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# The Use of Histological Techniques to Study the Reproductive Biology of the Hermaphroditic Mediterranean Fishes *Coris julis*, *Serranus scriba*, and *Diplodus annularis*

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***Abstract***

**The usefulness and importance of histological techniques in reproductive studies have been widely discussed for gonochoristic fish species. In the case of hermaphroditic fishes, histology is a particularly important tool for the proper identification of sexual pattern (i.e., sequential, simultaneous, or nonfunctional hermaphroditism). We used a histological approach to describe hermaphroditism, dynamics of follicle development, and spawning patterns in three species from the northwestern Mediterranean Sea (Balearic Islands, Spain): the Mediterranean rainbow wrasse *Coris julis* (sequential hermaphrodite), painted comber *Serranus scriba* (simultaneous hermaphrodite), and annular sea bream *Diplodus annularis* (nonfunctional hermaphrodite). Development of secondary growth follicles was asynchronous in all three species, and similar peaks in spawning activity occurred between May and July. However, notable differences in sexual cycle and egg production were found. For the painted comber, hydrated follicles were present in ovarian tissue almost every day during the peak of the spawning season, suggesting daily spawning and increasing the chances of autofertilization unless a mechanism to avoid this action is present in this species. Intersexual Mediterranean rainbow wrasses were identified, and the size and age at sex change were estimated to be 132 mm total length and 4 years, respectively. Previous investigators have concluded that the annular sea bream is a protandric hermaphrodite, but our results indicate nonfunctional hermaphroditism. These three species are of little commercial interest and are considered to be bycatch by the artisanal fleet, but they are vulnerable to the impacts of some recreational angling activities. Currently, no specific management plan has been developed for these species. We address the importance of describing sexual pattern and its implications for future conservation efforts.**

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To understand fish population dynamics, it is essential to study reproductive biology, especially in exploited species. One of the key aspects of the present study is the classification of species' sexual patterns, defined as the typical expression of sexuality in a strictly functional sense, as exhibited within the lifetime of individual fish in a population or species (Sadovy and Shapiro 1987; Sadovy and Domeier 2005; Sadovy de Mitcheson and Liu 2008). Teleost fishes are characterized by a diversity of sexual patterns. The number of families known to include hermaphroditic species has increased significantly from 13 (as reported by Atz 1965) to 27 (Sadovy de Mitcheson and Liu 2008), but most marine fishes of commercial importance are gonochoristic (Murua and Saborido-Rey 2003). Beyond its importance for phylogenetic purposes (Sadovy and Domeier 2005; Sadovy de Mitcheson and Liu 2008), the classification of sexual pattern should also be fundamental to the selection of fisheries conservation strategies because sex-changing species may be particularly prone to overexploitation by size-selective fishing (Punt et al. 1993; Alonzo and Mangel 2004; Molloy et al. 2007; Sattar et al. 2008). Other important aspects of reproductive biology that must be considered to determine the correct management strategies for a species are the follicle development process, reproductive cycle, and timing of spawning.

The usefulness and importance of histology techniques in reproductive studies have been widely illustrated for fish species (West 1990; Tyler and Sumpter 1996; Blazer 2002). Histology offers a powerful tool for reproductive studies and is routinely used for sex verification, assessment of reproductive phase, or quantification of atresia (Blazer 2002). Histology is particularly crucial for confirming the sexual pattern in hermaphroditic fishes. One or more of the following conservative criteria are used to diagnose functional hermaphroditism: (1) detailed gonadal histological slides that show several phases of sex change, (2) simultaneous occurrence of mature testicular and ovarian tissues in gonads, and (3) observations of functional sex change in identified individuals (Sadovy and Shapiro 1987; Sadovy and Domeier 2005; Sadovy de Mitcheson and Liu 2008). Other diagnostic criteria that are weak and therefore cannot be used alone include the presence of a bisexual gonadal phase, a sex ratio that is biased by sex and age, and sexual dimorphism (Sadovy de Mitcheson and Liu 2008).

To illustrate the coexistence of fishes with different hermaphroditic strategies in the same ecosystem, three characteristic species of the northwestern Mediterranean Sea were selected for study: the Mediterranean rainbow wrasse *Coris julis* (hereafter, "rainbow wrasse"; Labridae), painted comber *Serranus scriba* (Serranidae), and annular sea bream *Diplodus annularis* (Sparidae). In the Balearic Islands, Spain, these three species are of low commercial interest and are classified as byproduct or bycatch by the artisanal fleet (A. M. Grau, Direcció General de Pesca, Govern de les Illes Balears). Due to the past and present lack of commercial interest in these species, no specific management plan has been developed for them anywhere in the northwestern Mediterranean Sea. However, these fishes are the primary targets of recreational fishing (Morales-Nin et al. 2005) and have recently been reported as being vulnerable to the impacts of some recreational angling activities (Alós et al. 2008). Little information is available on the reproductive biology of painted comber within the study area (Tuset et al. 2005; Zorica et al. 2005). For the rainbow wrasse, previous studies were mainly focused on the sex reversal process (Bentivegna and Rasotto 1983; Bentivegna et al. 1985; Bruslé 1987; Lejeune 1987) and sexual pattern (Bacci and Razzauti 1958). Classification of the sexual pattern of the annular sea bream is still controversial (Buxton and Garratt 1990; Gonzalez Pajuelo et al. 2001; Matić-Skoko et al. 2007; Alós et al. 2010a).

The purpose of the present study was to use a histological approach to establish the functional sexual pattern of each species and then to delineate the organization of gonad development, the seasonal cycle of reproductive development, and the timing of spawning.

## METHODS

Samples of rainbow wrasses, painted comber, and annular sea bream were collected during fishing sessions conducted in Palma Bay and at the National Park of the Cabrera Archipelago, located off the south coast of Mallorca Island (northwestern Mediterranean Sea; Figure 1). This sample site was selected due to plentiful habitat with characteristics appropriate for the three study species—specifically, a 10–20-m depth with substrate dominated by beds of the seagrass *Posidonia oceanica*, a marine phanerogam that is characteristic of shallow sand substrate in the Mediterranean Sea. Individual fish were captured by use of hook-and-line methods with conventional recreational gear (rod and manual reel; six J-hooks with a 14-mm shank and 7-mm gape, each on a 15-cm leader; baited with 1-cm$^2$ pieces of shrimp) from December 2005 to August 2008 (Table 1). In total, 1,221 rainbow wrasses, 857 painted comber, and 918 annular sea bream were collected (Table 1). Total length (TL; nearest mm), gonad weight (nearest 0.01 g), and age were recorded for each individual. As an approximation of individual total weight ($W$; g), general weight–length relationships determined during the research project ROQUER (Ministerio de Educación y Ciencia, Spain) were used:

$$\begin{aligned}
\text{rainbow wrasse:}\quad & W = (3 \times 10^{-6})\text{TL}^{3.2457} \\
& (n = 748,\ P < 0.001, r^2 = 0.99), \\
\text{painted comber:}\quad & W = (9 \times 10^{-6})\text{TL}^{3.0918} \\
& (n = 840,\ P < 0.001, r^2 = 0.99),\ \text{and} \\
\text{annular sea bream:}\quad & W = (9 \times 10^{-6})\text{TL}^{3.1386} \\
& (n = 1,496,\ P < 0.001, r^2 = 0.99).
\end{aligned}$$

Sagittal otoliths were used for age determination (Gordoa et al. 2000; Alós et al. 2010a, 2010b). Whole otoliths were

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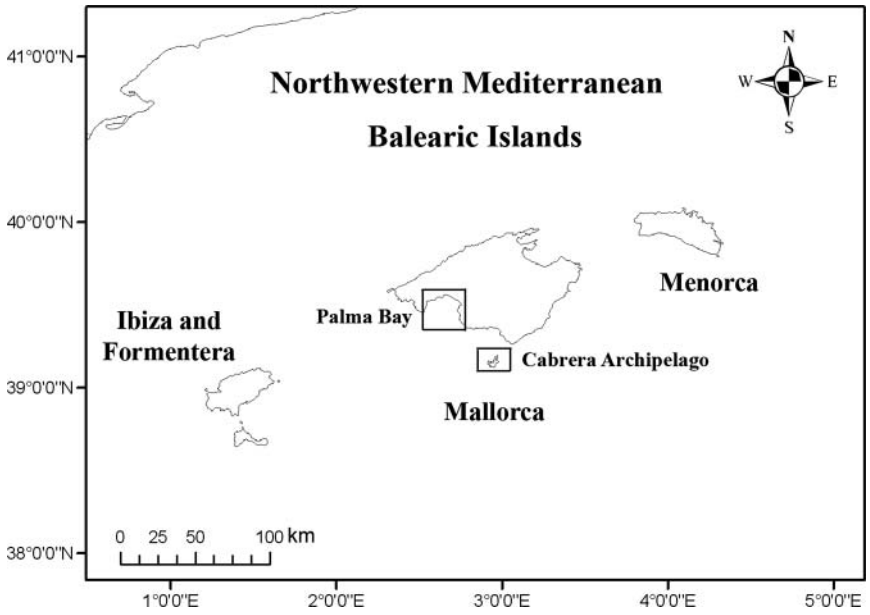



FIGURE 1. Location of fish sample collection areas in Palma Bay and at the National Park of the Cabrera Archipelago off the south coast of Mallorca Island, Spain (northwestern Mediterranean).

immersed in a glycerin–ethanol (50:50) solution to improve the visualization of annual increments, and each otolith was examined twice by a single reader; an interval of 2–3 months occurred between readings. If the two readings for a given otolith differed, a third reading was conducted; if this third reading was inconclusive, the otolith was discarded and considered unreadable. Gonads were also removed from all specimens and fixed immediately in a 10% solution of formalin buffered with $Na_2HPO_4 \cdot 2H_2O$ (molar concentration = 0.046 M) and $NaH_2PO_4 \cdot H_2O$ (0.029 M). The gonadosomatic index (GSI = [gonad weight/$W$] $\times$ 100) was estimated for sexually mature fish only.

Central portions of the fixed gonads were extracted, dehydrated, embedded in paraffin, sectioned at 3 μm, and stained with hematoxylin and eosin for microscopic analysis with a Leica Series RE digital microscope (Leica Microsystems, Wetzlar, Germany). To confirm sexual patterns, histological examination of specific gonadal features was conducted. In some cases, it was necessary to examine slides from several different locations within the selected gonads (see Sadovy and Shapiro

TABLE 1. Estimates of total length (TL) range for the Mediterranean rainbow wrasse, painted comber, and annular sea bream based on samples collected from the Balearic Islands, Spain, during the course of the reproduction assessment study (2005–2008).

| | Rainbow wrasse | | Painted comber | | Annular sea bream | |
|---|---|---|---|---|---|---|
| Month | *n* | TL range (mm) | *n* | TL range (mm) | *n* | TL range (mm) |
| Jan | | | | | | |
| Feb | 1 | 131–131 | | | | |
| Mar | 128 | 84–164 | 41 | 107–226 | 49 | 97–150 |
| Apr | 160 | 78–208 | 85 | 97–224 | 137 | 102–184 |
| May | 271 | 60–194 | 322 | 60–219 | 324 | 55–184 |
| Jun | 190 | 59–198 | 160 | 85–209 | 148 | 81–195 |
| Jul | 195 | 79–166 | 162 | 93–226 | 116 | 77–200 |
| Aug | 185 | 88–177 | 7 | 100–146 | 57 | 92–209 |
| Sep | 25 | 105–154 | | | 29 | 87–142 |
| Oct | 66 | 88–192 | 25 | 72–171 | 58 | 79–142 |
| Nov | | | | | | |
| Dec | | | 55 | 67–170 | | |
| Total | 1,221 | | 857 | | 918 | |

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1987). For each ovary, the follicles (oocytes and surrounding follicular layer) were classified into stages of development by using histological criteria (West 1990; Tyler and Sumpter 1996; Saborido-Rey and Junquera 1998; Murua and Saborido-Rey 2003). The stages assigned were primary growth follicle, cortical alveolar follicle, vitellogenic follicle, migratory nucleus stage, and hydrated follicle. Other ovarian structures, such as atretic follicles and postovulatory follicles, were also identified, and their presence was scored for each slide. In testicular tissue, germ cells were classified based on histological criteria into the stages of development described by Grier (1981). The assignment of female and male reproductive phases (for the rainbow wrasse and painted comber) was based on the criteria presented by Brown-Peterson et al. (2011, this special section). Testes in rainbow wrasses were identified histologically, but males were assigned to the primary or secondary male category based on fish external coloration. For annular sea bream, histological examination was carried out to determine only the sex of individuals and the sexual pattern of the species. Therefore, the GSI of sexually mature individuals was used to identify the spawning season of annular sea bream.

The daily spawning fraction of females was assessed from the prevalence of actively spawning females during the spawning season (calculated as the number of actively spawning females divided by the total number of mature active females in each month). Mature active females included the developing phase (early developing subphase excluded) and spawning capable phase (actively spawning subphase included); immature individuals and inactive mature fish were therefore excluded (Hunter and Goldberg 1980; Picquelle and Stauffer 1985; Macchi and Acha 2000). No work has yet been conducted in captivity to properly ascertain the duration of the histological indicators of spawning; thus, to determine the spawning fraction, it was assumed that the actively spawning subphase in females corresponded to the period of ovulation or within approximately 12 h of spawning (before or after) based on three histological indicators: late follicle maturation (i.e., completed germinal vesicle migration), ovulation, and newly collapsed postovulatory follicles (Lowerre-Barbieri et al. 2009). Once the spawning fraction was estimated, spawning frequency was calculated as the inverse of the spawning fraction (Macchi and Acha 2000). Immature females were excluded from this analysis. The size-frequency distribution of developing follicles was estimated by separating follicles in selected samples of each species according to the method of Lowerre-Barbieri and Barbieri (1993). The number of follicles in the sample was estimated by combining the gravimetric method with a computer-aided image analysis system that enumerated follicles and measured diameters in an approximately 0.050-g subsample of ovarian tissue. Measurements (one ovary from one individual of each species: 3,960 follicles in rainbow wrasse; 2,423 follicles in painted comber; 2,515 follicles in annular sea bream) were performed with Leica QWin software on a PC (AMD Athlon XP 3000+) connected to a Leica IC-A video camera on a Leica MZ6 stereomicroscope.

Estimation of TL and age at sex change in rainbow wrasses (i.e., TL and age at which 50% of the individuals had undergone the transition from the initial sex to the terminal sex) was conducted using generalized linear models with a binomial family function (Zuur et al. 2007). Data for these estimations were limited to the year 2007 because sampling did not occur throughout the year in 2006 or 2008. Statistical analyses were conducted by using R software version 2.7.2.

## RESULTS

### Mediterranean Rainbow Wrasse

As has been observed in previous studies, two colorations were found in the target rainbow wrasse populations: (1) a yellow-brown initial phase coloration ("giofredi livery") that is associated with females and a small proportion of small males called primary males (i.e., fish that are males at first maturation and do not undergo a sex change from functional female to functional male); and (2) a brightly colored terminal phase ("julis livery") found only in secondary males (i.e., fish that have transitioned from functional female to functional male). Among all rainbow wrasses sampled during this research ($n = 1{,}221$), only 10.4% of the individuals were classified as primary males.

The ovaries of this species are oval-shaped, displaying two lobes that are fused posteriorly; each lobe has a roughly hollow, cylindrical form and a lamellar configuration. Histological examination of the ovaries revealed that the rainbow wrasse exhibits an asynchronous development of secondary growth follicles (i.e., follicles at all stages of development are present without a dominant population; Figure 2a). This asynchrony was reflected by the follicle size-frequency distribution in the actively spawning subphase (Figure 3). The distribution was continuous except in ovaries where hydrated follicles were present; such ovaries had a separate mode of very large (diameter $> 600$ μm) hydrated follicles. Several modes were present within the continuous portion of the follicle size-frequency distribution, indicating the presence of several batches of follicles.

The testes of rainbow wrasses are lobular and show spermatogonial development along the germinal epithelium of the hollow lobules in reproductively active fish. Spermatogenesis is cystic, as developing germ cells are enclosed within germinal cysts formed by enveloping Sertoli-cell processes; within each cyst, the maturation of germ cells is synchronous (Figure 2b).

Intersexual individuals were clearly identified via histological examination. The first evidence of sex change was the presence of (1) degenerating ovarian tissue with an increasing number of atretic vitellogenic follicles and (2) spermatogonial nests distributed along the gonad wall (Figure 4a). Other structures were likely associated with sex reversal. For instance, the prevalence of eosinophilic cells in intersexual individuals could indicate a relationship between that cell type and the sex change process. Also, melanomacrophage centers were usually present as a result of follicle degeneration (Figure 4b). Some residual primary growth follicles could remain in the functional testes

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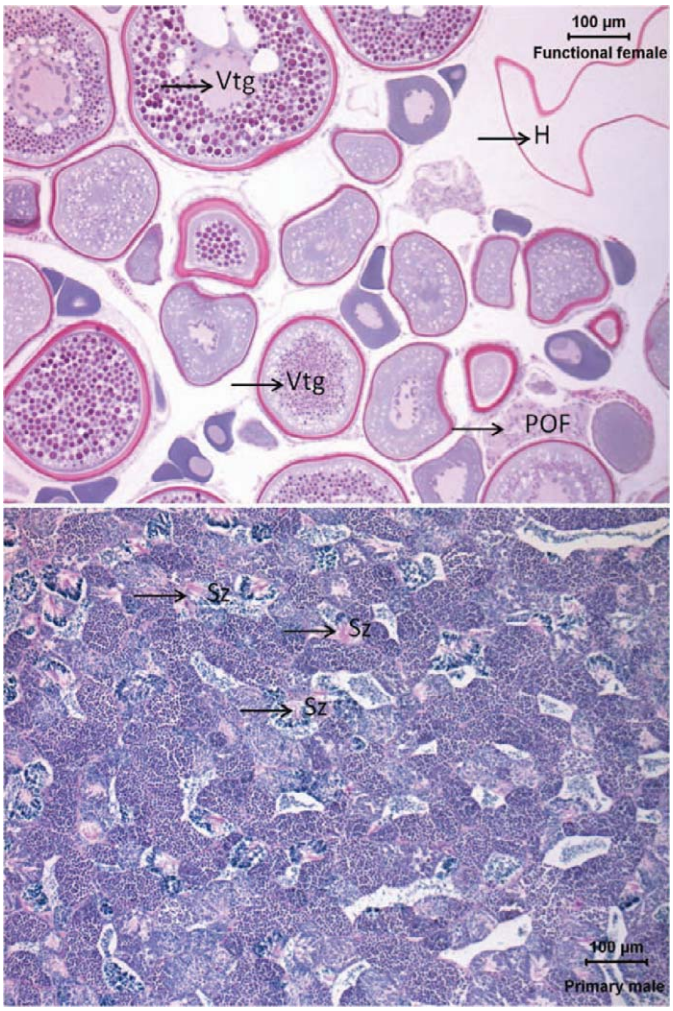



FIGURE 2. Transverse sections of Mediterranean rainbow wrasse: **(a)** actively spawning female (Vtg = vitellogenic follicle; H = hydrated follicle; POF = postovulatory follicle) and **(b)** mature primary male (Sz = spermatozoa).

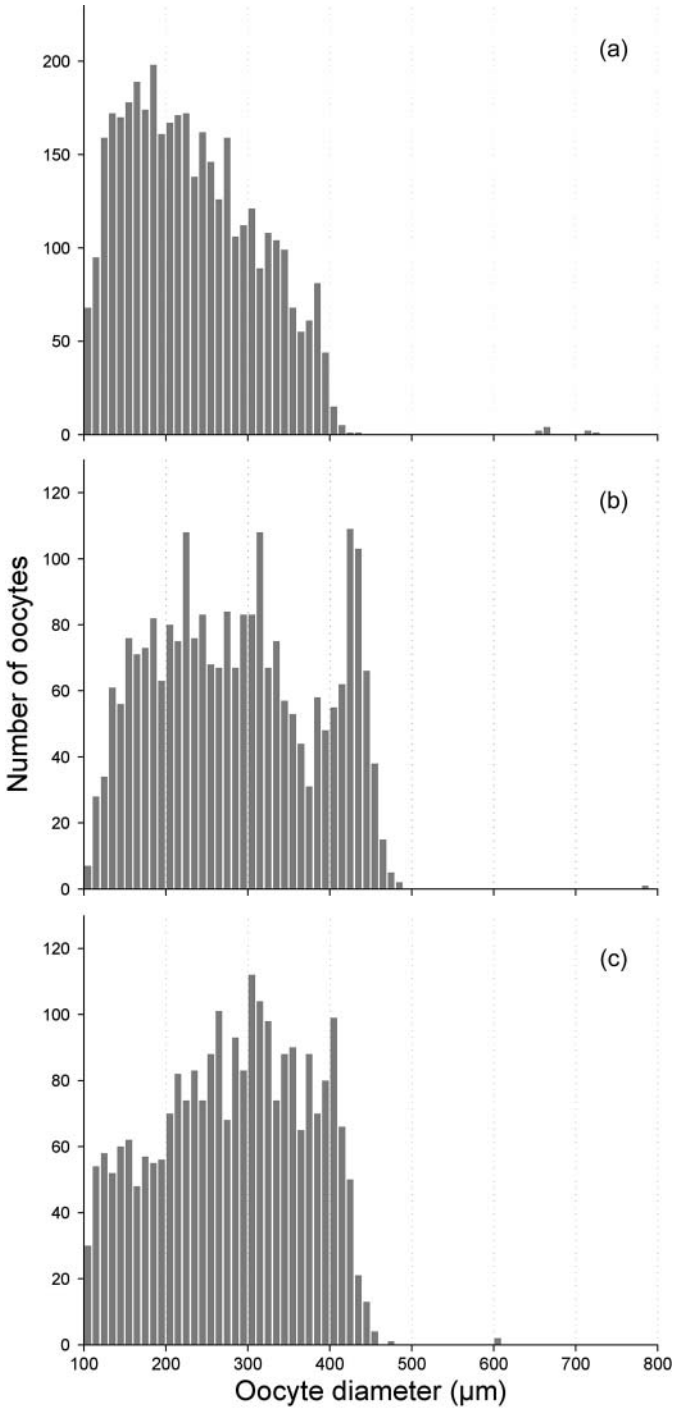



FIGURE 3. Size distribution of vitellogenic and larger follicles from an individual **(a)** Mediterranean rainbow wrasse, **(b)** painted comber, and **(c)** annular sea bream in the actively spawning subphase from collections obtained in 2007 (~0.05-g subsample).

(Figure 4c). In rainbow wrasses, testicular tissue proliferates from spermatogonial nests in the ovarian wall, spreading inward during sex change. As a result, the transformed gonad usually did not exhibit a clear lamellar organization, which made it difficult to distinguish the testes of primary and secondary males (Figure 5). Only two (0.5%) secondary males revealed clear evidence of previous lamellar organization projecting into a central cavity, likely corresponding to a residual ovarian lumen. A bimodal age-frequency distribution was evident; the mode for females (3 years) was at a younger age than the mode for secondary males (5 years). Primary males also exhibited a younger mode (3 years) than secondary males. The mode age of intersexual individuals occurred in the middle of the age-frequency distribution (Table 2; Figure 6a).

Once diandric protogyny was identified for rainbow wrasses, the TL and age at sex change were estimated for individuals sampled during 2007, excluding primary males and intersexual individuals. Length and age at sex change were 132 mm TL and 4 years, respectively (Table 3; Figure 7). Intersexual individuals ranged from 79 to 192 mm in TL ($n = 52$) and from 1 to 5 years in age ($n = 40$).

The reproductive cycle of rainbow wrasses was evaluated based on variation in mean GSI values and frequencies

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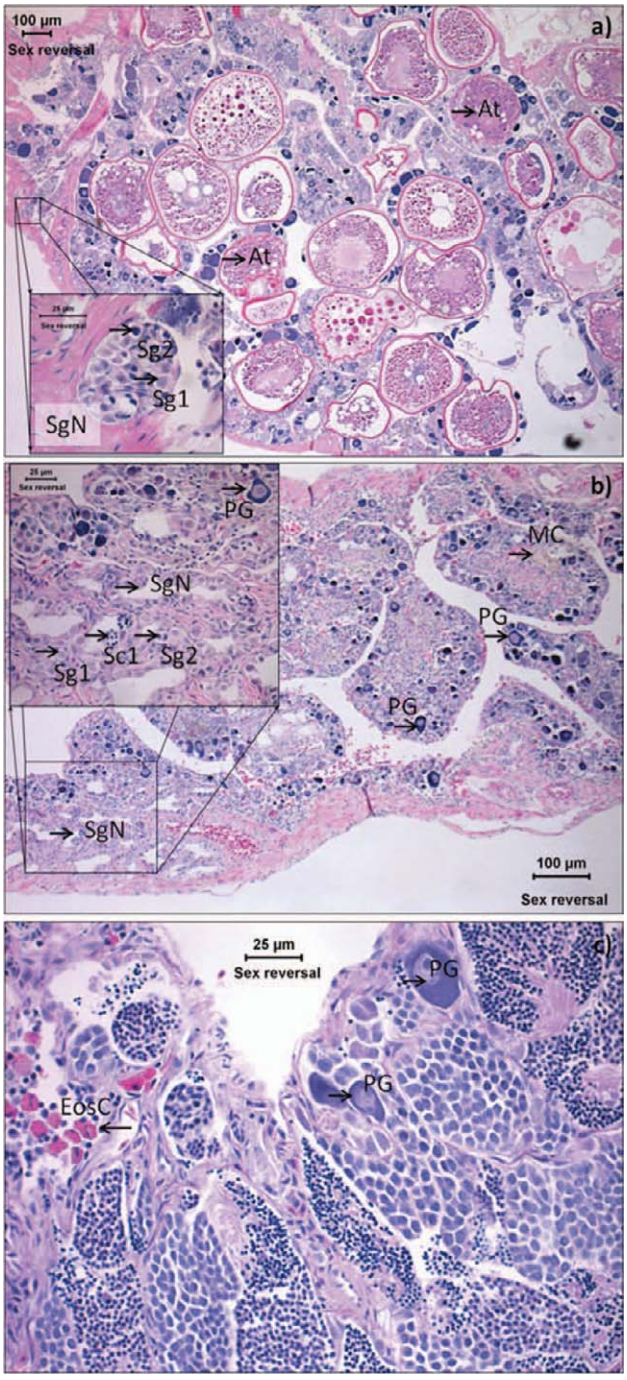



FIGURE 4. Transverse sections illustrating the sex reversal process in the Mediterranean rainbow wrasse: **(a)** initial stage of transition (ovarian degeneration; At = atretic follicle; SgN = spermatogonial nest; Sg1 = primary spermatogonia Sg2 = secondary spermatogonia), **(b)** spermatogonial spread (MC = melanomacrophage centers; PG = primary growth follicle; Sc1 = primary spermatocytes), and **(c)** functional testis (EosC = eosinophilic cells).

of reproductive phases (Figure 9a). The mean GSI values in females, primary males, and secondary males showed a peak of reproductive activity during May and June, which is corroborated by the large proportion of actively spawning females

TABLE 2. Summary of the age distribution for each sex of Mediterranean rainbow wrasse (mean, mode, and range are given in years).

| Sex | *n* | Range | Mean ± SD | Mode |
|---|---|---|---|---|
| Female | 480 | 0–6 | 3.12 ± 0.97 | 3 |
| Primary male | 96 | 1–5 | 3.08 ± 1.06 | 3 |
| Secondary male | 281 | 2–7 | 4.52 ± 0.95 | 5 |
| Intersexual | 40 | 1–5 | 3.85 ± 0.95 | 4 |

during these months. Some spawning activity was detected in April (24% of total mature females), but nearly all females were in the actively spawning subphase during May (83%) and June (91%), indicating that the peak of spawning extended from late spring through early summer. The rate of spawning declined in July (36%) and was negligible in August (3%). As determined by the prevalence of hydrated eggs in active mature ovaries during the spawning season, the spawning frequency was 1.2, 1.1, and 1.3 d (i.e., interval between spawning events) in May, June, and July, respectively. The highest percentages of intersexual individuals were found at the end of the spawning season during July, August, and September (18, 21, and 20%, respectively). Testes of primary and secondary males presented spermatozoa in the lumen, sperm duct, or both structures from March to August, meaning that these males were in the spawning capable phase or actively spawning subphase. From April to June, 100% of the mature population of primary and secondary males was in the spawning capable phase; in other months of the spawning season, this percentage ranged from 68% (August) to 99% (July). It is notable that primary males presented higher average GSI values than secondary males from April to June, which encompassed most of the spawning period.

Due to the small number of immature rainbow wrasses captured during the sampling period ($n = 4$; 62–131 mm TL), the length at 50% maturity was not calculated.

## Painted Comber

The histological study of the gonads in painted comber revealed that functional (reproductively active) ovarian and testicular tissues are simultaneously present in the same gonad. As was seen in the rainbow wrasse, an asynchronous development of secondary growth follicles is present in painted comber (Figure 3b, 10a). Testes are characterized by cystic spermatogenesis and are of the lobular type (Figure 10b, c). Presence of a functional ovotestis is sufficient evidence for a diagnosis of simultaneous hermaphroditism. Ovarian and testicular tissues are separated by connective tissue only (Figure 10c); thus, the germinal tissue configuration is of the delimited type.

The specimens examined in the present study ranged from 60 to 226 mm in TL (mean = 137.0 mm, SD = 32.4 mm, mode = 136 mm, $n = 883$) and from 0 to 11 years in age (mean = 4.1 years, SD = 2.2 years, mode = 3 years, $n = 687$; Figure 6b). Due to the relatively low number of immature painted comber captured during the sampling period ($n = 14$; TL = 60–134

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TABLE 3. Estimates of parameters for total length and age at sex change for the Mediterranean rainbow wrasse based on generalized linear models with a binomial family function. Length at sex change ($LSC_{50}$) is given in millimeters, and age at sex change ($ASC_{50}$) is given in years.

| Sex change by | Parameter | Estimate | SE | $z$ | $P$ | $LSC_{50}$ or $ASC_{50}$ |
|---|---|---|---|---|---|---|
| Length | Intercept | −15.878 | 1.046 | −15.190 | < 0.0001 | 132.32 |
| ($n = 872$) | Slope | 0.120 | 0.008 | 14.970 | < 0.0001 | |
| Age | Intercept | −6.381 | 0.480 | −13.280 | < 0.0001 | 4.15 |
| ($n = 705$) | Slope | 1.538 | 0.119 | 12.910 | < 0.0001 | |

mm; age = 0–4 years), it was not possible to calculate length or age at maturity.

Monthly variation in GSI values clearly showed a peak of reproductive activity in May and June, and despite a significant decrease in July the values remained relatively high during July and August (Figure 8b). As determined from the proportion of specimens with ovarian tissue in the actively spawning subphase, the spawning season extended from late spring (as in the rainbow wrasse) through midsummer. Actively spawning individuals were present in abundance throughout the spawning season (76, 97, 87, and 86% in May, June, July, and August, respectively; Figure 9b). Spawning occurred daily; estimates of spawning frequency were 1.1, 1.0, and 1.1 d in May, June, and July, respectively. Spermatozoa were present in the lumen, sperm duct, or both from March to August, indicating that the testicular tissue was in the spawning capable phase or actively spawning subphase. The percentage of these two phases combined ranged from 92% in April to 100% during the rest of the spawning period.

## Annular Sea Bream

The gonads of the annular sea bream are elongate and suspended by a dorsal mesentery in the posterior region of the visceral cavity. In general, both gonadal lobes are equally well developed. The annular sea bream shows asynchronous development of secondary growth follicles (Figure 3c, 11a), and testes are of the lobular type with cystic spermatogenesis (Figure 11b, c). In specimens with an ovotestis ($n = 37$), only one of the tissues—always the male tissue—was functional (Figure 11c), which indicated that the annular sea bream is not a simultaneous hermaphrodite. No intersexual individuals demonstrating degeneration of functional tissue (e.g., vitellogenic follicles or later stage) and proliferation of gonadal tissue of the other sex were found.

Female annular sea bream examined in the present study ranged from 65 to 209 mm in TL (mean = 125.8 mm, SD = 22.6 mm, mode = 123 mm, $n = 534$) and from 0 to 9 years in age (mean = 3.1 years, SD = 1.3 years, mode = 3 years, $n = 534$); males ranged from 55 to 195 mm in TL (mean = 119.3 mm, SD = 24.4 mm, mode = 118 mm, $n = 384$) and from 0 to 8 years in age (mean = 2.7 years, SD = 1.4 years, mode = 2 years, $n = 384$). Although the sex ratio was slightly biased toward females (except at ages 0 and 1), the age-frequency distributions for both sexes were similar (Figure 6c). When combined with the lack of intersexual individuals, nonfunctional hermaphroditism should be considered the sexual pattern of the annular sea bream.

The spawning season of female annular sea bream was assessed throughout the year via monthly variation in GSI only. May and June showed the highest reproductive activity and a sharp decrease was observed in July (GSI <

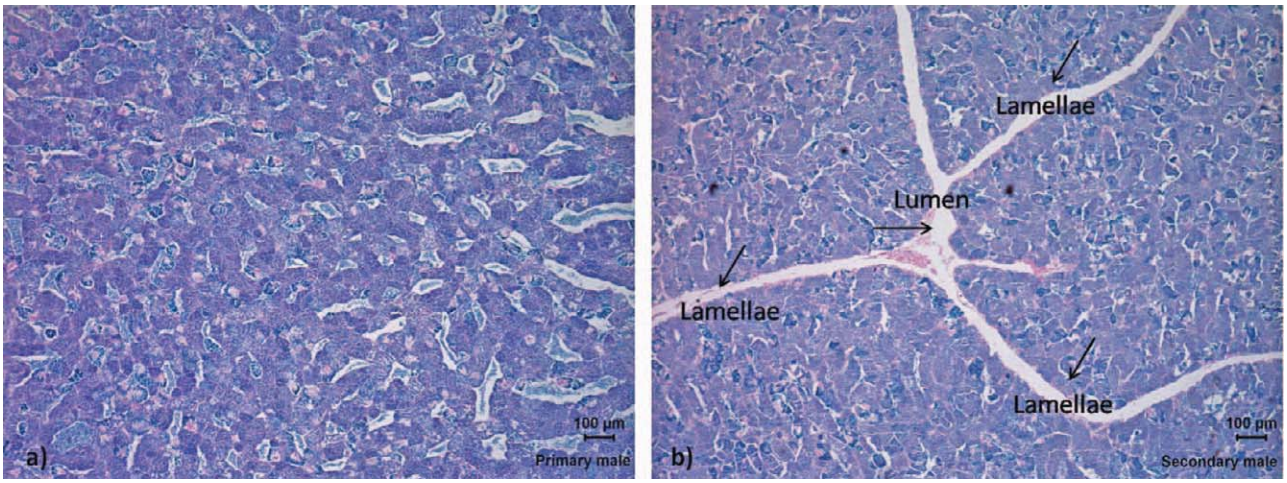



FIGURE 5. Testis morphology in two types of male Mediterranean rainbow wrasse: **(a)** primary male and **(b)** secondary male.

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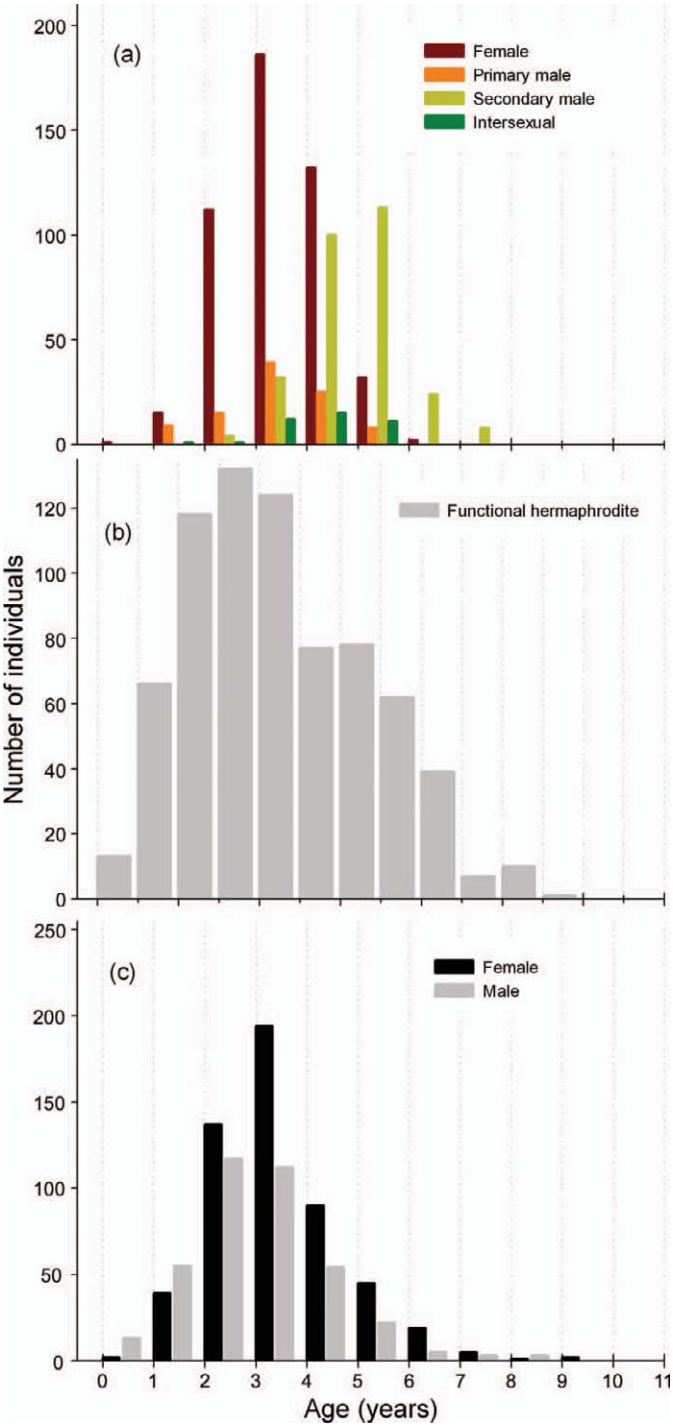



FIGURE 6. Age-frequency distributions of **(a)** Mediterranean rainbow wrasses, **(b)** painted comber, and **(c)** annular sea bream from the south coast of Mallorca Island (northwestern Mediterranean).

1), indicating that the spawning season occurred from late spring through early summer (Figure 8c) but mainly in May and June, as was found for the rainbow wrasse and painted comber.

## DISCUSSION

Recreational fishing constitutes an important use of coastal fisheries resources in the Balearic Islands, as nearly 10% of the population actively participates in recreational angling (Morales-Nin et al. 2005). Minimum legal landing sizes, maximum number of lines per rod, seasonal closures, marine protected areas, minimum hook sizes, and other management tools are used by the local fisheries administration to regulate the recreational fishery (Alós et al. 2009). Truly effective fishery management, however, requires insights into the reproductive biology of the fish species of interest. For sex-changing fishes, it is essential to consider the sex change pattern when crafting management policies; thus, the present study provides the scientific evidence necessary to improve the efficacy of management options for these three species (Punt et al. 1993; Huntsman and Schaaf 1994; Armsworth 2001; Alonzo and Mangel 2004; Molloy et al. 2007) because a better understanding of their life histories improves our comprehension of stock dynamics.

Histology is a particularly crucial tool for reproductive studies of hermaphroditic fish species since details of internal gonad morphology are only visible when histological techniques are used (Sadovy and Shapiro 1987; Sadovy and Domeier 2005; Sadovy de Mitcheson and Liu 2008). In the present study, a detailed histological examination of gonads of the three target species was conducted to delineate the organization of ovarian development, properly diagnose sexual pattern, and describe the seasonal reproductive cycle.

### Gonad Development

Histological examination of the ovaries revealed that all three species studied herein possess asynchronous development of secondary growth follicles (Wallace and Selman 1981; Murua and Saborido-Rey 2003). Asynchronous development of secondary growth follicles is usually associated with species exhibiting indeterminate fecundity (Murua and Saborido-Rey 2003), although a determinate fecundity strategy has been found in certain asynchronous species, including the Atlantic mackerel *Scomber scombrus*, bib *Trisopterus luscus*, and brown meagre *Sciaena umbra* (Greer Walker et al. 1994; Alonso-Fernández et al. 2008; Grau et al. 2009), and in a protogynous hermaphrodite, the dusky grouper *Epinephelus marginatus* (Reñones et al. 2010), and such a strategy could occur here as well. Further research is required to definitively determine the fecundity strategies of the three study species.

Testicular tissue of all three target species was of the lobular type (Takashima and Hibiya 1995), wherein spermatogonia are randomly distributed along the entire length of the lobule in reproductively active fish; this is equivalent to the unrestricted spermatogonial type described by Grier (1980, 1981). Spermatogenesis occurs within germinal cysts formed

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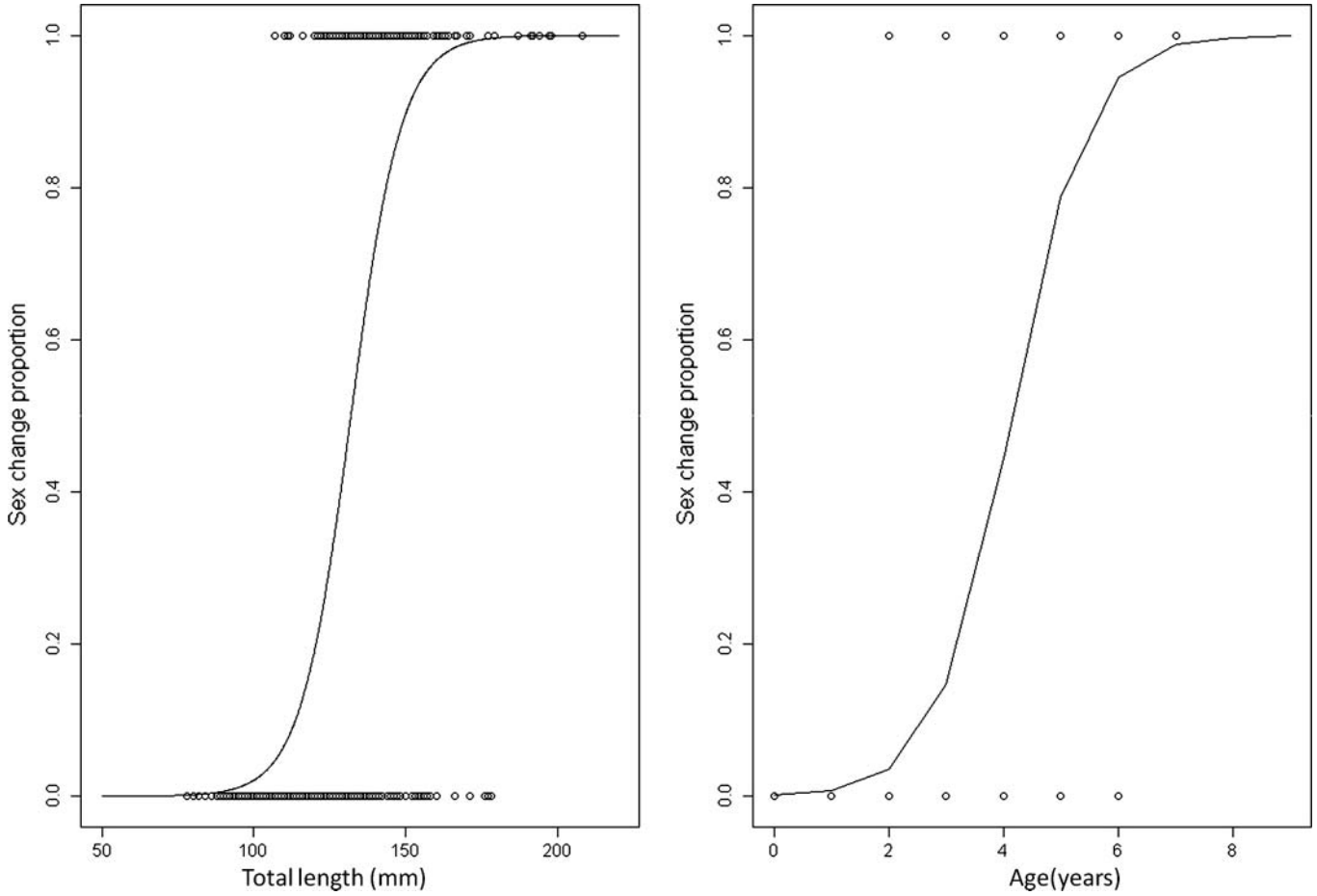



FIGURE 7. Generalized linear model (binomial family function) for the estimated percentages of sex-changed individuals (males) of the Mediterranean rainbow wrasse as a function of **(a)** total length ($n = 872$) and **(b)** age ($n = 705$).

by enveloping Sertoli-cell cytoplasmic processes; within each cyst, the maturation of germ cells is synchronous.

### Mediterranean Rainbow Wrasse

The rainbow wrasse was documented in previous studies to be a diandric protogynous hermaphrodite with two types of coloration (Bacci and Razzauti 1958; Bruslé 1987): (1) an initial phase associated with females and a variable proportion of males (Bentivegna and Rasotto 1983) and (2) a terminal phase found only in males. This sexual pattern was clearly confirmed in the present study by the presence of intersexual individuals with degenerating functional tissue of one sex (i.e., vitellogenic follicles or later-stage follicles) and proliferation of tissue of the other sex. The sexual pattern was also supported by the bimodal distribution of age frequencies in which the mode for females was at a younger age than the mode for secondary males. Histology also confirmed the existence of two male types, primary and secondary.

In the present study, photomicrographs illustrated the sex reversal process from ovarian degeneration and spermatogonial proliferation to the development of functional testes in secondary males. Bruslé (1987) also studied sex inversion in the rainbow wrasse; however, in their study the process was induced artificially by injection of hormones, whereas our study was based on samples collected from a wild population. Through histological examination, we also identified two types of leukocytes that were likely associated with sex reversal: eosinophils and melanomacrophage centers (yellow-brown bodies produced by differentiation of monocytes). The eosinophilic cells seem to be associated with ovary degeneration, as has been reported for other fish species, such as the red porgy *Pagrus pagrus* and gilthead bream *Sparus auratus* (Kokokiris et al. 1999; Liarte et al. 2007), and these cells could be used as secondary evidence for the presence of intersexual individuals. It has been suggested that melanomacrophage centers represent the late regressive stages of follicular atresia (Blazer 2002), but Sadovy and Shapiro (1987) stated that other processes not involving follicular atresia can produce very similar structures. Besseau and Faliex (1994) described melanomacrophage centers as resulting from the synergistic action of eosinophilic granulocytes and macrophages during gamete resorption. In the marbled swamp eel *Synbranchus marmoratus*, these structures may

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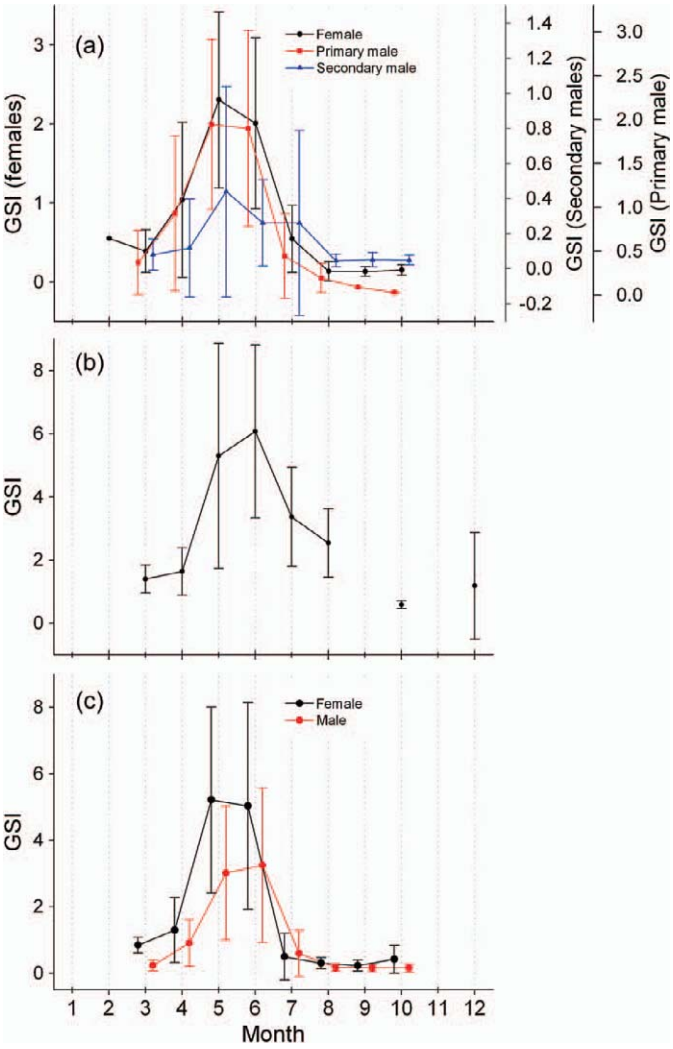



FIGURE 8. Mean (±SD) monthly gonadosomatic index (GSI) values for **(a)** Mediterranean rainbow wrasses ($n = 652$; note the different scales for primary and secondary males), **(b)** painted comber ($n = 826$), and **(c)** annular sea bream ($n = 907$).

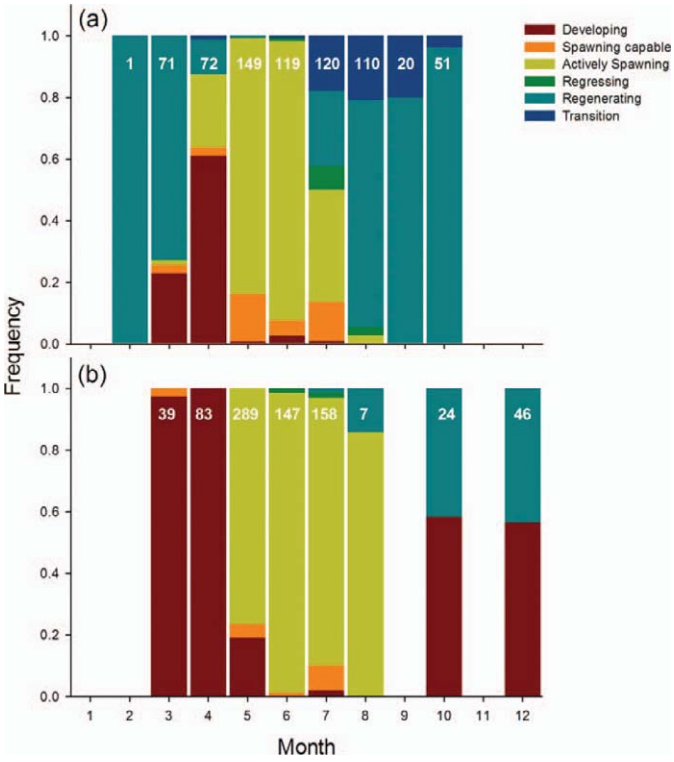



FIGURE 9. Frequency of reproductive phases in ovarian tissue from **(a)** Mediterranean rainbow wrasses and **(b)** painted comber by month of collection. The intersexual phase is only present in Mediterranean rainbow wrasses. Sample size ($n$) is noted within the frequency bars.

act as "atretic cell cleaners," among other functions (Ravaglia and Maggese 1995).

Although primary and secondary male rainbow wrasses have been described as differing with respect to several ovarian features present in the testes (e.g., lamellar configuration or presence of ovarian lumen; Bentivegna and Rasotto 1983), the present study showed that it was rarely possible to identify these two male types based only on histology. In rainbow wrasses, fish external coloration must be used to assign male category because nearly all male specimens appeared to have similar testis morphology, as has been demonstrated in some serranids, labrids, and scarids (Sadovy and Shapiro 1987; Sadovy and Domeier 2005; Sadovy de Mitcheson and Liu 2008). A karyotype analysis of rainbow wrasses (Duchac et al. 1982) revealed differences among male types, indicating that the karyotype of secondary males was identical to that of the majority of females. A surprising result in the present study was that primary males exhibited higher reproductive investment (i.e., higher GSI values) than did secondary males, perhaps suggesting the adoption of a sperm competition strategy. In this respect, a recent quantitative genetic study of the guppy *Poecilia reticulata* (Evans 2010) produced evidence that better sperm quality (i.e., for sperm competition) was developed in male guppies that predominantly attained matings by "sneaking," like the primary males of the rainbow wrasse (Duchac et al. 1982; Lejeune 1987).

The annual sexual cycle of rainbow wrasses had not been previously assessed for the Balearic Islands population. Reproductive activity at the population level is highly synchronous, showing a spawning season that extends from May to July. The spawning peak (May–June) occurs 1 month earlier than that reported for a Mediterranean population off the south coast of France (Bruslé 1987). Spawning in the Balearic Islands population occurs frequently during this peak, as a batch of eggs is released approximately every 1–2 d. This suggests a high rate of ovulation, and the sharp increase in GSI from April to May indicates that follicle yolk deposition occurs rapidly as well. Males in the actively spawning subphase were found during a larger portion of the year than were females of this subphase. Spermatogenesis is completed prior to spawning, as was found in the blackmouth angler *Lophiomus setigerus* and

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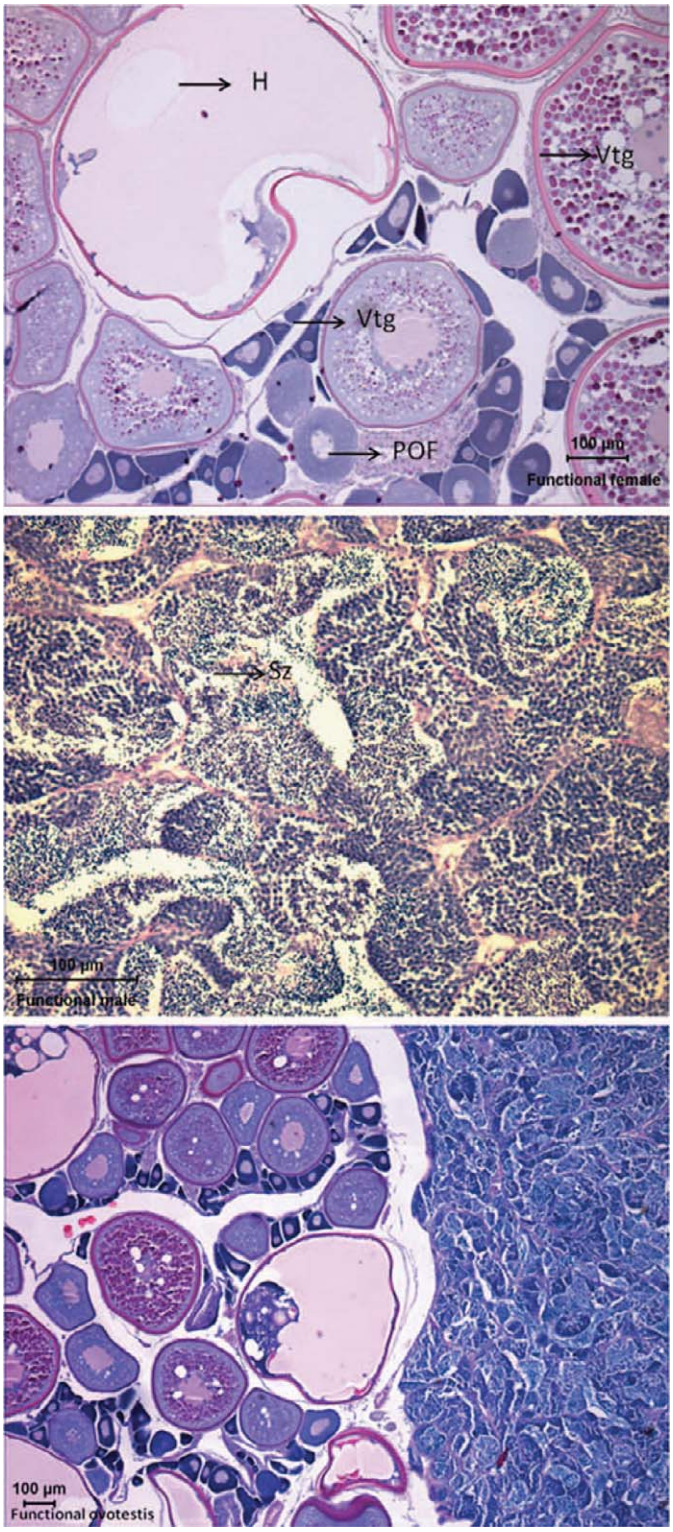



FIGURE 10. Transverse sections of gonads in painted comber: **(a)** detail of functional ovarian tissue (Vtg = vitellogenic follicle; H = hydrated follicle; POF = postovulatory follicle), **(b)** detail of functional testicular tissue (Sz = spermatozoa), and **(c)** functional ovotestis.

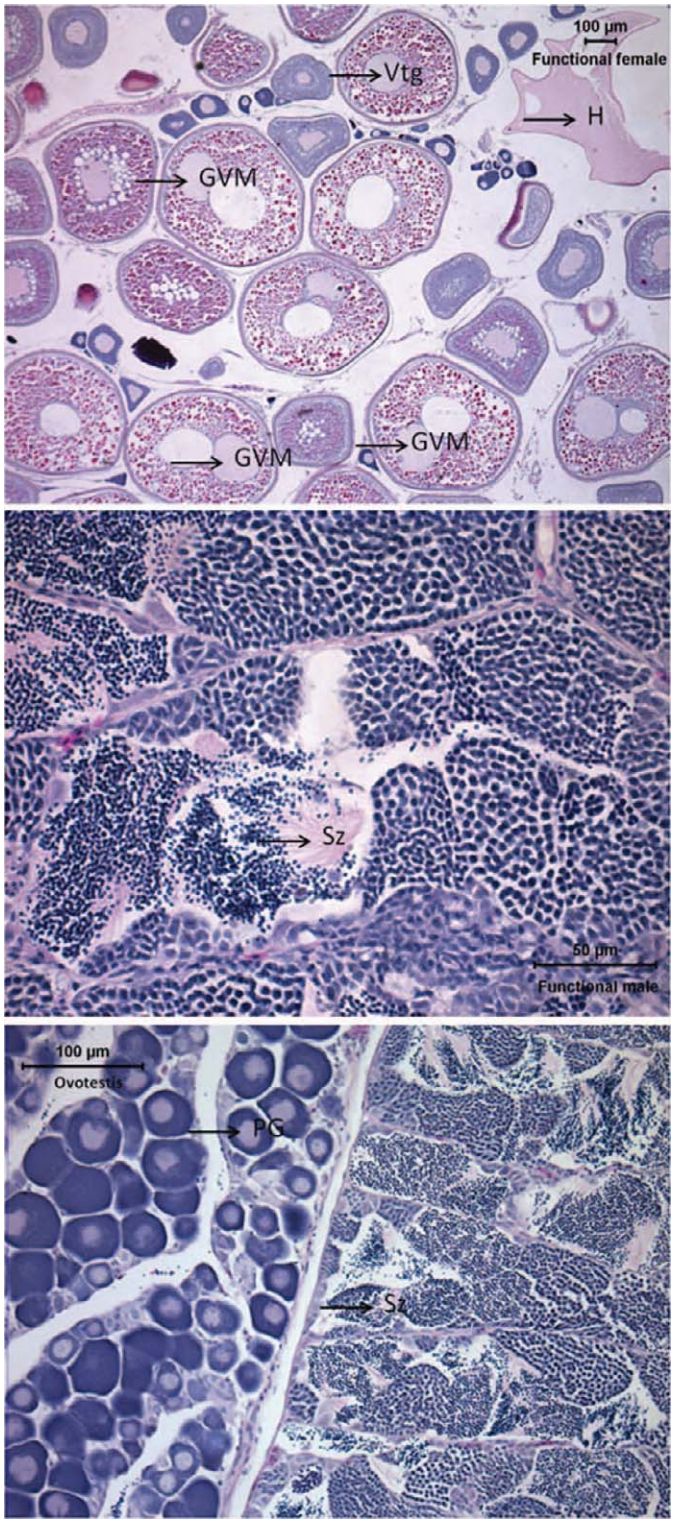



FIGURE 11. Transverse sections of gonads in annular sea bream: **(a)** actively spawning female (Vtg = vitellogenic follicle; H = hydrated follicle; GVM = germinal vesicle migration), **(b)** functional male (Sz = spermatozoa), and **(c)** ovotestis (only the male tissue is functional; PG = primary growth follicle).

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winter flounder *Pseudopleuronectes americanus* (Yoneda et al. 1998; Sonya and Burton 1999); in some ways, this guarantees reproductive success since active males are available to fertilize eggs during the entire spawning season. Sex change appears to take place primarily during the regenerating phase after the spawning season; however, previous studies have also reported that sex change occurs before the period of sexual reproduction (Bentivegna and Rasotto 1983).

In the study population, the length and age at sex change were 132 mm TL and 4 years, respectively; sex change therefore occurred within the length range (132–160 mm) found in a previous study conducted at Banyuls-sur-Mer along the south coast of France (Bruslé 1987). This knowledge of the species' biology provides critical information for the improvement of management strategies. In protogynous species, fishing activity that targets large individuals removes large males from the stock, altering both the sex ratio and the age structure of the population. The number of eggs produced or the biomass of female spawning stock can remain relatively stable regardless of high fishing mortality; however, sperm limitation may occur and must be considered (Brooks et al. 2008) since it could lead to decreases in fertilization rate and thus to a decline in population size. For a protogynous species, any measure of spawning that does not consider sperm limitation will probably underestimate the impact of fishing on the population (Punt et al. 1993; Huntsman and Schaaf 1994; Armsworth 2001; Alonzo and Mangel 2004). Therefore, knowledge of the sex change parameters of the species permits the establishment of informed, biologically relevant minimum and maximum legal catch sizes that reduce the impact of fishing activity on the reproductive potential of the population. A maximum size limit could be more effective in decreasing the effect of fishing on the sex ratio. An understanding of the species' annual sexual cycles is also crucial for proper scheduling of seasonal closures. Additionally, while the factors triggering the sex change in rainbow wrasses are not clear, evidence suggests that the transition is socially mediated (Lejeune 1987) as in other species like the dusky grouper (Zabala et al. 1997; Reñones et al. 2010). Lejeune (1987) concluded that sex change in the rainbow wrasse is controlled by availability of territory as the most important cue and that local stock density is a secondary factor. Removal of large males likely favors sex change in larger females, thus reducing the female population overall and the number of large females in particular and resulting in a loss of reproductive potential.

## Painted Comber

Some authors have indicated the coexistence of more than one sexual pattern in a species (Buxton and Garratt 1990); thus, the confirmation of sexual pattern through histological methods in different populations is of great interest. The painted comber was previously described as a simultaneous hermaphrodite by Tuset et al. (2005) based on histological examination of a population from the Atlantic Ocean (Canary Islands) and by Zorica et al. (2005) based on macroscopic observations in an eastern mid-Adriatic Sea population. However, Abd-el-Aziz and Ramadan (1990) pointed out the presence of females, males, and hermaphrodites (129, 4, and 59 specimens, respectively) in an Egyptian Mediterranean population. Although Abd-el-Aziz and Ramadan (1990) used histology to establish functional simultaneous hermaphroditism for this species based on the simultaneous occurrence of reproductively active testicular and ovarian tissues, their study also showed the existence of primary females (67% of the population) and primary males (2% of the population). Abd-el-Aziz and Ramadan (1990) speculated that the primary male fraction of the population does not take part in reproduction, whereas they assumed that cross-fertilization between primary females and hermaphrodites was possible. In the present study, no such sexual differentiation was found and the histological analysis of the gonads revealed functional testicular and ovarian tissues at the same time in all specimens examined, providing sufficient evidence to diagnose simultaneous hermaphroditism (Sadovy and Shapiro 1987; Sadovy de Mitcheson and Liu 2008).

The study population of painted comber presented a relatively protracted spawning season, which ran for 4 months (May–August). Similar results were found for the eastern mid-Adriatic population (Zorica et al. 2005), but in lower latitudes the spawning season is longer and can extend up to 9 months in the Canary Islands (Tuset et al. 2005). During the peak of spawning activity, the spawning frequency was close to 1, suggesting that these fish spawn almost daily and have a high chance of autofertilization. Although the occurrence of simultaneous hermaphroditism was discovered in the mangrove rivulus *Rivulus marmoratus*, which is found in tropical mangroves (Harrington 1961), cross-fertilization seems to be the rule and self-fertilization is the exception. The "hermaphrodite's dilemma" theory suggests that simultaneous hermaphrodite mating systems are based on reciprocation (Leonard 1990), and a mechanism to avoid self-fertilization may exist for these species. In serranids, egg trading seems to be the most common prevention mechanism (Leonard 1993); tobaccofish *Serranus tabacarius* show strong alternation of sexual roles between mating partners (Petersen 1995). However, further research is required to clarify this issue in the painted comber.

Because simultaneous hermaphrodites may be considered as species that do not change sex, their response to overfishing may be similar to the response of gonochoristic species because both egg production and sperm production decrease with increased fishing pressure (Alonzo and Mangel 2004). Therefore, similar conservation strategies may be appropriate.

## Annular Sea Bream

Historically, the diagnosis of sexual pattern in annular sea bream has been controversial (Buxton and Garratt 1990); this species has been described as a protandrous hermaphrodite

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(Tortonese 1975; Whitehead et al. 1986; Gonzalez Pajuelo et al. 2001) and as a nonfunctional hermaphrodite (Bini 1968; Matić-Skoko et al. 2007; Alós et al. 2010a). As mentioned by Buxton and Garratt (1990) and Sadovy de Mitcheson and Liu (2008), only histological assessment and behavioral observations will yield unambiguous results from a functional reproductive perspective. To our knowledge, this is the first histologically based research to have verified nonfunctional hermaphroditism in annular sea bream. Nonfunctional hermaphroditism was previously known as rudimentary hermaphroditism (see Buxton and Garratt 1990). The presence of inactive ovarian tissue in functional testes or vice versa is not a clear diagnostic of either sex change or simultaneous hermaphroditism. One type of definitive evidence for sex change consists of a histological series that includes the presence of degenerating functional tissue of one sex (e.g., vitellogenic or later-stage follicles) and the proliferation of tissue of the other sex (Sadovy and Shapiro 1987; Sadovy de Mitcheson and Liu 2008). Therefore, according to our results, the annular sea bream is considered to be a nonfunctional hermaphrodite because no histological evidence of functional simultaneous hermaphroditism or sex change was found. The similar age distribution for both sexes is supporting evidence for nonfunctional hermaphroditism (Sadovy and Shapiro 1987). From a functional perspective, this state is gonochorism (Sadovy de Mitcheson and Liu 2008), so conservation strategies similar to those used for gonochoristic species should be applied to the annular sea bream.

This species showed a shorter spawning period (May–June) than the other two species investigated in the present study. Comparisons of spawning in annular sea bream across different geographical areas suggest some variation, mainly with latitude. For example, in the central Adriatic Sea, GSI values revealed a spawning season lasting from the end of April to the end of August (Matić-Skoko et al. 2007), which is slightly longer than the spawning season in the present study. In the Canary Islands, spawning occurs from February to May and peaks in March–April (Gonzalez Pajuelo et al. 2001), which is 2 months earlier than in the present study.

In this work, we focused on the importance of proper identification of some key reproductive structures in the gonad to simply describe sexual pattern or to assess reproductive condition. Histology was utilized in the present study as the basic tool for a reproductive study of three hermaphroditic species. Although time consuming, histology is an essential tool because it has allowed development of definitive criteria to determine sexual pattern and to investigate other aspects of fish reproductive biology. The results of studies that utilize histology as an investigative tool can be informative in the future selection of fisheries conservation strategies and can allow improvements in the efficacy of management options.

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## REFERENCES

Abd-el-Aziz, S. H., and A. A. Ramadan. 1990. Sexuality and hermaphroditism in fishes. I. Synchronous functional hermaphroditism in the serranid fish *Serranus scriba* L. Folia Morphologica 38:86–100.

Alonso-Fernández, A., R. Domínguez-Petit, M. Bao, C. Rivas, and F. Saborido-Rey. 2008. Spawning pattern and reproductive strategy of female pouting *Trisopterus luscus* (Gadidae) on the Galician shelf of north-western Spain. Aquatic Living Resources 21:383–393.

Alonzo, S. H., and M. Mangel. 2004. The effects of size-selective fisheries on the stock dynamics of and sperm limitation in sex-changing fish. U.S. National Marine Fisheries Service Fishery Bulletin 102:1–13.

Alós, J., M. Palmer, A. Alonso-Fernández, and B. Morales-Nin. 2010a. Individual variability and sex-related differences in the growth of *Diplodus annularis* (Linnaeus, 1758). Fisheries Research 101:60–69.

Alós, J., M. Palmer, S. Balle, A. M. Grau, and B. Morales-Nin. 2010b. Individual growth pattern and variability in *Serranus scriba*: a Bayesian. ICES Journal of Marine Science 67:502–512.

Alós, J., M. Palmer, and A. M. Grau. 2009. Mortality of *Diplodus annularis* and *Lithognathus mormyrus* released by recreational anglers: implications for recreational fisheries management. Fisheries Management and Ecology 16:298–305.

Alós, J., M. Palmer, A. M. Grau, and S. Deudero. 2008. Effects of hook size and barbless hooks on hooking injury, catch per unit effort, and fish size in a mixed-species recreational fishery in the western Mediterranean Sea. ICES Journal of Marine Science 65:899–905.

Armsworth, P. R. 2001. Effects of fishing on a protogynous hermaphrodite. Canadian Journal of Fisheries and Aquatic Sciences 58:568–578.

Atz, J. W. 1965. Hermaphroditic fish. Science 150:789–797.

Bacci, G., and A. Razzauti. 1958. Protogynous hermaphroditism in *Coris julis* L. Nature (London) 181:432–433.

Bentivegna, F., P. Cirino, and M. B. Rasotto. 1985. Further investigations into sex reversal of *Coris julis* L. (Pisces, Labridae). Bolletino di Zoologia 52:355–358.

Bentivegna, F., and M. B. Rasotto. 1983. Anatomical features of sex inversion in the rainbow wrasse, *Coris julis*. Bolletino di Zoologia 50:73–78.

Besseau, L., and E. Faliex. 1994. Resorption of unemitted gametes in *Lithognathus mormyrus* (Sparidae, Teleostei): a possible synergic action of somatic and immune cells. Cell and Tissue Research 276:123–132.

Bini, G. 1968. Atlante dei pesci delle coste Italiane: Osteitti. [Atlas of fish of the Italian coast: Osteichthyes.] Mondo Sommerso Editrice 4:163.

Blazer, V. S. 2002. Histopathological assessment of gonadal tissue in wild fishes. Fish Physiology and Biochemistry 26:85–101.

Brooks, E. N., K. W. Shertzer, T. Gedamke, and D. S. Vaughan. 2008. Stock assessment of protogynous fish: evaluating measures of spawning biomass used to estimate biological reference points. Fishery Bulletin 106:12–23.

Brown-Peterson, N. J., D. M. Wyanski, F. Saborido-Rey, B. J. Macewicz, and S. K. Lowerre-Barbieri. 2011. A standardized terminology for describing reproductive development in fishes. Marine and Coastal Fisheries: Dynamics, Management, and Ecosystem Science [online serial] 3:52–70.

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Bruslé, S. 1987. Sex-inversion of the hermaphroditic, protogynous teleost *Coris julis* L. (Labridae). Journal of Fish Biology 30:605–616.

Buxton, C. D., and P. A. Garratt. 1990. Alternative reproductive styles in seabreams (Pisces: Sparidae). Environmental Biology of Fishes 28:113–124.

Duchac, B., F. Huber, H. Müller, and D. Senn. 1982. Mating behavior and cytogenetical aspects of sex-inversion in the fish *Coris julis* L. (Labridae, Teleostei). Cellular and Molecular Life Sciences 38:809–811.

Evans, J. P. 2010. Quantitative genetic evidence that males trade attractiveness for ejaculate quality in guppies. Proceedings of the Royal Society of London B: Biological Sciences 277:3195–3201.

Gonzalez Pajuelo, J. M., J. M. Lorenzo Nespereira, A. J. Gonzalez Ramos, M. Mendez-Villamil Mata, and J. Coca Saez de Albeniz. 2001. Sexuality and reproduction of annular seabream *Diplodus annularis* (L., 1758) (Pisces, Sparidae) of Gran Canaria (Canary Islands). Boletín del Instituto Español de Oceanografía 17:307–312.

Gordoa, A., M. Balbina, and N. Raventós. 2000. Growth performance of four wrasse species on the north-western Mediterranean coast. Fisheries Research 45:43–50.

Grau, A., M. Linde, and A. M. Grau. 2009. Reproductive biology of the vulnerable species *Sciaena umbra* Linnaeus, 1758 (Pisces: Sciaenidae). Scientia Marina 73:67–81.

Greer Walker, M., P. R. Witthames, and J. I. Bautista de los Santos. 1994. Is the fecundity of the Atlantic mackerel (*Scomber scombrus*: Scombridae) determinate? Sarsia 79:12–26.

Grier, H. J. 1980. Structural evidence for two different testicular types in teleost fishes. The American Journal of Anatomy 159:331–345.

Grier, H. J. 1981. Cellular organization of the testis and spermatogenesis in fishes. American Zoologist 21:345–357.

Harrington, R. W. 1961. Oviparous hermaphroditic fish with internal self-fertilization. Science 134:1749–1750.

Hunter, J. R., and S. R. Goldberg. 1980. Spawning incidence and batch fecundity in northern anchovy *Engraulis mordax*. U.S. National Marine Fisheries Service Fishery Bulletin 77:641–652.

Huntsman, G. R., and W. E. Schaaf. 1994. Simulation of the impact of fishing on reproduction of a protogynous grouper, the graysby. North American Journal of Fisheries Management 14:41–52.

Kokokiris, L., S. Bruslé, M. Kentouri, and A. Fostier. 1999. Sexual maturity and hermaphroditism of the red porgy *Pagrus pagrus* (Teleostei: Sparidae). Marine Biology 134:621–629.

Lejeune, P. 1987. The effect of local stock density on social behavior and sex change in the Mediterranean labrid *Coris julis*. Environmental Biology of Fishes 18:135–141.

Leonard, J. L. 1990. The hermaphrodite's dilemma. Journal of Theoretical Biology 147:361–371.

Leonard, J. L. 1993. Sexual conflict in simultaneous hermaphrodites: evidence from serranid fishes. Environmental Biology of Fishes 36:135–148.

Liarte, S., E. Chaves-Pozo, A. Garcia-Alcazar, V. Mulero, J. Meseguer, and A. Garcia-Ayala. 2007. Testicular involution prior to sex change in gilthead seabream is characterized by a decrease in DMRT1 gene expression and by massive leukocyte infiltration. Reproductive Biology and Endocrinology 5:20–35.

Lowerre-Barbieri, S. K., and L. R. Barbieri. 1993. A new method of oocyte separation and preservation for fish reproduction studies. U.S. National Marine Fisheries Service Fishery Bulletin 91:165–170.

Lowerre-Barbieri, S. K., N. Henderson, J. Llopiz, S. Walters, J. Bickford, and R. Muller. 2009. Defining a spawning population (*Cynoscion nebulosus*) over temporal, spatial, and demographic scales. Marine Ecology Progress Series 394:231–245.

Macchi, G. J., and E. M. Acha. 2000. Spawning frequency and batch fecundity of Brazilian menhaden, *Brevoortia aurea*, in the Rio de la Plata Estuary off Argentina and Uruguay. U.S. National Marine Fisheries Service Fishery Bulletin 98:283–289.

Matić-Skoko, S., M. Kraljević, J. Dulčić, and I. Jardas. 2007. Age, growth, maturity, mortality, and yield-per-recruit for annular sea bream (*Diplodus annularis* L.) from the eastern middle Adriatic Sea. Journal of Applied Ichthyology 23:152–157.

Molloy, P. P., N. B. Goodwin, I. M. Côté, M. J. G. Gage, and J. D. Reynolds. 2007. Predicting the effects of exploitation on male-first sex-changing fish. Animal Conservation 10:30–38.

Morales-Nin, B., J. Moranta, C. Garcia, M. P. Tugores, A. M. Grau, F. Riera, and M. Cerda. 2005. The recreational fishery off Majorca Island (western Mediterranean): some implications for coastal resource management. ICES Journal of Marine Science 62:727–739.

Murua, H., and F. Saborido-Rey. 2003. Female reproductive strategies of marine fish species of the north Atlantic. Journal of Northwest Atlantic Fishery Science 33:23–31.

Petersen, C. 1995. Reproductive behavior, egg trading, and correlates of male mating success in the simultaneous hermaphrodite, *Serranus tabacarius*. Environmental Biology of Fishes 43:351–361.

Picquelle, S. J., and G. Stauffer. 1985. Parameter estimation for an egg production method of northern anchovy biomass assessment. An egg production method for estimating spawning biomass of pelagic fish: application to the northern anchovy, *Engraulis mordax*. NOAA Technical Report NMFS 36:7–15.

Punt, A. E., P. A. Garratt, and A. Govender. 1993. On an approach for applying per-recruit methods to a protogynous hermaphrodite, with an illustration for the slinger *Chrysoblephus puniceus* (Pisces: Sparidae). South African Journal of Marine Science 13:109–119.

Ravaglia, M. A., and M. C. Maggese. 1995. Melano-macrophage centres in the gonads of the swamp eel, *Synbranchus marmoratus* Bloch, (Pisces, Synbranchidae): histological and histochemical characterization. Journal of Fish Diseases 18:117–125.

Reñones, O., A. Grau, X. Mas, F. Riera, and F. Saborido-Rey. 2010. Reproductive pattern of an exploited dusky grouper *Epinephelus marginatus* (Lowe 1834) (Pisces, Serranidae) population in the western Mediterranean. Scientia Marina 74:523–537.

Saborido-Rey, F., and S. Junquera. 1998. Histological assessment of variations in sexual maturity of cod (*Gadus morhua* L.) at the Flemish Cap (north-west Atlantic). ICES Journal of Marine Science 55:515–521.

Sadovy, Y., and M. L. Domeier. 2005. Perplexing problems of sexual patterns in the fish genus *Paralabrax* (Serranidae, Serraninae). Journal of Zoology 267:121–133.

Sadovy, Y., and D. Y. Shapiro. 1987. Criteria for the diagnosis of hermaphroditism in fishes. Copeia 1987:136–156.

Sadovy de Mitcheson, Y., and M. Liu, 2008. Functional hermaphroditism in teleosts. Fish and Fisheries 9:1–43.

Sattar, S. A., C, Jørgensen, and Ø. Fiksen. 2008. Fisheries induced evolution of energy and sex allocation. Bulletin of Marine Science 83:235–250.

Sonya, M. M., and M. P. M. Burton. 1999. Histological observations on spermatogenesis in winter flounder, *Pleuronectes americanus* Walbaum, from Conception Bay, Newfoundland, Canada. Canadian Journal of Zoology 77:1682–1689.

Takashima, F., and T. Hibiya, editors. 1995. An atlas of fish histology: normal and pathological features, 2nd edition. Kodansha, Tokyo.

Tortonese, E. 1975. Fauna d'Italia. Osteichthyes (Pesci ossei). [Fauna of Italy. Osteichthyes (bony fish).] Parte Seconda Edizioni Calderini 11:636.

Tuset, V. M., M. M. Garcia-Diaz, J. A. Gonzalez, M. J. Lorente, and I. J. Lozano. 2005. Reproduction and growth of the painted comber *Serranus scriba* (Serranidae) of the marine reserve of Lanzarote Island (central-eastern Atlantic). Estuarine, Coastal and Shelf Science 64:335–346.

Tyler, C. R., and J. P. Sumpter. 1996. Oocyte growth and development in teleosts. Reviews in Fish Biology and Fisheries 6:287–318.

Wallace, R., and K. Selman. 1981. Cellular and dynamic aspects of oocyte growth in teleosts. American Zoologist 21:325–343.

West, G. 1990. Methods of assessing ovarian development in fishes: a review. Australian Journal of Marine and Freshwater Research 41:199–222.

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Whitehead, P. J. P., M. L. Bauchot, J. C. Hureau, J. Nielsen, and E. Tortonese. 1986. Fishes of the north-eastern Atlantic and the Mediterranean. United Nations Educational, Scientific, and Cultural Organization, Paris.

Yoneda, M., M. Tokimura, H. Fujita, N. Takeshita, K. Takeshita, M. Matsuyama, and S. Matsuura. 1998. Reproductive cycle and sexual maturity of the anglerfish *Lophiomus setigerus* in the East China Sea with a note on specialized spermatogenesis. Journal of Fish Biology 53:164–178.

Zabala, M., P. Louisy, A. Garcia-Rubies, and V. Gracia. 1997. Socio-behavioural context of reproduction in the Mediterranean dusky grouper *Epinephelus marginatus* (Lowe, 1834) (Pisces, Serranidae) in the Medes Islands Marine Reserve (NW Mediterranean, Spain). Scientia Marina 61:79–98.

Zorica, B., G. Sinovcic, and V. Cikes Kec. 2005. Reproductive period and histological analysis of the painted comber, *Serranus scriba* (Linnaeus, 1758), in the Trogir Bay area (eastern mid-Adriatic). Acta Adriatica 46:77–82.

Zuur, A. F., E. N. Leno, and G. M. Smith. 2007. Analysing ecological data: statistics for biology and health. Springer, New York.

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