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FULL LENGTH ARTICLE

Reproductive biology, steroid and biochemical profiles of *Dentex dentex* ovaries in the Eastern Mediterranean in relation to histological structure

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Abstract This study focuses on reproductive biology in female *Dentex dentex*, which is a summer multiple spawner with the spawning period extending from late April to June. Females with body length over 35 cm were mature. The GSI value had peak values during May and June. The ova diameter ranged over nine groups (50–700 μm). The relative fecundity ranged from 510 to 1276 eggs per g gutted weight and from 20,409 to 22,595 eggs per 1 cm total length. The histological appearance of the ovarian cycle was divided into five periods. The histological structure of the maturing and ripe oocyte wall shows the presence of five different layers. Baseline of testosterone and estradiol levels was found during the immature and spent, whereas the peak value was found in prespawning and ripe-spawning period. While the maximum total lipid content was in the maturing ovary, the minimum was in muscles of prespawning female. The main polyunsaturated fatty acids n-3 (PUFA) for ovary, liver and muscles were docosahexaenoic (DHA, 22:6 n-3) and eicosapentaenoic (EPA, 20:5 n-3) acids, whereas n-6 (PUFA) were linoleic (18:2 n-6) and arachidonic (20:4 n-6). All together, the present observations gave basic data of the reproductive cycle of female *D. dentex* required for its successful spawning.

Introduction

The common dentex, *Dentex dentex* (Linnaeus 1758) is a bentho-pelagic sparid fish inhabiting hard sea beds in shallow water of less than 50 m depth. Common dentex is a high-valued fish in the Mediterranean and tropical areas (Pavlidis et al., 2000), and has been suggested as new candidate aquaculture species, since it showed a high growth rate (Loir et al., 2001) specially, when applying the current production technology to culture common dentex (Efthimiou et al., 1994). Common dentex is a gonachoristic species (D'ncona, 1949; Loir et al., 2001), protandric hermaphrodite (Glamuzina et al., 1989), with sex reversal unlikely to be obligatory (Morales-Nin and Moranta, 1997; Loir et al., 2001).

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Under farming aspects, the *D. dentex* showed high growth rates and fecundity make this species highly suitable to enter the mariculture system and supporting the intensive sparid production (Pavlidis et al., 2000).

Studying the reproduction is essential for a successful aquaculture and provides the basis of its propagation in captivity. The reproduction and sexuality of the common dentex has been documented (Pavlidis et al., 2000, and Loir et al., 2001). In Crete; common dentex, sexual differentiation is recorded at age between 5 and 12 months and the spawning season is found to be from end of March to end of May (Loir et al., 2001).

Oogenesis has been studied in many teleost fish and illustrated by several authors. This includes the work on *D. dentex* (Loir et al., 2001), *Agyromous regius* (Abou Shabana et al., 2012) and Red Lion fish *Pterois volitans* (Priyadharsini et al., 2013). Ultrastructure studies have been carried out on many teleost fish by a number of authors, *e.g. Caranx crysos* (Assem, 2000), *Pagellus erythrinus* (Assem, 2003) and *Merluccius merluccius* (Al-Absawey, 2010). Also the seasonal variations in steroids have been reported (Pavlidis et al., 2000), seasonal fluctuations in serum 17β-oestradiol, testosterone, 11-ketotestosterone, vitellogenin and thyroid hormones were reported through the annual reproductive cycle of *D. dentex* (Pavlidis et al., 2000).

The (n-3) highly unsaturated fatty acid requirements of *D. dentex* larvae and its antioxidant status were investigated during artemia feeding stage (Mourente et al., 2000). Advances have been made in understanding the key role of polyunsaturated fatty acids nutrition for marine fish larvae and broodstock. The total lipid and fatty acid content of broodstock ovaries were reported for many species, as for *Mugil cephalus* ovaries, in which difference in concentrations was detected during the reproductive cycle (Assem et al., 2008).

Dietary lipids are crucial energy sources and essential fatty acids for fish (NRC, 1993). The n-3 HUFA such as EPA and DHA are demanded by most marine fish for normal growth and development (Izquierdo et al., 1989). Therefore, it is critical to develop feed formulas with appropriate classes and levels of EFA to fulfil the fish nutritional requirements (Kim et al., 2002; Sargent et al., 1997).

The aim of the present work is to study the reproductive biology of female *D. dentex* in order to give a clear image about the spawning season, length at the first sexual maturity and to identify ovaries maturation using histological and fine structure analysis with respect to steroid hormones, lipid and fatty acids throughout one year period.

Materials and methods

Biological studies

91 female *D. dentex* were sampled from Alexandria Mediterranean waters over one year from November 2012 to October 2013. These ranged in total length and total weight from 17 cm to 60 cm and from 80 g to 2600 g, respectively. For each individual, the total length (mm) and the total weight (g) were recorded. Gonadosomatic index (GSI) was computed as a percentage weight of ovary to the gutted weight × 100. Morphological characteristics and duration of maturity stages were recorded.

Reproductive biology

The paired ovarian loops were weighted (0.1 g), for the ripe and spawning stages; ovaries were fixed in 4% neutral formalin. For the estimation of fecundity, the ovaries of mature females (19 fish) were weighed; three sub-samples were taken from each ovary and weighted. Then the total number of eggs in each ovary sub sample was proportionally estimated using the equation, Fa = (gonad weight × number of eggs in the sub-sample)/sub-sample weight (Yeldan and Avsar, 2000). Later, absolute fecundity for each female fish was calculated as the mean number of three sub-sample fecundities. Relative fecundity was calculated in relation to gutted weight or total length groups to get number of eggs per gram body weight and per cm of the body length. The egg diameters were split over nine diameter groups in every 0.1 mm: the first four groups were small (⩽0.3 mm) and transparent in shape, while the remaining ovae groups were yolky and ranging in diameter between (0.4 and 0.7 mm).

Histological examination

The fixed ovaries were washed in ethyl alcohol (70%) for two days prior to dehydration, then cleared followed by paraffin wax embedding. Ovarian Sections of 4–8 μm were cut and stained with Eirlich hematoxylin and eosin.

Fine structure studies

Small ovarian specimens were fixed overnight in 4% buffered glutaradialdehyde (at 4 °C), then in 1% osmium tetroxide. Samples were rinsed two times in cacodylate buffer, ethanol dehydrated, cleared in propylene oxide and embedded in epoxy resin. Glass and diamond knives were used to prepare ultrathin sections of one μm thickness and stained with uranyl acetate and lead citrate. Lastly, sections were examined using a transmission electron microscope.

Biochemical studies

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

Hormonal assay

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

Statistical analysis

The arithmetic means and standard deviation for numerical data were used to compare more than two groups, ANOVA test followed by LSD method were applied to calculate the significance between groups at <0.05.