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Incidence of early onset puberty in two-year-old female sea bass, *Dicentrarchus labrax* L.

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ABSTRACT

The incidence of early onset puberty in female aquaculture fish species is less well known than in males and, it merits to be taken into account in order to properly address its outcomes. The goal of this study was to compare the growth performance and reproductive physiology between 2-year-old female sea bass with early or late onset puberty. Fish from seven fertilized egg cohorts of sea bass were considered. Animals were individually tagged and histologically identified when terminally sampled over the first and second year of life. Ovaries from 1-year-old females were in the chromatin nucleolus (CN) or perinucleolar stage (PN), whereas 2-year-old females showed oocytes in the PN stage and early vitellogenesis (EV) (GSI $< 0.7\%$) or late vitellogenesis (LV) (GSI of $4.45 \pm 0.55\%$). Accordingly, 2-year-old females with less advanced reproductive development were considered as fish with late onset puberty (PN, EV), in contrast to their counterparts that were considered to be fish with early onset puberty (LV). Non-spawning females with early onset puberty were observed. The early onset puberty occurred in $18.1 \pm 6.4\%$ of fish in the population, although it was variable among the cohorts. Body size of early pubertal females was usually larger than that of fish with late onset puberty (28.7% heavier in weight and 7.9% greater in fork length). Differences in circulating levels of the insulin-like growth factor-1 (Igf-1) and those of 17-beta estradiol (E_2), follicle-stimulating hormone (Fsh) and vitellogenin (Vtg) one year before spawning, significantly contributed to explaining the total variance associated with the early onset puberty in this species. This study provides valuable information on the interplay that these factors might have at the onset of early puberty in fish and, in turn, its potential use as key indicators of this trait in the female sea bass.

1. Introduction

Puberty in teleosts is the period that marks the transition from sexual immaturity to maturity, and during this time, juvenile fish acquire adult reproductive functions. Puberty in males is characterized by the initiation of spermatogenesis, whereas in females, it is marked by the onset of vitellogenesis (Carrillo et al., 2015; Taranger et al., 2010). Under aquaculture conditions, accelerated growth of juvenile fish often results in the onset of puberty at a younger age. Thus, fast-growing prepubertal fish that exhibit large sizes usually exhibit gonads in advanced stages of development, whereas slow-growing fish that are smaller in size remain immature. The higher growth rates recorded in farmed fish as compared to those of wild fish are believed to be due to unlimited feed availability under aquaculture conditions, which leads to high levels of adiposity and in turn promotes the early onset of puberty (Carrillo et al., 2015; Rowe and Thorpe, 1991; Taranger et al., 2010). In species with short periods of quiescence before the onset of puberty, a certain proportion of fish are able to exhibit early maturation (precocity). This is the case of several teleost species, such as some salmonids, serranides, pleuronectiformes and cyprinids, among others, in which mainly males undergo complete spermatogenesis and spermiation earlier than expected (Begtashi et al., 2004; Campbell et al., 2003; Imsland and Jonassen, 2003, 2005; Rodríguez et al., 2019). In this sense, males are considered to be precocious fish and are initially heavier than their immature

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counterparts during their first year of life. However, they later fail to reach the body weight that fish attain during the subsequent reproductive period at the time of puberty. Therefore, precocious males are small in size at harvest, which has negative side effects for the aquaculture sector, as it compromises harvest efficiency and flesh quality (Aussanasuwannakul et al., 2011; Dupont-Nivet et al., 2010; Felip et al., 2006). The incidence of early onset puberty in females is less well known than in males and, it also merits consideration in fish aquaculture, as the outcomes of variable puberty onset and maturation might prove to be critical in terms of production. In the case of the protandrous barramundi (*Lates calcarifer*), the use of hormonal treatments has been used as an effective strategy to obtain precocious females for the management of broostock in the same generation (Banh et al., 2021). In female longtooth grouper (*Epinephelus bruneus*), oocyte development stages and changes of endocrine regulation factors have been analysed in order to characterize pubertal development progress in this species and control this process in breeding stock (Ryu et al., 2013). In teleosts, oogenesis is a complex and prolonged process by which oogonia transform into ovarian follicles that grow during the sexual reproductive cycle, culminating in ovarian maturation and the ovulation of fertilizable eggs coinciding with the spawning season (Berlinsky et al., 2020; Kagawa, 2013; Lubzens et al., 2010). Ovarian growth and development are controlled by a regulatory network in which several factors play specific roles, both internal and external to the follicle complex. The integration of gonadotropins and steroid hormone signals are known to be crucial for oocyte growth, acting mainly as endocrine hormones in the ovaries. However, other peptides, such as the insulin-like growth factors (Igfs), also play distinct roles in the oocyte growth, as local mediators through autocrine or paracrine signaling in the ovary in both mammals and fish (Chandrashekar et al., 2004; Reinecke, 2010).

The European sea bass (*Dicentrarchus labrax* L.), one of the most important commercial teleost fish raised in the Mediterranean area, is a gonochoristic species, with females usually being larger than males. In captivity, the sex ratio favors males, an important percentage of which mature prematurely at one year of age, which is one year earlier than expected (20–30% of 1-year-old males in the population) (Felip and Piferrer, 2018). Females generally sexually mature at three years of age, although the early onset of puberty also affects them, and some are able to initiate vitellogenesis during their second year of life (Brown et al., 2014; Crespo et al., 2013; Zanuy et al., 2008). Mature egg bearing females have been observed in sea cage populations of sea bass in all size classes (from 350 g to over 1 kg) of harvested fish at 19 months of age (Brown et al., 2014). On the other hand, Crespo et al. (2013) observed that 2-year-old hemi-castrated females that exhibited previtellogenic oocytes in September were able to attain oocyte maturation in February. Currently, the percentage of females with early onset puberty is not well known in this teleost fish. The aim of the present work was to evaluate the incidence of early onset puberty in 2-year-old females and assess the growth history and reproductive performance between fish with early or late onset puberty, from early development until their harvest time at the second year of life.

2. Materials and methods

2.1. Animals and rearing conditions

Seven cohorts of fertilized eggs were collected from the Institute of Aquaculture Torre de la Sal (IATS, Castellón, Spain) on different dates over the course of the spawning season (January-March) during two consecutive years (Table 1). Egg incubation (300 ml of eggs/cohort, 1 ml = 650–750 eggs) and larval rearing were carried out following standard procedures for sea bass aquaculture (Barnabé, 1991). Fish were maintained under natural photoperiod and temperature conditions (annual average 18.9 ± 0.16 ºC) at the IATS facilities (40º N and 0º E) and fed commercial pelleted food (protein 39–42%, lipid 20–24%, carbohydrate 18.8–24.8%, ash 5.8–7.8%, moisture 1.9–3.9%, DE 18.6–19.6 MJ kg^{-1}) (BioMar, Skretting) of an appropriate size to apparent satiety seven days a week (four times per day up to 5 g and twice from this weight onwards). Fish were kept in 2200-l fiberglass tanks supplied with a seawater dispenser on the top and 4–5 oxygen dispensers in the bottom to aerate the seawater (salinity=37–38‰). In order to determine the growth dynamic and reproductive performance of sea bass females in each cohort, a total of 1400 juveniles (> 47 g; approximately 200 fish per cohort and an initial stocking density of approximately 4.27 kg m^{-3}) were individually tagged with passive-integrated transponder (PIT) tags (Avid Mussic Chip Identification System, Inc. CA, USA) at 183 days post hatching (dph). All females in this study were histologically identified when fish were terminally sampled over the course of the first and second year of life. Animals were handled according to the guidelines for animal experiments set out in European legislation (ETS No. 123, 01/01/91).

2.2. Growth performance and body indexes

Fish were anesthetized with MS-222 (0.1 g l^{-1} of sea water) (Sigma, Chemical Co., St. Louis, MO) before each sampling, and the individual weight (W), fork length (L) and Fulton's condition factor (K) of the animals in each cohort were periodically measured. The specific growth rates for weight (SGR_W) and length (SGR_L) were also calculated (Escobar-Aguirre et al., 2020). Growth parameters of sea bass females were tracked to a specific point in time, since fish were individually tagged, based on their degree of ovarian development (Table 1; Supplementary

Table 1
Sample sizes (n) of female European sea bass with a late (L) and early (E) onset puberty during their second year of life used in this study.

					Body indexes / Incidence of early onset puberty[a]			Growth performance (183–708 dph)[b]			Circulating plasma levels (183–708 dph)[c]		
Cohort	Spawn date (m-dd-year)	Hatching date	Type	Initial sample size	n	L	E	n	L	E	n	L	E
1	02. 29.2016	3 March	Natural	200	39	22	17	ND	ND	ND	ND	ND	ND
2	03.03.2017	6 March	Induced	200	79	50	29	20	14	6	15	9	6
3	03.09.2017	12 March	Induced	200	17	13	4	15	13	2	8	6	2
4	03.03.2017	6 March	Induced	200	16	14	2	12	11	1	10	9	1
5	02.24.2017	27 February	Natural	200	14	13	1	8	7	1	ND	ND	ND
6	02.27.2017	1 March	Natural	200	61	59	2	ND	ND	ND	ND	ND	ND
7	02.24.2017	27 February	Natural	200	48	48	0	ND	ND	ND	ND	ND	ND
					274	219	55	**55**	45	10	**33**	24	9

[a] Sample sizes (n) indicate the number of fish used in each cohort for the estimation of body indexes and the rate of early onset puberty in 2-year-old females based on ovarian stages. It included 274 total number of females of which 219 fish were females with late onset puberty (L) (137 fish in the perinucleolar stage and 82 fish in early vitellogenesis stage; see Fig. 2) and 55 fish were females with early onset puberty (late vitellogenesis stage). [b]Sample sizes (n) indicate the number of fish used in Cohort 2, 3, 4 and 5 for the estimation of growth performance from 183–708 days post hatching (dph) (n = 55 total number of fish) between females with a late (n = 45) and early (n = 10) onset puberty. [c]Sample sizes (n) indicate the number of fish used in Cohort 2, 3 and 4 for the estimation of circulating plasma levels from 183–708 dph (n = 33 total number of fish) between females with a late (n = 24) and early (n = 9) onset puberty.

Data Table S1). As necessary, fish were sacrificed using an overdose of anesthetic and the gonadosomatic index (GSI), viscerosomatic (VSI) and hepatosomatic (HIS) indexes were calculated (Escobar-Aguirre et al., 2020). To this end, body indexes were determined in a total of 33 females from Cohort 1 during the first cycle of life (January-February). During the second year of life (November-February), a total of 274 fish from all seven cohorts were sacrificed to calculate these body indexes (Table 1). This time interval was assumed to be the potential putative ovary growth period, one year before spawning, in this species.

2.3. Histology

Small pieces of ovaries (< 0.1 g) were fixed for 24 h in 4% formaldehyde: 1% glutaraldehyde buffered saline for histological analyses (McDowell and Trump, 1976). Gonads were dehydrated in a 70–96% ethanol series, and then embedded in glycol methacrylate resin (Technovit 7100; Heraeus, Kulzer, Germany). Sections of 4 μm were stained with methylene blue/azure II/basic fuchsin (Bennett et al., 1976). Identification and characterization of the stage of oocyte development was determined according to the criteria described by Kagawa (2013) and Sullivan and Yilmaz (2018). Briefly, females were divided into four distinct groups: i) the stage of chromatin nucleolus (designed as the CN stage), ii) the perinucleolar stage of primary oocyte growth (designated as the PN stage), iii) females showing oocytes that were filled with lipid droplets and beginning to accumulate yolk granules peripherally (early vitellogenic oocytes, EV stage) and iv) females having oocytes with both lipid and yolked droplets inside and enclosed in a well-developed zona pellucida, which exhibited late vitellogenesis (designated as the LV stage). Accordingly, 2-year-old females in the LV stage were considered to achieve early onset puberty, whereas females in the PN and EV stages were considered to be fish with late onset puberty. Based on this, the rate of early onset puberty was assessed from ovarian samples taken from individual fish from each cohort (n = 274 total number of fish) during the second year of life (Table 1).

2.4. Circulating hormone level analysis

Blood was collected from the caudal vein, using heparinized syringes and 21-gauge needles. Plasma was separated by centrifugation at 4 °C and stored at − 20 °C until analysed. Plasma testosterone (T) and 17β-estradiol (E_2) were measured by a conventional enzyme immunoassay (EIA) which were validated for sea bass in our laboratory. Levels of sea bass follicle-stimulating hormone (Fsh) and vitellogenin (Vtg) were measured by a homologous competitive enzyme-linked immunosorbent assays (ELISA) developed for this species. Accordingly, circulating T levels were measured according to the method described by Rodríguez et al. (2000). The sensitivity of the assay was 0.028 ng/ml (Bi/B0 = 90%), with inter- and intra-assay coefficients of variability of 9.5% and 6.2%, respectively. Plasma E_2 was measured according to the method described by Molés et al. (2011), and the sensitivity of the assay was 0.201 ng/ml. The inter- and intra-assay coefficients of variability were 9.38% and 6.55%, respectively. The plasma levels of Fsh were determined according to Molés et al. (2012). The assay sensitivity of Fsh was 0.936 ng/ml with inter- and intra-assay coefficients of variability of 5.66% and 7.26%, respectively. Plasma Vtg was measured according to the method used by Mañanós et al. (1994), and the sensitivity of the assay was 3.756 ng/ml. The inter- and intra-assay coefficients of variability were 9.8% and 5.3%, respectively. Plasma Igf-1 was extracted by acid-ethanol cryoprecipitation (Shimizu et al., 2000), and the concentration of Igf-1 was measured by means of a generic fish Igf-1 radioimmunoassay (RIA) validated for Mediterranean perciform fish, according to Vega-Rubín de Celis et al. (2004). The sensitivity of the assay was 0.6 ng/ml to 50% of maximum binding. The inter- and intra-assay coefficients of variability were 7.0% and 3.0%, respectively. Circulating hormone levels were individually determined in 1-year-old females in a total of 33 fish from Cohort 1, including females in the CN (n = 8) and PN (n = 25) stages between 183 and 351 dph. Changes in circulating plasma levels of 2-year-old females were individually analyzed in a total of 33 fish from Cohorts 2, 3 and 4 to compare females with late onset puberty (n = 24) and early onset puberty (n = 9) during the first two years of life (183–708 dph) (Table 1).

2.5. Statistical analysis

Data are represented as the mean ± standard error of the mean (SEM). Growth performance and plasma hormonal levels were analyzed with R-project for mixed ANOVAs. The mixed ANOVAs were run using the function anova_test from the rstatix package, as the fish were repeat-sampled. When normality and homogeneity of variance were violated, a logarithm transformation of the data was performed. Post-hoc analysis was carried out according to Bonferroni's p-adjusted method. Body indexes and the relationship between the GSI and VSI variables were analyzed using IBM SPSS Statistics version 26.0. A bootstrap resampling was used when normality and homogeneity of variance were violated even with a logarithmic transformation of the data. A principal component analysis (PCA) of body weight and plasma levels of E_2, Vtg, Igf-1 and Fsh was used to explain the total variance of the two first components between female sea bass with a late and early onset puberty at 2 years of age, using the PCA function from the FactoMineR and FactoExtra packages of the R-project software. The PCA was validated by performing a MANOVA, using the manova function from the MANOVA.RM package. Statistical differences were considered to be significant when $P < 0.05$.

3. Results

3.1. Biometry, somatic indexes and plasma levels of 1-year-old females

In their first year of life, 24% of sea bass females were in the CN stage, whereas the remaining 76% were in the PN stage of primary oocyte growth. The mean values of GSI (0.16 ± 0.01%) and VSI (4.12 ± 0.25%) in females in the PN stage were significantly higher than in those in the CN stage (0.04 ± 0.01% and 3.14 ± 0.28%, respectively), while HSI was 2.60 ± 0.08% in both groups. No differences in growth performance were observed between females in the two stages, and comparable specific growth rates for weight and length were observed. Females gradually increased in weight from 47.88 ± 4.00 g at 183 dph (September) to 78.88 ± 1.29 g at 378 dph (March), whereas their length increased from 14.81 ± 0.39–18.07 ± 0.16 cm over the same period of time. Changes in the condition factor were similar between females in the CN and PN stages, with values that ranged from 1.28 ± 0.06–1.49 ± 0.03. Circulating levels of Fsh significantly increased in females in the CN stage, from 10.54 ± 1.70 ng/ml at 183 dph to 15.80 ± 2.16 ng/ml at 378 dph, whereas plasma levels in females in the PN stage ranged between 9.43 ± 0.73 ng/ml and 7.30 ± 0.40 ng/ml during the same period. Circulating levels of T and E_2 in females in the CN stage were 0.85 ± 0.11 and 0.85 ± 0.04 ng/ml, respectively, during early development (September). Later, plasma T was 0.53 ± 0.13 ng/ml and plasma E_2 was 0.27 ± 0.06 ng/ml during the first sexual cycle of life. In females in the PN stage, T and E_2 levels were 0.45 ± 0.04 and 0.46 ± 0.03 ng/ml, respectively, and 0.13 ± 0.02 and 0.12 ± 0.02 ng/ml, respectively, for the same periods of time. At this point, no differences were observed in circulating levels of Vtg (< 0.04 mg/ml) and Igf-1 (average value of 8.0 ± 0.34 ng/ml) between females in the two stages.

3.2. Ovarian stages and somatic indexes of 2-year-old females

The percentage of females with oocytes in PN was 50% throughout the putative ovary growth period (November-February), whereas females with oocytes in EV and LV reached 30% and 20%, respectively. The GSI values in females in the PN and EV stages were low and remained low throughout the ovarian development period, whereas GSI

in females in the LV stage attained maximum values in January-February (4.45 ± 0.55%) (Fig. 1A). The dynamic of ovary growth in the females reaching the LV stage differed from that of the remaining stages, although the differences were not statistically significant (Supplementary Data, Fig S1). VSI values decreased throughout the ovarian development period (Fig. 1B), they were significantly lower in females in the LV stage (Fig. 1A). VSI values sharply decreased in females in the LV stage, showing values of approximately 4.82% in November and attaining lower values from December onwards ($P < 0.05$) (Supplementary Data, Fig S1). A regression analysis was performed to examine the interaction between the independent variable logGSI and the three female stages (PN, EV and LV) over the dependent variable logVSI. GSI values significantly increased as VSI values decreased in females in the LV stage (Supplementary Data, Fig S2). Overall, HSI values were similar between females in the EV and LV stages (Fig. 1A), with an increase in mean HSI values until January, which then subsequently decreased (Fig. 1B). The pattern of HSI variation was similar between females in the EV and LV stages, although the values were higher in females reaching the LV stage, leading to early onset puberty in these fish (Supplementary Data, Fig S1).

3.3. Rate of early onset puberty in 2-year-old females

The incidence of females with early onset puberty was variable among the cohorts of sea bass (from 0% to 43.6%), with an average mean of 18.1 ± 6.4% of fish in the population (Fig. 2). Cohorts 1 and 2 showed the highest values of females with early onset puberty (43.6% and 36.7% LV stage, respectively), whereas a more reduced rate was observed in cohorts 3, 4, 5 and 6 (3.3–23.5%). No early pubertal females were observed in cohort 7.

3.4. Growth and reproductive performance in 2-year-old females with early and late onset puberty

There was substantial variation in the means of biometric parameters in each cohort. Females with late onset puberty displayed larger phenotypic coefficients of variation than females with early onset

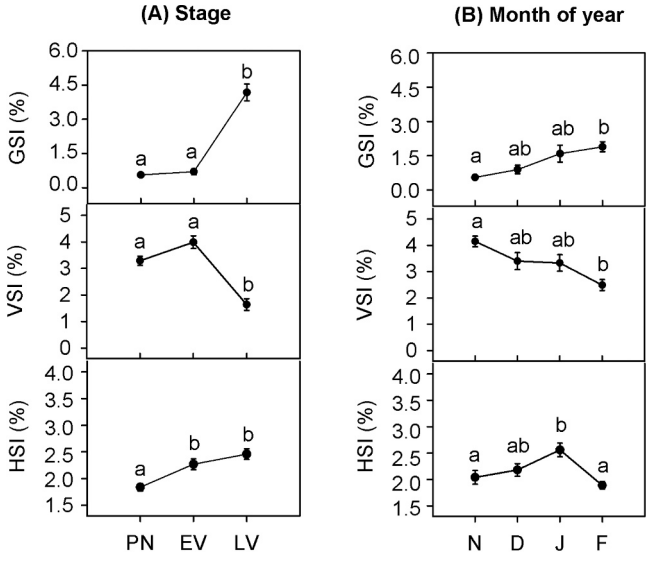

Fig. 1. Changes in gonadosomatic (GSI), viscerosomatic (VSI) and hepatosomatic (HSI) indexes of female European sea bass during the second cycle of life, one year before spawning, according to the ovarian stage (A) and month of the year (B). Females were divided into three stages: primary growth with perinucleolar oocytes (PN), secondary growth with early vitellogenic oocytes (EV) and late vitellogenic oocytes (LV) according to the criteria described by Kagawa (2013) and Sullivan and Yilmaz (2018).

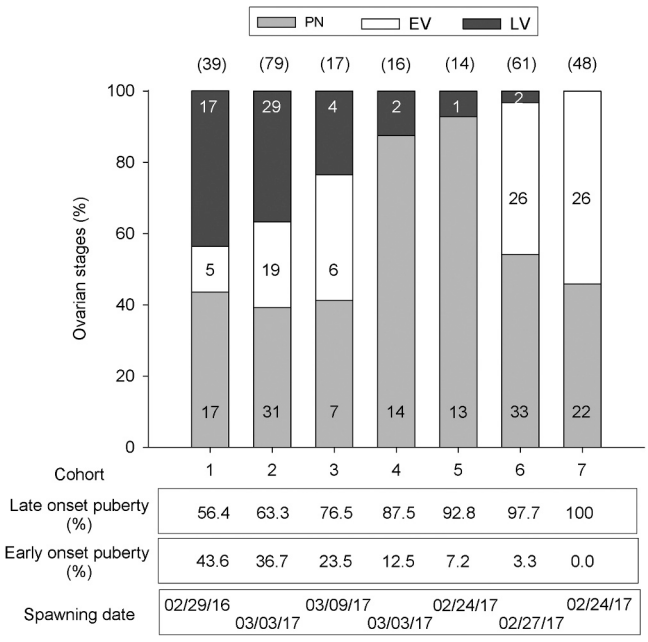

Fig. 2. Percentages of 2-year-old female European sea bass with late (PN, EV) and early (LV) onset puberty. The number of fish sampled in each cohort (November-February) is indicated in brackets. Ovarian stages as described in Fig. 1 and the number of fish in each stage is indicated in each cohort.

puberty (Supplementary Data, Table S1). Growth differences in favor of early pubertal females were observed from the early stages of development, and continued during their second year of life, with early pubertal females reaching greater weight and length, 527.20 ± 57.60 g and 32.20 ± 1.26 cm, respectively, than that of females with late onset puberty (376.13 ± 24.30 g and 29.65 ± 0.58 cm) at 2 years of age (Fig. 2A-B). Despite these divergences in growth, the interaction between the gonadal stage of fish and the month of the year did not show any statistical differences. No differences were found in the SGRw and SGRl between the two groups of females throughout the experiment, independently of the seasonal period considered. During the first year of age, the SGRw and SGRl was 0.45 ± 0.01 and 0.15 ± 0.01%, respectively, whereas values of 0.26 ± 0.04% in SGRw and 0.09 ± 0.01% in SGRl were observed at the end of the second year of age. Variations in K (Fig. 2C) showed a statistically significant interaction between the gonadal stage and age of fish at 427 and 488 dph and from 610 dph onwards ($P < 0.05$). Regarding to the circulating Fsh levels, although the interaction between the gonadal stage of fish and the month of the year did not show any statistical differences, significant higher levels were observed in females with early onset puberty throughout the experiment in comparison to those of their counterparts with late onset puberty according to the stage and age of fish ($P < 0.05$) (Fig. 3A). The temporal profile of Igf-1 did not show any significant differences between females with early and late onset puberty (Fig. 3B), in spite of the plasma levels were higher in early pubertal females from May to September, prior to the start of the putative ovary growth period in this species. Circulating plasma levels of T (Fig. 3C), an androgen considered as the precursor hormone of estrogens (E_2) for oocyte growth and development, were comparable between females in the two groups. Plasma levels of E_2 (Fig. 3D) showed a statistically significant interaction between the gonadal stage and the age of the animals ($P < 0.05$), with early pubertal females attaining the highest values during early development and later coinciding with the potential putative ovary growth period. Finally, circulating plasma levels of Vtg (Fig. 3E) were significantly elevated in early pubertal females, whereas those of females with a late onset puberty remained low throughout the experiment. The PCA showed that > 80% of total variance was explained by the first two

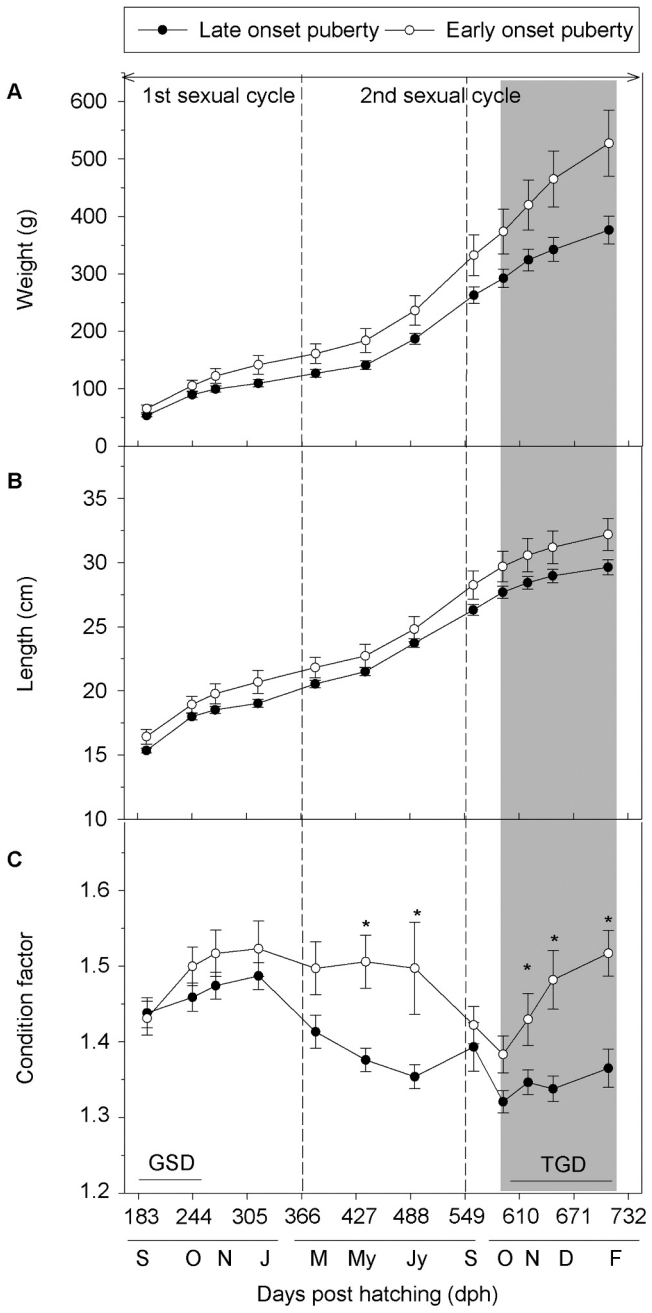

Fig. 3. Body weight (A), fork length (B) and condition factor (C) of female European sea bass with late (closed circles) and early (open circles) onset puberty during the first two years of life. The p-values for body weight showed significant differences acccording to the ovarian stage ($p = 0.033$) and age ($p = 3.68E\text{-}69$) but not for their interaction ($p = 0.228$). For the fork length, significant differences were observed acccording to the ovarian stage ($p = 0.074$) and age ($p = 3.63E\text{-}85$) but not for their interaction ($p = 0.476$), whereas condition factor showed significant differences for all these factors (stage, $p = 0.015$; age, $p = 4.61E\text{-}7$ and interaction, $p = 0.025$ indicated with asterisks). Shaded areas indicate the growth and the sex differentiation period (GSD) and the putative gonadal development (TGD) period, one year before spawning.

components (Fig. 4A) including, the gonadal stage that accounted for the changes along the X-axis (51.3% of total variance) and the Y axis (30.8% of total variance) that accounted for all the factors measured. The percentage of variation explained by each factor was 23% for weight and plasma levels of E_2, 21% for Vtg levels and 19% and 14% for Igf-1 and Fsh levels, respectively. Accordingly, females with an early and late onset puberty clustered into two groups, suggesting that these factors might be considered as non-lethal predictors (markers) of early onset puberty in female sea bass, at least, one year before spawning.

4. Discussion

This study reports that the incidence of early onset puberty in 2-year-old female sea bass is variable among cohorts, with an average value of 18.1 ± 6.4% of fish in the population. Early pubertal females increase their GSI, but they are not able to attain the high values that adult female sea bass have (GSI of 15–16%) during their third year of life when they usually mature under rearing conditions (Rocha et al., 2009). Nevertheless, the initial accumulation and later reduction of visceral fat reserves that occurs in LV females at 2 years of age evokes early onset puberty in fish, suggesting these changes might be enough to satisfy early gonadal growth demands during the putative ovary growth period, one year before spawning, in this species. The relationship that exists between both GSI and VSI variables supports the idea that ovary growth is a dynamic and energetically demanding process in which animals use energy stored prior to the reproductive period to deal with reproductive effort. In addition, the high HSI values that LV females have in comparison to those in the PN and EV stages throughout this critical period, confirm the high plasma levels of E_2 and increased production of Vtg that early pubertal females attain in order to elicit ovarian growth and thus leading to the early onset of puberty.

Under our experimental conditions, 2-year-old female sea bass underwent late vitellogenesis, but non-spawning fish were observed. The fish size attained by early pubertal females in this study might be a limiting factor affecting egg production and release. In agreement with this, precocious female maturation in sea cage operations in Cyprus produced many more spawning fish at larger sizes (>700 g) in this species (Brown et al., 2014). It has been argued that a threshold for body growth is needed to be reached for the initiation of gametogenesis in fish, as gamete production is an energetically demanding process that requires fish to accumulate enough lipid reserves to permit it (Rowe and Thorpe, 1991). In agreement with this notion, it has been reported that the onset of puberty in female zebrafish (*Danio rerio*) is strongly correlated with body growth, but not age (Chen and Ge, 2013). On the basis of the present data, females with an early onset puberty exhibited better body growth and body condition as compared to their counterparts, being 28.7% heavier in weight and 7.9% greater in fork length at the end of their second year of life. Nevertheless, it is probably that a higher threshold needs to be achieved, at least, in females, to fully complete gametogenesis and produce viable gametes. Of note, juvenile male sea bass that show a low weight and small size at the end of their first annual cycle present much reduced gonadal growth, and in turn, they are immature, whereas larger fish as much as 168.40 ± 3.18 g in weight and 22.61 ± 0.13 cm in length are precocious males, which produce and release sperm (Begtashi et al., 2004; Felip et al., 2006). Although both the body energy reserves (and hence body weight) and the metabolic stage of the organism in some way influence the onset of puberty in fish, reproductive needs might differ between sexes. In this sense, a better understanding of the signals involved in the metabolic control of reproduction is necessary in order to control testicular and ovarian growth. Additionally, under aquaculture conditions as well as experimental rearing conditions, where feed availability is unlimited, the accelerated growth of juvenile fish often results in a younger age at puberty with those fast-growing (large-sized) fish being usually mature fish, whereas slow-growing (small-sized) fish remain immature. These differences affecting body size and percentage of early onset of puberty both within and among fish populations (this study) lead to size dispersion and, in turn, a lower biomass. Therefore, this situation should be taken into account in aquaculture, as it might limit the cost effectiveness of farmed sea bass and the profitability of female dominant stocks in this teleost fish, as well the harvest efficiency of other aquaculture species.

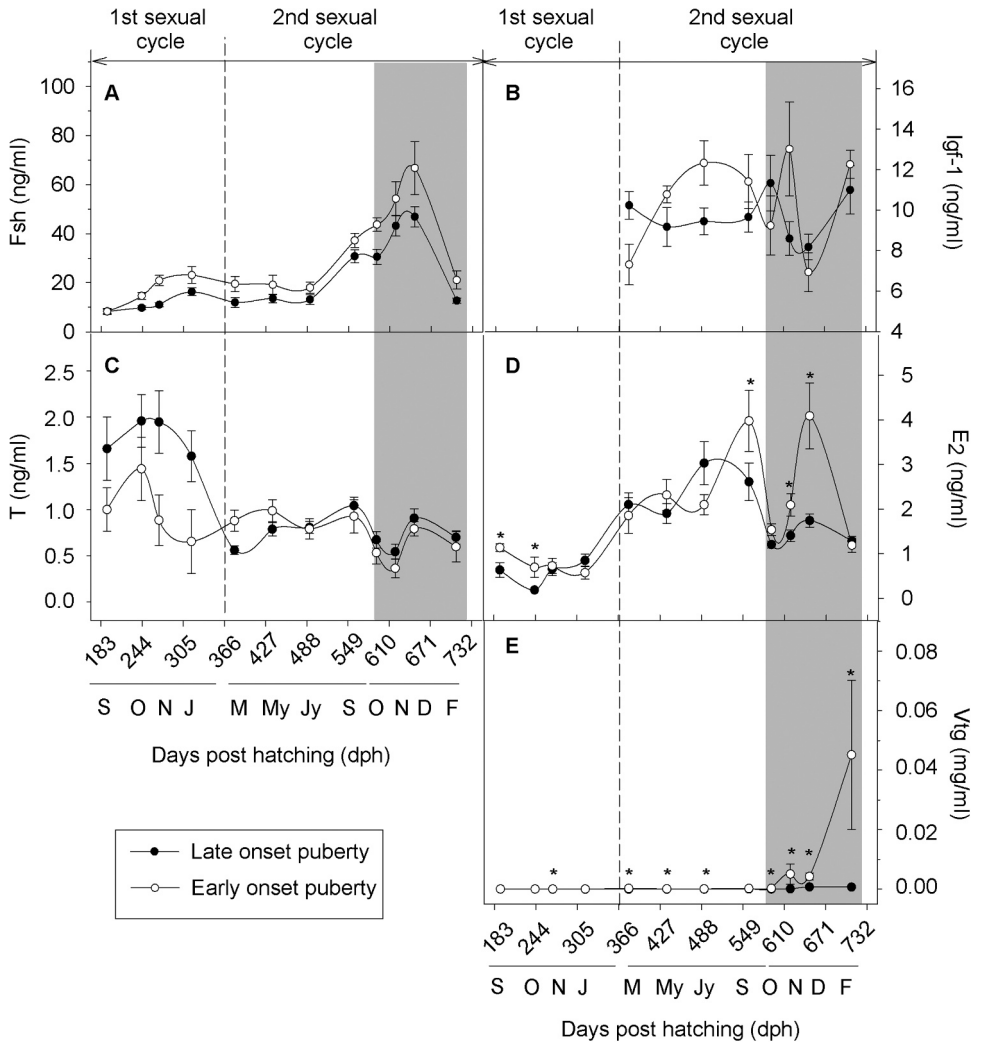

Fig. 4. Changes in the circulating levels of follicle-stimulating hormone, Fsh (A); insulin-like growth factor-1, Igf-1 (B); testosterone, T (C); 17b-estradiol, E_2 (D); and vitellogenin, Vtg (E) between females with late (closed circles) and early (open circles) onset puberty in European sea bass during the first two years of life. The p-values for Fsh showed significant differences acccording to the ovarian stage (p = 5.16E-5) and age (p = 6.58E-24) but not for their interaction (p = 0.598). For Igf-1, significant differences were observed acccording to the age (p = 0.046) but no significant differences were observed for the ovarian stage (p = 0.984) and their interaction (p = 0.054). The p-values for T showed significant differences acccording to the age (p = 9.48E-5) but not for the ovarian stage (p = 0.164) and their interaction (p = 0.056). The p-values for E_2 showed significant differences for all these factors (stage, p = 0.009; age, p = 5.1E-31 and interaction, p = 4.33E-4 indicated with asterisks) as well as for Vtg (stage, p = 3.98E-4; age, p = 3.76E-15 and interaction, p = 3.73E-14 indicated with asterisks). Shaded areas as described in Fig. 3.

These findings highlight the complexity of the maturation-growth relationship in teleosts. Although both traits are interrelated, the control of reproductive and growth axis in fish is multifactorial and it involves the participation of neuropeptides and endocrine hormones, as well as complex molecular mechanisms and gene regulation (Ma et al., 2020), among other factors. Results in male tiger pufferfish (*Takifugu rubripes*) have indicated that although maturation is inheritable, phenotypic variations might be partly due to genotype by environment interactions (Yoshikawa et al., 2019) or certain metabolic circumstances which might influence fish maturation (Carrillo et al., 2015; Taranger et al., 2010). In this sense, a comparison of the temporal profiles of plasma levels between females with a late and early onset puberty has revealed clear differences in some maturity indicators in 2-year-old female sea bass. Our results report that high plasma levels of Fsh during early gonadal development might be an indicator of individual females exhibiting early onset puberty. The Fsh signaling is one key regulator of sexual maturation as it plays a major role in follicular growth and development, and steroid production in teleost fish (Campbell et al., 2006; Luckenbach et al., 2008), including the European sea bass (Molés et al., 2008, 2012, 2021; Rocha et al., 2009). Accordingly, the levels of E_2 showed a significant increase during the putative ovary growth period in early pubertal female sea bass, which stimulated the liver to produce Vtg, and in turn, it promoted oocyte growth (Young et al., 2005). This study also reported a major increase of circulating levels of Igf-1 in females with an early onset puberty prior to the initiation of the putative gonad development period, during which time a significant increase in Fsh, E_2 and Vtg levels was observed. These results are in line with the idea that Igf-1 may play a major role in lipid droplet accumulation in fish, as previously observed in salmonids (Campbell et al., 2006). In addition, the level of plasma Igf-1 is considered to be an important component associated with body size, as it constitutes an important link between the somatotropic and reproductive axes in animals and humans (Chandrashekar et al., 2004). In salmonids, a positive relationship between plasma Igf-1 and other endocrine factors during critical periods of the life cycle suggests that this growth factor is involved in the mechanism by which body growth influences ovary development (Campbell et al., 2006). In cod, genes encoding Igf-binding proteins are upregulated during the late-vitellogenic stage, thus

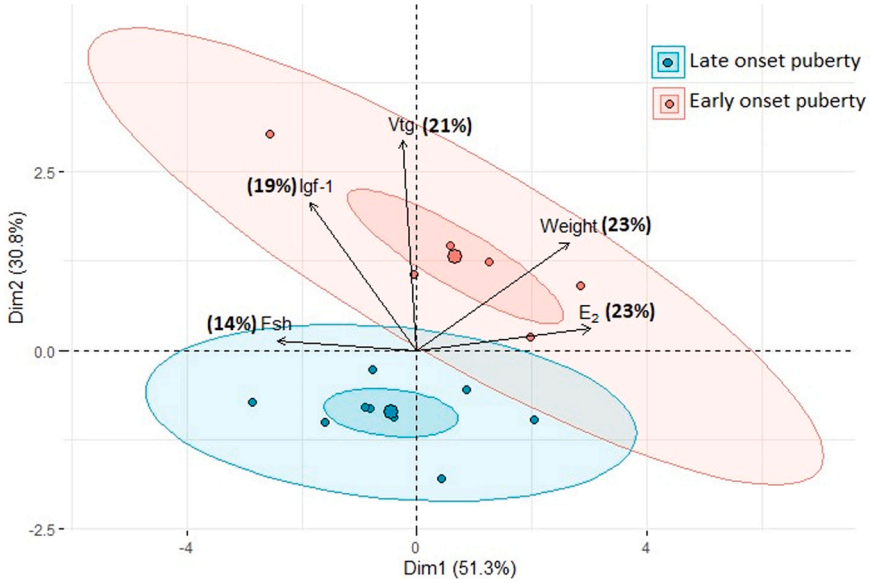

Fig. 5. Biplot of principal component analysis based on key factors measured in female European sea bass. Samples clustered into two groups, the females with late onset puberty (green circles) and those fish with early onset puberty (red circles). Percentage values represent the variation explained by each factor considered (p = 2.71E-05). The ovals represent the concentration ellipse (light color) and the confidence ellipse (dark color) that is ploted around of mean of each group with a security of 95%.

supporting the potential involvement of the Igf system in lipid accumulation in teleosts (Kleppe et al., 2014). Regarding sea bass, primary and lipid vesicle stage follicles have shown elevated *igf1* and *igf2* transcript levels, suggesting that they might be involved in lipid uptake in this species (García-López et al., 2011). So, the role of Igfs as endocrine factors on extra-ovarian sites has been considered in fish (Jia et al., 2019), but it requires further investigation. In zebrafish females, the initiation of puberty provides evidence of the importance of the growth axis in the modulation of fish reproduction (Chen and Ge, 2013). In salmonids, plasma levels of Igf-1, E_2 and Fsh are considered important factors through which body growth is associated with the rate of oocyte development during critical life cycle periods in female coho salmon, *Oncorhynchus kisutch* (Campbell et al., 2006). Furthermore, there is evidence of an upregulation of the fsh receptor, anti-müllerian hormone and gonadal soma-derived growth factor at the cortical alveoli stage, which suggests the role of Fsh and Tgf beta peptides in previtellogenic oocyte growth and the onset of puberty in female salmon (Luckenbach et al., 2008). Accordingly, further studies are necessary for a better understanding of the key factors involved in the crosstalk between the somatotropic and gonadotropic systems in the sea bass to improve aquaculture practices for fish breeding and management in this species.

Based on these findings, changes in the circulating plasma levels in female sea bass during the summer and fall months over the course of the second year of life might represent critical periods for oocyte growth in this teleost fish. The significant contribution of these factors to the total variance associated with the rate of oocyte development in females with late and early onset puberty provides evidence of the possible interplay among body weight and circulating plasma levels of E_2, Vtg, Igf-1 and Fsh during oocyte growth in the sea bass. Although some studies show that Igf-1 plays a role in follicle cell proliferation and oocyte maturation and ovarian steroidogenesis (Weber and Sullivan, 2000), the role of paracrine/autocrine and/or endocrine Igfs in gonad growth still remains unclear in fish. Therefore, in vivo and in vitro approaches are required to understand their specific biological actions as well as the interaction between Igf and gonadotropin-ovary systems during oocyte growth. It will be helpful to reveal the relationship between body growth and sexual maturation in fish in order to define breeding objectives and avoid unwanted side effects and reduced efficiency in aquacultured species (Gjedrem and Rye, 2018).

5. Conclusions

In this study, early onset puberty occurred in 18.1 ± 6.4% of fish in the population, although this percentage was variable among the cohorts of sea bass. Females with an early onset puberty underwent late vitellogenesis but they did not spawn. It was found that they were large-sized fish with higher circulating levels of Fsh, Igf-1, E_2 and Vtg in comparison to those of smaller fish with a late onset puberty. Therefore, these factors could be potentially used as non-lethal markers of the onset of puberty. Early onset puberty evokes both size dispersion and reduced biomass in the sea bass, which leads negative side-effects that needs to be avoided. To maximise commercial gain in the sector, management strategies should be taken into account for the control of the large proportion of fish with early onset puberty. This works provides valuble tools for the identification of this trait in this aquaculture marine fish.

CRediT authorship contribution statement

AF conceived of and supervised the investigation, and also participated in sampling, data processing, histological analysis and paper writing. LS participated in sampling, data processing, statistical analysis and paper writing. SI contributed to fish sampling and hormonal analysis. CM contributed to sampling and larval and fish rearing. GM contributed to the setup of the Fsh ELISA. LS, SP and JR contributed to the statistical analysis. JPS carried out the hormonal analysis of Igf-1. Finally, AF coordinated the work and took primary responsibility for the final content of the manuscript. All authors have read and approved the final manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data will be made available on request.

Acknowledgments

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.aqrep.2023.101834.

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