

Aspects of reproduction in pink dentex *Dentex gibbosus* (Rafinesque, 1810) from the Archipelago of Madeira in the northeast Atlantic

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This work describes and identifies the macroscopic, and corresponding microscopic, changes of gonads through the annual reproductive cycle of pink dentex, *Dentex gibbosus*, from the Madeira Archipelago. This new contribution focused on validating a macroscopic maturity scale for this species using a histological technique. A total of 906 individuals were collected from waters around the Madeira Archipelago between September 1997 and December 2008. A six-stage maturity scale based on macroscopic characteristics was used to classify the gonads. The overall ratio of males to females was 1:1.12. The annual gonad development, together with the analysis of monthly indices (gonadosomatic and hepatosomatic) and complementary histological observations allowed us to conclude that spawning takes place during the summer months, with a peak in May-June.

Key words: Sparidae, NE Atlantic, maturity stages, histology, spawning season

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INTRODUCTION

The pink dentex, *Dentex gibbosus* (Rafinesque, 1810), belongs to the Family Sparidae and is a bottom dwelling marine fish associated with a variety of temperate to subtropical habitats, occurring along the shelf on rocky and rubble bottoms, and on sand near rocks, between 20 and 400 m depth. This species is distributed throughout the Mediterranean and Eastern Atlantic coasts, from Portugal to Angola (Fernandez-Palacios et al. 1994; Pajuelo & Lorenzo 1995). Juveniles are found near shore whereas adults occur offshore in the vicinity of the continental slope. Adults present a marked protuberance on their forehead. The third and fourth spines of the dorsal fin are long and filamentous in juveniles (Fernández Palacios et al. 1994). Considered a carnivorous species, the pink dentex's diet is

composed mainly of crustaceans, fish and cephalopods (Bauchot et al. 1981; Bauchot & Hureau 1986).

This species is usually consumed fresh, captured by local artisanal boats fishing with bottom longlines and handlines, which operate around the Madeira and Porto Santo islands and occasionally off Desertas Island (Fig. 1). In 2010, 2.8 tonnes (€17 thousand) of pink dentex were landed in the Archipelago of Madeira, representing 5.4% and 6.9% of the demersal landings in weight and value, respectively.

Despite its abundance and commercial value, there have been few studies on the reproductive biology of pink dentex. Abdelkader & Ktari (1983) described the morphological characteristics of this species in Tunisia. Fernandez-Palacios et al. (1994) analysed embryonic and larval development under controlled conditions in Gran

Canaria. Pajuelo et al. (1995) studied biological parameters reflecting the current state of the population around the Canary Islands.

The type of hermaphroditism in this species is uncertain as different studies report conflicting evidence regarding this feature. Leon et al. (2007) concluded that this species is a rudimentary hermaphrodite in the Adriatic Sea. Pajuelo & Lorenzo (1995) concluded that the species displays protogynous hermaphroditism in the Canary Islands, whereas Bonnet (1969) reported that pink dentex off North-West Africa appeared to display protandrous hermaphroditism.

Understanding the life history of a species is an important and essential step towards the assessment of its potential as an exploitable resource. Two of the most important aspects of fish life-history are growth and reproduction because they are the biological processes that replenish the biomass taken by mortality. Accuracy and precision in biological evaluation are of crucial importance in stock assessment. The macroscopic

evaluation of gonads has been applied for many years to determine the individuals' stage of reproductive development. However, this method may be considered as ambiguous and subjective, and lead to incorrect classifications of fish reproductive status. Several studies in other species have shown that the most accurate method to evaluate gonad development in individual fish is histological analysis (Murua & Motos 1998; Saborido-Rey & Junquera 1998; Kjesbu et al. 2003; Tomkiewicz et al. 2003; Vitale et al. 2005).

The present study focused on the microscopic validation of a six-stage macroscopic maturity scale adapted from Holden & Raitt (1974) for *Dentex gibbosus*, using a histological technique (Ramos 1986). A small image collection of the microscopic assessment of gonad development is also provided. Monthly percentages of maturity stages, the gonadosomatic (GSI) and hepatosomatic (HSI) indices were calculated to complement the reproductive development assessment of pink dentex in the Madeira Archipelago.

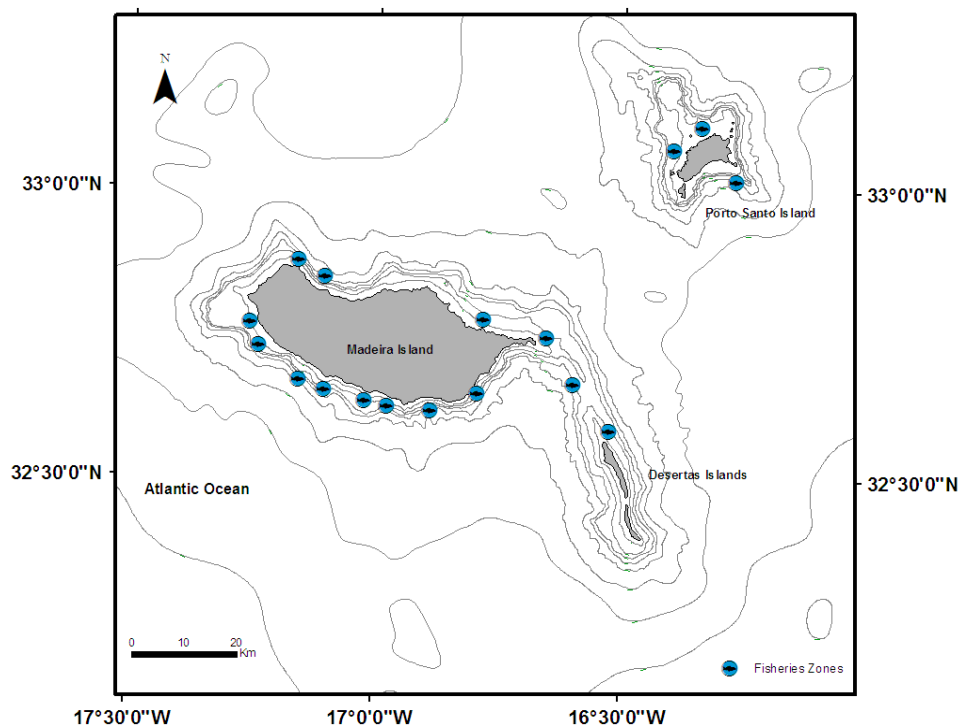


Fig. 1. Location of the main fisheries zones (black dots) for *Dentex gibbosus* in the Madeira Archipelago according to available positional data - Datum Base SE/Porto Santo - (DRP-Direcção Regional de Pescas da Madeira/Regional Directorate for Fisheries, Madeira).

MATERIAL AND METHODS

A total of 906 specimens of *Dentex gibbosus* were collected between September 1997 and December 2008. For the histology study, 106 individuals (50 males, 56 females) were selected for validating the macroscopic maturity scale. The majority of the pink dentex specimens (93.8%) sampled monthly were from commercial longline landings, while the remaining specimens (6.2%) were obtained from survey cruises on the *R/V SÃO ROQUE* around the Madeira Archipelago. For each specimen, total length (TL) was measured to the nearest millimetre and total wet weight (TW), gonadal weight (GoW) and liver weight (LW) were recorded to the nearest 0.01g.

The sexual maturity stage of each pink dentex was determined using the macroscopic maturity scale shown in Table 1, adapted from Holden & Raitt (1974), and includes six stages: 0 - Immature; I - Resting; II - Developing; III - Pre-spawning; IV - Spawning; V - Spent. Maturity stages were identified based on the appearance, degree of opacity, colouration and vascularisation of the gonads, and on the ease of seeing sperm and individual oocytes. Photographs of all different stages for both sexes were taken using a digital camera.

The spawning season was determined by following monthly changes in frequency of maturity stages (0, I, II, III, IV and V). Mean monthly gonadosomatic (GSI) and hepatosomatic (HSI) indices were calculated using equations 1 and 2, respectively.

$$\text{GSI} = (\text{GoW} / \text{TW}) \times 100 \quad (1)$$

$$\text{HSI} = (\text{LW} / \text{TW}) \times 100 \quad (2)$$

The sex-ratio of the sampled population was analysed by season and 2 cm length classes, and tested statistically for significant deviations from the expected 1:1 ratio using the chi-square test ($\alpha = 0.05$) (Zar 1996).

To validate the macroscopic evaluation of the gonads, a histological analysis was performed. The gonads were processed according to the histological technique described by Ramos (1986). Small portions of ovaries and testis were fixed with glutaraldehyde in 0.1M sodium cacodylate

buffer (pH 7.4), dehydrated through ascending concentrations of ethanol and embedded in resin (methacrylate). Gonads were sectioned at 5µm thickness and stained with Löffler's methylene blue solution. A LEICA DMLB microscope, LEICA DC 300 camera and LEICA Image manager 50 software were used for image acquisition and treatment.

RESULTS

Of the fish examined, 449 were females, 402 were males and the sex of the remaining 55 fish could not be determined macroscopically. Fish ranged in size from 16.0 to 99.0 cm TL, and in total weight from 46.5 g to 11100.0 g. Females ranged from 19.3 to 99.0 cm in length and 83.4 g to 11100.0 g in weight. Males ranged from 16.0 to 91.9 cm in length and 46.5 to 9607.0 g in weight.

The overall ratio of males to females was 1:1.12. Significant differences from 1:1 ratio were found only in the spring samples ($\chi^2=3.98$; $df=1$; $P<0.05$) (Table 1). In addition, none of the sex ratios by 2 cm length classes had significant departures from the 1:1 ratio ($\chi^2 > \chi^2_{1, 0.05} = 3.84$) (Fig. 2). A total of 448 females and 401 males were used for this analysis.

Table 1. Number of females (F) and males (M) by quarter, with comparison of sex-ratio to a 1:1 ratio by χ^2 analysis.

Quarter	F	M	Significance
Winter	117	124	$P>0.05$
Spring	128	98	$P<0.05$
Summer	59	61	$P>0.05$
Autumn	144	118	$P>0.05$

According to monthly percentage evaluation of maturity stages, females and males with pre-spawning gonads (stage III) were recorded between April and December, spawning females and males (stage IV) appeared in April, becoming dominant during the summer months and spent females and males (stage V) were recorded mainly in the autumn and winter months (Fig. 3).

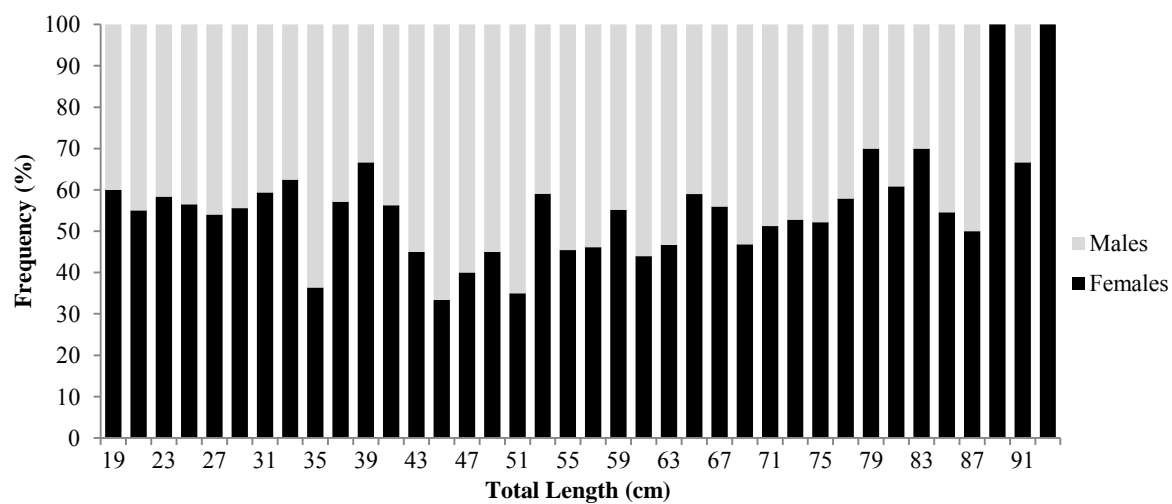


Fig. 2. Sex ratios of *Dentex gibbosus* off the Madeira Archipelago by 2 cm length-class.

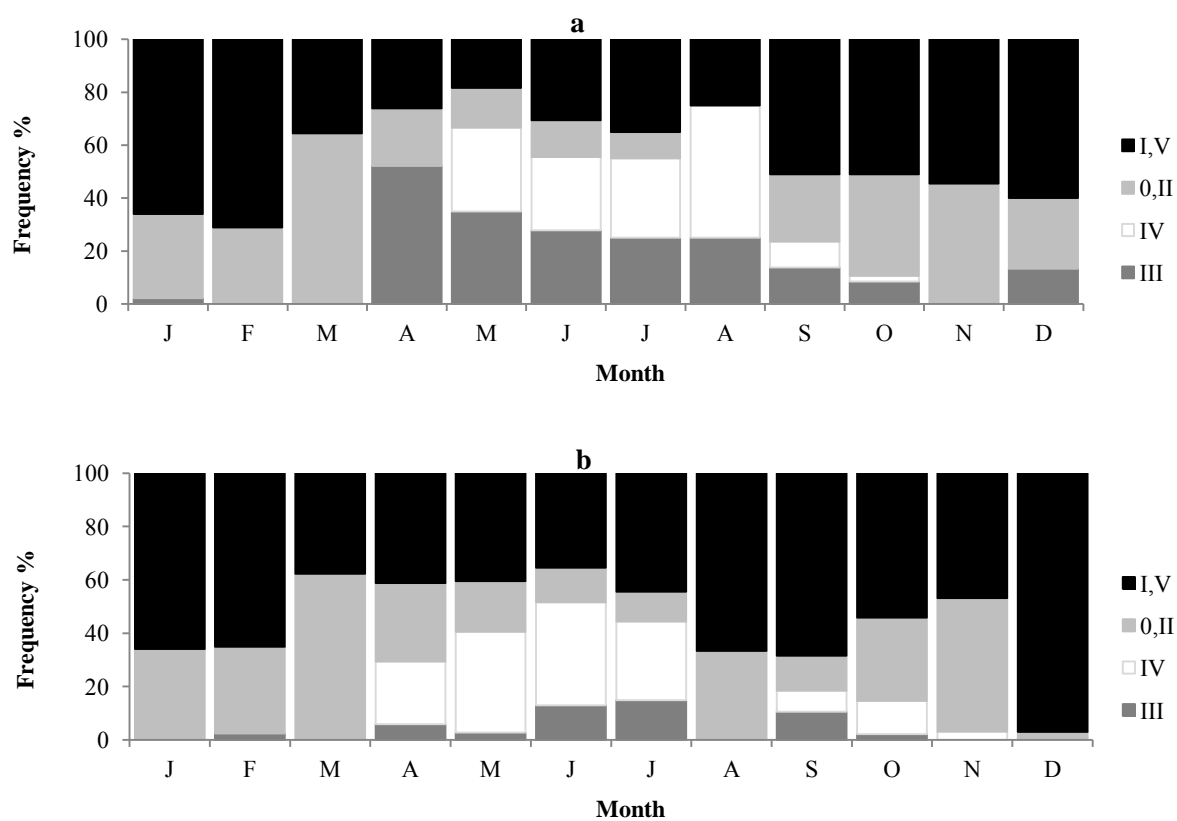


Fig. 3. Monthly percentages of maturity stages 0 to V obtained for (a) females and (b) males of *Dentex gibbosus*, between 1997 and 2008.

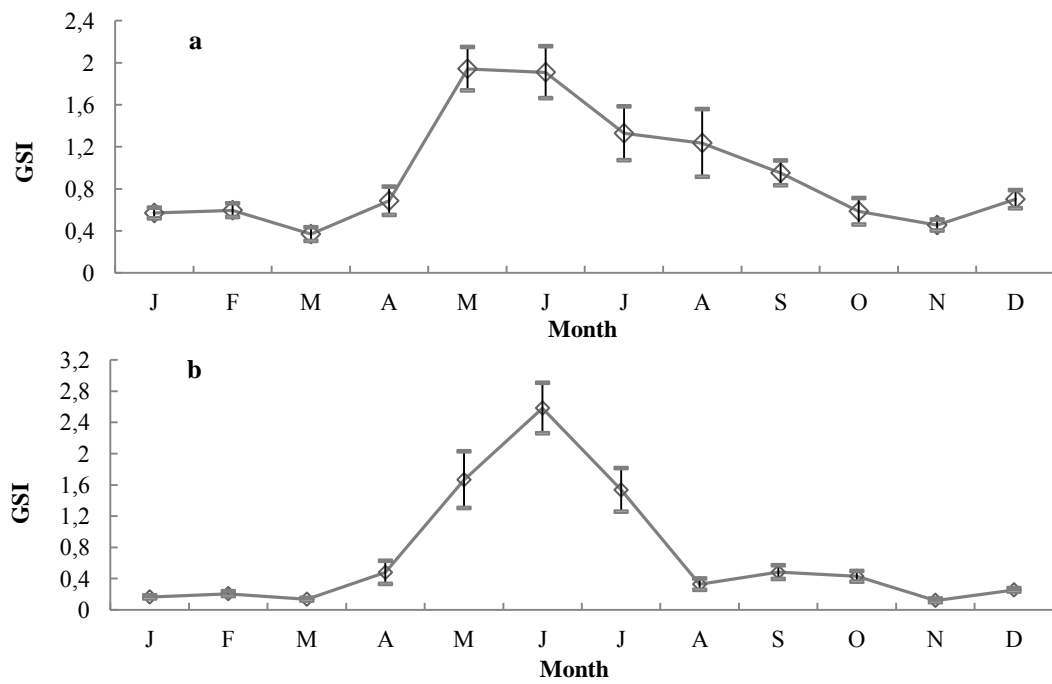


Fig. 4. Monthly evolution of the gonadosomatic index (GSI) for (a) females and (b) males of *Dentex gibbosus* caught off the Madeira Archipelago, between 1997 and 2008.

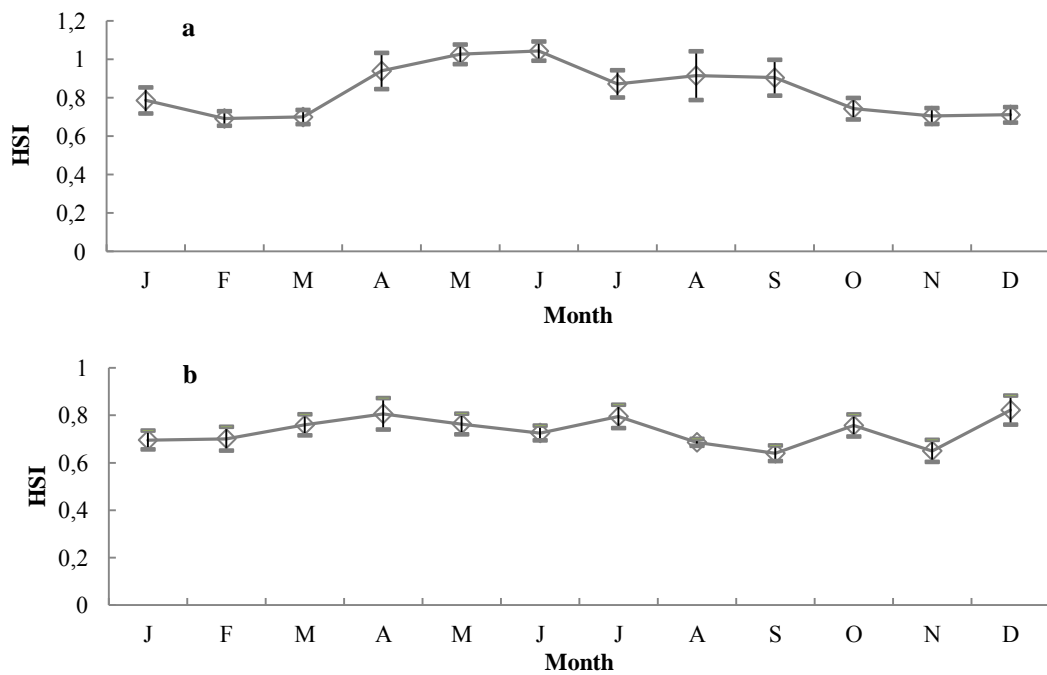


Fig. 5. Monthly evolution of the hepatosomatic index (HSI) for (a) females and (b) males of *Dentex gibbosus* caught off the Madeira Archipelago, between 1997 and 2008.

The lowest values of the GSI occurred from August to April and highest values between May and August, with a peak in May-June, for both sexes (Fig. 4). Mean HSI for females increased in April and remained high until September, indicating that pink dentex are summer spawners. For males, the maximum values of HSI were found in April and December (Fig. 5).

To facilitate a better understanding of the comparative analysis of all maturity stages for both sexes at a macroscopic and microscopic level, a summary of the main features of each stage at both levels are presented in Tables 2 and 3, later used for evaluating specimen maturity stages.

During macroscopic examination of the gonads, all maturity stages for both sexes were identified. Photographs of the macroscopic aspect of gonads are presented in Figure 6. A histological examination of the gonads was performed to study the reproductive cycle and to validate the macroscopic maturity scale. Microscopic analysis of the gonads verified a strong agreement between the observed maturity stages and the macroscopic classification. Macroscopically stage V was sometimes confused with stage I in males, and stage I with stage II in females. Photographs taken from histological sections for all stages are presented in Figures 7 (females) and 8 (males).

The presence of oocytes at different development stages were observed in ovaries, according to the gonadal maturity stage. Oocytes develop gradually through different stages of primary and vitellogenic growth until they complete their maturation and are released. In immature females (Fig. 7A) only the presence of hexagonal shaped oocytes, showing a nucleus with dense chromatin or with one or more nucleoli (primary growth stage), were observed. Some authors sometimes identify the presence of oogonias at this stage, but in our case these were not discernible. In developing stage females (Fig. 7B), oocytes were almost all at primary growth stage, with some in the perinucleolar stage already displaying circumnuclear oil droplets and cortical alveoli at the periphery of cytoplasm. Some oocytes were already in the beginning of the vitellogenesis stage. Pre-spawning ovaries (Fig. 7C) revealed the existence of some oocytes still in the primary growth stage, mixed with larger oocytes at different stages of

vitellogenesis, containing a cytoplasm filled with yolk granules and oil droplets, while follicle had already begun to take shape. In spawning females (Fig. 7D) a considerable amount of oocytes were in late maturation events before hydration, while in some individuals the presence of hydrated oocytes were visible, which is typical for this stage. Post-spawning ovaries (Fig. 7E) exhibited empty spaces between oocytes in different stages of development and the presence of atretic oocytes and post-ovulatory follicles. Resting ovaries (Fig. 7F) showed oocytes at chromatin nucleolus stage and early perinucleolar stage. At this stage, atretic oocytes can be observed.

The testicular organization is classic, with seminiferous tubules containing spermatogenic cysts where the presence of several spermatogenic stages: spermatogonia; spermatocytes; spermatids and spermatozoa, can be identified. The relative abundance of each, as well as, the level of organisation of seminiferous tubules is used to characterise each stage of maturity. Immature males (Fig. 8A) showed no differentiation in seminiferous tubules and the presence of spermatogonial activity could be observed. In testes in developing stage (Fig. 8B) seminiferous tubules were distinguishable, becoming individualised. Apart from spermatogonias, spermatocytes were also present at this stage. Pre-spawning males (Fig. 8C) showed seminiferous tubules already individualised, presenting cysts with all spermatogenic stages. Spermatozoa were not seen in every individual at this stage. In testes at spawning stage (Fig. 8D) spermatozoa predominated inside seminiferous tubules, which is typical for this stage. Sperm ducts were filled with spermatozoa. Post-spawning males (Fig. 8E) revealed an increase in the development of connective tissue between seminiferous tubules, which showed residual spermatozoa inside beginning to differentiate. Resting testes (Fig. 8F) presented no organisation and were at the beginning of spermatogonial activity, showing some spermatocytes development.

As for hermaphroditism, a condition that has been reported and described by several authors for this species, it should be noted that it was not observed in any of the individuals sampled in this study.

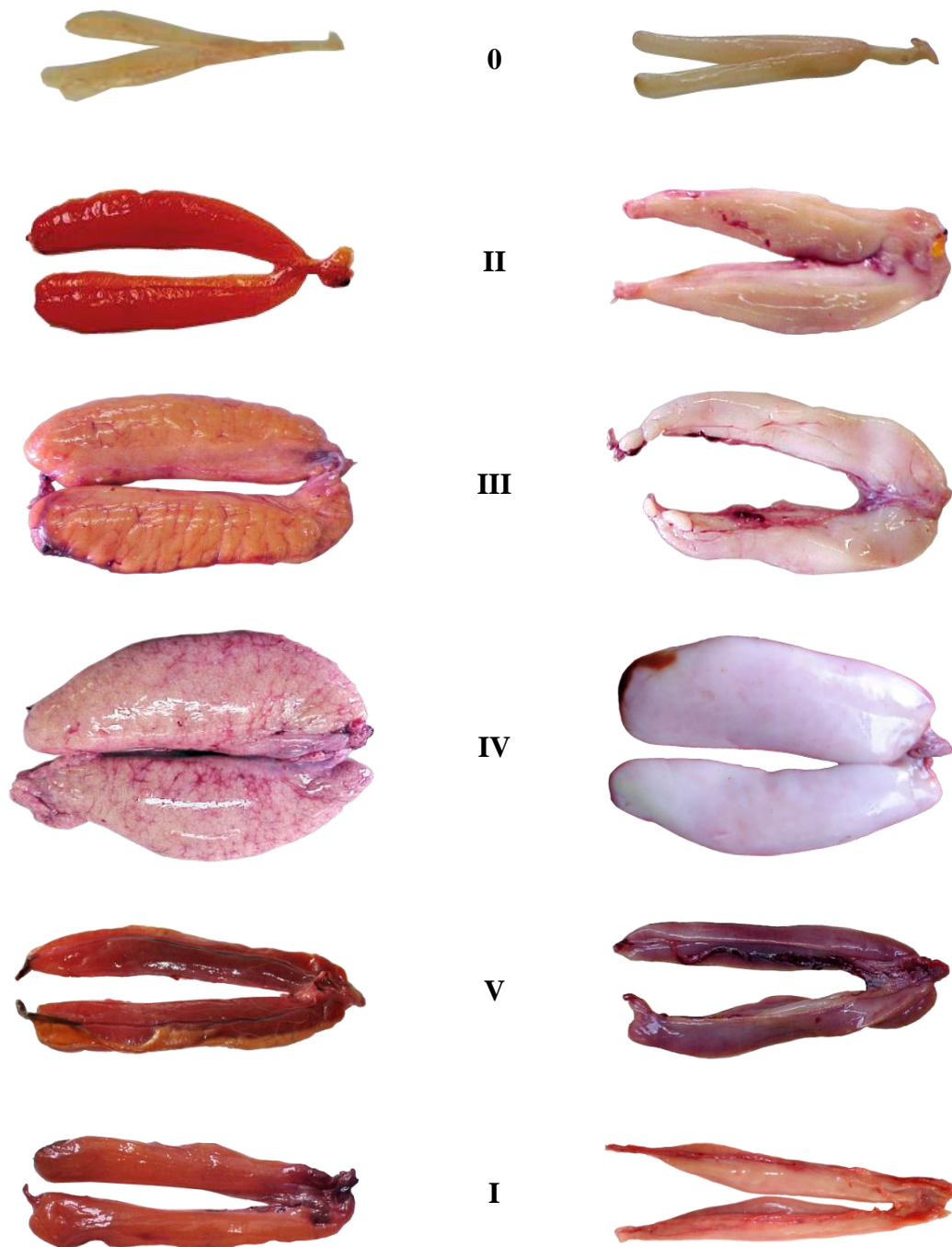


Fig. 6. Macroscopic appearance of ovary (left) and testis (right) maturity stages of *Dentex gibbosus* as described in Table 2 (a) and Table 3 (a).

Table 2. Summary of the main features of (a) macroscopic and (b) microscopic observation of the ovaries.

(a) Macroscopic		
0	Immature	Small, thin and translucent.
II	Developing	Firm, orange coloured, showing some vascularisation. The oocytes are barely visible to naked eye. There is a small increase in the diameter of the ovary.
III	Pre-spawning	Large and firm with a well developed vascularisation. The oocytes are clearly discernible to naked eye, with an orange colour.
IV	Spawning	Firm, larger in diameter, well vascularised and filled with yellow to translucent oocytes.
V	Spent	Flaccid, showing some vascularisation and reddish colour.
I	Resting	Thin and firm, with reddish to orange colour. The oocytes are not visible to naked eye.
(b) Microscopic		
0	Immature	With hexagonal shaped primary oocytes only, with a densely stained cytoplasm and a large central nucleus. Thin ovarian wall and little space between oocytes. No atresia.
II	Developing	Most oocytes in primary growth stage with a central nucleus where numerous small nucleoli are present at its periphery. Some oocytes are already at the beginning of vitellogenesis.
III	Pre-spawning	Many oocytes in vitellogenesis phase with cytoplasm displaying small lipids droplets and yolk granules. Follicle has already begun to take shape.
IV	Spawning	Many vitellogenic oocytes are in late maturation events before hydration. Presence of hydrated oocytes.
V	Spent	With empty spaces and residual oocytes at different development phases. Atretic oocytes and post-ovulatory follicles are present at a variable number.
I	Resting	Oocytes at primary growth, with one or more nucleoli at a central position in nucleus.

Table 3. Summary of the main features of (a) macroscopic and (b) microscopic observation of the testes.

(a) Macroscopic		
0	Immature	Small, thin and translucent.
II	Developing	Larger, firm and white, with no release of sperm when the gonads are pressed
III	Pre-spawning	Wider and firm, well developed, white and with visible sperm.
IV	Spawning	Wider, with a whitish cream colour and sperm that runs easily when the gonad is pressed.
V	Spent	Flaccid, with bloody aspect and some sperm.
I	Resting	Small, thin and flaccid with a whitish to light pink colour. Occasionally it has residual sperm.
(b) Microscopic		
0	Immature	Presence of spermatogonia and little spermatocyte development and no clear distinction of seminiferous tubules.
II	Developing	With development of cysts containing spermatogonia, spermatocytes and spermatids.
III	Pre-spawning	With seminiferous tubules clearly distinguishable and spermatozoa already present.
IV	Spawning	With predominance of spermatozoa inside seminiferous tubules.
V	Spent	Presence of residual spermatozoa in seminiferous tubules. Development of connective tissue.
I	Resting	Beginning of spermatogonia activity, with little or no spermatocyte development. There are residual spermatozoa in spermatic ducts, although less than in spent stage.

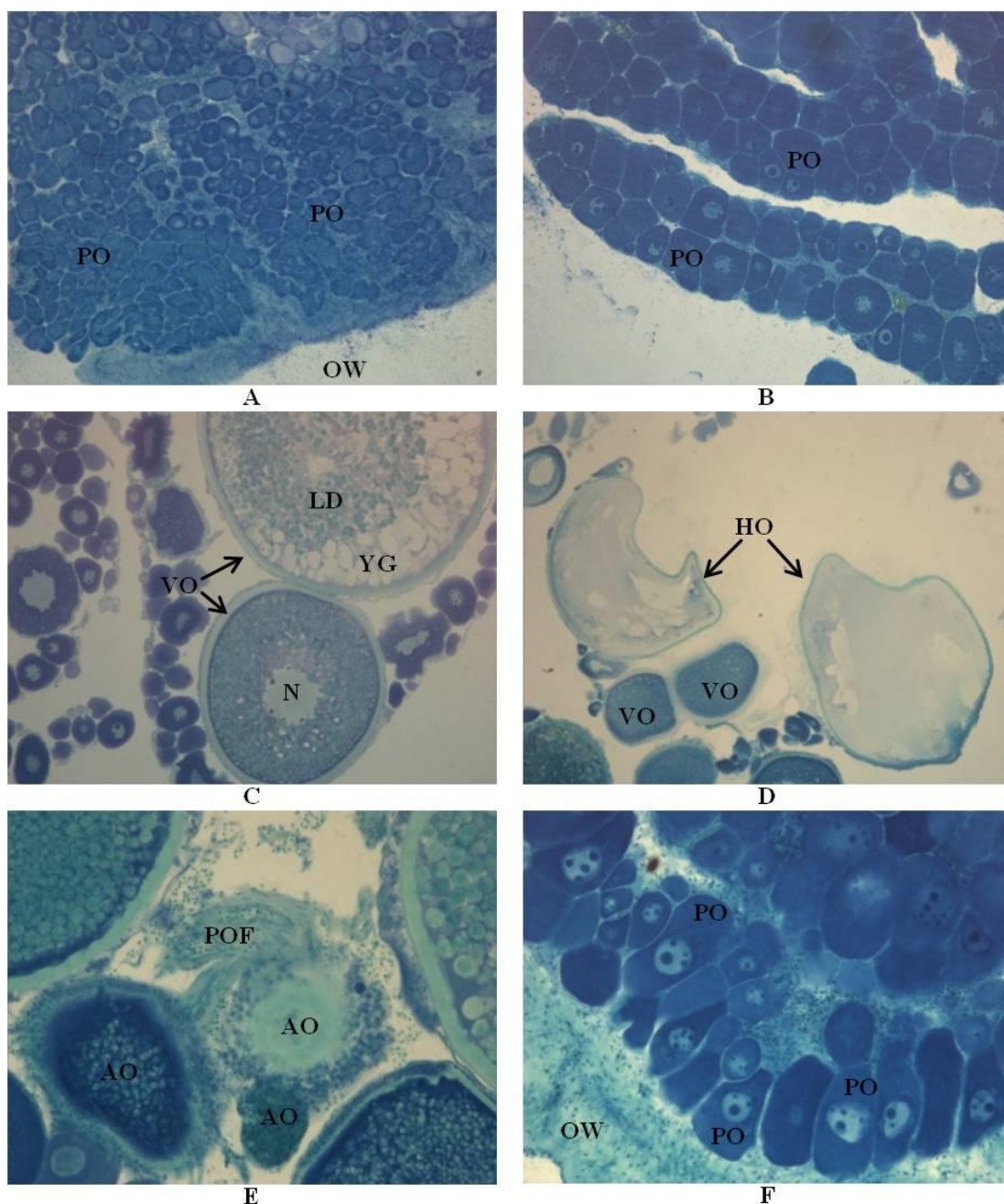


Fig. 7. Microscopic appearance of ovary maturity stages of *Dentex gibbosus*. A - Immature ovary, with primary oocytes (100 x); B - Ovary at developing stage, with some oocytes at the beginning of vitellogenesis (100 x); C - Ovary at pre-spawning stage, with oocytes at different stages of vitellogenesis (100 x); D - Ovary at spawning stage, with the presence of hydrated oocytes (50 x); E - Ovary at spent stage, with the presence of atretic oocytes (200 x); F - Ovary at resting stage, with primary oocytes (100x). (AO - Atretic oocytes; HO - Hydrated oocytes; LD - Lipid droplets; N - Nucleus; PO - Primary oocytes; POF - Post-Ovulatory Follicles; OW - Ovarian wall; VO - Vitellogenic oocytes; YG - Yolk granules).

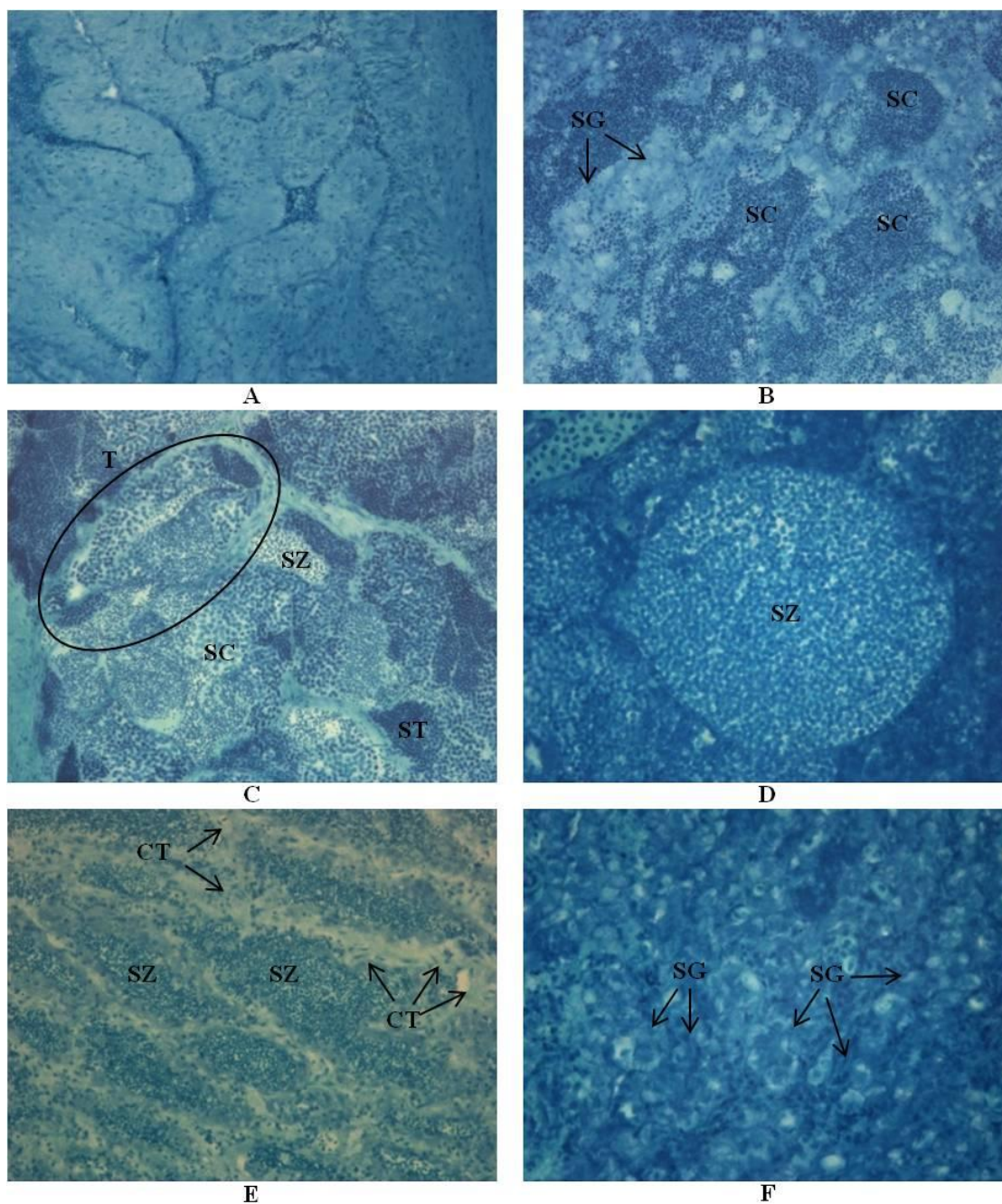


Fig 8. Microscopic aspects of testis maturity stages of *Dentex gibbosus*. A - Immature testis, with no clear distinction of seminiferous tubules (200 x); B - Testis at developing stage, with evident spermatogonial activity and presence of spermatocytes (200 x); C - Testis at pre-spawning stage, with distinguishable seminiferous tubules and presence of spermatozoa (200 x); D - Testis at spawning stage, with predominance of spermatozoa in seminiferous tubules (400 x); E - Testis at spent stage, with residual spermatozoa and development of connective tissue (200 x); F - Testis at resting stage, at the beginning of spermatogonia activity (400x). (CT - Connective tissue; SC - Spermatocytes; SG - Spermatogonia; ST - Spermatids; SZ - Spermatozoa; T - Spermatid tubule: inside black line).

DISCUSSION

The reproductive season of pink dentex in the Madeira Archipelago extends from boreal spring to summer (April-August). The mean GSI and HSI proportions of individuals at pre-spawning and spawning stages (III and IV respectively), increased in April and remained high until August. GSI calculated over the entire year indicated the approximate reproductive cycle. The low values of GSI towards the winter months sustained the end of the reproductive season, as was indicated by high rates of atresia present in ovaries of females caught during this period, and observed histologically.

These results, together with complementary observations from the application of a histological technique, lead us to conclude that in Madeira, the spawning period takes place during the summer months with a peak in June, as found by Pajuelo & Lorenzo (1995) off Canary Islands. The overall sex-ratio of males to females was 1:1.12, which differs from that obtained by Pajuelo and Lorenzo (1995), reporting a 1:1.45 sex-ratio for Canary Islands.

Macroscopic maturity classification of gonads based on a quick visual inspection is somewhat uncertain as several features are not easily identified by the naked eye during certain phases of the development process. Furthermore, when a gonad advances to the recovery phase after the spawning season, there may be bias towards classifying them as immature. This is confirmed by the monthly bias trend, as shown in Figure 3, which illustrates the risk of misjudgement when the macroscopic classification used is influenced by the time of the year at which gonads are collected. Vitale et al. (2006) suggested that the best time to perform maturity surveys, in order to obtain reliable results and to reduce the risk of misjudgement, is about a month before the spawning season. In the case of the pink dentex, this corresponds to May.

The use of histology in maturity studies has become more widespread as it is more consistent and reliable (Kjesbu et al. 2003; Murua et al. 2003). Thus, only by means of histological analysis it is possible to more accurately identify maturity stages.

Despite the agreement verified between macroscopic maturity scale and histology analysis, there were some misjudgements between stages V and I in males, and stages I and II in females. These stages are hardly discernible to the naked eye and consequently, most susceptible to misclassification. It should be noted that steps were taken to achieve a better characterisation of the stages mentioned, thus, enabling a more accurate classification. This allowed for the calibration of the macroscopic scale, with all stages now better described and illustrated. Further, histology assessment demonstrated that it is unnecessary to include any extra stage to the scale.

In our case, it is too early to draw a conclusion on the type of hermaphroditism, because no sample revealed this condition, hence, the importance of monitoring the reproductive cycle of this species and the use of the histological technique. However, a constraint of this method is that the results of a relatively small sample have to be extended to a large population.

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