Gonadal development and spawning cycle in the digynic hermaphrodite sharpsnout seabream Diplodus puntazzo (Sparidae) off the Canary Islands, northwest of Africa

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Summary

Diplodus puntazzo is a species characterized by a rudimentary hermaphroditism, with a low level of protandry. The study found that the overall sex ratio was one male to 2.03 females, diverging significantly from 1 : 1, and that there was a ratio of one male to 0.11 transitional fish. The sex ratio varied with season: during the reproductive period the ratio was 1 : 1.86 (male : female), significantly lower than in the non-reproductive period in which the sex ratio was approximately 1 : 2.26. Both sexes had similar size distributions; however, females were predominant in all size intervals, especially in the larger ones. No significant difference in mean total length (TL) between females (330 mm), males (319 mm) or transitional (321 mm) was found. The smallest functional female and male analysed were 159 and 157 mm TL, and the largest were 520 and 510 mm TL, respectively. No significant difference in length at first level of maturity, attained at the second year of life, was found between males (281 mm) and females (292 mm), respectively. A protracted spawning season was identified for the species: from September to February, with a peak in November. GSI values followed the same pattern for males and females, with values of females being similar to those of males; the highest values occurred between September and December, with a maximum in November. Occurrence of transitional fish was significantly higher during the month with highest values of GSI than in the resting period. The study found that vitellogenesis did not begin synchronously for all females, and the simultaneous presence of oocytes in all stages of development indicated an asynchronous mode of ovarian development. The presence of postovulatory, together with transitional fish, was significantly higher during the month with highest values of GSI than in the resting period. The study found that vitellogenesis did not begin synchronously for all females, and the simultaneous presence of oocytes in all stages of development indicated an asynchronous mode of ovarian development.

Introduction

The family Sparidae (seabream) consists of 106 species worldwide, with the highest diversity in the Northeast Atlantic Ocean and in the Mediterranean Sea, where 24 species have been found. Seabream are of great interest for fisheries and, in recent years, have gained considerable importance in aquaculture because of their outstanding eating quality.

Diplodus is one of the genus in the family Sparidae in which hermaphroditism has been reported (Buxton and Garratt, 1990). This genus comprises ten species and seven subspecies, which vary in sexual pattern and body size (de la Paz, 1975). While hermaphroditism and reproductive characteristics have been investigated in the majority of these species, little is known about these aspects in the sharpsnout seabream Diplodus puntazzo (Cetti, 1777). Sharpsnout seabream is a marine fish inhabiting rocky bottoms and sea grass beds. A common species throughout the Mediterranean and the Eastern coasts of the Atlantic Ocean from Gibraltar to Sierra Leone, it is rare in the Black Sea and in the North Atlantic Ocean (Bay of Biscay), and present in the Canary Islands and the Cape Verde (Bauchot and Hureau, 1990). The sharpsnout seabream is particularly interesting for farm production, where it has been cultured for > 10 years (Greco et al., 1993). Despite the growing interest in this species, many aspects of the wild biology remain unknown or appear contradictory. This is particularly worrying if we consider that the correct management of biological resources should be based on the most complete information about the ecology of the species. Data on its reproductive biology is based on observations carried out on specimens caught sporadically in the Mediterranean waters or grown in culture (Micale et al., 1996). D’Ancona (1949) found a lack of sex inversion in this species despite the occurrence of a bisexual gonad. Lissia-Frau and Pala (1968) indicated that protandrous individuals can be found alongside rudimentary hermaphrodites. Micale et al. (1996) found that D. puntazzo is an ambisexual species. Other data on biological aspects of this species concerning the age and growth (Domínguez-Seoane et al., 2006) and feeding (Atienza et al., 2004) have been published.

In recent years, it has become increasingly important to analyse sex reversal in exploited populations. As sex reversal leads to size differentials between the sexes, size-selective fisheries are capable of unbalancing sex ratios, thus reducing the reproductive potential of certain species (Garratt, 1986). D. puntazzo forms part of the Canary Islands coastal benthic fishery; the objective of the present study is to establish the pattern of its sexual development and to determine the main reproductive characteristics of the species.

Materials and methods

In total, 695 specimens of sharpsnout seabream were collected monthly between March 2001 and March 2003 in the Canarian archipelago. The total length L T (mm) and total weight W T (g) were recorded. After the gonads were removed and weighed, the gutted weight W g was taken to the nearest 0.001 g. The sex and the stage of maturation were assessed visually. Stages of maturation were classified as follows: I, immature; II, resting; III, ripe; IV, ripe and running; V, spent. Although any individuals showed evidence of hermaphroditism, fish were sexed macroscopically according
to the dominant tissue in the gonad. Fish were assessed as transitional when female and male tissues occurred in similar proportions.

Identifying the sex macroscopically in individuals <180 mm was not possible. Gonads were also fixed and preserved in 4% buffered formaldehyde and subsequently processed histologically. Slices of tissue were embedded in paraffin, sectioned at 5 µm and stained with Harris haematoxylin followed by eosin counter stain. Sectioned and stained tissue was subsequently examined under a light microscope. The sex and the stage of maturation were determined from histological sections. The growing oocytes were classified into eight histological stages (Table 1) based on criteria used by Buxton (1990) and Micale et al. (1996). Testes were classified according to the most advanced stage of spermatogenesis observed. Spermatogenesis was classified into four histological stages: spermatogonia, spermatocytes, spermatids, and spermatozoa, based on criteria used by Buxton (1990) and Micale et al. (1996). The spermatogenic cells were only identified because their description requires electron microscopy to detect the morphological changes.

The overall sex ratio (males : females) and the sex ratios by size intervals (10 mm) were determined. Differences in the sex ratio between the reproductive and the non-reproductive periods were compared. The periodicity of reproduction was examined on the basis of monthly changes in the histology of females and males gonads and on the temporal evolution in the percent frequency of the maturity stages (MS). The reproductive period was also described by analysing the gonadosomatic index (GSI). The length at first maturity was determined as the proportion of reproductively active fish in each size class and by fitting a logistic ogive: 

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P = \frac{100}{1 + e^{-\frac{C0 - Lm}{r}}} \]

where \(P\) is the

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Table 1

Stages of ovary development and maturity categories based on histological criteria for Diplodus puntazzo

<table>
<thead>
<tr>
<th>Development stage</th>
<th>Maturity category</th>
<th>Histological criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Oogonia (Oo)</td>
<td>Immature and Resting</td>
<td>Only primary growth oocytes present</td>
</tr>
<tr>
<td>II. Perinuclear oocytes (PO)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preperinuclear (PPO)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Early perinuclear (EPO)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Late perinuclear (LPO)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>III. Primary vesicle oocytes (PVO)</td>
<td>Ripe</td>
<td>Ovaries with primary growth and cortical alveoli. Cortical alveoli located on periphery of oocyte and the developing chorion eosinophyllic</td>
</tr>
<tr>
<td>IV. Secondary yolk vesicle oocytes (SYVO)</td>
<td>Ripe</td>
<td>Ovaries with primary growth, cortical alveoli and yolked oocytes; no major atresia. Yolk accumulation initiated within eosinophilic spheres, located at oocyte periphery; ooplasm residing adjacent to nucleus</td>
</tr>
<tr>
<td>V. Tertiary yolk vesicle oocytes (TYVO)</td>
<td>Ripe</td>
<td>Ovaries with dominating yolked oocytes; primary growth and yolked oocytes also present. No major atresia of oocytes. Yolk spheres enlarge and become predominant component within oocyte; ooplasm displaced from nucleus</td>
</tr>
<tr>
<td>VI. Hydrated oocytes (HO)</td>
<td>Ripe and running</td>
<td>Ovaries with primary growth to advanced yolked oocytes. All yolk fused, obscuring oocyte nucleus. Oocytes hydrated and located in ovary lumen</td>
</tr>
<tr>
<td>VII. Post ovulatory follicle (POF)</td>
<td>Ripe and running</td>
<td>Post ovulatory follicles present</td>
</tr>
<tr>
<td>VIII. Atretic oocytes (AO)</td>
<td>Spent</td>
<td>Primary growth oocytes with major atresia of partially yolked and advanced yolked oocytes. Massive atretic oocytes present</td>
</tr>
</tbody>
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Fig. 1. Transverse section through:
(a, b) ovotestis of a mature functional male; (c, d) transitional individual showing a developed testis with first signs of atrophy and early development of the ovary with presence of late primary growth oocytes and with well-organized ovigerous lamellae. TT, testicular tissue; OT, ovarian tissue; SP, spermatogonia; SZ, spermatozoa; (e) ovotestis of a functional female showing a testicular tissue densely packed with normal spermatogonia with no sign of regression; (g) ovotestis of an inactive functional female showing early development of testis; (f, h) ovotestis of a functional female showing the ovary and degenerating testis with atrophied and degenerated spermatogonia isolated among a well-developed fibrous stroma.
percentage of fish mature at length $L_t$; $L_{m}$ the length at first maturity, and $r$ the model parameter.

**Results**

The gonads were two symmetrical, separate hollow lobes lying in a caudocranial direction. Out of a total of 695 histologically sexed specimens, 373 were functional females, 183 functional males and 20 transitional individuals. The remaining 123 fish showed ovotestes without any sign of development.

Histologically, the gonads were ovotestes consisting of dorsomedial ovarian tissue and ventro-lateral testicular tissue, distinctly separated by a layer of connective cells and fibres. The testicular tissue in the ovotestes of the functional male showed all states of spermatogenesis (Fig. 1a,b), while the ovarian tissue appeared as a thin filament lining the testicular portion dorsally and constituted oogonia and perinuclear oocytes. The testicular tissue in the ovotestes of the functional females appeared as a tiny filament or small area, lining the ovarian portion ventrally (Fig. 1g). In approximately 30% of these ovotestes, the testicular tissue appeared regressed with

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**Fig. 2.** Size distributions of male ($n=183$), female ($n=373$), transitional ($n=20$) and immature ($n=123$) *Diplodus puntazzo* off the Canary Islands collected over two complete reproductive cycles (2001–2003)

**Fig. 3.** Sexual maturity ogive for male and female *Diplodus puntazzo* off the Canary Islands. $L_{50}$ = length at first maturity, $r^2$ = correlation coefficient, and $n$ = number of fish
Reproductive aspects of Diplodus puntazzo

### Table 2: Histological description of oocyte stages of development and observed period

<table>
<thead>
<tr>
<th>Oocyte stage</th>
<th>Histological description</th>
<th>Observed period</th>
</tr>
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<tbody>
<tr>
<td><strong>Oogonia</strong></td>
<td>High nucleus to cytoplasm ratio, small size and lightly basophilic cytoplasm (&lt;40 μm)</td>
<td>All year-round</td>
</tr>
<tr>
<td><strong>Perinuclear oocytes</strong></td>
<td>Polygonal in shape, few basophilic yolk, proliferation of yolk in the nucleolus (70–140 μm)</td>
<td>September to December</td>
</tr>
<tr>
<td><strong>Pre-perinuclear</strong></td>
<td>More oval in shape, basophilic yolk, proliferation of yolk in the nucleolus (40–80 μm)</td>
<td>All year-round</td>
</tr>
<tr>
<td><strong>Early perinuclear</strong></td>
<td>Oocytes had yolk vesicles which appeared empty that gradually increased in size and number, were termed lipidiophores.</td>
<td>September to December</td>
</tr>
<tr>
<td><strong>Primary vesicle oocytes</strong></td>
<td>Lipid vesicles were surrounded by a granular perinuclear zone, cytoplasm, nuclei, became flattened against the nuclear membrane (130–180 μm).</td>
<td>November and December</td>
</tr>
<tr>
<td><strong>Secondary yolk vesicle oocytes</strong></td>
<td>Yolk appeared as granules first, changing to globules later, within and between the lipid droplets.</td>
<td>December and November</td>
</tr>
<tr>
<td><strong>Tertiary yolk vesicle oocytes</strong></td>
<td>The zona radiata appeared homogenous. The zona radiata, followed by the appearance of yolk vesicle stage oocytes</td>
<td>January and November</td>
</tr>
<tr>
<td><strong>Hydrated oocytes</strong></td>
<td>Oocytes were completely filled with yolk, resembling the extracellular yolk vesicle stage oocytes (SYVO)</td>
<td>December and November</td>
</tr>
<tr>
<td><strong>Atretic oocytes</strong></td>
<td>Appearance of clear eosinophilic zones within the cytoplasm, followed by dissolution of the nuclear membrane and the zona radiata breakdown.</td>
<td>January and November</td>
</tr>
</tbody>
</table>

Yolk accumulation continued until it obscured the cortical alveoli, eventually filling the cytoplasm in the tertiary yolk vesicle stage or granule stage (Table 2; Fig. 5c–h). Histological

The overall sex ratio was one male to 2.03 females, diverging significantly from 1:1 ($X^2 = 4.92 > X^2_{1,0.05} = 3.84$), and there was one male for 0.11 transitional fish. Sex ratio varied with season. During the reproductive period the sex ratio was 1:1.86 (male : female), significantly lower ($P < 0.05$) than in the non-reproductive period in which the sex ratio was approx. 1:2.26. Males and females showed the same length range; however, females predominated in all size intervals, especially in the larger ones (Fig. 2). No significant differences in mean TL between females (330 mm) and males (319 mm) were found ($t = 1.89$, $P > 0.05$). Sex ratios by size intervals had significant deviation from 1:1 ratio for the size categories over 240 mm ($X^2 > X^2_{1,0.05} = 3.84$). The mean $X_T$ of females or males and transitional (321 mm) were not significantly different (Females-transitional, $t = 0.58$; and Males-transitional, $t = 1.73$; $P > 0.05$).

The smallest functional female and male analysed were 159 and 157 mm TL and the largest 520 and 510 mm TL, respectively (Fig. 2). The length at which 50% of males and females were sexually mature was 281 and 292 mm, respectively (Fig. 2). The length at which 50% of males and females were sexually mature was 281 and 292 mm, respectively (Fig. 3). No significant difference in length at first maturity was found between sexes ($t$-test, $t = 1.72 < t_{0.05,234} = 1.96$; $P > 0.05$).

Histologically, all perinuclear oocytes were without yolk (Table 2; Fig. 4a). Oogonia were most frequently observed in the peripheral regions of the ovigerous lamellae and predominated after the spawning season (Fig. 4b). Three types of oocytes could be distinguished within this stage (Table 2): the pre-perinuclear oocytes (PPO), the early perinuclear oocytes (EPO) and the late perinuclear oocytes (LPO) (Fig. 4b–e). Primary growth oocytes were present year-round in all samples examined, and this was the only stage present from February to August.

The formation of the follicular epithelial layer or zone granulose below the theca cells, the appearance of the zona radiate at the oocyte surface, between the cytoplasm and the zona granulose, marked the end of the primary growth phase and the beginning of the vitellogenesis. This phase was followed by the appearance of primary vesicle oocytes (PVO) or cortical alveoli in the cytoplasm (Table 2; Fig. 5a). Cortical alveoli oocytes were first observed in September and persisted through December.

Vitellogenesis was initiated by the formation of acidophilic secondary yolk vesicle oocytes (SYVO) which first appeared in the region of the primary vesicles, but later as extra vesicular yolk throughout the cytoplasm (Table 2; Fig. 5a,b). Vitellogenic oocytes were present in addition to the other early developmental stages and were observed mainly between September and December.

Yolk accumulation continued until it obscured the cortical alveoli, eventually filling the cytoplasm in the tertiary yolk vesicle stage or granule stage (Table 2; Fig. 5c–h). Histological
examination of hydrated oocytes was unsatisfactory, with oocytes collapsing during tissue dehydration. In the early phase oocytes the dissolution of the nucleus occurred, and the yolk globules started to coalesce to form yolk plates (Table 2; Fig. 5i). Late vitellogenic oocytes and hydrated oocytes were present in addition to the other developmental stages and were observed mainly in late November and December, indicating a near-spawning condition. The presence of postovulatory follicles in December indicated that individuals had already spawned.

Atresia of both perinucleolus and yolk vesicle stage oocytes was found in the resting ovaries (Table 2; Fig. 6a,b,f). Atretic oocytes were occasionally found in the ovaries of maturing and gravid females, affecting the advanced vitellogenic oocytes and starting the breakdown of the zona radiata (Fig. 6c,e). Massive atresia was observed in January.

Spermatogenesis was initiated by the division of the spermatogonia which were characterized by large size and a basophilic nucleus. Spermatogonias were transformed into spermatocytes. Spermatids were visible as tiny, round cells with a basophilic nuclei. Spermatozoa were characterized by their small size and basophilics heads (Fig. 7a–c).

The proportion of the macroscopic maturity stage for males and females varied with season (Fig. 8). Males and females
with ripe gonads were first observed in September. Ripe and running individuals began to appear in October, and increased in proportion during November, whilst the proportion of ripe individuals decreased. The ripe and running period extended throughout the early winter. Spent individuals were observed from November to January.

The GSI values followed the same pattern for males and females, with values of females being similar to those of males; the highest values occurred between September and December, with a maximum in November (Fig. 9). The occurrence of transitional fish was significantly higher (P < 0.05) during the month with the highest values of GSI than in the resting period.

**Discussion**

*D. puntazzo* off the Canary Islands is hermaphrodite. This characteristic has also been observed in this species in the Mediterranean (D’Ancona, 1949; Lissia-Frau and Pala, 1968; Micale et al., 1996). The similar size distribution of males and females, and the presence of a percentage of individuals with well-formed ovaries and residues of degenerated testes, indicate a rudimentary hermaphroditism with low levels of protandry. D’Ancona (1949), Lissia-Frau and Pala (1968) and Micale et al. (1996) indicated that the gonads of *D. puntazzo* in their initial development are always bisexual, with both sexes present at the same time. After this initial phase, individuals present a gonadal evolution in a gonochorism direction, called late gonochorism or rudimentary hermaphroditism (Micale et al., 1996). From an ecological perspective, this sexual typology is supported by the fact that both sexes have similar length ranges, and by the gregarious behaviour displayed by the species. In their natural environment, large individuals are often found together over extended periods of time. This suggests that reproduction is independent of length: the benefits derived from the sexual succession decrease from the point of view of the size advantage model. The formation of schools of individuals of similar sizes reduces the tendency for polygamy or polyandry, which is associated with sex reversal (Buxton and Garratt, 1990). However, a partial protandry has also been observed in *D. puntazzo* from the Canary Islands. Related to this, Lissia-Frau and Pala...
Fig. 8. Seasonal changes in maturity stages for male (n = 183) and female (n = 373) Diplodus puntazzo off the Canary Islands followed over two complete reproductive cycles (2001–2003). Maturity stages: (II) resting, (III) ripe, (IV) ripe and running and (V) spent.

Fig. 9. Seasonal changes in gonadosomatic index (GSI) for male (n = 183) and female (n = 373) Diplodus puntazzo off the Canary Islands over two complete reproductive cycles (2001–2003). Values represent means (dots) and standard deviations (bars) of monthly samples.
(1968) pointed out the possibility that a small section of the population undergoes sexual inversion (partial protandry). This fraction of the population may develop a polygamous mating system because Diplodus appear to be pelagic spawners with pelagic larvae (Vigliola et al., 1998), and exhibit no parental care.

 Gonads showing ovarian and testicular tissues, both growing in size and developing, with well organized ovigerous lamellae, indicating true transitional fish, were observed in a small group of individuals from the Canary Islands. Sex inversion begins with the atrophy and degeneration of the testicular tissue and the resurgence of the oogenesis (strongest evidence of protandry). The ovarian tissue of transitional individuals contains oocytes at the perinuclear stage. The development of the perinuclear oocytes alongside simultaneous atrophy and degenerative changes in testicular tissues, are the initial phase of sexual change. This change culminates in the complete degeneration of the testicular tissue and the appearance of primary vesicle oocytes in the ovary. The presence of primary vesicle oocytes (vitellogenesis) in the gonads indicates ovarian maturation (Micale et al., 1996).

 The transitional individuals are mainly between 260 and 380 mm in length, indicating that sexual succession takes place mainly between the second and the fourth years of life (Domínguez-Seoane et al., 2006). No transitional individuals were observed by Micale et al. (1996) in captive D. puntazzo during the third year of life. The sex-ratio for 3-year-old specimens of D. puntazzo in the Canary Islands was approximately 1 : 2. This result differs from that reported by Micale et al. (1996) who found a 1 : 1 sex ratio in captive D. puntazzo. The fish examined by Micale et al. (1996) did not show sex inversion. Failure to reach sexual development has also been reported in intensively reared species, and is not unusual in captive populations which very often need hormonal stimulation to spawn, or to reach the maturity stage.

 The similar size at sexual maturity between the sexes observed in this study is explained adequately by the sexual pattern (rudimentary hermaphroditism and partial protandry), with males and females attaining sexual maturity in their second year of life. This agrees with Micale et al. (1996), who found recovering spent mature individuals in captive D. puntazzo during the third year of life, indicating that the fish were already reproductively active at 2 years.

 Spawning occurs during autumn and early winter, from September to February. The initial period of spawning coincides with the warm phase of the water temperature cycle (24°C) and continues throughout early winter, ceasing when mean water temperature reaches the annual lowest values (17°C). The peak of spawning activity occurs when the water temperature starts to decrease. The gonadosomatic index is closely associated with the seasonality of the maturity stages, assigned macroscopically, and with water temperature. The relationship between spawning activity and mean water temperatures indicates optimal spawning temperatures range between 18 and 20°C. Mean monthly gonadosomatic indices do not vary between males and females during the pre-spawning and spawning periods, indicating that ovary and testis sizes are identical. Similar results have been reported by Micale et al. (1996) in D. puntazzo in captivity during the third year of life.

 Literature on the reproduction of the Diplodus species shows that spawning seasonality appears to be correlated with food availability and sea temperature (Morato et al., 2003; Matic-Skoko et al., 2007). In this study, D. puntazzo spawn when the sea temperature started to decrease. The spawning of D. puntazzo in culture conditions in the Mediterranean Sea takes place from August to October (Tortonese, 1975; Micale et al., 1996). The differences observed with the spawning period of D. puntazzo in the Mediterranean Sea may be due to the fact that the breeding season in the Canaries is timed to ensure that the settlement of juveniles on coastal water avoids the settlement period of the other Diplodus species present in the archipelago. In this sense, D. vulgaris spawns in December–January, D. sargus cadenati in January–February, D. annularis in March–April, and D. cervinus cervinus in May–June (Pajuelo and Lorenzo, 2001, 2004; Pajuelo et al., 2003, 2006). Similar reproductive and settlement patterns have also been reported for Diplodus species in the Mediterranean (Biagi et al., 1995; Vigliola et al., 1998).

 The protracted spawning season of D. puntazzo recorded in this study indicates serial spawning behaviour, which has been observed for other Diplodus species (Mann and Buxton, 1998; Gonçalves and Erzini, 2000; Pajuelo et al., 2006). The study shows that vitellogenesis does not begin synchronously for all females, and the simultaneous presence of oocytes in all stages of development indicates an asynchronous mode of ovarian development in D. puntazzo. In addition, the co-presence of postovulatory, together with tertiary yolk vesicle oocytes, indicates that D. puntazzo is a multiple spawner.

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 References

Mann, B. Q.; Buxton, C. D., 1998: The reproductive biology of Diplodus sargus capensis and Diplodus cervinus hottentotus (Sparidae) off the south-east Cape coast, South Africa. Cybium 22, 31–47.


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