EVALUATION OF MACROSCOPIC MATURITY ANALYSIS WITH HISTOLOGY IN THE DIGYNIC PROTANDROUS HERMAPHRODITE WHITE SEABREAM DIPLodus Sargus Sargus (PISCES, SPARIDAE)

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ABSTRACT. – Gonads of 111 individuals of a digynic protandrous hermaphrodite fish species, white seabream (Diplodus sargus sargus), were sampled along the coast of NW Sicily (southern Tyrrhenian Sea). Theses gonads were analyzed to evaluate a macroscopic maturity scale widely used in fisheries studies, through histological examination of the gonadal tissue, to observe the microscopic morphological features. A 5-phase microscopic classification scale was produced based on macroscopic staging for both males and females. A mismatch was found between macro and microscopic sexing due possibly to the difficulties met in correct sex identification of bisexual individuals. Likewise a mismatch was found between macro and microscopic reproductive phase identifications. Partial matching was detected between the two analysis methods as regards the monthly occurrence of different reproductive phases, with a peak of spawning capable individuals in spring. Gonadosomatic index and sea surface temperature were used along with reproductive phases to describe the reproductive cycle on a monthly basis. The seasonal trend of gonadosomatic index followed closely that of reproductive phases, showing a peak in spring preceded and followed by consistently low gonad weight. Despite the small sample size, microscopic validation of a macroscopic reproductive phase evaluation proved useful to identify the weaknesses of the latter method and widened the knowledge on reproductive biology of white seabream in the Tyrrhenian Sea.

INTRODUCTION

Knowledge of reproductive biology, besides being an important aspect of basic biology, is key in management of fish populations. Identification of spawning season and maturity phase, estimation of length at first maturity, as well as characterization of sex change in hermaphroditic species, all increase our understanding of spatial and temporal patterns in the reproductive cycle of exploited finfish and shellfish. Moreover, such parameters provide key-elements used in stock assessment programs and population dynamics models. For this reason, fishery commissions strongly recommend to produce accurate methodologies for sex and maturity determination, to standardize them in order to obtain reliable data sets, as well as to minimize differences in data quality when the same species are studied in different areas by different teams (ICES 2014).

For most exploited marine species, the determination of sex and reproductive phase is traditionally based on visual inspection of macroscopic features of whole gonads (West 1990). This method allows reproductive status of individuals to be determined immediately and large amounts of data needed by fisheries scientists to be collected rapidly and inexpensively. However, macroscopic analysis alone may lead to a misclassification of reproductive phases because it relies upon subjective judgement of gonad size, shape, and color that may only partially correspond with actual gonadal development (Ferreri et al. 2009, McPherson et al. 2011, Midway & Scharf 2012, Klibansky & Scharf 2015). Histological analysis provides detailed information about changes occurring in gonadal tissue and allows researchers to distinguish precisely between immature, maturing and fully mature individuals (Murua & Saborido-Rey 2003). This method can be successfully used as a powerful tool for validating a macroscopic scale by linking categories of microscopic development to macroscopic features (Bianchini et al. 2010, Prista et al. 2014, Sieiro et al. 2014), possibly coupled to a gonadosomatic index (Barreto et al. 2008, McPherson et al. 2011).

The white seabream, Diplodus sargus sargus (L., 1758), is a high-value sparid fish targeted by artisanal and recreational fishers throughout the Mediterranean (Harmelin-Vivien et al. 1995). This species has been reared in aquaculture farms in Greece and in a few other countries (FAO 2014) and has been the object of marine ranching experiments (D’Anna et al. 2004, Santos et al. 2006, Pereira et
Despite its high economic value, few studies have focused on the reproductive ecology of this species, especially in the Mediterranean Sea where sexual activity in wild white seabream has been addressed only in Tunisian waters (Mouine et al. 2007). Family Sparidae includes several types of hermaphroditism among its species (Sadovy de Mitcheson & Liu 2008). The white seabream is a rudimentary hermaphroditic species featuring partial digynic protandry; it undergoes a bisexual gonadal phase during juvenile development after which males develop at first maturity, while females may arise either at first maturity (primary females), or from males undergoing sex reversal (secondary females) (Micale et al. 1987, Micale & Perdichizzi 1994, Mouine et al. 2007).

In the present study, gonadal maturation in wild white seabream was investigated to validate the reproductive phases assigned using a macroscopic scale by means of microscopic examination of histology slides of gonad tissue. Body and reproductive conditions were also assessed to fill the knowledge gap on timing of sexual maturity cycle in relation to sea temperature.

**MATERIALS AND METHODS**

White seabream samples were collected monthly from January to December 2010, except in August, by professional fishermen along the western coast of the Gulf of Castellamare (northwestern Sicily, southern Tyrrenian Sea, 38°03’N 12°55’E) by means of longline and trammel net (Fig. 1). During the same dates, sea surface temperature (SST) was measured using a multiparameter probe at two stations within the fishing area (Fig. 1).

Fishes were processed in laboratory by the same technician within 6 hours from catch. For each individual, total length (TL, to the nearest 1 mm), total weight (TW, to the nearest 1 g), and somatic weight (SW, TW-gonad weight, to the nearest 1 g) were recorded. The abdominal cavity was dissected to obtain gonadal weight (GW, to the nearest 0.01 g), and sex determination by visual examination, *i.e.* male (M), female (F) and, when male and female tissues of the gonads appeared both well visible, the individual was considered to be a bisexual (B). Moreover, for males and females, reproductive phase was assigned according to a five-phase macroscopic scale (Holden & Raitt 1974).

Gonads were fixed in 4% buffered formalin for about two or three months, then gonadal tissues were embedded in paraffin, cut at 5 μm and stained with hematoxylin and eosin (Hunter & Maciewicz 1985). Both sexes were microscopically staged for reproductive phase implementing the five-phase macroscopic scale presented by Holden & Raitt (1974), modified by the authors according to the histological characteristics, but adopting the standardized terminology proposed by Brown-Peterson et al. (2011). Reproductive phases for ovaries were selected according to the most advanced developing stage of oocytes, as usually adopted for multiple spawners (West 1990).

Percentage of correct matching (PCM), *i.e.* the proportion of agreement between macro and microscopic examination, was calculated for each reproductive phase, joining M + Mf and F + mF (where Mf is referred to bisexual gonads classified as functional primary males, and mF is referred to bisexual gonads classified as functional primary females—see Results section for more details). The analyses on PCM, between macro and microscopic identifications of reproductive phase, were made excluding fishes for which the results showed a mismatched sex recognition between two evaluation methods, *i.e.* fish macroscopic identified as M but F without evidence of hermaphroditism at the histological analysis, and vice versa.

Mean gonadosomatic index (GSI) was calculated on a monthly basis for each sex grouping M + Mf and F + mF:

\[
GSI = \frac{GW}{SW} \times 100
\]

where SW is the somatic weight, and GW is the gonadal weight.

Chi-square test was used to detect differences between macro- and microscopic gonadal classifications for each sex (F, M, and B) and reproductive phase. The relationship between

![Fig. 1. – Map of the study area. The oval region indicates the area of fish collection, asterisks (*) indicate the sea surface temperature (SST) sampling stations.](image)
RESULTS

Overall, 111 individuals of white seabream (mean monthly no. = 10.09 ± 7.98) ranging from 165 to 380 mm TL and from 65 to 1025 g TW were processed. Details of monthly catches are shown in Table I.

Comparison of macro versus microscopic analysis

Histology revealed the following gonad types: I) ovaries with no male tissue, classified as females (F); II) testes with no female tissue, classified as males (M); III) bisexual gonads (ovotestes) containing a predominantly testicular portion at different developmental stage and an ovarian portion showing only oogonia and primary growth oocytes, classified as functional primary males (Mf); IV) bisexual gonads (ovotestes) containing a predominantly ovarian portion at different developmental stage and a degenerating or atrophied testicular portion, classified as functional primary females (mF). In both bisexual gonads (Mf and mF), the non-functional sex was observable only in a confined, limited portion of the histological slides.

All gonads were evaluated histologically and sex and maturity phase was assigned to each fish (Table II; Figs 2, 3). Immature females are characterized by the presence of only primary growth oocytes (PG) in the ovaries (Fig. 2A), while only spermatogonia (SG) are present in immature testes (Fig. 3A). Developing phase showed the appearance of cortical alveoli (CA) in females (Fig. 2B) and spermatocytes (SC) in males (Fig. 3B); spawning capable displayed vitellogenic oocytes (VO), or hydrated ones (H) when spawning occurred in females (Fig. 2C, D), while the presence of spermatotaxa (SZ) is typical of phase 3 males (Fig. 3C) in both sexes, the last two spawning phases (regressing and regenerating), both representing conditions at the end of reproductive season, are characterized by the new presence of first development stage like PG and SG, and in females degenerative processes of follicles, as atresia (A) and post-ovulatory follicle (POFs) (Figs 2E, F, 3D, E). Those individuals presenting microscopic evidence of hermaphroditism (Fig. 4) were sexually classified taking into account only the functional tissue in bisexual gonad (N = 62; Table III). The sex frequency values assigned to all individuals dif-

Table I. – Number (N), mean total length (TL) and mean total weight (TW) of white seabreams caught each month and sea-surface temperature (SST) monthly average values. Standard deviation values are reported in parentheses.

<table>
<thead>
<tr>
<th>Month</th>
<th>N</th>
<th>TL (mm)</th>
<th>TW (g)</th>
<th>SST (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>January</td>
<td>14</td>
<td>282 (43)</td>
<td>431 (223)</td>
<td>14.34 (0.42)</td>
</tr>
<tr>
<td>February</td>
<td>8</td>
<td>262 (48)</td>
<td>348 (200)</td>
<td>14.30 (0.06)</td>
</tr>
<tr>
<td>March</td>
<td>16</td>
<td>234 (40)</td>
<td>254 (144)</td>
<td>14.18 (0.04)</td>
</tr>
<tr>
<td>April</td>
<td>17</td>
<td>251 (21)</td>
<td>299 (77)</td>
<td>16.15 (0.18)</td>
</tr>
<tr>
<td>May</td>
<td>8</td>
<td>234 (44)</td>
<td>243 (126)</td>
<td>18.72 (0.04)</td>
</tr>
<tr>
<td>June</td>
<td>3</td>
<td>275 (63)</td>
<td>434 (267)</td>
<td>19.95 (0.29)</td>
</tr>
<tr>
<td>July</td>
<td>1</td>
<td>325 (-)</td>
<td>663 (-)</td>
<td>23.18 (0.08)</td>
</tr>
<tr>
<td>August</td>
<td>0</td>
<td>–</td>
<td>–</td>
<td>23.90 (0.14)</td>
</tr>
<tr>
<td>September</td>
<td>28</td>
<td>222 (32)</td>
<td>203 (94)</td>
<td>22.55 (0.49)</td>
</tr>
<tr>
<td>October</td>
<td>3</td>
<td>273 (13)</td>
<td>358 (77)</td>
<td>19.96 (0.20)</td>
</tr>
<tr>
<td>November</td>
<td>7</td>
<td>256 (32)</td>
<td>316 (125)</td>
<td>17.48 (0.06)</td>
</tr>
<tr>
<td>December</td>
<td>6</td>
<td>305 (28)</td>
<td>511 (136)</td>
<td>15.36 (0.01)</td>
</tr>
</tbody>
</table>

Fig. 2. – Histological sections of different reproductive phases in white seabream females. A: Immature (phase 1); B: Developing (phase 2); C: spawning capable (phase 3); D: spawning capable, with hydrated oocytes (phase 3); E: regressing (phase 4); F: regenerating (phase 5). PG = primary growth, CA = cortical alveoli, VO = vitellogenic oocytes, H = hydrated, POF = post-ovulatory follicles, A = atresia. See Table 2 for more details about each reproductive phase.

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fered significantly between the two methods \( (n = 111, \chi^2 = 163.09; \text{df} = 2, p < 0.0001) \). The comparison between two analyses confirmed the sex for 50% of females, correctly identified by naked eye as revealed by histology, while PCM was 22% for males and 100% for bisexual fishes, although only two seabreams with macroscopic evidence of hermaphroditism were caught (Table III). Grouping individuals with and without evidence of hermaphroditism led to a much higher PCM value in both sexes \( (F + mF = 84 \% ; M + Mf = 81 \%) \).

A significant difference in reproductive phase identification was detected between the two classifications for both sexes \( (n = 53, \chi^2 = 65.00; \text{df} = 4, p < 0.001, \text{for females}; n = 58, \chi^2 = 44.81; \text{df} = 4, p < 0.001, \text{for males}) \). Higher PCM values were observed generally in females (Table IV); although the macroscopic evaluation of spawning capable (phase 3) males appeared highly correct (PCM = 95.45%), females reached PCM ≥ 80% in phases 3, 4 and 5. A large error arose for females macroscopically identified as developing (phase 2), because 66.67% of them were classified as regenerating (phase 5) from histological analysis (Table IV). In males, the
biggest misclassification was that 92.31% of fish macroscopically identified as phase 2 were microscopically classified as phase 3. Correct macroscopic evaluation rates were ≤ 20% for all other male phases (Table IV).

Table III. – Percentage of matching between macroscopic (Macro) and microscopic (Micro) analyses of sex in white seabream. Grey boxes indicates the correct matching percentage (PCM). In parentheses, the number of white seabream specimens per sex: female (F), male (M), macroscopically bisexual (B), functional female bisexual (mF) and functional male bisexual (Mf) white seabreams. Mf and Fm was defined by microscopic analyses results.

<table>
<thead>
<tr>
<th>Micro</th>
<th>F</th>
<th>mF</th>
<th>Mf</th>
<th>M</th>
</tr>
</thead>
<tbody>
<tr>
<td>Macro</td>
<td>F</td>
<td>50 (25)</td>
<td>34 (17)</td>
<td>6 (3)</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>100 (2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>10 (6)</td>
<td>9 (5)</td>
<td>59 (35)</td>
</tr>
</tbody>
</table>

Reproductive biology of white sea bream

The monthly percent distribution of reproductive phases showed differences between macro and microscopic analyses for both sexes, especially in females. The annual reproductive cycle evolution is most distinct in female histological data. Although macroscopic examination recognized spawning capable (phase 3) females in January and February, microscopic analysis of ovaries showed that the spawning season was restricted to the spring, with a peak in April as highlighted also by more abundant hydrated oocytes than in other spring months. Although macroscopic analysis identified developing females (phase 2) in October (66.67% of individuals), histological data showed that ovaries did not begin to mature until January. Macro and micro reproductive phase evaluations agreed at 100% in only two months, in April (100% of fishes are spawning capable, phase 3) and in June (100%
of females are regressing, phase 4) indicating the end of the spawning period.

According to microscopic analysis, male seabreams started to mature in November (100 % of fishes are developing, phase 2) and were spawning capable (phase 3) from December to May, when 60 % of individuals were in phase 3 and 40 % in phase 5. According to macroscopic evaluation instead, they appeared able to spawn from January (60 % of individuals are developing and 40 % are spawning capable). As in the case of females, macroscopic observations detected 100 % of male seabreams as regressing (phase 4) in June, but histological data confirmed all these fishes were regenerating (phase 5) and the presence of regressing was only in March (10 % of individuals) and in April (14.29 % of individuals).

Trend of mean GSI highlighted a peak in April for both sexes preceded by a gradual increase started in December and followed by an abrupt fall in May, and by very low values between June and November (Fig. 6). The GSI versus SST monthly trend showed that the index reached a peak when the temperature began to increase after the winter minimum (Fig. 6). Significant negative correlations between GSI and SST were evident for both females \((n = 10; r = -0.66; p < 0.05)\) and males \((n = 10; r = -0.78; p < 0.05)\).

### DISCUSSION

The macroscopic determination of sex and reproductive phase by visual examination of gonads is a rapid, inexpensive method for determining the reproductive status in fishes. However, such method offers low accuracy because staging process is affected by subjective judgment (Stratoudakis et al. 2004, Gerritsen & McGrath 2006, Ferreri et al. 2009). Moreover, in species with hermaphroditism and/or asynchronous gonadal maturation, it is often difficult to set discrete categories and define distinct reference points based on macroscopic criteria, because maturation is a continuous and gradual process also during the spawning season.

Although the use of commercial vessels, but also artisanal fishery, to collect samples for scientific purposes, and particularly for study on reproductive biology, is a commonly applied practice (e.g. Alonso-Fernández et al. 2008, Khoufi et al. 2014, Carbonara et al. 2015, Jaziri et al. 2015), such sampling strategy shows some limits, particularly in relation to regularity on samples collection. However, despite the small number of sampled fishes (Table I), explained above all by sampling method, this study provides some useful knowledge on the reproductive biology of the white seabream in central Mediterranean. The obtained results on both reproductive phase evaluation and reproductive cycle description appeared well supported by the analysis and method applied, and in agreement with the literature. Histology is accepted as the best practice to study reproductive biology and to validate the gonad maturity evaluations, particularly in species showing sex reversal phenomena and/or asynchronous ovary development (Sadovy & Shapiro 1987, Sadovy & Domeier 2005, Ferreri et al. 2009, Costa 2010, Khoufi et al. 2014). Moreover, the presence of actively reproductive individuals and the spikes of the gonadosomatic index were recorded in the same period, as shown by similar studies in south Mediterranean Sea (Mouine et al. 2007).

### Comparison of macro versus microscopic analysis

Macroscopic sex identification represents a difficult task in hermaphroditic species. A better correspon-
dence between macro and microscopic examination was reported for females (50 %) than for males (22 %). When the histological definition of male and female was defined based on the predominant tissue type present in the gonad, the PCM rose to more than 80 %, probably because, in the early sex reversal process, any evidence of such phenomenon is not apparent in macroscopic features. Although the literature recommends the use of histology as the best methodology to recognize evidences of hermaphroditism in a gonad (Sadovy & Shapiro 1987), present results on sex identification can be evaluated as acceptable for macroscopic determination according to previous observations. A study carried out on two protogynous hermaphrodite species confirmed that macroscopic evaluation is a good tool for sex recognition, when only the prevalent male or female tissue was considered (Klibansky & Scharf 2015). Otherwise, in the Gulf of Tunis, the proportion of bisexual white seabream was very low when fishes were sexed according to macroscopic features (6.4 %). But it was considerably higher after histological sexing (81.2 %) (Mouine et al. 2007). Similarly, in northern Spain only 5.6 % of bisexu-als were correctly recognized after macroscopic sexing (Martínez-Pastor & Villegas-Cuadros 1996).

Histology is a fundamental aid in reproductive biology studies of hermaphroditic fish because it allows to obtain otherwise invisible details of internal gonad morphology (Sadovy & Shapiro 1987, Sadovy & Domeier 2005). In the case of white seabream, like in other hermaphroditic fishes, histology provided support for functional hermaphroditism in the species by allowing to identify individuals that likely function as both sexes at some time during their lives (Sadovy de Mitcheson & Liu 2008). Other diagnostic criteria of hermaphroditism (i.e. sexual dimorphism, sex ratio biased by sex and age, etc.) are weak and cannot be used alone (Sadovy & Shapiro 1987, Sadovy & Domeier 2005).

Observation of PCM between macro and microscopic methods within each sex yielded more problematic results. Apart from reproductive phase 3 males, the highest PCM were reached in females (phases 3, 4 and 5), while a highly variable level of agreement resulted in all other reproductive phases for both sexes. These results suggested that the macroscopic maturity scale gives slightly better results for females than for males. In many fish species, macroscopic misclassification between immature (phase 1) and regenerating (phase 5) females represents the most common error (Saborido-Rey & Junquera 1998, Domínguez-Petit 2007, Costa 2009), because in both phases the oocytes are not visible by naked eye. This occurred also in the present study, in which macroscopic analysis failed to identify more than 50 % stage 5 females, which were incorrectly assigned to stage 1 or 2, resulting in an underestimation of their number in the population. Similarly to females, both phase 1 and 5 males appeared not well recognized. Such misclassification has an impact on the estimation of reproductively active proportion of fish population because, unlike immature individuals, regenerating fish have already contributed to the stock spawning biomass of that year. Klibansky & Scharf (2015) showed similar results for Centropristis striata and Pagrus pagrus, highlighting a possible mistake in the estimate of size at maturity, when only macroscopic data were used, especially outside of the spawning season when regenerating fishes are very abundant.

The most easily recognizable macroscopic phase in both sexes is generally the spawning capable (phase 3), especially in females when ovaries are hydrated: big size and translucent aspect of hydrated oocytes represent a clearly identifiable feature also for less expert technicians (Domínguez-Petit 2007, Costa 2009, Ferreri et al. 2009). This proved true also in the present study, as demonstrated by correct attribution of macroscopic stage 3 to 80 % of females and the remaining 20 % misclassified with developing (phase 2). However, microscopic anal-
ysis identified a larger number of stage 3 males (95 %), suggesting that the macroscopic description by Holden & Raitt (1974) may be more adequate to detect spawning capable males than females in white seabream.

Incorrect recognition of reproductive phase can lead to problems and errors in fisheries management because it affects the assessment of the spawning population, the length at first maturity, the spawning period, etc. (Murua et al. 2003, Stratoudakis et al. 2004, Ferreri et al. 2009). The discrepancies generally observed among macroscopic and histological classifications may be due to one or more of the following causes: operator subjectivity and/or inexperience, unclear description of reproductive phases in reference scales, time elapsed from catch to sample examination before tissue degradation processes become significant. Misclassification of maturity phases in white seabream may also be due to the lack of species-specific scales or of histologically validated macroscopic scales. Several studies described and categorized the reproductive phases of different hermaphrodite fishes (e.g., *Pagrus pagrus*, Kokokiris et al. 1999, *Diplodus sargus sargus*, Mouine et al. 2007, *D. puntazzo*, Pajuelo et al. 2008 *Coris julis*, Serranus scriba, and *D. annularis*, Alonso-Fernández et al. 2011), while no studies addressed wild white seabream. The very few studies on this species proposed a scale with description of macroscopic features for the related species *D. sargus capensis* in the South Africa (Mann & Buxton 1998) and histological classification in hatchery-reared specimens (Micale et al. 1987, Micale & Perdichizzi 1994).

**Reproductive biology of white sea bream**

Despite the small number of sampled fish, particularly from June to October, the reproductive seasonality in white seabream in this study is rather unequivocal, due to the presence of maturing, spawning and post-spawning phases in both sexes, and the clear seasonal trend in GSI. Moreover, present results are consistent with previous studies on the same species, as can be argued by the timing of spawning peak. Monthly trend of gonadosomatic index confirmed such seasonality, in agreement with pattern observed in the Gulf of Tunis by Mouine et al. (2007), and to a lesser extent with the one described in the Azores by Morato et al. (2003). Although some authors questioned the usefulness of the gonadosomatic index as a proxy of maturity state and reproductive potential (DeVlaming et al. 1982, West 1990, Yoneda et al. 2013), several studies demonstrated its applicability for such purposes (Trippel 1997, Marshall et al. 1998), highlighting also its validity as a tool for studying the reproductive seasonality in batch spawning species (i.e. Basilone et al. 2006, Koufi et al. 2014), including some showing hermaphroditism (Mann & Buxton 1998, Mouine et al. 2007, Alonso-Fernández et al. 2011; Klibansky & Scharf 2015). GSI trend showed by the present samples did not differ between sexes, as well as for white seabream inhabiting the Gulf of Tunis (Mouine et al. 2007). The high values observed in March and April followed by a drastic decrease suggested that in May most of the population has already spawned. Although the results of the two different methods of gonad analysis overlap only partially, the comparison of temporal trends between both maturity evaluation methods and GSI confirmed the general pattern of reproductive seasonality. The concurrence of the presence of developing females (F + mF in phase 2) and GSI increase, although not showing much high values, suggested that female seabreams began maturation in January, reaching the spawning peak in March/April (F + mF in phase 3). Although all females appeared spawning capable from March-May, the GSI data is substantially higher in April than in either other month, thus indicating an increasing energy investment into egg production at the population level. The GSI values confirmed a spawning peak in early spring also for males, although spawning capable males were observed for a longer period than females. Taking all the data together, all the males would be able to spawn already in January and February, but probably they are not producing a lot of sperm, as suggested by low GSI. Such behavior may be explained by the fact that the females are not producing any eggs during the first months of the year, so they are not fertilizing anything. Low production of sperm (i.e. maintained low GSI) appeared a good strategy because it would be energy wasted (Aristizábal 2007).

In other species, including gonochorists, spawning capable males are present year round, despite the well defined spawning period in females, i.e. *Mullus* spp. (Mahé et al. 2013, Carbonara et al. 2015).

The spring peak in the prevalence of spawning capable individuals appeared significantly related to the rise in temperature after the winter minimum, and within a temperature range comparable with the data collected in the Azores (Morato et al. 2003 and reference therein) and in the Gulf of Tunis (Mouine et al. 2007). It is well known that sexual activity and duration of spawning in many fish species with asynchronous gonadal development are governed by temperature and photoperiod (i.e. Bye 1984, Motos et al. 1996, Van Der Kraak & Pankhurst 1996, Holt & Riley 2000).

**CONCLUSIONS**

The present study highlights the difficulties inherent to the identification of bisexual gonads, particularly in early transition periods from a sex to the other one, and to the macroscopic determination of reproductive phases in white seabream. The partly contrasting results obtained from macroscopic and microscopic evaluation methods of gonadal analysis suggested that histological validation should be used where possible in order to fine tune
the macroscopic analysis, which is usually applied in fish stock assessment programs.

Histological observation of gonads was used here for the first time in a reproductive study of white seabream in the Tyrrhenian Sea. Although expensive and time consuming, microscopic analysis proved an essential tool to develop definitive criteria to describe the maturity phases, and to investigate other aspects of the reproductive biology in white seabream, such as the identification of a spawning peak.

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