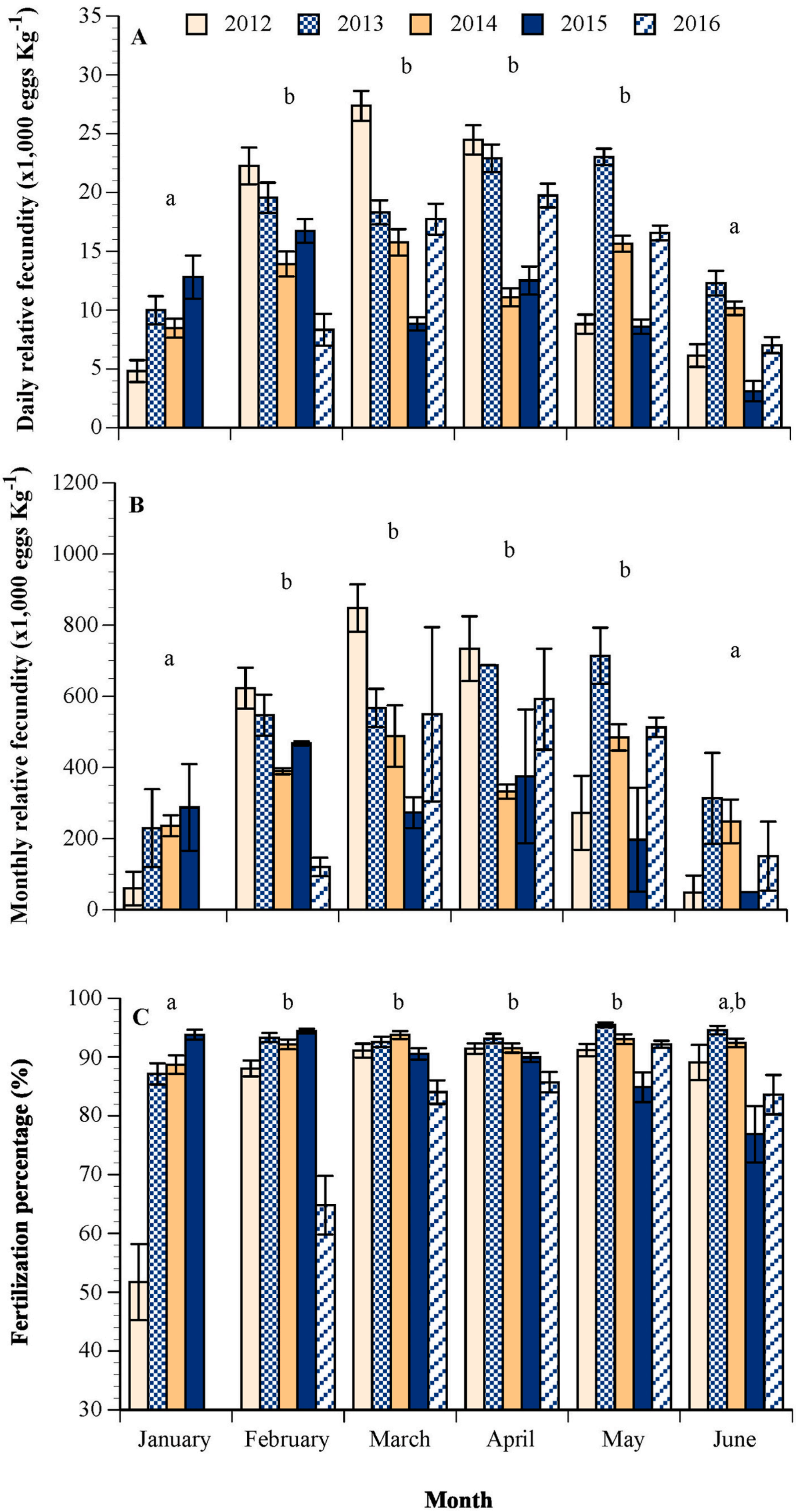

Fig. 4. A. Mean ± S.E.M. Daily relative fecundity (eggs/kg female wet weight) of gilthead seabream (*Sparus aurata*) broodstocks (n = 2) during 5 consecutive spawning seasons (2012–2016). **B.** Mean ± S.E.M. Monthly relative fecundity (eggs/kg female wet weight) of gilthead seabream (*Sparus aurata*) broodstocks (n = 2) during 5 consecutive spawning seasons (2012–2016). **C.** Mean ± S.E.M. fertilization success (%) of gilthead seabream eggs (n = 2) during 5 consecutive spawning seasons (2012–2016). Different Latin characters denote statistically significant differences between months (ANOVA, Tukey's HSD, $P < 0.05$).

season (Barbaro et al., 1997; Zohar et al., 1995), and markedly higher compared to other species of the Sparidae family. For example, the red porgy reproduces for 90–120 days per year (Aristizabal, Suárez, Vega, & Bargas, 2009; Mihelakakis, Yoshimatsu, & Tsolkas, 2001; Mylonas, Papadaki, Pavlidis, & Divanach, 2004), the blackhead seabream for 30 days (Gonzalez, Umino, & Nagasawa, 2008; Leu, 1994), the common dentex for 70–99 days (Abellan, 2001; Loir et al., 2001) and the white seabream for 140 days (Mann & Buxton, 1998; Mylonas et al., 2011).

Temperature and photoperiod effects on fish reproduction have been studied in different species (Bromage, Porter, & Randall, 2001; Munro, Scott, & Lam, 1990), including other members of the Sparidae family. For example, the spawning period of the blackhead seabream *Acanthopagrus schlegelii*, both in nature and in captivity lasts from April until early June when temperature ranges between 12 and 20 °C (Foscarini, 1988; Watanabe & Kiron, 1995), but in warmer waters (15–22 °C) the season starts earlier (Kato, Aoki, Nishimura, & Murai, 1985). In the red porgy, spawning lasts from March until May at temperatures between 15 and 19 °C in the Mediterranean (Kokokiris, Menn, Kentouri, Kagara, & Fostier, 2001), whereas in the white seabream *Diplodus sargus* it lasts between January and June at temperatures between 13 and 18 °C (Micale & Perdichizzi, 1994; Micale, Perdichizzi, & Santangelo, 1987; Morato, Afonso, Lourinho, Nash, & Santos, 2003; Mouine, Francour, Ktari, & Chakroun-Marzouk, 2007; Mylonas et al., 2011). The sharpsnout seabream spawns from September until December at 19–21 °C (Georgiou & Stephanou, 1995; Papadaki, Papadopoulou, Siggelaki, & Mylonas, 2008) and the common pandora from April until August at temperatures of 19–24 °C (Güner, Özden, Altunok, Koru, & Kizak, 2004; Mylonas et al., 2011; Pajuelo & Lorenzo, 1998; Somarakis & Machias, 2002; Valdéz et al., 2004). In the blackspot seabream *Pagellus bogaraveo* the spawning period under captivity is in March-May in Italy (Micale, Maricchiolo, & Genovese, 2002), and February-March in the north-west coast of Spain (Peleteiro, Olmedo, & Alvarez-Blázquez, 2000). Even though in aquaculture operations fish are maintained under a combined controlled photoperiod and temperature scheme, recent studies have shown that the reproductive function in gilthead seabream is more sensitive to photoperiod than to water temperature and fish can reproduce under constant temperatures for multiple years when exposed to the appropriate photoperiod (Mylonas et al., 2011). The two studied broodstocks of the present study were exposed all year-round to simulated natural photoperiod and constant well-water temperature (18–20 °C), which permitted (presumably) the long duration of the spawning period, for five consecutive years.

Relatively high fecundity values were observed in gilthead seabream in the present study, throughout the five spawning seasons, at least partly as a result of a more prolonged spawning season. Mean daily relative fecundity was 10 700–18 400 eggs/kg female WW, with total annual relative fecundity values reaching 1 480 000–3 100 000 eggs/kg female WW. These values are higher than the ones reported in other studies in Sparidae; however, direct comparisons with other studies are not always valid, due to differences in the genetic origin, fish rearing density, age and size, nutrition, water temperatures and other parameters than cannot be controlled or described. For example, in other studies with gilthead seabream, mean daily relative fecundity was reported to be 40 000 eggs/kg female WW (Fernández-Palacios et al., 1997; Mylonas et al., 2011), which is much higher than our results. On the other hand, other studies reported similar total annual relative fecundity of 2 000 000 eggs/kg female WW (Zohar et al., 1995). The red seabream *Pagrus major* under natural photoperiod spawned around 1 900 000 eggs during the spawning season (Matsuura, Furuichi, Maruyama, & Matsuyama, 1988). The red porgy can produce 20 000 eggs per day (Mihelakakis et al., 2001) and was reported to produce 440 000 eggs/kg female WW per reproductive season (Mylonas et al., 2004), a value that makes this species one of the less fertile of the Sparidae family. The sharpsnout seabream has a mean daily relative fecundity between 42 000 and 62 000 eggs/kg female WW and mean annual relative fecundity between 2 360 000 and 4 950 000 eggs/kg female WW (Papadaki et al., 2008), making it one of the most fecund sparid species. Regarding the apparent trend towards a reduction in fecundity during the last two reproductive seasons in the present study, this may be partly related to the removal of larger females, and the spawning of (a) the remaining smaller females and (b) the sex reversed males in the following year, which were even smaller than the existing females. However, this does not explain why during the first two reproductive seasons total relative fecundity was significantly higher, even as mean female body weights were significantly lower.

Variations in egg quality parameters (*i.e.* fertilization, embryo survival and hatching) among consecutive reproductive seasons are common in fish (Kjesbu, Witthames, Solemdal, & Walker, 1998) and genetic, physiological and environmental parameters have been shown to be involved. For example, growth (Kraus, Müller, Trella, & Köuster, 2000), temperature (Tveiten, Solevac, & Johnsen, 2001), feeding regime (Tyler & Sumpter, 1996) and broodstock sex ratio (Pavlidis, Greenwood, & Scott, 2004) have been shown to explain these variations. In the present study, mean annual fertilization success remained very high (87%–92%) over the years, with a significant -though still small numerically- reduction only in the last reproductive season. The values obtained in the present study agree with other studies of gilthead seabream and of other relative species. More specifically, mean annual fertilization success was reported to be 80%–85% in the gilthead seabream (Barbaro et al., 1997; Mylonas et al., 2011; Zohar et al., 1995), 37% and 69% in two successive reproductive seasons in the red porgy (Mylonas et al., 2004), between 72% and 84% in the yellowfin seabream *Acanthopagrus latus* (Leu, 1997), and 97% in the common pandora (Güner et al., 2004). In the present study, in 2015 older females were removed and younger males were added in the broodstocks, and at the same time older males changed sex to females. This could explain -at least in part- the fact that the fertilization success decreased significantly the following year, as sex change of older males could have resulted in reduced sperm quantity or quality exhibited by the remaining younger males in the population (Brown, 2003).

The decreasing trend in total annual fecundity and fertilization success that was observed in the last spawning seasons, may be interpreted -perhaps- as a negative effect of the longer-than-natural reproductive seasons that were stimulated by the constant water temperatures, as the females may have become progressively “exhausted”. This may not be conclusively determined by the present experimental design, since control broodstocks exposed to annual thermal fluctuations were not included. However, studies with red porgy (Mylonas et al., 2004) and sharpsnout seabream (Papadaki et al., 2008), also demonstrated that the highest fecundity and fertilization success were obtained in the first two spawning seasons. It has been argued that egg production decreases with female age (Kjørsvik, 1994), though the exact relation is still unclear (Zastrow, Houde, & Saunders, 1989); nevertheless it has been reported that there are differences in egg production between young first-time spawners and older aged females in the Atlantic halibut *Hippoglossus hippoglossus* (Evans, Parish, Brown, & Davis, 1996), the Atlantic wolffish *Anarhichas lupus* (Tveiten et al., 2001), and the sharpsnout seabream (Papadaki et al., 2008). The time when “aging” can be blamed for reductions in egg production (quantity and quality) is not known in gilthead seabream, but it is unlikely that it happens already when the fish are only 5 years old. Regardless of the reason for the reduced fecundity and fertilization, maintaining gilthead seabream broodstocks under constant water temperature has significant advantages for commercial hatcheries. Firstly, most large gilthead seabream hatcheries are operating selective breeding programmes (Janssen, Chavanne, Berentsen, & Komen, 2016), and replacement of breeding stocks for production is expected to take place after four to five spawning seasons while the annual fecundity and fertilization success is still quite high, based on the results of the present study. So, even if maintaining gilthead seabream breeders under constant water temperature throughout their life results in a progressive reduction in productivity, this is expected to have a minimal effect on hatchery operations, if the breeders are changed every 4–5 years. Secondly, using well water without controlling the temperature reduces the energy costs of the hatchery for heating the water in the summer and/or cooling it in the winter. Thirdly, a longer spawning season means that a hatchery can produce eggs on a year-round basis with a smaller number of photoperiod-shifted broodstocks, in the present case with only two stocks spawning for 5–6 months each, as opposed to three-four stocks spawning for 4 months each, which is the typical arrangement in gilthead seabream hatcheries (personal communication). Finally, maintaining fewer stocks, means a smaller total breeding biomass, thus additional savings for facilities, water, energy, feed and person hours.

In conclusion, the male percentage in gilthead seabream was reduced during the first two reproductive seasons and remained stable thereafter at ~20%. An attempt to increase male percentage to 50% by removing the large females and adding younger males was followed by sex reversal in the very next spawning period of the larger males and a re-establishment of a ~20% male percentage. Egg production and quality was high for five consecutive years and no variations were observed in the duration of the spawning period following the spontaneous and manipulated shifts in the sex ratio of the broodstocks. A trend towards a small, though statistically significant, reduction in total relative fecundity and fertilization success during the last years of the study could be related to (a) a progressive female "exhaustion" due to the longer-than-natural reproductive periods stimulated by the constant water temperatures, (b) an aging effect on the broodstock, (c) the removal of the large females or (d) a combination of all three previous parameters. According to the results of the present study, year-round egg production of gilthead seabream can be achieved by maintaining two broodstocks with a 6-month difference in annual photoperiods and constant water temperature (18–20 °C). High fecundity and fertilization success may be expected without any modification of the spontaneously established and expectedly low male percentage. This protocol offers significant economic advantages to commercial hatcheries, due to savings in heating and/or cooling costs, and the maintenance of fewer stocks and a smaller total breeding biomass.

CRediT author statement

CC Mylonas designed the experiment. The fish husbandry was carried out by ES and samplings were performed by ES, IF, MP and DK. Histological evaluations were carried out by DK and MP. Data analysis was performed by DK and MP. The manuscript was written by MP, DK and CCM. All authors read and approved the final manuscript.

Declaration of competing interest

The authors declare no conflict of interest.

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