Egg and sperm production and quality of sharpsnout sea bream (*Diplodus puntazzo*) in captivity

Maria Papadaki[1], Mihaela Papadopoulou[1,2], Irini Siggelaki[1] and Constantinos C. Mylonas[1,*]

[1]Institute of Aquaculture, Hellenic Center for Marine Research, P.O. Box 2214, Iraklion 71003, Crete, Greece

[2]University of Crete, Department of Biology, P.O. Box 2208, Iraklion 71409, Crete, Greece

*Corresponding author: tel +30 2810 337878, fax +30 2810 337875, mylonas@her.hcmr.gr

Abstract

Egg production from captive-reared sharpsnout sea bream (*Diplodus puntazzo*) was monitored during the spawning seasons of 2003 to 2005, and sperm production during 2003. Spawning took place between September and December under water temperatures of 21.0 - 18.5°C. Daily fecundity varied without a consistent trend and peak egg production occurred in October. Mean (± S.E.M.) number of spawning days per month was 20 ± 3 in 2003, and 14 ± 3 in 2004 and 2005. A significant (ANOVA, DNMR, $P < 0.01$) drop was observed in mean total annual relative fecundity from 4.9 ± 0.08 million eggs Kg^{-1} female body weight in 2003, to 2.4 ± 0.07 million eggs Kg^{-1} in 2004 and 2005. Mean monthly fertilization success also dropped significantly from 81 ± 1% in 2003 to 76 ± 2% in 2004 and 78 ± 2% in 2005. Annual hatching success did not vary significantly and was around 86 ± 2%. Finally, 5d larval survival decreased significantly from 85 ± 2% in 2003 to 44 ± 3% in 2004. The first spermiating males were found in August and sperm production continued until December. Mean total volume of expressible sperm was maximal in November (3 ± 1 ml Kg^{-1}) and the gonadosomatic index (GSI) ranged between 0.6 and 2.0% in spermiating fish, reaching its peak in September - November. Sperm motility (%) remained unchanged during the season, whereas motility duration (6.2 ± 1.7 min) and sperm density (2.7 ± 0.2 x 10^{10} spermatozoa ml^{-1}) peaked in October. Mean sperm survival ranged between 9 and 13 d during most of the spawning season, and decreased significantly to 5 d in December. The study suggests that egg production is stable for the first 3 months of the spawning season, with relatively unchanged egg quality. On the contrary, sperm production and quality peaks in the middle of the reproductive season in October.

Keywords: *Diplodus*, sharpsnout sea bream, egg quality, spermiation, reproduction

1. **Introduction**

The sharpsnout sea bream (*Diplodus puntazzo*, Cetti 1777) is a demersal marine fish widely distributed in the Black and Mediterranean Seas, and the Atlantic Ocean. It has been reared in aquaculture for more than 10 years (Abellan and Basurco, 1999; Divanach and Kentouri, 2000) and has good consumer acceptance (Hernández et al., 2002). Studies on larval rearing, development and morphogenesis (Bodington, 2000; Palma and Andrade, 2002; Papandroulakis et al., 2004); growth and pathology (Favarolo and Mazzola, 2000; Pastor et al., 2000; Hernández et al., 2003; Tramati et al., 2005); and egg quality criteria (Lahnsteiner and Patarnello, 2004; Lahnsteiner and Patarnello, 2005) have provided important information on the performance of this species in captivity. Nevertheless, there still exist some problems concerning its nutritional demands and optimal environmental rearing conditions (Abellan and Basurco, 1999). In addition, there is still limited information on its reproductive performance, egg and sperm production during the reproductive season, and gamete quality in captivity (Faranda et al., 1985; Georgiou and Stephanou, 1995).

The quality of eggs and larvae produced in hatcheries are considered an important limiting factor in fry production (Kjørsvik et al., 1990) and, consequently, in the development of the aquaculture industry (Bromage, 1995). Sperm quality is equally important, as it can affect fertilization success and production of viable eggs (Bromage, 1995). Egg quality has been defined as the potential of eggs to produce viable fry (Kjørsvik et al., 1990; Brooks et al., 1997) and depends on various factors which may change frequently during spawning, such as the endocrine status of females during oogenesis, physico–chemical water parameters, broodstock management, etc. (Bromage, 1995; Brooks et al., 1997). Changes may also occur in sperm quality characteristics during the

reproductive period (Billard, 1986; Fauvel et al., 1999; Shangguan and Crim, 1999) and stress imposed on fish under captivity can have negative results on reproductive function and gamete quality (Schreck et al., 2001). Therefore, it is of utmost importance for each new candidate species for aquaculture to examine the existence of seasonal changes in egg and sperm quality (Mylonas et al., 2003a; Mylonas et al., 2003b; Mylonas et al., 2004). Such information will assist commercial facilities in organizing and, if possible, optimizing egg and fry production.

The sharpsnout sea bream is a protogynous hermaphrodite fish and its gonads contain both ovarian and testicular tissue (Micale et al., 1996). Puberty is reached at 2 years of age (Georgiou and Stephanou, 1995), the spawning period begins in October–November, and the fish appear to have a very narrow temperature optimum for spawning at 21 ± 0.5°C (Marangos, 1995; Micale et al., 1996). The aim of the present study was to acquire further knowledge on the spawning kinetics and performance of sharpsnout sea bream in captivity, by studying egg and sperm production and quality characteristics, and their variation during the spawning period in captivity. For this purpose, egg production and quality was evaluated for three consecutive spawning seasons (2003-2005), and spermiation and sperm quality for one spawning season (2003).

2. Materials and methods

2.1 Broodstock management

Two groups of experimental fish of wild origin (males >3 years old, females > 4 years old) were maintained in 5-m^3 rectangular tanks placed side by side and exposed to the same simulated photothermal regime between 2003 and 2005 (Figure 1). The simulated

photoperiod was employed in order to exaggerate an identifiable sign of the arrival of winter, by providing a more rapid reduction in daylight duration after the summer solstice. Based on previous experience, this modified photoperiod-temperature regime resulted in the production of eggs of good quality, one-month earlier than the normal season. Temperature was reduced gradually after mid July and was kept stable at 21± 0.5°C by chilling the incoming water, but after November the water temperature was ambient as no heating was possible (Figure 1). Fish were fed daily at satiation with industrial food during the non-reproductive season (A4 ICHTHYS, Perseus A.E., Greece), and 2 months before and during the reproductive season they were fed with a combination of an industrial maturation feed (Vitalis Repro, Hendrix, Italy) every day and frozen squid every other day. Each tank was fitted with an overflow egg collector and spontaneously spawned eggs were collected daily during three consecutive spawning seasons (2003, 2004 and 2005). In 2003, the first group of fish (tank 1) consisted of 3 females and 9 males of mean weight ± SD of 1029 ± 185 g and 850 ± 114 g respectively, and the second group (tank 2) contained 5 females and 5 males weighing 1021 ± 55 g and 998 ± 107 g, respectively. In 2004, after some mortality and sex inversion, tank 1 contained 4 females and 8 males of mean weight of 1102.5 ± 225 g and 1099 ± 165 g and tank 2 contained 5 females and 4 males of mean weight of 1182 ± 90 g and 1125 ± 143 g, respectively. In 2005, after some mortality, tank 1 contained 4 females and 7 males of mean weight of 1215 ± 165 g and 1116 ± 157 g, respectively, and tank 2 contained 4 females and 3 males of mean weight of 1260 ± 129 g and 1275 ± 144 g, respectively. Although sex ratio has been reported to influence spawning quality in other fishes (Pavlidis et al., 2004), we chose not to interfere with sex ratio during the three years of this study. This was done because earlier experiments indicated that sex inversion may take place

throughout the year in sharpsnout sea bream, including the spawning period (unpublished data). Therefore, it could not be assured that addition of males or females at the beginning of the spawning season would maintain the desired sex ratio. More importantly, such addition of fish could disrupt the social structure of the broodstock, thus further affecting spawning behaviour and egg production and quality. In addition it was intended to allow fish to spawn without such interference with broodstock structure, in order to obtain also some data on the natural evolution of a broodstock's sex ratio, due to sex inversion and mortality.

For the study of sperm quality, individually tagged (PIT tag, AVID, UK) 2-year-old fish (n = 64, mean weight of 299 ± 5 g) of unknown sex, produced from a wild stock in 2001 were removed from a mix-sexed population and placed in two 2-m^3 circular tanks, just before the spawning period of 2003 (July 2003). The tanks were supplied with surface seawater chilled at 21 ± 0.05°C until November, which was then allowed to decrease according to ambient temperature thereafter (Fig. 1). Fish were fed daily with industrial feed (Trouvit 3rd Period and A4 ICHTHYS, Perseus A.E., Greece), and were starved two days before handling, in order to prevent faecal contamination during sperm collection. Handling of the fish was carried out according to the "European Union Directive for the protection of animals used for experimental and other scientific purposes" (86/609EEC), and the "Guidelines for the treatment of animals in behavioral research and teaching" (Anonymous, 1998).

2.2 Evaluation of egg quality

Eggs were obtained from the egg collectors and were initially placed in a 10-l bucket in order to estimate fecundity and fertilization percentage after sub-sampling of 10 ml. In

order to monitor embryo and larval survival, eggs from 2 - 3 random spawns every week were individually placed in 96-well microtiter plates (in replicates) according to the procedure of Panini et al. (2001), with some modifications. Briefly, floating (>90 % fertilized) eggs from the 10-l bucket were taken in a 250 μm mesh filter and were rinsed with sterilized seawater and poured in a 2-l beaker. A Petri dish was used to scoop 100-200 eggs from the beaker and only fertilized eggs were taken one by one with a micropipette set to 200 μl and transferred to the wells of the microtiter plates (one egg per well). The microtiter plates were then covered with a plastic lid, placed in a controlled-temperature incubator and maintained for 5 d at 20 ± 0.5°C. Using a stereoscope, embryonic and early larval development was evaluated once a day, recording the number of 1-d embryos, hatched larvae and viable larvae on day 5 after egg collection.

For analysis, various egg quality parameters were calculated. Fertilization success was evaluated daily as soon as the eggs were obtained from the egg collectors and was calculated as the number of fertilized eggs / total number of eggs spawned. Embryo survival was evaluated the day after egg collection (1 d), and was calculated as the number of eggs having live embryos / number of fertilized eggs initially loaded in the microtiter plates. Hatching success was calculated as the number of hatched larvae / the number of live 1-d embryos, and 5d larval survival was calculated as the number of live larvae 5d after egg collection / the number of hatched larvae. Estimating percentage survival (%) by using in the denominator the number of individuals that survived to the previous developmental stage is considered as a more independent evaluation of survival within specific developmental stages, without the potential of a masking effect of the previous stage (Mylonas et al., 1992; Mylonas et al., 2004).

2.3. Evaluation of sperm quality

For each sampling (approximately every 2 weeks), 10 fish were randomly obtained from one of the two tanks (in sequence) and all males (n = 3-8) were sampled for sperm. The data collected in the different samplings were grouped according to month for statistical analysis and presentation. At three times during the spermiation period (August, October and November), the total volume of expressible sperm was collected in order to monitor sperm production. For sperm collection, fish were initially tranquilized in their tank (0.01 mL L^{-1} clove oil) and then transferred to an anaesthetic bath for complete sedation (0.04 ml l^{-1} clove oil) (Mylonas et al., 2005). They were then washed with clean seawater in order to avoid any kind of contact between the anesthetic and collected sperm. The genital pore was carefully blot dried and gentle abdominal pressure was applied to force the sperm out of the testes, avoiding contamination of the samples with faeces or urine. Small volumes of sperm were collected (50 - 100 μl) in order to avoid influencing the quality of the sperm during subsequent collections. The collected sperm was stored on ice until transferred to a 4°C refrigerator. In one sampling per month, gonads were excised for the calculation of the gonadosomatic index (GSI; [gonad weight / total body weight] x 100) and further histological analysis. On the sampling dates that total expressible sperm was also collected, GSI was calculated after adding the total sperm volume collected to the weight of the gonad. For histological processing, testicular pieces were fixed in 4% formaldehyde: 1% glutaraldehyde (McDowell and Trump, 1976), dehydrated in a 70–95% ethanol series and embedded in glycol methacrylate resin (Technovit 7100, Heraeus Kulzer, Germany). Serial sections were obtained at a thickness of 3–5 μm on a microtome (Biocut 2035, Reichert

Jung, Germany) using disposable blades. After drying, slides were stained with methylene blue/azure II/basic fuchsin (Bennett et al., 1976) and examined under a light microscope (Olympus CH-2, Germany).

Spermiation index was evaluated during each sampling, measured on a subjective scale from 0 to 3 (0 = no sperm released, 1 = only a drop of sperm was released after multiple stripping attempts, 2 = sperm was easily released after the first stripping attempt, 3 = copious amounts of sperm flowing with the slightest abdominal pressure) (Mylonas et al., 2003a). Sperm quality parameters that were evaluated included total volume of expressible sperm (ml), sperm density (number of spermatozoa ml^{-1} of sperm), percentage of spermatozoa showing forward motility (sperm motility, %) and duration of forward sperm motility of at least 10% of the spermatozoa in the field of view (motility duration, min). Total expressible sperm volume was determined by using the graduations marked on the collecting tube. Sperm density was estimated after a 5271-fold dilution with seawater using a Neubauer haematocytometer under 200x magnification (in duplicate). Sperm motility and motility duration were evaluated on a microscope slide (x 400 magnification) after mixing 1 μl of sperm with a drop of about 50 µl of saltwater (in duplicate). After evaluations on the day sperm was collected, it was stored at 4°C without the addition of any extenders. Sperm motility was evaluated 1 d after sperm collection and every 2 d thereafter, until cessation of any forward motility of the spermatozoa, thus allowing for the evaluation of the duration of sperm survival (days) at 4°C.

206 ***2.4 Statistical analysis***

Differences in spawning and egg quality parameters between reproductive seasons
208 and between months were analyzed by two-way analysis of variance (ANOVA, year x month), followed by Duncan's New Multiple Range test (DNMR) at a minimum significance
210 of $P < 0.05$. Differences in sperm quality parameters during the reproductive period were analyzed by one-way ANOVA, followed by DNMR ($P < 0.05$). Data of individual fish on
212 spermiation condition were analyzed by a non-parametric statistics test (Kruskal-Wallis). Percentage data were Arcsine transformed prior to statistical analyses to normalize variances.
214 Unless otherwise mentioned, results are presented as means ± S.E.M. Statistical analyses were performed with linear statistics software (SuperAnova, Abacus Concepts, Inc., USA).
216

3. Results

218 **3.1 Spawning and egg quality**

The spawning season of sharpsnout sea bream begun the last week of August or the
220 first week of September, and ended in the second or third week of December during the three years of monitoring (Fig. 2). Although the spawning period was slightly longer in 2005,
222 spawning after November was scarcer than the two previous spawning seasons. There was a large variation in daily relative fecundity without a consistent trend during the three
224 spawning seasons, except from a slight and gradual increase in September. Fertilization success was high during all the three spawning seasons with some occasional variations (Fig.
226 2).

Mean number of spawning days per month were similar for the first three months (Fig.
228 3A), with a slight increase in October in all three spawning seasons, followed by a significant

drop in December (2-way ANOVA, DNMR, $P < 0.01$). Mean daily relative fecundity varied among months, exhibiting its highest values in October in all three years (2-way ANOVA, DNMR, $P < 0.001$), while significant differences were also observed between years (Fig. 3B). Mean fertilization success exhibited different trends among the three years (2-way ANOVA, $P < 0.001$), varying very little during the season in 2003, having a dramatic reduction in December 2004, while the lowest values in 2005 were observed in November (Fig. 3C). Mean hatching percentage also exhibited different trends between the two years examined (2-way ANOVA, $P < 0.001$), reaching its highest values in the middle of the 2003 spawning season, whereas in 2004 the highest values were observed in September and December (Fig. 3D). Finally, mean 5d larval survival also exhibited significant differences between spawning seasons (2-way ANOVA, $P < 0.001$), with lowest values in September in 2003, whereas in 2004 lowest values were observed in December (Fig. 3E).

In considering the data throughout the three-year study (Table 1), mean daily relative fecundity differed significantly (ANOVA, DNMR, $P < 0.001$) among the three spawning seasons, with 2003 having the highest fecundity (62,000 ± 3,000 eggs Kg^{-1} female $spawn^{-1}$), compared to 42,000 ± 2,000 eggs Kg^{-1} in 2004 and 44,000 ± 2,000 eggs Kg^{-1} in 2005. The number of spawning days was not different between the three years and ranged between 14 and 20 days $month^{-1}$. As with daily relative fecundity, mean total annual fecundity was significantly higher for 2003 compared to 2004 or 2005 (ANOVA, DNMR, $P < 0.05$). Mean fertilization success was significantly higher in 2003 (ANOVA, DNMR, $P < 0.05$), compared to 2004 and 2005 that had similar values. Finally, hatching success was not different among the two years of monitoring, whereas 5d larval survival dropped significantly in 2004 (ANOVA, DNMR, $P < 0.001$).

252

3.2 Spermiation and sperm quality

254 At the first sampling (July 2003, data not shown) no sperm was produced from any of the fish. The first two spermiating males were observed in August. The spermiation index 256 increased significantly (ANOVA, DNMR, $P < 0.01$) in September and decreased in December, when sperm could be collected from only three of five males (Fig. 4A). Mean 258 sperm motility percentage was high (>80%) and remained unchanged throughout the spawning season (Fig. 4B). Sperm density increased after August reaching mean values of 260 $>2 \times 10^{10}$ spermatozoa mL^{-1} until October (Fig. 4C), when it reached 27.1×10^{9} spermatozoa ml^{-1}, and decreased gradually thereafter (ANOVA, DNMR, $P < 0.05$) reaching values of 262 about 1.2×10^{10} spermatozoa mL^{-1} (Fig. 4C). Here it should be noted that the lack of data for September was due to an error in the preparation of sperm, where distilled water was used 264 instead of physiological saline, causing the rupture of spermatozoa. The duration of forward motility exhibited a significant increase (ANOVA, DNMR, $P < 0.05$) towards the middle of 266 the spermiation period in October, with a significant reduction thereafter (Fig. 4D). On the other hand, mean sperm survival at $4^{\circ}C$ was similar between August and November, ranging 268 between about 8 and 12 days, and decreased dramatically and significantly (ANOVA, DNMR, $P < 0.01$) in December (Fig. 4E). The amount of sperm collected in December was 270 very small, thus not allowing the evaluation of motility duration.

Total expressible sperm volume increased significantly (ANOVA, DNMR, $P < 0.05$) in 272 October and remained unchanged in November, reaching a mean value of 3 ± 1 ml Kg^{-1} (Fig. 5). Similarly, GSI % varied significantly between 0.1 – 2.0% during the spawning season, 274 reaching its highest values in September-October and decreasing thereafter (Fig. 5).

Late meiotic stage testes were observed in fish in August, where all types of germ cells were observed, including some spermatozoa (Fig. 6A). In September the lobule lumen was filled with spermatozoa and the occurrence of other types of germ cells was declining (Fig. 6B). In October, fish were in the functional maturation stage when spermatozoa occupied almost the whole testis and only a few other germ cells were observed (Fig. 6C). In November, the testes still contained a large amount of spermatozoa occupying the testes, but somatic cell hypertrophy also begun, pointing towards the end of the spermiation period (Fig. 6D). Finally, in December testes were at the spent stage, when almost all spermatozoa had disappeared from the testes and spermatogonial proliferation was prevalent (Fig. 6E).

4. Discussion

In the present study, spawning lasted from September to mid December in all three years examined. In another location at the same geographical latitude (Cyprus), sharpsnout sea bream spawned from the end of October to the end of December (Georgiou and Stephanou, 1995) or from mid October to mid November (Marangos, 1995). In Italy, spawning lasted between the end of October and mid December (Lahnsteiner and Patarnello, 2004; Lahnsteiner and Patarnello, 2005). The one month earlier starting of the spawning season in the present study was attributed to the exposure of fish to a faster declining photoperiod and to the appropriate spawning temperature of 21°C already in September. The importance of temperature and photoperiod in fish reproduction has been amply demonstrated in many fishes (Munro et al., 1990; Bromage et al., 2001), including other members of the same family (Sparidae), such as the gilthead sea bream (*Sparus aurata*)(Kadmon et al., 1985) and the common dentex (*Dentex dentex*) (Pavlidis et al., 2001).

298 Especially in regards to the spawning season, earlier studies have shown that the sharpsnout sea bream is extremely sensitive to temperature (Georgiou and Stephanou, 1995; Marangos,
300 1995). According to these studies, spawning occurs between 19 and 21°C, with 21°C being the optimal, whereas significant deviations from this temperature cause non-reversible
302 ceasing of spawning. In the present study, spawning proceeded on an almost daily basis as long as water temperature was maintained at 21°C. When temperature decreased towards the
304 end of November, spawning also started to decrease. Other studies reported that the most favorable temperature for fertilization and hatching was 22°C (Faranda et al., 1985), whereas
306 embryonic development has been found to be optimal at 24°C (Firat et al., 2005). Our results support the range of 21-19°C as the optimal for the spawning of sharpsnout sea bream.
308 Mean daily relative fecundity in the present study was 42,000-62,000 eggs kg^{-1} body weight, giving annual relative fecundity values of 2,36 - 4,95 million eggs kg^{-1} body weight.
310 These values are much higher than those reporter in other Sparidae broodstocks, though direct comparisons with other studies are not always feasible or appropriate, given
312 differences in stocking densities, nutrition, age, water temperature, etc. For example, relative fecundity $spawn^{-1}$ of gilthead sea bream was reported to be around 40,000 eggs {Fernández-
314 Palacios, 1995 #3708} and total annual production has been estimated to be >2 million eggs kg^{-1} (Zohar et al., 1995). In common dentex, hormonal therapy induced spawning of 97,000
316 eggs kg^{-1} (Glamuzina et al., 1989), while during spontaneous spawning in captivity, annual relative fecundity was reported to be 600,000-700,000 eggs kg^{-1} in 50 spawnings, or about
318 12,000-14,000 eggs kg^{-1} $spawn^{-1}$ (Loir et al., 2001). In our facilities, daily relative fecundity of red porgy ranged between 1,000 - 30,000 eggs kg^{-1} in two consecutive spawning seasons
320 (Mylonas et al., 2004).

Variations in egg quality parameters between spawning seasons are common (Kjesbu et al., 1998) and have been attributed to both genetic and environmental factors, such as growth (Kraus et al., 2000), temperature (Tveiten et al., 2001), as well as the feeding level of females (Tyler and Sumpter, 1996) and the sex ratio of the broodstock (Pavlidis et al., 2004). In the present study, both total spawning days month^{-1}and relative fecundity decreased in 2004 and 2005, compared to 2003. However, the number of spawning days of sharpsnout sea bream in the present study falls within the commonly observed values for different sparids in captivity. For example, mean spawning days were 63 for common dentex (Pavlidis et al., 2001) and 96, 65 and 43 for silver bream (*Rhabdosargus sarba*) (Leu, 1994; Mihelakakis and Kitajima, 1995), whereas in our facilities, mean total spawning days season^{-1} are 75 for the white sea bream (*Diplodus sargus*), 62 for the red porgy (*Pagrus pagrus*), 107 for the gilthead sea bream and 59 for the common pandora (*Pagellus erythrinus*) (unpublished data). Variation in mean relative fecundity values in our broodstock has been also shown for the red porgy (Mylonas et al., 2004). An explanation for the decrease of fecundity values of sharpsnout sea bream in 2004 and 2005 could be the general assumption that egg quality diminishes with fish age (Kjørsvik, 1994). In the existing literature, the exact relationship between female age, and egg size and quality remains poorly understood (Zastrow et al., 1989), although differences in egg quality parameters between first–time and repeat spawners in Atlantic halibut (*Hippoglossus hipolossus*) and in common wolfish (*Anarhichas lupus*) (Evans et al., 1996; Tveiten et al., 2001) have been attributed to the age of the fish (Evans et al., 1996). In the present study, it is possible that an aging effect resulted in a decrease in egg quality after females exceeded 4 years of age.

Fertilization percentage, on the contrary, did not vary between years and was very high. In earlier studies of the reproduction of sharpsnout sea bream, mean fertilization percentage was found to be 25 – 65% (Faranda et al., 1985) and 42% (Abellan and Basurco, 1999), whereas the fertilization percentage of red porgy reared in captivity for two consecutive spawning seasons was found to be 37 (±2)% and 69 (±3)% (Mylonas et al., 2004). Mean (±S.E.M.) fertilization percentages for other sparids in our facilities range from 69 (±3)% for red porgy (Mylonas et al., 2004), to 71 (±5)% for white sea bream, 82 (±3)% for gilthead sea bream and 88 (±3)% for common pandora (unpublished data). The values obtained for sharpsnout sea bream in the present study are within the same range for these species. As far as other egg quality parameters are concerned, both hatching and 5d survival percentages did not exhibit a uniform trend during the spawning seasons. As it has been reported for Baltic herring (*Clupea harengus membras*), differences in egg mortality and total hatching success between years and between batches from different females are not uncommon and can be explained by differences in female size, condition or fat content (Laine and Rajasilta, 1999). Annual variation of these egg quality parameters can also be affected by the male (Laine and Rajasilta, 1999), as sperm quality can also vary annually. Overall, sharpsnout sea bream exhibits high fertilization percentages in captivity, but further studies are needed in order to examine the effect of various environmental and physiological parameters on egg quality variation.

It is always of interest for commercial hatchery managers, to have predictive indices for evaluating the quality of a batch of eggs, in terms of its potential for producing larvae of good quality and high survival. With this in mind, the ratio of the maximal and minimal diameter of the lipid vesicle was correlated with larval survival in sharpsnout se bream

366 (Lahnsteiner and Patarnello, 2005). In another study in the same fish, embryo survival was significantly correlated with larval survival (Lahnsteiner and Patarnello, 2004). Similarly,
368 significant correlations were found between 1-d embryo survival and fertilization percentage, and between hatching percentage and 1-d embryo survival in red porgy (Mylonas et al.,
370 2004), and between hatching and 1-d embryo survival and between larval survival and hatching in European sea bass (*Dicentrarchus labrax*)(Mylonas et al., 2003b). No such
372 correlations were found in the present study among fecundity, fertilization and hatching success or larval survival (data not shown), suggesting that these parameters can not be used
374 as early indicators of larval survival for the sharpsnout sea bream.

The spermiation period for the sharpsnout sea bream begun at the end of August, a few
376 days before the onset of the spawning period of the females, with the spermiation index reaching high values between September and November. This was confirmed histologically,
378 since in August testes contained all types of germ cells but full spermiogenesis was achieved in September when spermatozoa were the most abundant type of germ cell present. These
380 histological observations are in accordance with an earlier study of 2-year old fish (Micale et al., 1996). In another study, it was observed sperm release could be obtained even some
382 months after the female spawning season (Faranda et al., 1985). In accordance to the spermiation and the histological data, the GSI of sharpsnout sea bream was highest in
384 September - October, in agreement with earlier studies (Micale et al., 1996; Hernández et al., 2003). The GSI values in the present study (0.06–1.86%) were similar to the 1.14% found in
386 another mixed-sex population (Hernández et al., 2003), but lower than the 4.47% reported from an all-male population (Micale et al., 1996). On the other hand, total expressible sperm
388 volume reached its highest value of 3 ml Kg^{-1} in November, one month later than the peak of

the GSI and sperm index. Similar values of sperm volume have been observed in turbot (*Scophthalmus maximus*) (Suquet et al., 1994) and black sea bass (*Centropristis striata*) (DeGraaf et al., 2004), with higher values observed in salmonids, carp (*Cyprinus carpio*) and Atlantic halibut (see review by Suquet et al., 1994).

Sperm quality evaluations are very important in fish reproduction studies, as percentage and duration of forward motility can differ among species (Billard, 1986) and during the year (Mylonas et al., 2003a), influencing fertilization success and seed production (Chauvaud et al., 1995). Sperm motility percentage of sharpsnout sea bream was high and unchanged during the spawning season, as it has been shown for haddock (*Melanogrammus aeglefinus*) (Rideout et al., 2004), but in contrast to the European sea bass (Billard et al., 1977a) and the black sea bass (DeGraaf et al., 2004), where motility percentage was shown to decrease as the spawning season progressed. Sperm density, on the other hand, decreased after October, in accordance to what has been found in other species, such as the rainbow trout (*Oncorhynchus mykiss*) (Büyükhatipoglu and Holtz, 1984), the Atlantic salmon (*Salmo salar*) (Aas et al., 1991) and the European sea bass (Fauvel et al., 1999), but contrary to what has been observed for captive haddock (Rideout et al., 2004), cod (*Gadus morhua*) (Rakitin et al., 1999) and turbot (Suquet et al., 1998). The lowest value of sperm density in the present study was observed in December, when female spawning ceases. The range of sperm density values observed during the season was similar to values reported for other fishes, for example, 5 – 10 x 10^9 spermatozoa ml^{-1} in the whitefish (*Coregonus clupeaformis*), 5 – 18 x 10^9 spermatozoa ml^{-1} in the rainbow trout (*Oncorhynchus mykiss*), 8 - 23 x 10^9 spermatozoa ml^{-1} in the red porgy (Mylonas et al., 2003a), 5 – 55 x 10^9 spermatozoa ml^{-1} in the European sea bass (Sorbera et al., 1996; Fauvel et al., 1999; Rainis et al., 2003), 32 - 44 x 10^9

412 spermatozoa ml^{-1} in the turbot (Suquet et al., 1992), and 38 – 45 x 10^9 spermatozoa ml^{-1} in the yellow perch (*Perca flavescens*) (Ciereszko and Dabrowski, 1993). The above results
414 suggest that the number of motile spermatozoa may increase towards the middle of the spawning season in the sharpsnout sea bream, making this the most appropriate time for
416 collection of sperm for cryopreservation and artificial insemination procedures (Suquet et al., 2000).

418 In a study on sharpsnout sea bream spermatozoa (Taddei et al., 2001), it was observed that sperm continues to move for more than 300 sec. Motility duration in the present study
420 lasted from 1.9 to 6.2 min. High spermatozoa motility durations have also been reported for the red porgy (Mylonas et al., 2003a) and turbot (Suquet et al., 1994), where spermatozoa
422 movement lasts from 1 – 17 min; and the common bogue (*Boops boops*) and white sea bream, where about 10% of the spermatozoa remain motile for 2 – 3 hours after activation
424 (Lahnsteiner et al., 1998). In a study on Atlantic herring (*Clupea harengus*) (Geffen, 1999), motile spermatozoa could be observed in one sample 45 min after activation. According to
426 the same study, individual spermatozoa can be observed moving for only about 5 min, suggesting that the observed long motility durations in sperm samples may be attributed to
428 different individual spermatozoa activation times. Although such a phenomenon may explain the extreme duration of forward motility of spermatozoa observed in some samples,
430 we believe that the long duration of spermatozoa motility observed in the present study is not due to such an artifact. A longer duration of spermatozoa motility in this species may be an
432 adaptation to the extremely high fecundity of the females, which is one of the highest reported in a cultured member of the family Sparidae in the Mediterranean.

434 The duration of spermatozoa motility in the sharpsnout sea bream decreased at the end of the spermiation period, in accordance to studies with the European sea bass (Billard et al., 436 1977a; Sorbera et al., 1996), Atlantic halibut (Methven and Crim, 1991) and turbot (Suquet et al., 1998), but in contrast to the rainbow trout (Liley et al., 2002). A decrease at the end of 438 the spermiation period was also observed for sperm survival at 4^{o}C. Alterations of the spermatozoa plasma membrane at the end of the female spawning season could explain the 440 decrease in the storage capacity of spermatozoa, as shown for turbot (Suquet et al., 1998) and the European sea bass (Billard et al., 1977b), explaining part of the ageing phenomenon of 442 fish spermatozoa (Suquet et al., 1998). Still, the study demonstrates that sperm collected from sharpsnout sea bream can be maintained viable without the need for special storage 444 conditions for many days. This can simplify artificial insemination procedures, as sperm can be collected before the induction of ovulation of the females, and can be made readily 446 available for a long period of time, allowing insemination of the eggs obtained from females ovulating at slightly different times after induction.

448 In conclusion, it appears that good quality eggs can be produced throughout the spawning season of captive-reared sharpsnout sea bream, while the reported fecundity is 450 among the highest observed in Mediterranean cultured sparid fishes. On the other hand, although sperm quality values are comparable to the ones obtained in other cultured marine 452 fishes, there seems to be a deterioration of sperm quality as the spermiation period progresses. Therefore, the best time for the collection of sperm for cryopreservation and 454 artificial fertilization purposes is suggested to be the first two months of the spermiation period. Further research should focus on the effect of broodstock age and sex ratio on the 456 production and quality of eggs.

458 **Table 1**. Cumulative spawning and egg quality parameters of sharpsnout sea bream broodstocks (n =2) during two or three consecutive spawning seasons (2003–2005) as
460 described in figures 2 and 3. Significant differences between the three years (1-way ANOVA) are indicated with their P values in the last column, whereas lack of significance is
462 indicated by "ns" (not significant) and lack of available data is indicated by "nd" (no data).

Mean ± S.E.M.	n value (2003/2004/2005)	2003	2004	2005	P <
Daily relative fecundity ($x10^3$ eggs kg^{-1})	161/112/112	62 ± 3	42 ± 2	44 ± 2	0.001
Number of spawning days ($month^{-1}$)	8/8/8	20 ± 3	14 ± 3	14 ± 3	ns
Total annual fecundity ($x10^3$ eggs kg^{-1})	2/2/2	4949 ± 76	2371 ± 64	2360 ± 78	0.05
Fertilization (%)	161/112/112	81 ± 1	76 ± 2	78 ± 2	0.01
Hatching (%)	156/120/nd	86 ± 2	86 ± 1	nd	ns
5d larval survival (%)	156/120/nd	85 ± 2	44 ± 3	nd	0.001

464

466

Figure Legends

Figure 1. Annual daylength (natural and simulated) and water temperature variations during a representative year (2003). The bar at the bottom indicates the duration of the spawning season during the three years of the study (2003-2005).

Figure 2. Mean (+ S.E.M.) daily relative fecundity ($x10^3$ eggs Kg^{-1} female biomass) and fertilization success (%) of spawns from sharpsnout sea bream broodstocks (n = 2) during three spawning seasons (2003 – 2005). Fish were maintained in 5-m^3 rectangular tanks supplied with chilled surface seawater. Total female biomass for tank 1 and 2 broodstocks was 3.28 and 6.08 Kg in 2003, 4.41 and 5.91 Kg in 2004, and 4.86 and 5.1 Kg in 2005, respectively. The approximate time of the year (month) is shown below the x-axis.

Figure 3. Mean (± S.E.M.) values of various egg production parameters (spawning days, relative fecundity and fertilization success) of captive-reared sharpsnout sea bream collected during three consecutive spawning seasons (2003 – 2005), and hatching percentage and 5d larval survival collected during two consecutive spawning seasons (2003 - 2004). The ANOVA table shows the statistically significant effect of year, month and year x month interaction (2-way ANOVA, DNMR, * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$). Absence of statistical significance is indicated by "ns" (not significant). In the case of absence of a significant interaction between year and month, significant differences among months are indicated by different letter superscripts.

Figure 4. Mean (± S.E.M.) sperm quality parameters of captive–reared sharpsnout sea bream during the reproductive season of 2003. The number of males sampled and included in the statistical analyses (n) is indicated in a box on each bar. An error in the

dilution procedure caused the lack of sperm density data in September, whereas in December the very small amount of sperm collected did not allow for the evaluation of motility duration (indicated by "nd", no data). Sperm index was analyzed with a non-parametric test (Kruskar-Wallis) and the rest of the parameters with parametric ANOVA, followed by DNMR. Different letter superscripts indicate means that were significantly different (lower letters $P < 0.05$, capital letters $P < 0.01$).

Figure 5. Mean (± S.E.M.) gonadosomatic index (GSI; [testes weight / body weight] * 100]) and total expressible sperm volume (ml Kg^{-1} body weight) of captive-reared sharpsnout sea bream males during the reproductive season of 2003. Different letter superscripts indicate means that were significantly different (ANOVA, DNMR, $P < 0.05$).

Figure 6. Histological sections of sharpsnout sea bream testes showing the progression of reproductive maturation. A. Immature testis in August at the beginning of the spawning season, having germ cells at all stages of development, including spermatocytes (sc), spermatids (st) and spermatozoa (sz). B. Maturing testis of in September with increasing occurrence of spermatozoa, in addition to all other types of germ cells. C. Mature testis spermiating males in October, almost completely filled with spermatozoa. D. Mature spermiating testis in November, showing tubules still containing large amounts of spermatozoa, but with a marked hypertrophy of the somatic cells and appearance of spermatogonia (arrows). E. Spent or regressing testis in December, showing massive proliferation of spermatogonia (sg). Some residual spermatozoa and germ cells in other developmental stages are also present. Scale bar indicates 100 μm.

References

Aas, G.H., Refstie, T., Gjerde, B., 1991. Evaluation of milt quality of Atlantic salmon. Aquaculture 95: 125-132.

Abellan, E., Basurco, B., 1999. Options Méditerranéennes, No. 24: Marine Finfish Species Diversification; Current Situation and Prospects in Mediterranean Aquaculture. C.I.H.E.A.M., Zaragoza, Spain, 1-139 pp.

Anonymous, 1998. Guidelines for the treatment of animals in behavioural research and teaching. Animal Behaviour 55: 251-257.

Bennett, H.S., Wyrick, A.D., Lee, S.W., McNeil, J.H., 1976. Science and art in preparing tissues embedded in plastic for light microscopy, with special reference to glycol methacrylate, glass knives, and simple stains. Stain Technology 51:71-94.

Billard, R., 1986. Spermatogenesis and spermatology of some teleost fish species. Reproduction Nutrition Development 26: 877-920.

Billard, R., Dupont, J., Barnabé, G., 1977a. Diminution de la motilité et de la durée de conservation du sperme de *Dicentrarchus labrax* (poisson Téléostéen) pendant la période de spermiation. Aquaculture 11: 363-367.

Billard, R., Richard, M., Breton, B., 1977b. Stimulation of gonadotropin secretion after castration in rainbow trout. General and Comparative Endocrinology 33: 163-165.

Bodington, P., 2000. Enterprise experiences in the culture of new sparids. In: Basurco, B., Pedini, M. (Eds.), Cahiers Options Méditerranéennes, vol. 47: Recent Advances in Mediterranean Marine Aquaculture Finfish Species Diversification, C.I.H.E.A.M., Zaragoza, Spain, pp. 135-139.

536 Bromage, N., Porter, M., C., R., 2001. The environmental regulation of maturation in farmed finfish with special reference to the role of photoperiod and melatonin. Aquaculture
538 197: 63-98.

Bromage, N.R., 1995. Broodstock management and seed quality - general considerations. In:
540 Bromage, N.R., Roberts, R.J. (Eds.), Broodstock Management and Egg and Larval Quality, Blackwell Science, Oxford, pp. 1-24.

542 Brooks, S., Tyler, C.R., Sumpter, J.P., 1997. Egg quality in fish: what makes a good egg? Reviews in Fish Biology and Fisheries 7: 387-416.

544 Büyükhatipoglu, S., Holtz, W., 1984. Sperm output in rainbow trout (*Salmo gairdneri*)-effect of age, timing and frequency of stripping and presence of females. Aquaculture
546 37: 63-71.

Chauvaud, L., Cosson, J., Suquet, M., Billard, R., 1995. Sperm motility in turbot,
548 *Scophthalmus maximus*: initiation of movement and changes with time of swimming characteristics. Environmental Biology of Fishes 43: 341-349.

550 Ciereszko, A., Dabrowski, K., 1993. Estimation of sperm concentration of rainbow trout, whitefish and ellow perch using a spectrophotometric technique. Aquaculture 109: 367-
552 373.

DeGraaf, J.D., King V, W., Benton, C., Berlinsky, D.L., 2004. Production and storage of
554 sperm from the black sea bass *Centropristis striata* L. Aquaculture Research 35: 1457-1465.

556 Divanach, P., Kentouri, M., 2000. Hatchery techniques for specific diversification in Mediterranean finfish larviculture. In: Basurco, B., Pedini, M. (Eds.), Cahiers Options
558 Méditerranéennes, vol. 47: Recent Advances in Mediterranean Aquaculture Finfish

Species Diversification, CIHEAM, Instituto Agronomico de Zaragoza, Zaragoza, Spain, pp. 75-95.

Evans, R.P., Parish, C.C., Brown, J.A., Davis, P.J., 1996. Biochemical composition of eggs from repeat and first-time spawning captive Atlantic halibut (*Hippoglossus hippoglossus*). Aquaculture 139: 139-149.

Faranda, F., Cavaliere, A., Lo Paro, G., Manganaro, A., Mazzola, A., 1985. Preliminary studies on reproduction of *Puntazzo puntazzo* (Gmelin 1789) (Pisces, Sparidae) under controlled conditions. Aquaculture 49: 111-123.

Fauvel, C., Savoye, O., Dreanno, C., Cosson, J., Suquet, M., 1999. Characteristics of sperm of captive seabass in relation to its fertilization potential. Journal of Fish Biology 54: 356-369.

Favarolo, E., Mazzola, A., 2000. Meristic character analysis and skeletal anomalies during growth in reared sharpsnout seabream. Aquaculture International 8: 417-430.

Firat, K., Saka, S., Ozden, O., 2005. Effect of temperature on embryonic development in sharpsnot seabream (*Diplodus puntazzo*) eggs. The Israeli Journal of Aquaculture 57 (2): 105-114.

Geffen, A.J., 1999. Variations in sperm motility of the Atlantic herring *Clupea harengus*. Marine Biology 134: 637-643.

Georgiou, G., Stephanou, D., 1995. Contribution to the study of maturation and spawning problems of the sharpsnout seabream (*Puntazzo puntazzo*). In: Abellan, E., Basurco, B. (Eds.), Cahiers Options Méditerranéennes, vol. 24: Marine Finfish Species Diversification; Current Situation and Prospects in Mediterranean Aquaculture, C.I.H.E.A.M., Zaragoza, Spain, pp. 47-50.

582 Glamuzina, B., Jug-Dujakovic, J., Katavic, I., 1989. Preliminary studies on reproduction and larval rearing of common Dentex, Dentex dentex. Aquaculture 77:75-84.

584 Hernández, M.D., Egea, M.A., Rueda, F.M., Martínez, F.J., García García, B., 2003. Seasonal condition and body composition changes in sharpsnout seabream (*Diplodus*
586 *puntazzo*) raised in captivity. Aquaculture 220: 569-580.

Hernández, M.D., Martínez, F.J., García García, B., 2002. Sensory evaluation of farmed
588 sharpsnout seabream (*Diplodus puntazzo*) Aquaculture International 9: 519-529.

Kadmon, G., Yaron, Z., Gordin, H., 1985. Sequence of gonadal events and oestradiol levels
590 in *Sparus aurata* (L.) under two photoperiod regimes. Journal of Fish Biology 26: 609-620.

592 Kjesbu, O.S., Witthames, P.R., Solemdal, P., Greer Walker, M., 1998. Temporal variations in the fecundity of Arcto-Norwegian cod (*Gadus morhua*) in response to natural changes
594 in food and temperature. Journal of Sea Research 40: 303-321.

Kjørsvik, E., 1994. Egg quality in wild and broodstock cod *Gadus morhua* L. Journal of the
596 World Aquaculture Society 25: 22-29.

Kjørsvik, E., Mangor-Jensen, A., Holmefjord, I., 1990. Egg quality in fishes. Advances in
598 Marine Biology 26: 71-113.

Kraus, G., Müller, A., Trella, K., Köster, F.W., 2000. Fecundity of Baltic cod: temporal and
600 spatial variation. Journal of Fish Biology 56: 1327-1341.

Lahnsteiner, F., Berger, B., Weismann, T., Patzner, R.A., 1998. Determination of semen
602 quality of the rainbow trout, *Oncorhynchus mykiss*, by sperm motility, seminal plasma parameters, and spermatozoal metabolism. Aquaculture 163: 163-181.

604 Lahnsteiner, F., Patarnello, P., 2004. Biochemical egg quality determination in the gilthead seabream, *Sparus aurata*: reproducibility of the method and its application for
606 sharpsnout seabream, *Puntazzo puntazzo* Aquaculture 237: 433-442.
Lahnsteiner, F., Patarnello, P., 2005. The shape of the lipid vesicle is a potential marker for
608 egg quality determination in the gilthead seabream, *Sparus aurata*, and in the sharpsnout seabream, *Diplodus puntazzo* Aquaculture 246: 423-435.
610 Laine, P., Rajasilta, M., 1999. The hatching success of Baltic herring eggs and its relation to female condition. Journal of Experimental Marine Biology and Ecology 237: 61-73.
612 Leu, M.Y., 1994. Natural spawning and larval rearing of silver bream, *Rhabdosargus sarba* (Forsskål), in captivity. Aquaculture 120: 115-122.
614 Liley, N.R., Tamkee, P., Tsai, R., Hoysak, D.J., 2002. Fertilization dynamics in rainbow trout (*Oncorhynchus mykiss*): effect of male age, social experience, and sperm concentration
616 and motility on in vitro fertilization. Canadian Journal of Fisheries and Aquatic Sciences 59: 144-152.
618 Loir, M., Le Gac, F., Somarakis, S., Pavlidis, M., 2001. Sexuality and gonadal cycle of the common dentex (*Dentex dentex*) in intensive culture. Aquaculture 194: 363-383.
620 Marangos, C., 1995. Larviculture of the sheepshead seabream, *Puntazzo puntazzo* (Gmelin 1789) (Pisces, Sparidae). C.I.H.E.A.M., Zaragoza, Spain, 41-46 pp.
622 McDowell, E.M., Trump, B.F., 1976. Histologic fixatives suitable for diagnostic light and electron microscopy. Archives of Pathology and Laboratory Medicine 100: 405-414.
624 Methven, D.A., Crim, L.C., 1991. Seasonal changes in spermatocrit, plasma sex steroids, and motiity of sperm from Atlantic halibut (*Hippoglossus hippoglossus*). Proceedings of the
626 4th International Symposium on Reproductive Physiology of Fish, Sheffield. pp. 170

Micale, V., Perdichizzi, F., Basciano, G., 1996. Aspects of the reproductive biology of the
628 sharpsnout seabream *Diplodus puntazzo* (Cetti, 1777). I. gametogenesis and gonadal cycle in captivity during the third year of life. Aquaculture 140: 281-291.
630 Mihelakakis, A., Kitajima, C., 1995. Spawning of the silver sea bream *Sparus sarba* in captivity. Japanese Journal of Ichthyology 42: 53-59.
632 Munro, A.D., Scott, A.P., Lam, T.J., 1990. Reproductive Seasonality in Teleosts: Environmental Influences. CRC Press, Inc., Boca Raton, Florida, 254 pp.
634 Mylonas, C.C., Hinshaw, J.M., Sullivan, C.V., 1992. GnRHa-induced ovulation of brown trout (*Salmo trutta*) and its effects on egg quality. Aquaculture 106: 379-392.
636 Mylonas, C.C., Papadaki, M., Divanach, P., 2003a. Seasonal changes in sperm production and quality in the red porgy *Pagrus pagrus* (L.). Aquaculture Research 34: 1161-1170.
638 Mylonas, C.C., Sigelaki, I., Divanach, P., Mañanos, E., Carillo, M., Afonso-Polyviou, A., 2003b. Multiple spawning and egg quality of individual European sea bass
640 (*Dicentrarchus labrax*) females after repeated injections of GnRHa. Aquaculture 221: 605-620.
642 Mylonas, C.C., Papadaki, M., Pavlidis, M., Divanach, P., 2004. Evaluation of egg production and quality in the Mediterranean red porgy (*Pagrus pagrus*) during two consecutive
644 spawning seasons. Aquaculture 232: 637-649.
Mylonas, C.C., Cardinaletti, G., Sigelaki, I., Polzonetti-Magni, A., 2005. Comparative
646 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
648 different temperatures. Aquaculture 246: 467-481.

Palma, J., Andrade, J.P., 2002. Morphological study of *Diplodus sargus*, *Diplodus puntazzo*, and *Lithognathus mormyrus* (Sparidae) in the Eastern Atlantic and Mediterranean Sea. Fisheries Research 57: 1-8.

Panini, E., Mylonas, C.C., Zanuy, S., Carrillo, M., Ramos, J., Bruce, M., 2001. Incubation of embryos and larvae of marine fish using microtiter plates. Aquaculture International 9: 189-196.

Papandroulakis, N., Kentouri, M., Maingot, E., Divanach, P., 2004. Mesocosm: a reliable technology for larval rearing of *Diplodus puntazzo* and *Diplodus sargus sargus*. Aquaculture International 12: 345-355.

Pastor, E., Grau, A., Riera, F., Pou, S., Massuti, E., Grau, A.M., 2000. Experiences in the culture of new species in the 'Estacion de Acuicultura' of the Balearic Government (1980-1998). In: Basurco, B. (Ed.) Cahiers Options Méditerranéennes, vol. 47: Mediterranean Marine Aquaculture Finfish Species Diversification, C.I.H.E.A.M., Zaragoza, Spain, pp. 371-379.

Pavlidis, M., Keravec, L., Greenwood, L., Mourot, B., Scott, A.P., 2001. Reproductive performance of common dentex, *Dentex dentex*, broodstock held under different photoperiod and constant temperature conditions. Fish Physiology and Biochemistry 25: 171-180.

Pavlidis, M., Greenwood, L., Scott, A.P., 2004. The role of sex ratio on spawning performance and on the free and conjugated sex steroids released into the water by common dentex (*Dentex dentex*) broodstock. General and Comparative Endocrinology 138: 255-262.

Rainis, S., Mylonas, C.C., Kyriakou, Y., Divanach, P., 2003. Enhancement of spermiation in European sea bass (*Dicentrarchus labrax*) at the end of the reproductive season using GnRHa implants. Aquaculture 219: 873-890.

Rakitin, A., Ferguson, M.M., Trippel, E.A., 1999. Spermatocrit and spermatozoa density in Atlantic cod (*Gadus morhua*): correlation and variation during the spawning season. Aquaculture 170: 349-358.

Rideout, R.M., Trippel, E.A., Litvak, M.K., 2004. Relationship between sperm density, spermatocrit, sperm motility and spawning date in wild and cultured haddock. Journal of Fish Biology 65: 319-332.

Schreck, C.B., Contreras-Sanchez, W., Fitzpatrick, M.S., 2001. Effects of stress in fish reproduction, gamete quality, and progeny. Aquaculture 197: 3-24.

Shangguan, B., Crim, L.W., 1999. Seasonal variations in sperm production and sperm quality in male winter flounder, *Pleuronectes americanus*: the effects of hypophysectomy, pituitary replacement therapy, and GnRHa treatment. Marine Biology 134: 19-27.

Sorbera, L.A., Mylonas, C.C., Zanuy, S., Carillo, M., Zohar, Y., 1996. Sustained administration of GnRHa increases milt volume without altering sperm counts in the sea bass. The Journal of Experimental Zoology 276: 361-368.

Suquet, M., Omnes, M.H., Normant, Y., Fauvel, C., 1992. Assessment of sperm concentration and motility in turbot (*Scophthalmus maximus*). Aquaculture 101: 177-185.

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. Aquatic Living Resources 7: 283-294.

694 Suquet, M., Dreanno, C., Dorange, G., Normant, Y., Quemener, L., Gaignon, J.L., Billard, R., 1998. The ageing phenomenon of turbot spermatozoa: effects on morphology,
696 motility and concentration, intracellular ATP content, fertilization, and storage capacities. Journal of Fish Biology 52: 31-41.
698 Suquet, M., Dreanno, C., Fauvel, C., Cosson, J., Billard, R., 2000. Cryopreservation of sperm in marine fish. Aquaculture Research 31: 231-243.
700 Taddei, A.R., Barbato, F., Abelli, L., Canese, S., Moretti, F., Rana, K.J., Fausto, A.M., Mazzini, M., 2001. Is cryopreservation a homogeneous process? Ultrastructure and
702 motility of untreated, prefreezing, and postthawed spermatozoa of *Diplodus puntazzo* (Cetti). Cryobiology 42: 244-255.
704 Tramati, C., Savona, B., Mazzola, A., 2005. A study on the pattern of digestive enzymes in *Diplodus puntazzo* (Cetti, 1777) (Osteichthyes, Sparidae): evidence for the definition of
706 nutritional protocols. Aquaculture International 13: 89-95.
Tveiten, H., Solevac, S.E., Johnsen, H.K., 2001. Holding temperature during the breeding
708 season influences final maturation and egg quality in common wolffish. Journal of Fish Biology 58: 374-385.
710 Tyler, J.R., Sumpter, J.P., 1996. Oocyte growth and development in teleosts. Reviews in Fish Biology and Fisheries 6: 287-318.
712 Zastrow, C.E., Houde, E.D., Saunders, E.H., 1989. Quality of striped bass (*Morone saxatilis*) eggs in relation to river source and female weight. Rapp. P. -v. Réun. Cons. int. Explor.
714 Mer. 191: 34-42.

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.