Effect of Seasonality on Oocyte Growth, Oocyte Maturity Stages, and Reproductive Capacity in the Gilthead Sea Bream (Sparus aurata) in Relation with Depth

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Abstract

This study aimed to investigate the role of seasonal environmental variations in oocyte growth, oocyte maturity stages, and reproductive capacity of Sparus aurata. To model this in the field, two groups of fish were placed for a 1-year period at two depths: 0-2 m and 10-12 m. Environmental parameters such as temperature, salinity, pH, dissolved oxygen, and water transparency were monitored, and oocyte number was estimated using standard gravimetric techniques. Oocytes were categorized as primary, cortical alveoli, vitellogenic, hydrated, and atretic and were analyzed according to their shape and diameter. Oocyte maturation period was found to initiate in September and peak in November. During this process, the oocytes appeared to shift from more irregular to elliptical shape. Differences in the oocytes of the two groups, mainly concerning the higher vitellogenic and atretic oocyte number of the 10-12 m group compared with the 0-2 m, supported with gonadosomatic index and hepatosomatic index, might reflect the light intensity and temperature effects. Moreover, using the principal components analysis, we observed a possible link between the environmental temperature and light and the onset of spawning. Findings of this study provide a better understanding of S. aurata breeding pattern and may benefit the management of this highly commercial species.

Keywords: Atresia, light, oocytes, seasonality, teleosts, temperature

Introduction

Studies on fish reproduction, such as those on maturity size assessment, reproductive season duration, daily spawning behavior, and spawning fraction, permit the quantification of their reproductive capacity (Murua et al., 2003), which is an essential component of effective fisheries management. The importance of understanding the reproductive success and population reproductive potential has been previously highlighted in the studies of Kjesbu (2009) and Lowerre-Barbieri (2009). Furthermore, changes in environmental factors, especially temperature, have a direct effect on fish reproduction and behavior (McBride et al., 2016). The energy demanding process of reproduction is largely affected under environmental stress conditions that result in metabolic depression. Under these conditions, energy allocation for the investment in reproduction can result in decrease in the lifespan of fish or increase in mortality risks (McBride et al., 2016). Reproduction capacity in most teleosts is reflected in fecundity (F), calculated as the ratio of oocyte density (oocyte number per gram of ovarian tissue) to the total weight (gravimetric method) or volume (volumetric method) of the ovaries of known weight or volume, respectively, to body weight (Kurita and Kjesbu, 2009; Murua et al., 2003). A very accurate, precise, and up-to-date method is the autodiametric method, which provides the estimates of total fecundity in the determinate spawners taking into account the ovary weight and oocyte density (Alonso-Fernández et al., 2009; Hunter and Goldberg, 1980; Hunter et al., 1992; Klibansky and Juanes, 2008; Witthames et al., 2009).
The gilthead sea bream (*Sparus aurata*) is a eurythermal species, largely farmed along the Mediterranean coastline owing to its commercial value (Colloca and Cerasi, 2005). Gilthead sea bream is a protandric hermaphrodite species, maturing as male during the first or second year of age and as a female after the second or third year of age (Bauchot et al., 1981; Buxton and Garratt, 1990). Spawning occurs from October to December, with continuous spawning during the whole period. To our knowledge, although extended literature exists on the reproduction of *Sparus aurata* (Emre et al., 2009; Somarakis et al., 2013), and we have previously observed a qualitative seasonal variation of oocytes at different maturity stages in this species (Feidantsis et al., 2013), no literature is available on the effect of seasonality on the quantitative aspects of oocyte growth, different oocyte maturity stages, and reproductive capacity in this species of high economic importance. Herein, we aimed to better understand how environmental factors such as seasonality, temperature variability, and depth, including all the related changes in salinity, dissolved oxygen, pH, and water transparency, can influence these aspects of reproduction in 1-year acclimatized aquacultured *S. aurata*. Importance of temperature and light intensity changes on the reproductive physiology of this species was evaluated by placing two fish groups in different depths. Thus, this study is an in-depth field analysis of oocyte maturation and reproduction capacity in *S. aurata*, which can be exploited for the management of this highly commercial species.

**Materials and Methods**

**Animals, experimental design, and tissue sampling**

Farmed gilthead sea bream *S. aurata*, with mean (±standard deviation [SD]) weight of 294.5±4.5 g, 2 years of age, designated as males at the beginning of the experimental procedure, and growing under natural conditions in the field were transferred as males at the beginning of the experimental procedure, in a sea farm (Horozoglou, S.A. Fisheries) in Chalkidiki, North Greece, in the year 2012. To model two different conditions, fish were immersed and acclimatized for a month in cages at 0-2 m and 10-12 m depth. Fish were fed regularly (daily) and until satiety, without seasonal changes. Mechanical (blower) feeders were used for this purpose. Divers were recruited in order to inspect and confirm adequate feeding for 0-2 m and 10-12 m fish. Sea water temperature, salinity, oxygen, pH, and water transparency were measured daily in the field (per day, one measurement conducted in the night, and one in the day time). Monthly mean±SD are shown in Table 1. Specifically, water physicochemical parameters were measured for both the depths (0-2 m and 10-12 m) using a Multiparameter Water Quality Meter (Model WQC-24, DKK-TOA Company). Monthly variation±SD in light transparency was measured using Secchi disk. One month after placement in the cages, 10 individuals/fish cage/sampling were collected monthly. Anesthesia and sampling process are presented in detail in the study by Feidantsis et al. (2013). Fish were weighed (Table 2) and dissected, and the liver was removed, weighed, and stored at -80°C. Gonads were also removed, weighed, and fixed in Bouin solution. Seasonal variation of gonadosomatic index (GSI) = [gonad weight (g)/total tissue weight (g)] × 100 and hepatosomatic index (HSI) = [liver weight (g)/total tissue weight (g)] × 100 in different depths were also recorded as stated in Feidantsis et al. (2013). Histology was performed thereafter in order to detect the stages of gonad maturation. Monthly gonad samplings allowed estimation of the oocyte number and size at different stages: primary, cortical alveoli, vitellogenic, hydrated, and atretic (Brown-Peterson et al., 2011). The estimation of stages was performed according to the observed oocyte morphology (Ferreri et al., 2009; McBride et al., 2016; Mylonas and Zohar, 1995; Press et al., 2014; Schismenou et al., 2012). Oocyte shape changes were also estimated to define the variations occurring in the gonads during the reproductive cycle.

**Table 1.** Monthly recorded physicochemical water parameters: temperature, salinity, oxygen, and pH at the sampling area

| Parameter   | Temperature (°C) | Salinity (g/L) | Oxygen (mg/L) | pH       |
|-------------|-----------------|---------------|--------------|----------|
|             | 0-2 m           | 10-12 m       | 0-2 m        | 10-12 m  |
| Jul         | 28.37±0.12*     | 27.6±0.1**    | 31.6±0.04*   | 31.07±0.51* | 6.07±0.02* | 6.45±0.2* | 8.08±0.02* |
| Aug         | 28.8±0.02*      | 27.6±0.8**    | 31.78±0.08*  | 31.22±0.17* | 6.05±0.06* | 6.4±0.75* | 8.14±0.01* |
| Sep         | 24.23±0.07*     | 24.3±0.07*    | 31.2±0.06*   | 30.9±0.06* | 6.66±0.02* | 6.69±0.02* | 8.04±0.01* |
| Nov         | 17.63±0.05*     | 17.4±0.05*    | 32.33±0.05*  | 31.9±0.32* | 7.86±0.03* | 7.77±0.36* | 8.15±0.01* |
| Transparency (m) |            |               |             |          |
| Jul         | 4.23±0.39*      |               |             |          |
| Aug         | 5.49±0.44*      |               |             |          |
| Sep         | 4.24±0.57*      |               |             |          |
| Nov         | 3.88±0.33*      |               |             |          |

Values are mean±SD of everyday measurements. Small letters indicate significant differences between different sampling months (j for July, a for August, s for September, and n for November). * indicate significant difference between the different sampling depths.
Animals received proper care in compliance with the Guidelines for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH publication No 85–23, revised 1996) and the Principles of Laboratory Animal Care published by the Greek Government (160/1991) on the basis of the European Union (EU) regulations and directive (2010/63/EU). The protocol as well as the surgery and sacrifice conditions were approved by the Committee on the Ethics of Animal Experiments of the Directorate of Veterinary Services of Prefecture of Thessaloniki under the license number EL 54 BIO 05.

Estimation method for oocyte maturity stage

The gonads were prepared for histology using standard procedures (Presnell and Schreibman, 1997). For larger gonads, separate portions from the anterior, middle, and posterior parts were embedded. For smaller gonads, the entire gonad was prepared. At least 10 cross-sections were evaluated from each segment (≥30 sections evaluated per gonad) for determination of sex, sperm abundance, ovarian staging, intersex status, and any abnormalities (Blazer, 2002; Nolan et al., 2001). Oocytes of different maturity stage were estimated gravimetrically. Histological sections allow the quantification of cells in different maturity stages, i.e., oocytes that are either at the primary, cortical alveoli, vitellogenic, hydrated, or atretic stage, by applying the principles of stereology. In this study, although the hydrated oocytes were observed, they were not plotted because their quantification is not reliable, considering that histological sections with dehydration and rehydration steps might alter the real shape and size of these oocytes. The stereological method of Emerson et al. (1990) is based on the Delesse principle (1847), which states that the fractional volume (Vi) of a component (i) is proportional to its fractional cross-sectional area (Ai). An underlying assumption of this principle is that the component is distributed randomly and evenly through the tissue. The abundance of a specific type of oocyte is defined by the morphological criteria. The diameter and the area occupied from each cell per section per oocyte stage were counted using the image analysis program ImageJ, and the results were processed using the following formula (1) according to Weibel et al. (1966).

\[
N_v = K \frac{N_i^2}{V_i^{1/2}}
\]

The number of oocytes per unit volume (Nv) equals to the number of oocytes transections per unit area (Ni) to the volume occupied by oocytes (Vi). Two coefficients were calculated in order to correct for shape and size distribution: β and K, respectively. The total number of oocytes per lobe was calculated as Nv times the ovarian volume for each of the two lobes per animal.

K, which has to be determined for each ovary, was calculated using the following formula (2) (Williams, 1977):

\[
K = \left( \frac{M_1}{M_i} \right)^{3/2}
\]

where \( M_1 \) is the mean oocyte diameter (OD), i.e.,

\[
M_i = \frac{D_1 + D_2 + \ldots + D_n}{n}
\]

D being the diameter of an individual oocyte; n, the number of particles counted; and \( M_j \), the cube root of the third moment about the mean of the distribution, i.e.,

\[
M_j = \left( \frac{(D_1)^3 + (D_2)^3 + \ldots + (D_n)^3}{n} \right)^{1/3}
\]

Images were obtained using a Zeiss Imager.Z2 microscope (Carl Zeiss AS, Norway) supplied with a Zeiss AxioCam MRC 5 camera (Carl Zeiss AS, Norway), and quantification, as well as area measurements and size parameters were evaluated using ImageJ. After estimating the numbers of oocytes per maturity stage, we determined the number of cells for each oocyte stage and total, according to the depth and the month of the samplings.

Table 2. Seasonal variation of gonadosomatic index—GSI, hepatosomatic index—HSI (Feidantsis et al., 2013), and body weight (g) in different depths

|       | 0-2 m  | 10-12 m | 0-2 m  | 10-12 m |
|-------|--------|---------|--------|---------|
| GSI   | 0.378±0.072 | 0.488±0.057 | 3.781±0.849 | 3.287±0.911 |
| HSI   | 0.543±0.055 | 0.564±0.044 | 2.892±0.574 | 2.548±0.667 |
| Body weight (g) | 4.175±1.193 | 4.881±1.144 | 7.05±1.531 | 4.332±0.225 |

Values are mean±SD; n=10 preparations from different animals. Small letters indicate significant differences between different sampling months (j for July, a for August, s for September, and n for November), * indicate significant difference between the different sampling depths.

For the estimation of volume fraction (Vi), the following procedure was followed: each image was analyzed using ImageJ,
and after adjusting for a known scale, the perimeter of each oocyte was drawn, thus generating the corresponding regions of interest (ROI). For each ROI, ImageJ automatically estimated the oocyte surface ($A_o$) and the aspect ratio $\beta$, which equals to the ratio of the larger to the smaller diameter of an oocyte and marks how different it is from a circular shape. The total area of the section was also estimated as $A_{sl}$. According to Delesse stereological principle, $V_f$ equals $\frac{\Sigma A_o}{A_{sl}}$.

**Statistical analysis**

Seasonal changes in the physicochemical parameters (Table 1) were tested for significance at 5% level by using two-tailed $p$ value test and one-way (GraphPad Instat 3.0) analysis of variance (ANOVA). Seasonal changes in the maturation and different ovary cell types were tested for significance at 5% level by using one-way (GraphPad Instat 3.0) or two-way (GraphPad Prism 5.0) ANOVA depending on the number of factors tested each time (depth and sampling month), with sampling dates and depth as fixed factors. Values are presented as mean±SD Post-hoc comparisons were performed using Bonferroni test followed by Dunn’s post-test.

Principal components analysis (PCA) in the FactoMineR package in R was employed to assess the patterns of possibly correlated variables, and more specifically, to detect how oocyte parameters varied between physicochemical parameters.

**Results**

**Gonadal histology**

Gonads were immature and sexually undifferentiated in the first five sampling dates in February, March, April, May, and June, as stated in Feidantsis et al. (2013). In the following months, however, the different maturity stages (Figure 1) were determined by estimating the different type of oocytes: primary, cortical alveoli, vitellogenic, hydrated, and atretic. More specifically, the primary stage oocytes had dark cytoplasm and did not contain any yolk material; cortical alveoli oocytes contained cortical alveoli globules; vitellogenic oocytes had granular cytoplasm but appeared significantly lighter in color than those in the primary or cortical alveoli stages, with a very thick zona radiata; atretic oocytes were lighter in color than “healthy” ones, had no zona radiata, mainly had non-spherical shape, and had no germinal vesicle, and the cytoplasm was characterized by large, translucent globules. Fish were designated as intersex if the microscopic evaluation determined the simultaneous occurrence of both ovarian and testicular tissue (July and August). Fish were designated as female when the microscopic evaluation determined the occurrence of ovarian tissue only (September and November).

**Seasonal variation of gonad weight**

Gonad weight was measured over time for two different depths of 0-2 m and 10-12 m (Figure 2a). In July and August, the weights did not vary much and were relatively small, less than 2 g, with a minimum increase in August for both of the depth groups. A statistically significant increase was observed in September, especially for the animals of the 10-12 m depth group, demonstrating a ~3-fold weight increase. By the time of gonad maturation, in November, gonads weighed ~14 g, and no statistical difference was observed between the two depth groups (Figure 2a). As stated in the study by Feidantsis et al. (2013), although not statistically significant, seasonal variations of GSI (Table 2) followed the same trend. The HSI (Feidantsis et al., 2013; Table 2) underlined the possible metabolic differences in the fish acclimatized at 10-12 m, which in September and November had a significantly lower HSI than 0-2 m acclimatized fish.
Seasonal variation of total oocytes

The seasonal variations in the total number of oocytes were estimated using the stereological method described in Methodology (Figure 2b). In July, the number of oocytes ranged ~8000-10000 per ovary or ~35-38 per g of body weight and was almost the same in August with a small increase for both the depth groups of the range of ~2,000 oocytes per ovary or ~2 per g of body weight per group. In September, the oocyte numbers were significantly higher by ~5000 oocytes per ovary or ~15 oocytes per g of body weight for the fish that were sampled from 10 to 12 m depth. A significant decrease was observed in the 0-2m fish group by ~5000 oocytes per ovary or ~15 oocytes per g of body weight. In November, the number of the oocytes peaked to ~25,000 and ~27,000 per ovary or ~25 and ~27 oocytes per g of body weight, for 0-2 m and 10-12 m fish groups, respectively.

Seasonal variation of oocyte diameters

OD was measured, and seasonal variation was quantified for each oocyte stage during the time (Figure 3). Randomly selected 100 oocytes from our analysis using randomization formula in excel were sorted according to size and then plotted to show the variability of OD. No statistically significant differences were observed between the fish immersed in the two depths (data not shown). Diameter ranges were estimated: primary oocytes had <0.5 mm diameter; cortical alveoli oocytes ranged 0.35-0.6 mm in diameter; vitellogenic oocytes had 0.6-1 mm diameter; and atretic oocytes varied 0.6-0.7 mm in diameter. However, while not significantly different, between July and September, the ODs for the 10-12 m group were larger, but in November, the opposite pattern was observed. Primary stage oocytes ranged between 0.18 and 0.43 mm and decreased in size over time. Cortical alveoli oocytes, the following maturity stage, appeared first time in September and their diameter was ~0.4-0.7 mm. Vitellogenic oocytes with ~0.7-0.9 mm diameter also appeared in September. All the maturity stages were apparent in November, including atretic ones, with diameters of ~0.6-0.7 mm.

Seasonal variations of oocyte number per cell type

To better understand how seasonality affects oocyte maturation, we quantified oocytes per maturity stage (Figure 4). In July, primary oocytes ranged ~9-12,000 per ovary or ~30-40 per g of body weight, and their numbers did not increase significantly in August. Primary oocytes increased 3-fold from September to November when ~18,000 primary oocytes per ovary or ~50 per g of body weight were measured. Cortical alveoli oocytes increased 3-fold in November compared with September. Animals of the 0-2 m group had ~500 more cortical alveoli oocytes per ovary or ~2 per g of body weight than animals of the 10-12 m group. Vitellogenic oocytes increased

Figure 3. Seasonal variation of ODs in different maturity stages (Pr: primary, CA: cortical alveoli, Vit: vitellogenic, Atr: atretic) in two different depths. Small letters indicate significant difference of same stage oocytes between the different sampling months (j for July, a for August, s for September, and n for November)

Figure 4. a, b. Seasonal variation of (a) oocytes/ovary and (b) oocytes/g body weight in different maturity stages (Pr: primary, CA: cortical alveoli, Vit: vitellogenic, Hyd: hydrated, Atr: atretic) and different depths. Values are mean±SD; n=10 preparations from different animals. Small letters indicate significant difference of same stage oocytes between different sampling months (j for July, a for August, s for September, and n for November), * indicate significant difference between the different sampling depths (darker bars 10-12 m, lighter bars 0-2 m) at the same sampling month (p<0.05)
significantly from September to November ~10,000 per ovary or ~35 per g of body weight. The least apparent oocytes were the atretic ones. These oocytes were only found in November and were the least abundant ~200 oocytes per ovary or ~0-25-0.5 per g of body weight. Differences between the two examined depths were observed only for vitellogenic and atretic oocytes, with depth 10-12 m being significantly higher than those in 0-2 m. Hydrated oocytes were first time observed in November.

Oocyte shape changes as oocytes mature
We then investigated the relation between the shape parameter $\beta$ and the diameter of primary, cortical alveoli, and vitellogenic oocytes. The Vf for both vitellogenic and cortical alveoli oocytes increased with OD (Figure 5). The statistically significant ($p<0.05$) link between the shape parameter $\beta$ and the diameter of cortical alveoli and vitellogenic oocytes suggests a cell shape change from irregular to more elliptic as maturation progressed.

Multivariate analysis
The PCA analysis (principal components as extraction method) was applied to statistically define the differences in the oocyte parameters. PC1 explained 36.55% of the variance. Specifically, the oocyte variables that were positively correlated with scores on PC1 were HSI, GSI, hydrated and atretic oocytes, and gonad weight. Temperature and transparency were correlated with individual scores on PC2, which explained 19.66% of the variance. The cumulative value of PC1 and PC2 was 56.21 (Figure 6).

Discussion
For species with commercial value such as the sea bream, correlation between the number of oocytes and fecundity allows better fisheries management. Quantification of S. aurata oocytes using stereology in this study showed that there was an increasing estimated number of total oocytes per ovary and/or per g of body weight, which peaked in its spawning month, November (Zohar et al., 1978). Oocyte maturity stages were determined with morphological criteria alongside size differences, as previously reported in similar studies (Ferreri et al., 2009; McBride et al., 2016; Mylonas and Zohar, 1995; Press et al., 2014; Saber et al., 2016; Schismenou et al., 2012). In S. aurata, distinct oocyte batches at various maturity stages appear first in July and are thereafter evident through November. Smaller, primary oocytes decrease over time, and more mature populations start appearing in September, demonstrating how fish are being prepared during summer for the first batch disposition early in the fall, during which a shift of the shape parameter $\beta$ and subsequently of Vf indicates a switch from irregular to elliptic oocytes along maturation. Vf has been demonstrated before for determinate (e.g., Clupea harengus: Kurita and Kjesbu (2009)) and indeterminate spawners (e.g., Paralichthys olivaceus: Kurita and Kjesbu (2009) and Merluccius merluccius: Korta et al., 2010) and tends to be greater for determinate (~90%) than indeterminate (60-80%) spawners. Such variability reflects the simultaneous existence of premature oocytes with more mature ones. In S. aurata, we found a trend of 88%-94% indicative of a determinate spawning strategy. The ovarian morphology of the gilthead sea bream as presented here aligns with its multiple batch spawning, i.e., batches of oocytes develop and undergo final oocyte maturation, as suggested by the presence of oocytes ranging in size from 0.1 to 1 mm in diameter (Meiri et al., 2002). Collectively, our data show that S. aurata has distinct groups of oocytes for a long period and that the process of development...
maturation takes place in a time-defined and controlled manner. This strategy differs from another commercially important family, the Clupeidae, in which the distinction of batches takes place only at the hydration stage (Galias et al., 2004).

In this study, the coexistence of a high number of vitellogenic parallel to the atretic oocytes seems to support the fact that atresia does not affect the fertilization rate and fecundity (Devlaming, 1983; Miranda et al., 1999). Although some suggest that because atretic oocytes seem adjacent to the normal oocytes at the ovulation, atresia can result in a reduction in fecundity (Kjesbu et al., 1991; Palmer et al., 1995). As Kurita et al. (2003) have also shown, our data suggest that atresia takes place principally in later stages of vitellogenesis. However, this difference might be species specific and requires further investigation. Several stress factors such as temperature, light-photoperiod, fasting, and confinement can induce atresia (Nagahama, 1983; Wood and van Der Kraak, 2002). Therefore, temperature changes during pre-spawning and spawning time may reflect in atresia levels (Bell et al., 1992; Livingston et al., 1997). In S. aurata, atresia rates also peak in November in oocytes of diameter 0.6-0.7 mm (Figure 5). Similarly, Kjesbu et al. (2010a) have observed atretic diameters of 0.6 mm in Atlantic cod (Gadus morhua) and 0.9 mm in Atlantic herring (Clupea harengus) (Kurita et al., 2003). During this period, increased accumulation of oil drops in the mature gonads is observed (especially in November), a fact coinciding with our previous results of increased activity levels of 3-hydroxyacyl CoA dehydrogenase (HOAD) tissues like heart, liver, and red and white muscle (Feidantsis et al., 2018). As a key enzyme of β-oxidation, increased HOAD activity indicates that lipid metabolism in this fish shifts in order to cover high-energy demands for reproduction. Although maturation of primary oocytes may appear energetically inexpensive, their growth to final vitellogenic stages is energy demanding, and fish incorporate atresia as an energy regulating mechanism before spawning (Kjesbu et al., 1991). Thus, all these energy shifts can be also reflected to variations in follicular atresia (Bell et al., 1992; Livingston et al., 1997), which is the breakdown and reabsorption of gametes before ovulation for energy and cellular component recycle in order to promote homeostasis (Hussein, 2005; Kennedy, 2002; Murua et al., 2003; Saidapur, 1978; Santos et al., 2008).

Although all females in this research had atretic oocytes coexisting with vitellogenic and hydrated ones, suggesting that they would not skip spawning because of environmental conditions in the sea farm where this study was conducted; there is evidence of fish undergoing complete reabsorption in field-caught samples (Skjæraasen et al., 2010). Similar to our study, Rizzo and Bazzoli (1997) have observed that in captivity, atresia is more frequent in vitellogenic oocytes. Icterous fish, such as the gilthead sea bream, may skip spawning, with retention of ripe eggs, because of the conditions experienced during the spawning season. Such factors can be fish density, mate availability, pollution, failure to start vitellogenesis, or stress fish experienced before the spawning season, primarily temperature and pollution (Johnson et al., 1996).

Although GSI may be an imprecise proxy for the gonadal stage because it assumes isometry between somatic mass and gonad mass as well as correspondence between total gonad mass and gonad developmental stage (Devlaming et al., 1982; Packard and Boardman, 1988), it remains a useful indicator for sorting fish into gross reproductive categories when significant gonad growth occurs at distinct developmental stages (e.g., vitellogenesis). Therefore, GSI is considered a common metric of reproductive allocation and reproductive condition in fisheries biology (Devlaming et al., 1982). Similar to other studies such as of Hadj-Taieb et al. (2013) and Emre et al. (2009), in S. aurata, the highest GSI values are observed in November, alongside the onset of pre-spawning season. In accordance to GSI increased values, reduction in HSI in November (Feidantsis et al., 2013) reflected the liver energetic reserves and metabolic activity (Lenhardt et al., 2009). Such findings support energy allocation changes by mobilization of hepatic reserves for gonads maturation (Zin et al., 2011). As liver is a source of lipids, significantly increased vitellogenic oocyte production and high GSI in the 10-12 m group could be the reason of lower HSI in these fish. Moreover, and according to PCA analysis, both GSI and HSI seem to be correlated not only with oocyte characteristics but also with environmental factors such as temperature and light transparency, which indicate the strong effect of such factors in fish physiology and metabolic processes.

Although most of the examined physicochemical parameters differed significantly between the two depths nearly at all sampling months, correlation according to PCA analysis between temperature, oocyte characteristics, GSI and HSI, and the two depths seems to explain the effect of temperature on oocyte maturity. Because environmental temperature affects all the levels of biological organization (biochemical, physiological, biogeographical) in marine organisms (Pörtner, 2014), it is of great importance to interpret how water temperature affects cellular and organismal functions (Bozinovic and Pörtner, 2015; Somero, 2010). Temperature plays an important role in the maturation of oocytes because the process of progressive increase in the diameter of oocytes is strongly dependent on the environmental temperature (Kjesbu et al., 1998; 2010b). Beside temperature, light intensity variations might also explain some of the abovementioned differences observed between the two examined depths. As shown in 1, the deepest water transparency (observed around 5.5 m and recorded in August) was 5 m above the deepest immersed group of 10-12 m. PCA analysis has shown correlation of oocyte characteristics with PC1 scores and transparency with PC2 scores. Photoperiod affects several physiological fish processes, such as melatonin secretion, behavior, and reproduction (Boulcott et al., 2005; Downing, 2002; Downing and Litvak, 2001; Head et al., 2000; Marchesan et al., 2005; Ruchin, 2001, 2004). Even low intensity artificial night light can influence melatonin and gonadotropin...
gene expression in fish (Brüning et al., 2016). The connection between light spectrum and fish physiology lays on brain se-rotonergic and catecholaminergic activity, which is involved in cortisol secretion and in the control of hypothalamic-pitu-itary–inter-renal axis (Hoglund et al., 2001; Øverli et al., 2001; Winberg and Nilsson, 1993; Winberg et al., 1997). Rapid release of stress hormones (catecholamines and cortisol) are caused by both light intensity and temperature fluctuations, and they influence transcription and translation of multiple genes and proteins (Grad and Picard, 2007) in mammals (Murphy et al., 1996; Paroo and Noble, 1999; Udelsman et al., 1993) and fish (Currie et al., 2008). Thus, glucocorticoids can either inhibit or stimulate reproductive physiology depending on the timing of the annual cycle of species (Pottinger and Carrick, 1999). In this study, environmental parameters did not have any effect on the early stages of oocyte maturation, but later differences were observed between the two groups. In the late stages of the reproductive cycle, the number of vitellogenic and atretic oocytes was higher in the 10-12 m fish group. Taken together, these data suggest that lower temperature and lower light intensity promote the oocyte maturation of the gilthead sea bream.

**Conclusion**

Summarizing, we showed here that *S. aurata* exhibits distinct oocyte batches per maturity stage according to qualitative criteria, cell quantification, and estimation of size parameters such as diameter and β factor. Additionally, according to the PCA analysis, it seems that differences in the vitellogenic and atretic oocyte rates depending on the depth indicate a connection between the environmental temperature and light and the onset of spawning. Consequently, under the prism of energy allocation, seasonal growth and energy storage cycles are common in temperate fish and are related to environmental production cycles, usually reflecting the impact of reproduction on physiological condition (Aristizabal, 2007; Callow, 1985; Stearns, 1992).

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