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

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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, 5 day 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 ($2.7\pm0.2\times10^{10}$ spermatozoa ml^{-1}) peaked in October. Mean sperm survival ranged between 9 and 13 days during most of the spawning season, and decreased significantly to 5 days 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.
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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, 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

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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,b, 2004). Such information will assist commercial facilities in organizing and, if possible, optimizing egg and fry production.

The sharpsnout sea bream is a rudimentary hermaprodite fish with partial protandry (Pajuelo et al., 2008) 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 (Fig. 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, 1 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 (Fig. 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

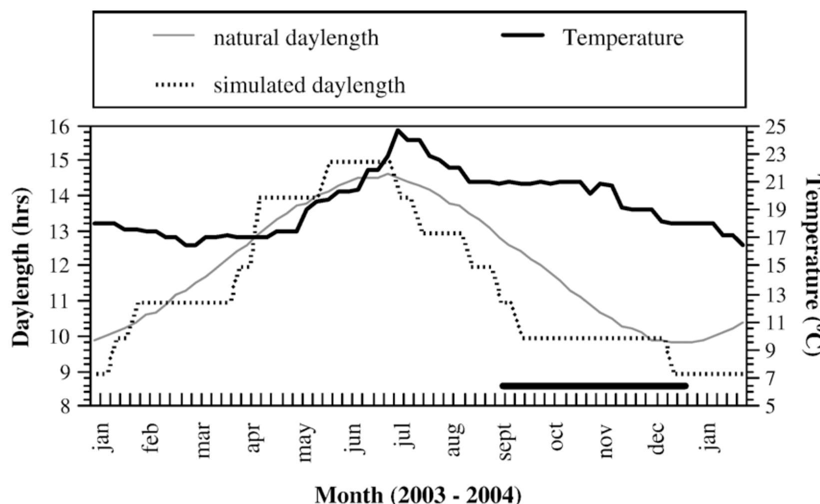

Fig. 1. Annual daylength (natural and simulated) and water temperature variations during a representative year (2003). The bar at the bottom indicates the duration of the spawning season during the 3 years of the study (2003–2005).

starved 2 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 behavioural 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 days at 20 ±0.5 °C. Using a stereoscope, embryonic and early larval development was evaluated once a day, recording the number of 1-day 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 day), 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-day embryos, and 5 day larval survival was calculated as the number of live larvae 5 days 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, 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

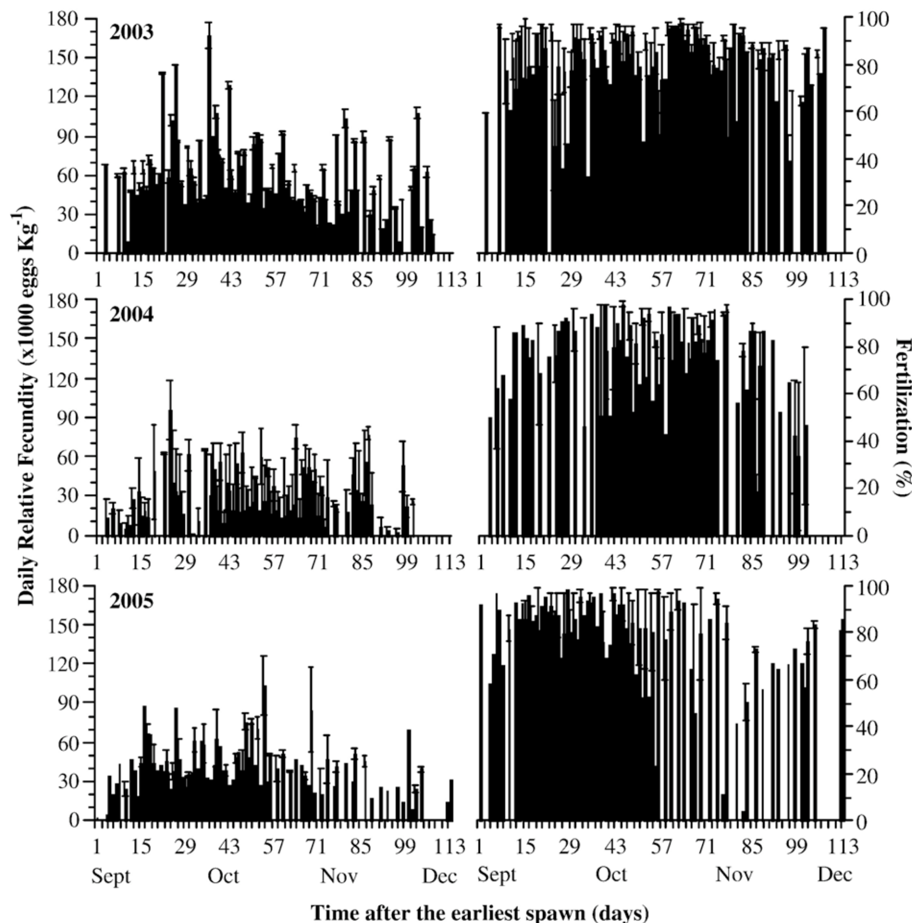

Fig. 2. Mean (+S.E.M.) daily relative fecundity (×10^3 eggs kg^{-1} female biomass) and fertilization success (%) of spawns from sharpsnout sea bream broodstocks (n=2) during three spawning seasons (2003–2005). Fish were maintained in 5-m^3 rectangular tanks supplied with chilled surface seawater. Total 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.

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] × 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 200× magnification (in duplicate). Sperm motility and motility duration were evaluated on a microscope slide (× 400 magnification) after mixing 1 µl of sperm with a drop of about 50 µl of saltwater (in duplicate). 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 day after sperm collection and every 2 days 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.

2.4. Statistical analysis

Differences in spawning and egg quality parameters between reproductive seasons and between months were analyzed by two-way analysis of variance

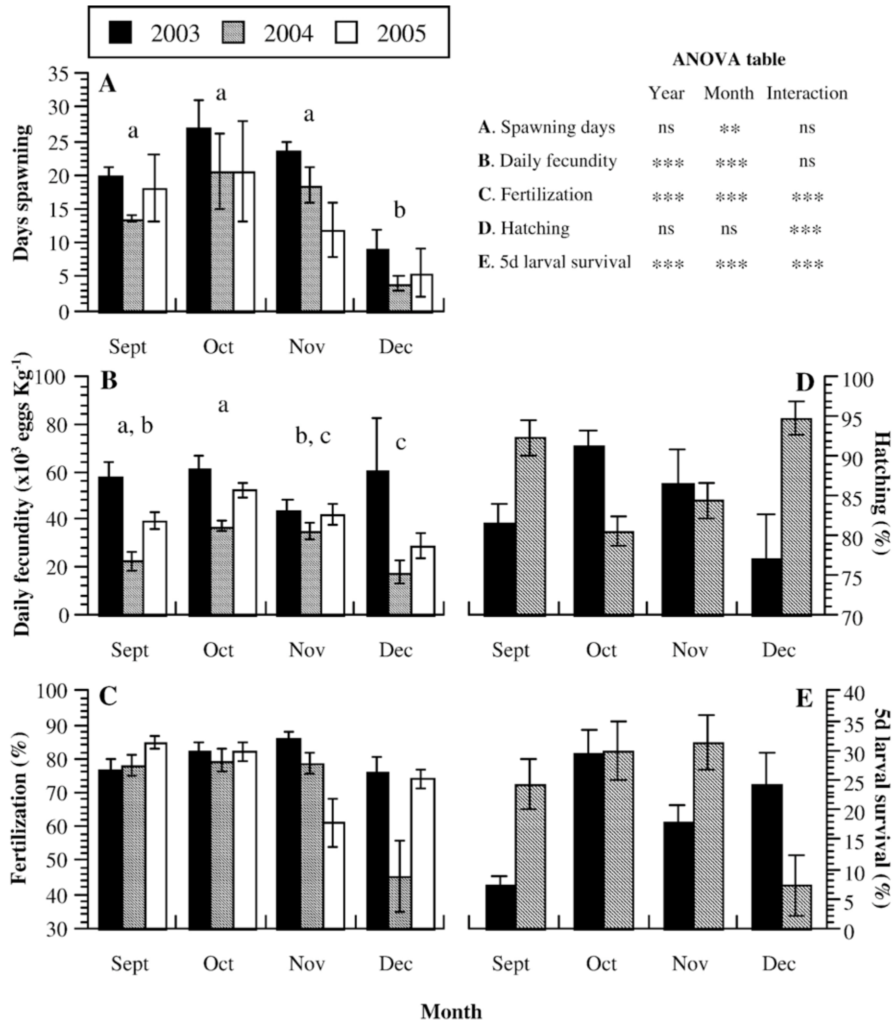

Fig. 3. Mean (±S.E.M.) values of various egg production parameters (spawning days, relative fecundity and fertilization success) of captive-reared sharpsnout sea bream collected during three consecutive spawning seasons (2003–2005), and hatching percentage and 5 day larval survival collected during two consecutive spawning seasons (2003–2004). The ANOVA table shows the statistically significant effect of year, month and year × 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.

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 described in Figs. 2 and 3

Mean ± S.E.M.	n value (2003/2004/2005)	2003	2004	2005	$P<$
Daily relative fecundity ($\times 10^3$ 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 ($\times 10^3$ 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
5 day larval survival (%)	156/120/nd	85±2	44±3	nd	0.001

Significant differences between the 3 years (1-way ANOVA) are indicated with their P values in the last column, whereas lack of significance is indicated by "ns" (not significant) and lack of available data is indicated by "nd" (no data).

(ANOVA, year × month), followed by Duncan's New Multiple Range test (DNMR) at a minimum significance 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 spermiation condition were analyzed by a non-parametric statistics test (Kruskal–Wallis). Percentage data were Arcsine transformed prior to statistical analyses to normalize variances. Unless otherwise mentioned, results are presented as means ± S.E.M. Statistical analyses were performed with linear statistics software (SuperAnova, Abacus Concepts, Inc., USA).

3. Results

3.1. Spawning and egg quality

The spawning season of sharpsnout sea bream begun the last week of August or the first week of September, and ended in the second or third week of December during the 3 years of monitoring (Fig. 2). Although the spawning period was slightly longer in 2005, spawning after

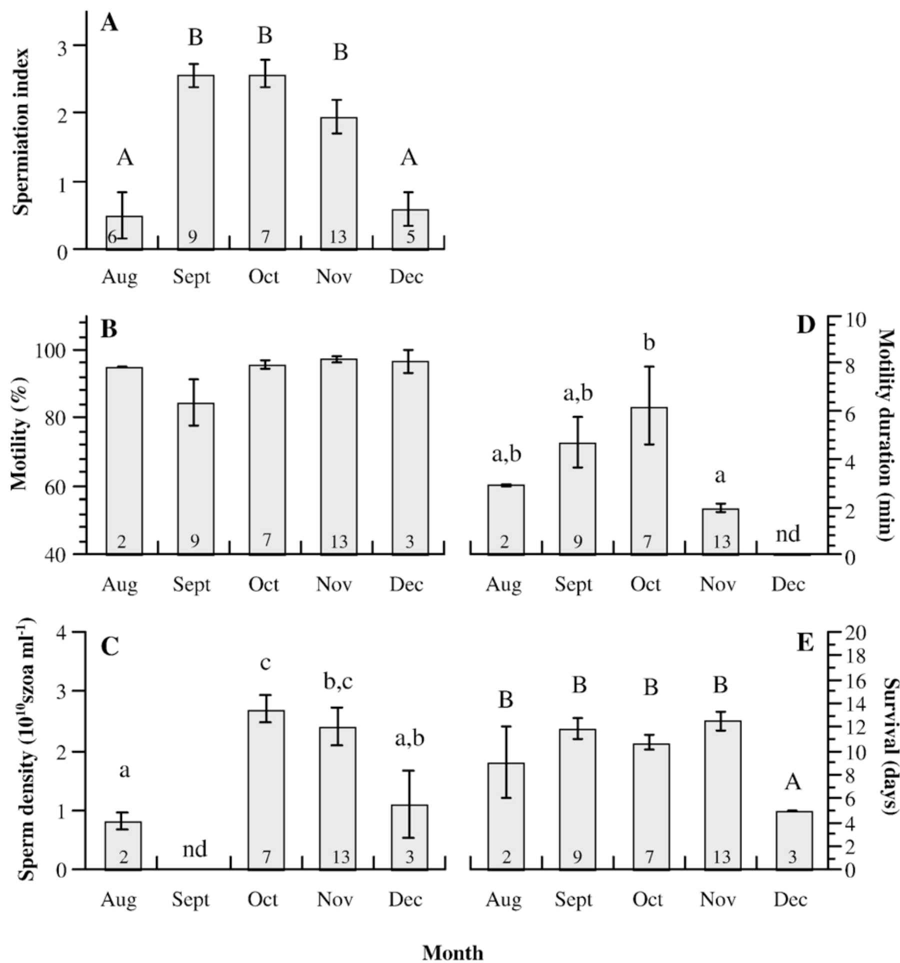

Fig. 4. Mean (±S.E.M.) sperm quality parameters of captive-reared sharpsnout sea bream during the reproductive season of 2003. The number of males sampled and included in the statistical analyses (n) is indicated in a box on each bar. An error in the dilution procedure caused the lack of sperm density 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 $P<0.01$).

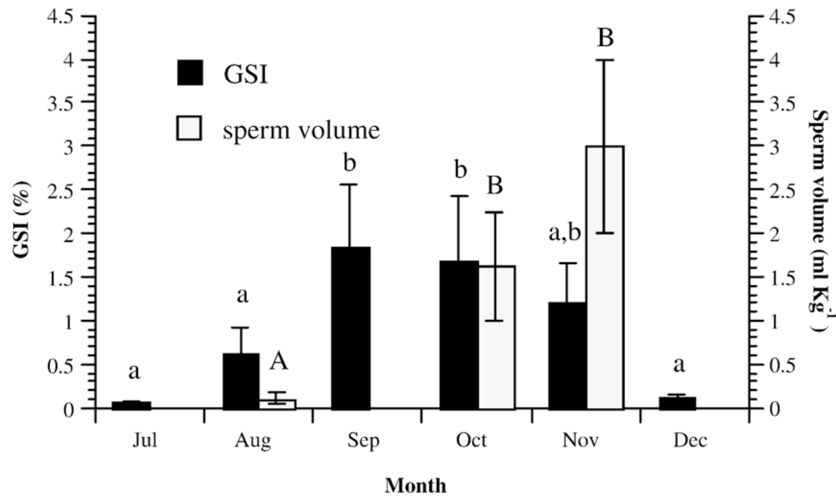

Fig. 5. Mean (±S.E.M.) gonadosomatic index (GSI; [testes weight/body weight] * 100]) and total expressible sperm volume (ml kg^{-1} body weight) of captive-reared sharpsnout sea bream males during the reproductive season of 2003. Different letter superscripts indicate means that were significantly different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

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 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. 2).

Mean number of spawning days per month were similar for the first 3 months (Fig. 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 3 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 3 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

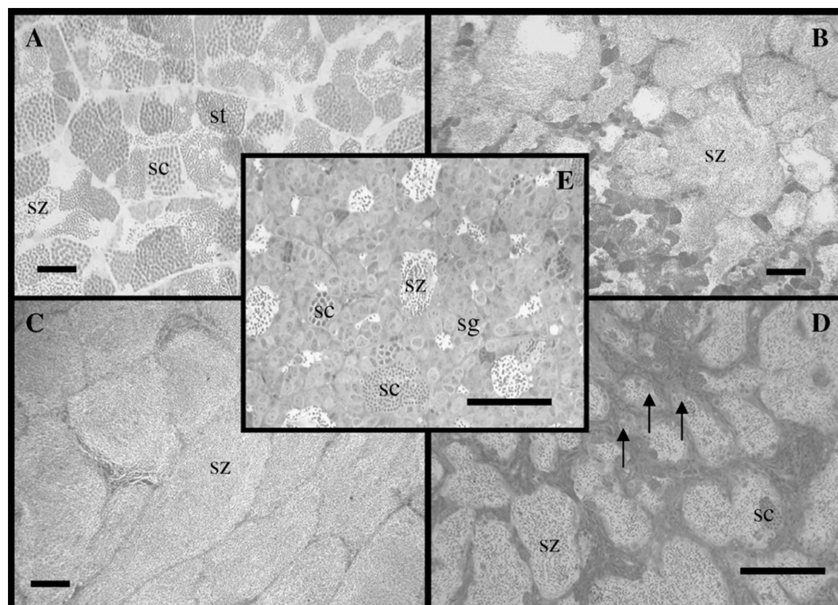

Fig. 6. Histological sections of sharpsnout sea bream testes showing the progression of reproductive maturation. A. Immature testis in August at the beginning of the spawning season, having germ cells at all stages of development, including spermatocytes (sc), spermatids (st) and spermatozoa (sz). B. Maturing testis in September with increasing occurrence of spermatozoa, in addition to all other types of germ cells. C. Mature testis of 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.

2004 the highest values were observed in September and December (Fig. 3D). Finally, mean 5 day 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±3000 eggs kg^{-1} female $spawn^{-1}$), compared to 42,000±2000 eggs kg^{-1} in 2004 and 44,000±2000 eggs kg^{-1} in 2005. The number of spawning days was not different between the 3 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 5 day larval survival dropped significantly in 2004 (ANOVA, DNMR, $P<0.001$).

3.2. Spermiation and sperm quality

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 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 sperm motility percentage was high (>80%) and remained unchanged throughout the spawning season (Fig. 4B). Sperm density increased after August reaching mean values of $>20.0\times10^9$ 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 about 12.0 $x10^9$ 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 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 the spermiation period in October, with a significant reduction thereafter (Fig. 4D). On the other hand, mean sperm survival at 4 °C was similar between August and November, ranging 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 very small, thus not allowing the evaluation of motility duration.

Total expressible sperm volume increased significantly (ANOVA, DNMR, $P<0.05$) in 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, 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 3 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, 2005). The 1 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). 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, 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 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 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 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.

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. These values are much higher than those reported in other Sparidae broodstocks, though direct comparisons with other studies are not always feasible or appropriate, given 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-Palacios et al., 1995) 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 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 12,000–14,000 eggs kg^{-1} $spawn^{-1}$ (Loir et al., 2001). In our facilities, daily relative fecundity of red porgy ranged between 1000 and 30,000 eggs kg^{-1} in two consecutive spawning seasons (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 5 day 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 sea bream (Lahnsteiner and Patarnello, 2005). In another study in the same fish, embryo survival was significantly correlated with larval survival (Lahnsteiner and Patarnello, 2004). Similarly, significant correlations were found between 1-day embryo survival and fertilization percentage, and between hatching percentage and 1-day embryo survival in red porgy (Mylonas et al., 2004), and between hatching and 1-day embryo survival and between larval survival and hatching in European sea bass (*Dicentrarchus labrax*) (Mylonas et al., 2003b). No such 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 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 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, 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 histological observations are in accordance with an earlier study of 2-year-old fish (Micale et al., 1996). In another study, it was observed that sperm release could be obtained even some 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 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 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 volume reached its highest value of 3 ml kg^{-1} in November, 1 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, $5–10 \times 10^9$ spermatozoa ml^{-1} in the whitefish (*Coregonus clupeaformis*), $5–18 \times 10^9$ spermatozoa ml^{-1} in the rainbow trout, $8–23 \times 10^9$ spermatozoa ml^{-1} in the red porgy (Mylonas et al., 2003a), $5–55 \times 10^9$ spermatozoa ml^{-1} in the European sea bass (Sorbera et al., 1996; Fauvel et al., 1999; Rainis et al., 2003), $32–44 \times 10^9$ spermatozoa ml^{-1} in the turbot (Suquet et al., 1992), and $38–45 \times 10^9$ spermatozoa ml^{-1} in the yellow perch (*Perca flavescens*) (Ciereszko and Dabrowski, 1993). The above results 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 collection of sperm for cryopreservation and artificial insemination procedures (Suquet et al., 2000).

In a study on sharpsnout sea bream spermatozoa (Taddei et al., 2001), it was observed that sperm continues to move for more than 300 s. Motility duration in the present study 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 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 h after activation (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 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 different individual spermatozoa activation times. Although such a phenomenon may explain the extreme duration of forward motility of spermatozoa observed in some samples, 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 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.

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., 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 the spermiation period was also observed for sperm survival at 4 °C. Alterations of the spermatozoa plasma membrane at the end of the female spawning season could explain the 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 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 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 available for a long period of time, allowing insemination of the eggs obtained from females ovulating at slightly different times after induction.

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 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 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 artificial fertilization purposes is suggested to be the first 2 months of the spermiation period. Further research should focus on the effect of broodstock age and sex ratio on the production and quality of eggs.

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