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FULL LENGTH ARTICLE

Reproductive biology spermatogenesis and biochemical characteristics of male sparid fish *Dentex dentex* from the south eastern Mediterranean coast

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KEYWORDS

Dentex dentex;
Spermatogenesis;
Ultrastructure;
Fatty acids;
Steroid hormones

Abstract The present study focuses on reproductive biology parameters and histological and fine structure investigations of testes maturation, in respect to steroid hormones and fatty acids' profile. All males over 30 cm in length were found to be mature. Gonadosomatic index (GSI) of males increased progressively to reach a peak value in May and June. In *Dentex dentex*, the spermatogonia were detected throughout the year in the peripheral zone of the testes. Spermatocytes are characterized by large nuclei with higher electron density and a layer of cytoplasm. The nuclei of spermatids were characterized by a condensed chromatin material. The early spermatid had a central nucleus and a large number of mitochondria. One big mitochondrion lies beneath the head of the sperm. The seasonal change of serum testosterone correlates with gonadal development. The presence of nearly ripe and ripe male *D. dentex* coincides with the surge of testosterone and the decrease in estradiol continues throughout the spawning periods. In males, higher GSI was accompanied by the highest lipid content of the testis. The high concentration of polyunsaturated fatty acids n-3 (PUFA) for testis, liver and muscles was as follows: docosahexaenoic (DHA, 22: 6 n-3) and eicosapentaenoic (EPA, 20:5 n-3) acids. The higher concentration of polyunsaturated fatty acids n-6 (PUFA) for testis, liver and muscles was as follows: linoleic (18:2 n-6) and arachidonic acids (20:4 n-6) with significant differences in relation to maturation stages at ($P < 0.001$).

Introduction

The common dentex (*Dentex dentex*) is a highly valued table fish in the Mediterranean and the tropical area. Furthermore, it is considered a prime new candidate for the aquaculture species over the last decade. According to the available literature, this

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species has been scarcely investigated, with most of the relevant studies referring to its distribution, growth, feeding (Tibaldi et al., 1996) and reproduction aspects (Pavlidis et al., 2000).

Examination using the microscope is an easy and inexpensive method of estimating the reproductive state of species in situ (TomkieWicz et al., 2003). Several studies revealed that there are discrepancies between microscopic and histological examinations of the gonads and have emphasized the inaccuracy of macroscopic inspection (Costa, 2009; Pavlov et al., 2011; McBride et al., 2013). Clearly, histological examination is important to verify visual observations.

All sexual cycles and gametogenesis of common dentex reared in captivity are lacking or conflicting (Loir et al., 2001). Studying spermatogenesis and spermiogenesis enhances the understanding of the germ cell differentiation and gives fresh insights into the relationship between teleosts groups, especially those of the same family (Medina et al., 2003; Gwo et al., 2006).

Spermatogenesis process is associated with the steroidogenic action of leydig and sertoli cells (Ee-Yung, 2008). Sertoli cells have a high phagocytic activity in the sea bream testis. Besides, they are considered to be the only cell type involved in the phagocytosis of germ cells in this species (Elena et al., 2005).

The essential role of reproductive biology, hormonal and environmental control of spawning and broodstock management toward the development of *D. dentex* for Mediterranean mariculture, was previously presented by Pavlidis et al. (2000).

Hormonal control of the reproductive cycle in *D. dentex* has been studied by Pavlidis et al. (2000). They reported that in common dentex the maturity stages and seasonal fluctuations related differences in serum 18 β-oestradiol testosterone, 11 ketotestosterone, vitellogenin and thyroid hormones that were found during the annual reproductive cycle.

The present study aims to study the reproductive biology of male *D. dentex* in order to give a clear image of the spawning season, length at the first sexual maturity and identification of gonadal maturation using histological and fine structure analysis in respect to steroid hormones, lipid and fatty acids throughout different maturity stages.

Material and methods

D. dentex (180 individuals),ranging in body length from 17 to 60 cm and total weight from 150 gm to 2500 gm, were collected at intervals twice a month during the period from November 2012 to October 2013 from the south eastern Mediterranean at the Alexandria coast. For each sampled fish, the date of capture, total body length (mm), total weight and gutted weight (gm) sex and maturity stage were recorded morphologically (Table 1). Testes were carefully removed and weighed then fixed in 10% formal saline solution. Gonadosomatic index was computed as the percentage weight of the testes to the gutted weight of the fish.

Histological and ultrastructure examinations

For histological studies, the fixed testes were washed in 70% ethyl alcohol then dehydrated, cleared and embedded in paraffin wax. Sections were stained with Ehrlich hematoxylin and Eosin (6 μm thick). For the ultrastructure examination of each testis, small blocks were fixed in 4% buffer glutaraldehyde made in 0.12 M phosphate buffer then in 1% osmium tetroxide and embedded in epoxy resin. Ultra thin sections were prepared with 2% uranyl acetate followed by lead citrate then examined by transmission electron microscope.

Table 1 Morphological characteristics and duration of maturity stages for testes cycle of *D. dentex*.

Identification	Stages of maturity
The testes are of thin width and transparency. This stage is noticed in fish with total length less than 21 cm and is detected throughout the year	I. Immature stage
At this stage the testes start to increase in length and width. This stage is detected along the whole year	II. Maturation Stage
Testes increase in thickness and become whitish in color. This stage is detected in March and April	III. Nearly ripe stage
The testes show maximum development then spawning takes place at intervals, a considerable amount of sexual products was discharged during the course of the spawning. This stage is noticeable along the period from late April to late June	IV. Ripe & running stage
Testes are reduced in size, pinkish in color. This stage is detected in July and August	V. Spent stage

Biochemical studies

Total lipid and fatty acids extractions

Lipids were extracted from the sample by chloroform–methanol–water (2:2:1.8) as described by Bligh and Dyer (1959). Fractions of lipid were methylated to obtain fatty acids methyl esters as recorded by Gallagher et al. (1984). The methyl esters were analyzed by using the Shimadzu gas chromatography 4CM equipped with flame ionization detector.

Hormonal assay

Serum was kept at −20 °C until used. Testosterone, progesterone and estradiol hormones were estimated using HPLC (Wang and Stapleton, 2010). Mean values were calculated ($n = 5$ mean ± SEM; Standard Error of Means).

Statistical analysis

Arithmetic means and standard deviation of numerical data were used to compare more than two groups. They were followed by the ANOVA test then by the LSD method, to calculate the significance between groups at < 0.05.

Results

Biological studies:

Monthly distribution of maturity stages

As shown in Fig. 1 the immature and mature male stages were present during the whole year and their relative abundance

fluctuated continuously reaching a maximum of 66% in October (immature) and 71% in February (mature). Nearly ripe testes started to appear in March (20%) and then increase in April to reach 53%. The ripening and running male dominated in May (82%) and continued in June (77%). The spent stage started to appear from early July (48%) and reached the maximum percentage in August (69%).

Length at first sexual maturity

Males of *D. dentex* smaller than 17 cm were all immature, the mature individuals appeared in the group of 21 cm body length (38%). All males longer than 30 cm were sexually mature (Fig. 2).

Gonadosomatic Index

The monthly fluctuations of the GSI values of *D. dentex* revealed that spawning occurs in May and June, when the mean value of GSI for male reached its highest level (3.53 and 3.9), a considerable drop in July and August (0.31) was then observed. Thereafter, the indices remained almost constant till December (0.36). From January, the value of GSI increased gradually till March and April at the pre-spawning period (1.74) (Fig. 3).

Histological and ultrastructure examination of testes in D. dentex

Histological studies of the testes showed that the course of spermatogenesis and the gradual changes in its development are as follow:

- *Immature stage*: The immature stages are marked by nests of spermatogonia, (Fig. 4a) each spermatogonium ranges from 8 μm to 13 μm in diameter with a spherical nucleus that differs from 5 μm to 8 μm in diameter. Spermatogonia are known from the faintly stained cytoplasm and their homogenous nuclei. The larger spermatogonia have conspicuous boundaries and nuclear outlines with a dark stained nucleolus in the central zone. The small spermatogonia can be distinguished in the electron micrograph by the presence of clusters of chromatin material and scanty faintly stained cytoplasm (Fig. 4b). Sertoli cells were scattered between the interlobular connective tissue. Also, sertoli cells are characterized by faintly stained cytoplasm with small nucleus that contains a granular chromatin material.

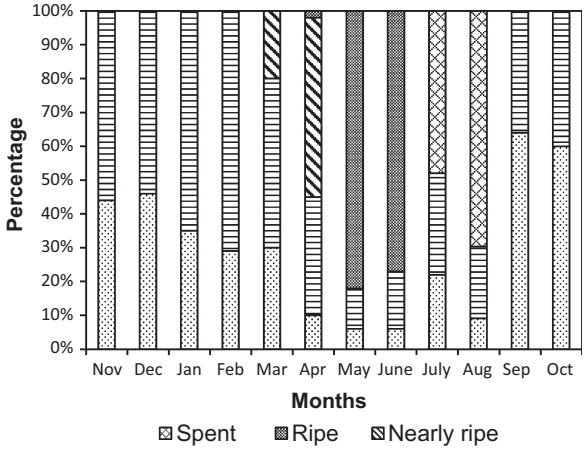

Figure 1 The monthly variation of maturity stages in male *D. dentex* throughout the period from November 2012 to October 2013.

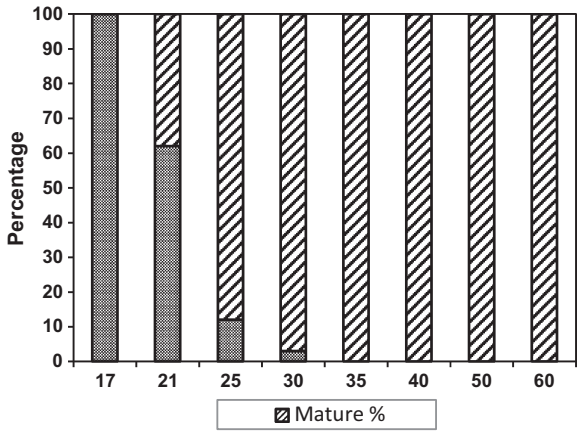

Figure 2 Percentage distribution of immature and maturing individuals of male *D. dentex* throughout the period from November 2012 to October 2013.

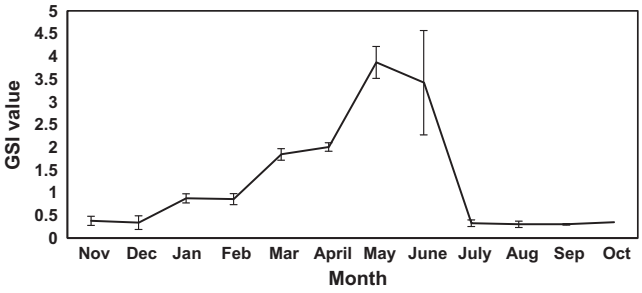

Figure 3 Monthly variation of gonadosomatic indices (GSI) in male *D. dentex* throughout the period from November 2012 to October 2013.

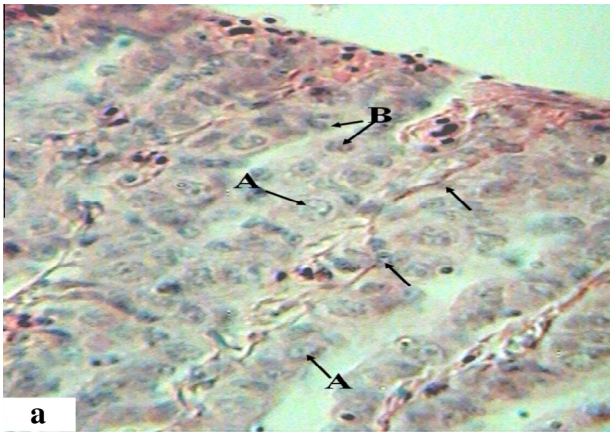

Figure 4a Photomicrograph of a cross section (CS) in testes at immature stage showing, (A) small and (B) large spermatogonia, (arrow) interlobular connective tissue. Staining with Hematoxylin and Eosin (H & E). x 100.

- *Maturation stage*: The maturing testis sets the beginning of active spermatogenesis. Nests of spermatogonia, spermatocytes and a few nests of spermatids can be detected. Concerning the spermatocytes, neither the nucleoli nor the cell membrane can be differentiated using the light microscope. The diameters and the comparatively little cytoplasm helped in distinguishing the primary and secondary spermatocytes. As for their diameters, the primary spermatocytes measured 6 μm and the secondary spermatocytes measured about 5 μm in diameter. The spermatids measured about 2 μm in diameter. Ultrastructurally, they were characterized by their compact and dense chromatin (Fig. 5a). Spermatocytes were oval and had nucleus with clump of centrally located chromatin (as irregular strands) with little chromatin at the nuclear periphery. Microtubules seemed to duplicate on the outside of the nuclear envelope. Ribosomes, large number of mitochondria and endoplasmic reticulum occupied the cytoplasm. Late spermatocytes were characterized by condensed chromatin materials. Well developed sertoli and leydig cells were located near the cysts containing spermatogenic generations, each leydig cell is cuboidal and contains an elongated nucleus. (Figs. 5b and c).
- *Nearly ripe stage*: Spermatogenesis and more active spermiogenesis processes were recognized in the nearly ripe testes at all stages of development (Fig. 6a). All generations of spermatogenesis, nests of primary and secondary spermatocytes, spermatids and a few numbers of spermatozoa were easily detected. A few numbers of spermatogonia were noticed in the periphery of the testes. Ultrastructurally, the spermatids were seen in different stages of spermiogenesis, as indicated by the degree of chromatin condensation. In the early spermatid stage, nuclei are oval or round; they then become smaller and show denser chromatin (Fig. 6b).
- *Ripe and running stage*: Seminiferous tubules showed a fair quantity of spermatozoa with little spermatids (Fig. 7a). Moreover, the morphology of the spermatid nucleus gradually changed. As for the centrioler complex, it appeared laterally to the nucleus as to form the flagellum and some mitochondria that were near the mid piece. The mature spermatozoan is a simple elongated cell composed of a

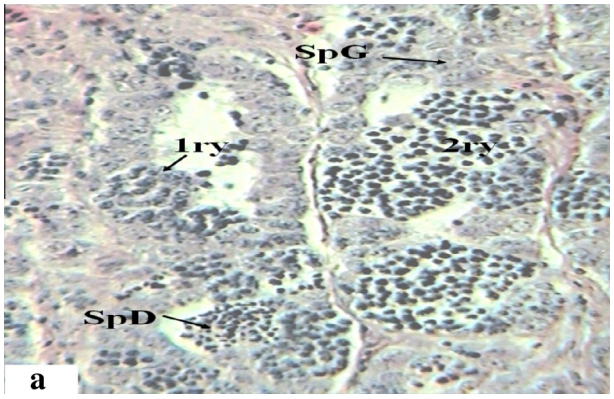

Figure 5a Photomicrograph of a (CS) in maturing testes showing few nestes of spermatids (SPD) and large number of spermatocytes nests (1ry & 2ry) and spermatogonia (spg).(H & E). x 250.

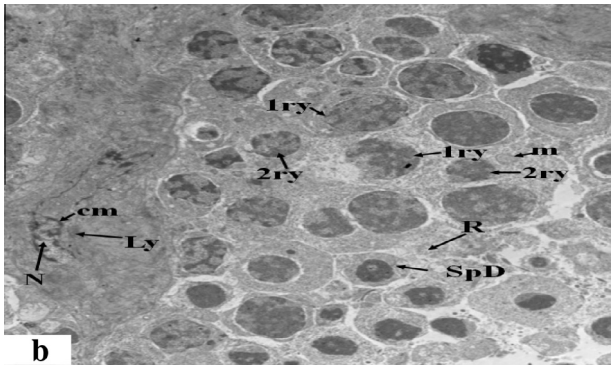

Figure 5b Photo-electrongraph of (CS) in maturing testes showing, (1ry) primary and (2ry) secondary spermatocytes,(SPD) spermatids, (cm) chromatin material, (m) mitochondria (Ly) lydig cell with elongated nucleus (N).

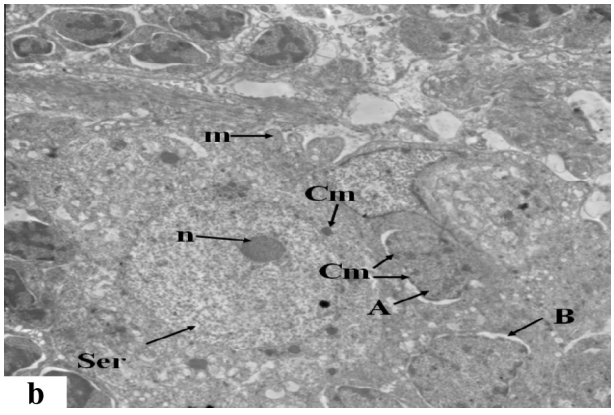

Figure 4b Photo-electrongraph of a (CS) in early spermatogonia of immature testes showing, small (A) and large (B) spermatogonia, chromatin material (cm), mitochondria (m), nucleus (N), sertoli cells (ser) with nucleolus (n). x 1500.

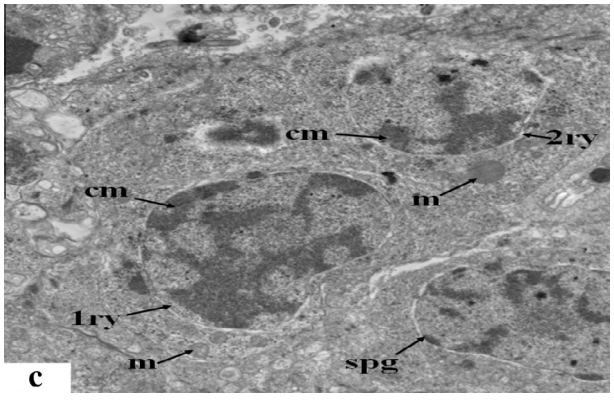

Figure 5c Magnification of spermatogonia (SPG), primary (1ry), secondary (2ry) spermatocytes, chromatin material (cm) and mitochondria (m). x 2500.

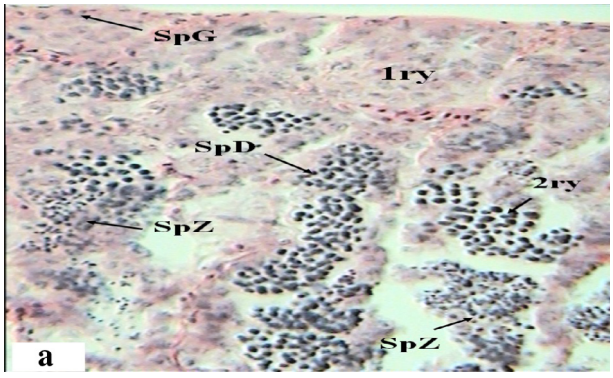

Figure 6a Photomicrograph in (CS) of nearly ripe testes showing few number of spermatozoa nests (spz), spermatogonia (SPG), large number of spermatid nests (spd) and small number of spermatocytes (1ry&2ry).(H & E) x 400.

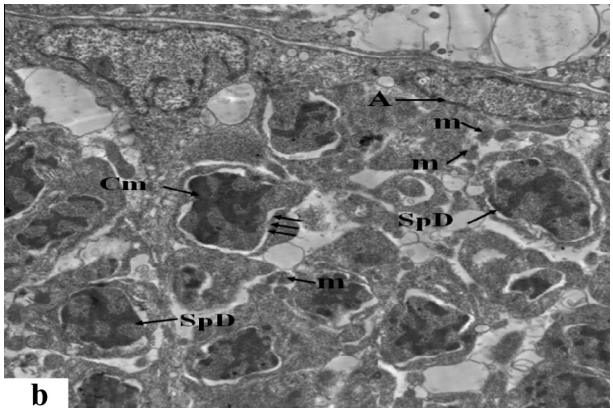

Figure 6b Photo-electrongraph of longitudinal section in late spermatid showing the basal part of spermatid form a depression (arrow), large number of small mitochondria (m) and interlobular connective tissue (A). X10000.

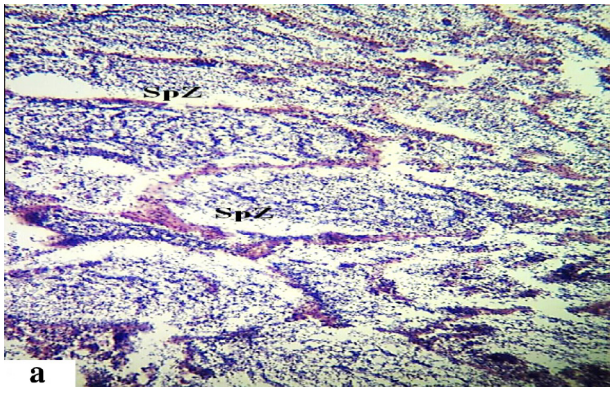

Figure 7a Photomicrograph in (CS) of ripe testes showing, fair quantity of spermatids (spd) and spermatozoa (spz) cells, (H &E) (X400).

head, a short mid piece and a long tail or flagellum (Fig. 7b). The mid piece is short and contains a mitochondrial ring. The flagellum is surrounded by the flagellum plasma membrane. The axoneme consists of nine double outer tubules and two single central microtubular constructions (Fig. 7c). During the running stage, discharge of a considerable quantity of sperms was naturally accompanied by a decrease in the width of the seminiferous lobules. Despite this, there was still a considerable quantity of spermatozoa.

- *Spent stage*: At this stage, the spermatogenesis and spermiogenesis processes completely stopped. The lobules were distorted and vacuolated, they also contained the remains of non-discharged spermatozoa. At the end of the spent period, new generations of spermatogonia and trapped spermatozoa were formed (Fig. 8).

Hormonal control of the reproductive cycle in male D. dentex

Fluctuations of serum steroid hormone and related maturity stages were detected; differences in serum estradiol and testosterone were found during the annual reproductive cycle in male common dentex. The minimum values of estradiol (0.067 ± 0.040 ppm) and testosterone (1.413 ± 0.387 ppm) concentration are found during the spent period (July and August) (Table.2).

According to the seasons, the androgens of the male common dentex (testosterone) serum levels were well correlated with gonadal development, with the exception of those in the immature males (32.35 ± 1.959 ppm). Gradual increase in serum testosterone levels was recorded in mature males (9.22 ± 1.034 ppm) with a slight increase in nearly ripe males (13.737 ± 1.56 ppm). It reached the maximum concentration in spermiating males (49.20 ± 2.96 ppm), but decreased again to its minimum levels for spent males (1.413 ± 0.387 ppm). A gradual decrease was recorded in male serum estrogen (estradiol) values, they started in mature males (4.737 ± 0.512 ppm) and showed a slight decrease in pre-spawning males (3.347

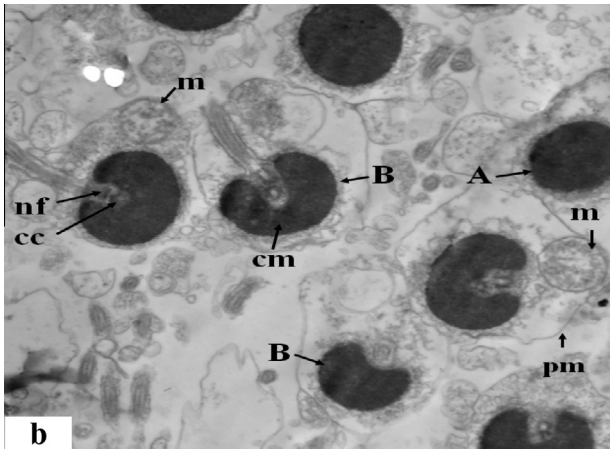

Figure 7b Photo-elecrongraph in (CS) of ripe testes showing, TS in spermatozoa (A), LS in spermatozoa (B), chromatin material (cm), mitochondria (m), centriolar complex (CC) plasma membrane (PM),and the nuclear fossa (nf). X 4000.

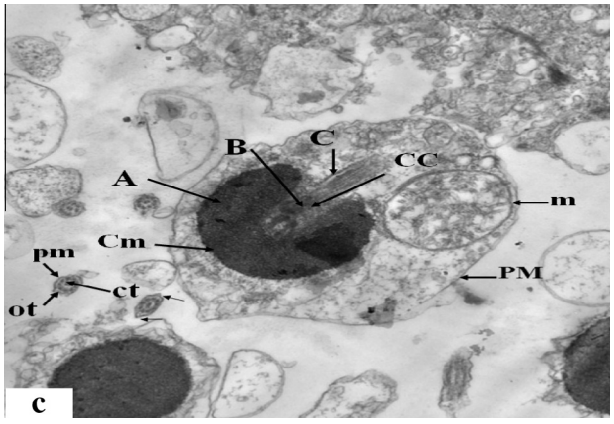

Figure 7c Photo-electongraph of longitudinal section (LS) in spermatozoa showing the bullet shape head (A) large mitochondria (m), midpiece (B) and tail region (C). centriolar complex (CC), plasma membrane (PM), central microtubule (CT) and outer microtubule (OT) with two lateral projections (arrow) .X 50000.

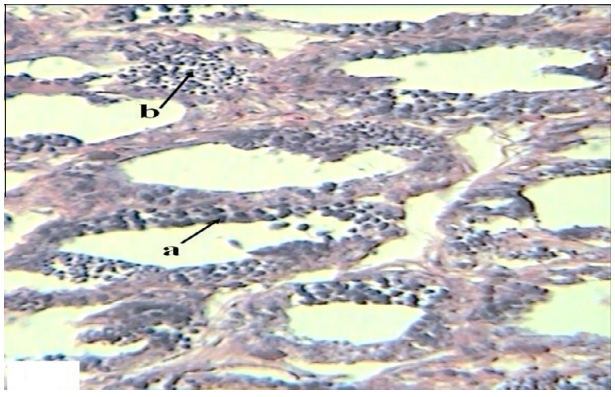

Figure 8 Photomicrograph of (CS) of early spent testes showing (a) new generation of spermatogonia and (b) trapped spermatozoa. (H &E) X 250.

Table 2 Fluctuation in serum steroid hormones (Estradiol and testosterone) in male *D. dentex* in relation to maturation stages throughout a period from November 2012 to October 2013. Values within a row with different superscript letters were significantly different at ($P \leqslant 0.05$).

Estradiol (ppm)	Testosterone (ppm)	Stages of maturity
2.27 ± 0.626[a]	32.35 ± 1.959[a]	Immature
4.737 ± 0.512[b]	9.22 ± 1.034[b]	Maturing
3.347 ± 0.715[b]	13.737 ± 1.56[b]	Nearly ripe
2.707 ± 0.451[a]	49.20 ± 2.96[c]	Ripe & running
0.067 ± 0.040[c]	1.413 ± 0.387[d]	Spent
24.65	22.98	F
0.0001*	0.001*	p

± 0.715 ppm), then another decrease in spermiating male (2.707 ± 0.451 ppm). They decreased to the lowest levels in spent males (0.067 ± 0.040 ppm).

Total lipid and fatty acids contents of testis, liver and muscles of D. dentex and their relationship to the maturity stages

The total lipid content in the testis, liver and muscles of *D. dentex* fluctuated in relation to stages of maturity to reach a maximum value in the nearly ripe testis (5.1 ± 0.52 mg/100 gm), while the minimum values were noticed in the muscles of maturing and livers of immature fish (0.1 ± 0.013 mg/100 gm) as shown in Table 3.

The maximum contents of saturated fatty acid were detected in palmitic acid (C16:0) at all maturation stages of testis, liver and muscles with significant correlation at $P < 0.001$. The main concentration of monounsaturated fatty acid for testis, liver and muscles was recorded in palmitoleic (C16:1) and oleic (C18:1 w9). The highly concentrated polyunsaturated fatty acids n-3 (PUFA) for testis, liver and muscles are docosahexaenoic (DHA, 22: 6 n-3) and eicosapentaenoic (EPA, 20:5 n-3) acids. The highly concentrated polyunsaturated fatty acids n-6 (PUFA) for testis, liver and muscles were linoleic (18:2 n-6) and arachidonic (20:4 n-6) acids with significant differences in relation to maturation stages at ($P < 0.001$) as shown in (Table 4a–c).

Discussion

The present study focuses on reproductive biology parameters, histological and ultrastructure investigations of testes maturation in respect to steroid hormones and fatty acids profile. Morphology, spermatogenesis, cyclic changes and fine structures during spermatogenesis are investigated in many teleost fish by many authors like: perch by Lahnesteinr et al. (1995); *Caranx crysos* and *Pagellus erythrinus* by Assem (1999, 2003); *Merluccius merluccius* by Al-Absawey (2008) and *Diplodus cervinus cervinus* by Abou-Shabana (2012).

The pattern of the development of testes in this study (immature, maturation, nearly ripe, ripe – running and spent) conforms to that shown for most teleosts as reported by Zaki et al. (1995) and Assem (2003).

Monthly distribution of maturity stages revealed that *D. dentex* have short spawning periods that extend from late April to late June. *D. dentex* showed simple and smooth testes surfaces throughout the year. Diverse forms and appearances of teleost testes have been reported by several investigators during the breeding season. Zaki et al. (2005) and Priyadharsini (2013) observed a simple and smooth surface in the testes of *Boops boops* and *Pterois volitans* respectively. Assem (1999) observed lobulation in the testes of carangid male *C. crysos*.

In the present study, all males over 30 cm in length were found to be mature. All male *D. dentex* smaller than 17 cm were found to be immature. Loir et al. (2001) stated that all *D. dentex* fish had differentiated gonads at the age of 12 months; the maximal weight of the gonads increased with age with a reaction of 70–75 g in 46-month-old fish.

Assem (2003) stated that all male *P. erythrinus* longer than 18 cm were sexually mature. Al-Absawey (2008) pointed that all male *M. merluccius* over 34 cm in length were mature. Therefore, it is clear that length at first sexual maturity varies according to species.

In *D. dentex*, the results obtained from monthly fluctuations in the GSI agree with the findings obtained from the monthly fluctuations of maturity stages. Thus, it was observed that the GSI of males increased progressively to reach a peak

Table 3 Average total lipid content (mg/100 gm) ± SD in testis, liver and muscles of *D. dentex* throughout the period from November 2012 to October 2013. Values within a row with different superscript letters were significantly different at ($P \leqslant 0.05$).

F	*p*	Total lipid content (mg/100 gm) ± SD					Tissue
		Spent	Ripe & running	Nearly-Ripe	Maturing	Immaturation	
16.52	0.001*	4.1 ± 0.42^{c}	0.5 ± 0.05^{b}	5.1 ± 0.52^{c}	0.6 ± 0.05^{b}	8.6 ± 0.91^{a}	Testes
4.25	0.012*	0.35 ± 0.032^{b}	0.5 ± 0.042^{c}	0.4 ± 0.032^{bc}	0.3 ± 0.041^{b}	0.1 ± 0.013^{a}	Liver
12.6	0.001*	0.1 2 ± 0.013^{b}	0.12 ± 0.01^{b}	1.1 ± 0.12^{c}	0.1 ± 0.013^{b}	0.6 ± 0.035^{a}	Muscles

value in May and June. An increase in the percentage of ripe individuals toward the spawning season was also spotted. In agreement with our GSI results, several authors mentioned that for many species there is only one peak during the spawning period: Zaki et al. (1995) for *Oblada melanura*, Loir et al. (2001) for *D. dentex*, Assem (2003) for *P. erythrinus*, Al-Absawey (2008) for *M. merluccius* and Priyadharsini (2013) for *P. volitans*.

Spermatogenesis had progressively taken place during the annual reproductive cycle. The seasonal testicular cycle in the *D. dentex* was divided into five stages: 1. Immature, 2. Maturation, 3. Nearly ripe, 4. Ripe and running stage, and 5. Spent stage. The spermatogenic activity in *D. dentex* started in late April. Throughout the period from late April to late June, the testes were in the ripe and spawning stages. From early July to late August, most of the testes were in spent and recovery stages. From August to February, the spermatogenesis passed through a period of quiescence.

Loir et al. (2001) indicated that in some male *D. dentex* the spermatogenic activity resumed gradually throughout the period, lasting from post spawning period until the following January. Assem (2003) stated that throughout the period from late July to November, most of the testes of *P. erythrinus* were in the spent and recovery periods and the spermatogenesis passed through a period of quiescence.

Al-Absawey (2008) found that spermatogenesis of *M. merluccius* passed through a period of resting (from October to January). Abou-Shabana (1998) did not find a quiescent period in the testes of *Diplodus cervinus cervinus*, spermatogonial proliferation throughout winter and summer months.

In *D. dentex*, spermatogonia were detected throughout the year in the peripheral zone of the testes. However, they reduced greatly in number during the breeding season. The position of the spermatogonia was reported to be common in many fishes *e.g.* Assem (2003) for *P. erythrinus*, Al-Absawey (2008) for *M. merluccius,* and Abou-Shabana (2012) for *Diplodus cervinus cervinus*.

The spermatogonia are large rounded cells with electron lucent cytoplasm and large round to oval nucleus. Primary spermatogonia are larger in size than secondary spermatogonia. Distinctive changes were observed in *the D. dentex* spermatogonia as they developed into spermatic cysts, especially in the nuclei that illustrated electron lucent nucleoplasm with a few clumps of electron dense chromatin.

D. dentex spermatocytes are characterized by large nuclei with higher electron density and the appearance of synaptonemal complex displaying diplosomal structure. Grier (1992) proved that sertoli cells and primary spermatogonia are always sequestered from interstitial tissues. The current results coincide with the findings on *Boleophthalmus pectinirostris* by Ee-Yung (2008).

In *D. dentex*, fine structures of the spermatocytes nuclei are known by the appearance of clumps of chromatin and a thin layer of cytoplasm; the spermatids nuclei also had a condensed chromatin material. This conforms with what had been previously observed by Assem (2003) and Al-Absawey (2008).

Young spermatid of *D. dentex* has proven to possess a central nucleus and scattered mitochondria throughout the cytoplasm. The distal centriole differentiates into the basal body that rises to the flagellum.

Depending on the orientation of the centriolar complex to the nuclear fossa, Mattei (1991) classifies teleostean sperm into two types I and II. In type I, the centriolar complex lies inside the nuclear fossa. *D. dentex* centriolar complex lies deeply inside the nuclear fossa (type I) as pre-mentioned in other sparid fish (Zaki et al., 2005 and Abou-Shabana, 2012). However in type II the centriolar complex lies outside the nuclear fossa.

D. dentex has one big mitochondrion that lies beneath the head of the sperm as reported in other sparids; this is a characteristic feature of this family (Gwo et al., 2004; Zaki et al., 2005; Abou-Shabana, 2012). The flagellum of *D. dentex* has the common microtubular structure 9 + 2 which is observed in many teleosts (Assem, 2003; Al-Absawey, 2008; Abou-Shabana, 2012).

Seasonal changes of serum testosterone are well correlated with gonadal development. The presence of nearly ripe and ripe male *D. dentex* coincides with the surge of testosterone and the decrease in estradiol that lasted until the end of the spawning period. In agreement with the present results, Fostier et al. (2000) indicated that serum 11Kt and testosterone levels were higher in male 3 and male 4 *D. dentex*. That's why results were in accordance with the established role of androgens in initiating and maintaining testicular development. The high level of serum testosterone during immature male *D. dentex* may be related to recruitment (proliferation) of germ cells as indicated by Heidari et al. (2010) for *Rutilus frisii kutum*. Rinchard et al. (1993) mentioned that in other teleosts such as gudgeon, and *Gobio gobio* there was not any decrease in the E_2 level during maturation.

In the present study on male *D. dentex*, the serum testosterone reached its maximum concentration value in ripe male, whereas the estradiol showed a much lower concentration level; this comes in agreement with the present results. Abou-Shabana et al. (2012) stated that the levels of plasma steroids in the male *Argyrosomus regius* showed a fluctuation in the mean concentration as the testosterone had showed a massive increase during testes maturation and ripening while the plasma progesterone and estradiol levels displayed different patterns in the mean concentration during the developmental stages.

In male *D. dentex*, higher GSI at maturing, pre-spawning and ripe-running was accompanied by higher lipid content of testis, which indicates the transport of energy resources to

Table 4 Average total fatty acids content (mg/100gm) ± SD in (A)testes, (B) liver and (C) muscles of *D. dentex* throughout the period from November 2012 to October 2013. Values within a row with different superscript letters were significantly different at ($P \leqslant 0.05$).

Stages of maturity											
p	V Spent		IV Ripe & Running		III nearly ripe		II maturing		I immature		Fatty acid
	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	
A – Testes											
											Saturated fatty acid
0.001*	0.15	1.5[a]	0.02	0.2[a]	0.03	0.3[ab]	0.01	0.1[b]	0.05	0.5[a]	C6:0
0.001*	0.63	5.2[b]	0.22	2.1[a]	0.12	1.3[a]	0.16	1.5[a]	0.25	2.0[a]	C8:0
> 0.05	0.04	0.4	0.01	0.1	0.00	0.04	0.01	0.1	0.00	0.02	C10:0
> 0.05	0.02	0.2	0.00	0.04	0.00	0.02	0.01	0.1	0.00	0.01	C11:0
0.032*	0.05	0.4[b]	0.06	0.5[b]	0.01	0.1[a]	0.05	0.5[b]	0.01	0.08[a]	C12:0
0.022*	0.17	1.6[c]	0.02	0.2[b]	0.01	0.09[a]	0.07	0.7[b]	0.01	0.1[a]	C13:0
0.001*	0.10	0.9[b]	0.55	5.3[a]	0.53	5.5[a]	0.11	1.1[b]	0.57	4.6[a]	C14:0
0.001*	0.09	0.9[a]	0.14	1.4[a]	0.84	6.9[b]	0.08	0.8[a]	0.10	0.9[a]	C15:0
0.001*	1.71	17.1[b]	2.08	18.1[b]	2.28	24.6[a]	2.33	21[a]	2.72	26.7[a]	C16:0
> 0.05	0.05	0.5	0.06	0.6	0.06	0.6	0.05	0.5	0.08	0.7	C17:0
0.001*	1.41	13.3[b]	1.27	11.7[ab]	1.01	9.3[a]	1.15	10.9[a]	0.88	9.1[a]	C18:0
> 0.05	0.33	3.1	0.36	3.9	0.21	2.1	0.26	2.1	0.20	2	C20:0
											Monounsaturated FA
0.002*	0.12	1[b]	0.04	0.4[a]	0.02	0.2[a]	0.04	0.4[a]	0.02	0.2[a]	C14:1
> 0.05	0.05	0.5	0.08	0.6	0.01	0.1	0.04	0.4	0.03	0.3	C15:1
0.001*	0.12	1.1[c]	0.67	5.4[b]	0.89	8[a]	0.31	3.3[b]	0.81	7.8[a]	C16:1
0.001*	0.11	1.1[a]	0.11	1.1[a]	0.02	0.2[b]	0.14	1.1[a]	0.12	1.1[a]	C17:1
0.001*	0.51	4.6[b]	0.63	6.9[b]	0.53	4.8[b]	0.98	9.5[a]	0.96	8[a]	C18:1ω9c
0.001*	0.00	0	0.41	4.3[a]	0.20	1.9[b]	0.38	3.9[a]	0.00	0	C20:1
0.001*	0.00	0	0.20	2[c]	0.95	8.2[b]	0.00	0	0.09	0.9[b]	C22:1
											Polyunsaturated FA
0.001*	0.55	5.8[c]	1.11	9.2[b]	1.44	15.6[a]	0.84	8.6[b]	1.66	16.3 [a]	C18:2ω6c
> 0.05	0.02	0.2	0.01	0.1	0.05	0.5	0.02	0.2	0.05	0.5	C18:3ω3
0.001*	0.00	0	0.00	0	0.33	3.3	0.00	0	0.00	0	C20:2
0.001*	1.41	14.8[c]	0.47	4[a]	0.22	1.9[a]	0.28	2.7[a]	0.40	3.8[a]	C20:3ω3
0.001*	0.00	0	0.00	0	0.00	0	0.00	0	0.17	1.9	C20:3ω6
0.036*	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0.02	C20:4ω6
> 0.05	0.04	0.4	0.06	0.6	0.02	0.2	0.02	0.2	0.02	0.2	C20:5ω3
0.001*	0.00	0	0.00	0	0.94	8.2[b]	0.00	0	0.31	3.1[a]	C22:2
0.001*	3.09	25[b]	2.41	21.2[b]	0.24	2.0[c]	2.81	30.3[b]	0.89	8.5[a]	C22:6ω3
> 0.05	5.48	45.5	5.14	44.2	4.32	44.9	4.02	39.4	5.64	47.4	SFA
> 0.05	5.40	54.5	5.47	55.8	5.30	55.1	5.61	60.6	5.21	52.6	UFA
0.0021*	0.83	8.3[b]	2.59	20.7[a]	2.13	23.4[a]	1.74	18.6[a]	2.03	18.3[a]	MUFA
> 0.05	4.71	46.2	3.34	35.1	3.83	31.4	4.00	42	3.77	34.3	PUFA
0.001*	4.16	40.4[d]	3.24	25.9[b]	0.43	4.6[c]	3.41	33.4[b]	1.57	13[a]	PUFAs-ω3
0.001*	0.69	5.8[c]	1.14	9.2[b]	1.49	15.6[a]	0.91	8.6[b]	2.25	18.22[a]	PUFAs-ω6
B – Liver											
											Saturated fatty acid
0.01*	0.11	0.9[b]	0.09	0.9[b]	0.00	0	0.01	0.1[b]	0.04	0.4[a]	C6:0
0.002*	0.55	6[d]	0.82	6.7[d]	0.12	1[c]	0.02	0.2[b]	0.23	2.3[a]	C8:0
0.013*	0.03	0.3[a]	0.03	0.3[a]	0.06	0.6[b]	0.01	0.1[a]	0.01	0.1[a]	C10:0
> 0.05	0.01	0.1	0.01	0.1	0.02	0.2	0.00	0.03	0.00	0.03	C11:0
0.012*	0.03	0.3[a]	0.07	0.6[b]	0.05	0.5[b]	0.01	0.1[a]	0.01	0.1[a]	C12:0
0.036*	0.10	0.8[b]	0.01	0.1[a]	0.06	0.6[b]	0.04	0.4[b]	0.01	0.07[a]	C13:0
0.001*	0.14	1.2[b]	0.49	4.1[c]	0.25	2.3[b]	0.26	2.6[b]	0.02	0.2[a]	C14:0
0.011*	0.05	0.4[b]	0.13	1.4[a]	0.16	1.8[a]	0.10	1[a]	0.10	0.9[a]	C15:0
0.001*	1.92	20.7[a]	2.33	19.1[a]	2.47	24.7[a]	5.18	46.6[b]	2.79	25.4[a]	C16:0
> 0.05	0.08	0.7	0.10	1	0.06	0.5	0.11	1.2	0.08	0.7	C17:0

Table 4 (*continued*)

Stages of maturity											
p	V Spent		IV Ripe & Running		III nearly ripe		II maturing		I immature		Fatty acid
	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	
0.001*	1.03	9[a]	0.98	10.3[a]	0.93	10.2[a]	2.59	20.7[b]	1.14	9.8[a]	C18:0
0.001*	0.19	2[a]	0.12	1.1[a]	0.03	0.3[b]	0.04	0.4[b]	0.18	1.9[a]	C20:0
											Monounsaturated FA
> 0.05	0.05	0.4	0.03	0.3	0.15	1.5	0.03	0.3	0.02	0.2	C14:1
0.023*	0.02	0.2[a]	0.05	0.5[a]	0.17	1.5[b]	0.03	0.3[a]	0.02	0.2[a]	C15:1
0.001*	0.28	2.6[c]	0.48	4.8[b]	0.43	4.2[b]	0.56	4.8[b]	0.75	7.6[a]	C16:1
0.022*	0.10	1[b]	0.03	0.3[b]	0.02	0.2[b]	0.09	1[b]	0.12	1.2[b]	C17:1
0.001*	0.48	4.9[a]	0.55	6.1[a]	1.11	9.3[b]	0.75	6.1[a]	0.61	6[a]	C18:1ω9c
0.001*	0.73	6.4[b]	0.41	3.9[a]	0.00	0	0.14	1.5	0.11	1	C20:1
> 0.05	0.00	0	0.00	0	0.00	0	0.01	0.1	0.08	0.9	C22:1
											Polyunsaturated FA
0.001*	0.59	5.1[b]	0.77	7.7[b]	0.59	6.3[b]	0.84	9.1[b]	1.89	20.2[a]	C18:2ω6c
> 0.05	0.02	0.2	0.02	0.2	0.05	0.5	0.03	0.3	0.04	0.4	C18:3ω3
–	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	C20:2
0.001*	0.44	4.5[a]	0.32	3.3[a]	0.82	8[c]	0.04	0.3[b]	0.42	3.5[a]	C20:3ω3
0.001*	0.00	0	0.00	0	0.00	0	0.00	0	0.16	1.7	C20:3ω6
–	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	C20:4ω6
> 0.05	0.02	0.2	0.03	0.3	0.00	0	0.12	1.1	0.02	0.2	C20:5ω3
> 0.05	0.00	0	0.00	0	0.00	0	0.02	0.2	0.00	0	C22:2
0.003*	3.30	32[d]	2.55	26.8[c]	2.77	25.8[c]	0.14	1.5[b]	1.78	14.4[a]	C22:6ω3
0.001*	3.97	42.5[a]	4.87	45.8[a]	4.03	42.7[a]	6.92	73.4[b]	5.06	42.5[a]	SFA
0.0032*	6.85	57.5[a]	5.42	54.2[a]	5.51	57.3[a]	3.13	26.6[b]	7.01	57.5[a]	UFA
> 0.05	1.45	15.5	1.71	15.9	1.65	16.7	1.42	14.1	2.06	17.1	MUFA
0.001*	4.83	42[a]	4.79	38.3[a]	4.23	40.6[a]	1.21	12.5[b]	4.75	40.4[a]	PUFA
0.001*	3.88	36.9	2.94	30.6	3.99	34.3	0.32	3.2	1.78	18.5	PUFAs-ω3
0.0051*	0.50	5.1[b]	0.92	7.7[b]	0.72	6.3[b]	0.91	9.1[b]	2.52	21.9[a]	PUFAs-ω6
C – Muscles											
											Saturated fatty acid
0.036*	0.05	0.5[b]	0.00	0	0.04	0.3[a]	0.00	0	0.01	0.1	C6:0
0.012*	1.38	14.2[c]	0.05	0.4[b]	0.21	2.3[a]	0.10	1[b]	0.43	3.4[a]	C8:0
0.036*	0.26	2.7[b]	0.03	0.3[a]	0.01	0.1[a]	0.06	0.6[a]	0.02	0.2[a]	C10:0
0.042*	0.06	0.7[b]	0.02	0.2[a]	0.00	0.03[a]	0.05	0.5[b]	0.01	0.1[a]	C11:0
0.015*	0.12	1.3[b]	0.05	0.5[a]	0.01	0.1[a]	0.08	0.9[b]	0.01	0.1[a]	C12:0
0.001*	0.12	1.2[a]	0.15	1.6[a]	0.02	0.2[c]	0.39	4.1[b]	0.11	1.1[a]	C13:0
0.001*	0.32	3.1[e]	0.25	2.7[d]	0.61	6.7[c]	1.53	13.5[b]	0.09	0.8[a]	C14:0
0.013*	0.33	3.3[b]	0.20	1.7[a]	0.14	1.2[a]	0.49	4.8[b]	0.07	0.7[a]	C15:0
0.001*	2.30	19.1[a]	1.87	18.1[a]	3.45	30[b]	2.27	18.8[a]	2.61	23[a]	C16:0
> 0.05	0.00	0	0.03	0.3	0.06	0.5	0.03	0.2	0.07	0.7	C17:0
> 0.05	1.24	10.2	1.23	10.7	1.46	11.8	0.86	7.1	0.83	9	C18:0
0.001*	0.00	0	0.13	1.2[a]	0.26	2.4[a]	1.18	12[b]	0.18	2[a]	C20:0
											Monounsaturated FA
0.015*	3	3	0.11	1.1	0.03	0.3	0.25	2.4	0.09	0.9	C14:1
0.022*	1.5	1.5	0.11	1.1	0.02	0.2	0.15	1.6	0.07	0.6	C15:1
0.001*	0.7	0.7	0.59	5.5	0.73	7.5	0.25	2.4	0.93	7.6	C16:1
0.021*	0.7	0.7	0.06	0.6	0.03	0.3	0.05	0.4	0.13	1.2	C17:1
0.001*	5.7	5.7	1.07	10.8	0.67	7	1.14	10.5	1.00	9.2	C18:1ω9c
0.001*	0	0	0.46	4.8	0.00	0	0.00	0	0.09	0.8	C20:1
0.0021*	0	0	0.00	0	0.00	0	0.06	0.6	0.00	0	C22:1
											Polyunsaturated FA
0.001*	2.8	2.8	1.05	9.1	1.21	12.1	0.65	6.5	1.29	13.8	C18:2ω6c

(*continued on next page*)

Table 4 (*continued*)

Stages of maturity											
p	V Spent		IV Ripe & Running		III nearly ripe		II maturing		I immature		Fatty acid
	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	
0.021*	0	0	0.06	0.6	0.05	0.5	0.01	0.1	0.05	0.5	C18:3ω3
0.032*	0	0	0.00	0	0.00	0	0.04	0.4	0.00	0	C20:2
0.001*	2.5	2.5	0.23	2	0.74	6.5	0.07	0.6	0.34	3.3	C20:3ω3
0.013*	0	0	0.00	0	0.00	0	0.00	0	0.22	2.1	C20:3ω6
-	0	0	0.00	0	0.00	0	0.00	0	0.00	0	C20:4ω6
0.046*	0	0	0.03	0.3	0.03	0.3	0.00	0	0.03	0.3	C20:5ω3
0.001*	0	0	0.00	0	0.48	3.9	0.00	0	0.00	0	C22:2
0.002*	26.8	26.8	2.47	26.4	0.59	5.8	1.25	11	2.12	17.8	C22:6ω3
0.001*	56.3	56.3	4.33	37.7	5.98	55.6	7.38	63.5	4.76	41.9	SFA
0.001*	43.7	43.7	6.29	62.3	4.19	44.4	3.65	36.5	6.68	58.1	UFA
0.003*	11.6	11.6	2.34	23.9	1.80	15.3	1.74	17.9	1.85	20.3	MUFA
0.0119*	32.1	32.1	4.36	38.4	2.97	29.1	2.14	18.6	4.34	37.8	PUFA
0.0022*	29.3	29.3	3.08	29.3	1.62	13.1	1.26	11.7	2.05	21.9	PUFAs-ω3
0.001*	2.8	2.8	0.94	9.1	1.39	12.1	0.61	6.5	1.45	15.9	PUFAs-ω6

the maturing gonads. This observation was cited for other species by different authors such as sea bass (Cerda et al., 1995), striped bass (Lund et al., 2000), mummichog (Jensen and Taylor, 2002), trout (Wallaert and Babin, 1994) and *D. dentex* (Chatzifotisa et al., 2004). During gonad maturation of male *D. dentex*, insignificant differences in lipid concentration of muscles were observed, but a similar pattern of their reduction could easily be attributed to environmental factors such as temperature and food consumption (Chatzifotisa et al., 2004).

When it comes to energy sources during maturation, the role of liver and muscle tissue seems to be a minor part. Lipids were not reduced during maturation indicating that liver energy resources were well preserved. Liver's importance as lipid depot organ differs between species. In red drum (Craig et al., 2000); sand smelt (Tomasini and Laugier, 2002) and burbot (Mustonen et al., 2002), liver plays a massive role as a lipid supplier to the maturing fish, but in blue fin tuna and Japanese cat fish, liver has no importance as a lipid storage organ (Mourente et al., 2002). Tissue samples for fatty acids analysis were taken throughout the year.

In *D. dentex,* the polyunsaturated fatty acids (*e.g.* DHA) are essential for tissue synthesis, therefore a steady increase in DHA concentration was observed in muscle, liver and testis in the period of accelerated growth. (Chatzifotisa et al., 2004; Izquierdo et al., 2001) stated that polyunsaturated fatty acids are very important for gonad maturation and also larval growth (Tulli and Tibaldi, 1997). At spawning of marine fish, that cannot synthesize PUFA, seem to maintain them in their tissues, since there is a preference for mobilization of saturated and mono-unsaturated fatty acids over PUFA to compensate the maturing ovaries needs (Zohar et al., 1995).

Conflict of interest

The authors state that there's no conflict of interest to declare.

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