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Recent advances in reproductional aspects of *Pagrus pagrus*

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SUMMARY – Most of the knowledge about *Pagrus pagrus* was related to sampling in the wild, and farming conditions needed to be explored. The present experiment took place at the Aquaculture Research Station of the Institute of Marine Biology of Crete (IMBC, Iraklion, Greece). Fish were caught from the wild as about 6 months old juveniles (mean body weight = 15.8 ± 4.2 g) from nursery beaches in the Gulf of Iraklion and then reared for 6 years in the IMBC facilities. *Pagrus pagrus* is a protogynous hermaphrodite. Gonadal development proceeds through three different ways. In the first way, immature fish (less than 3 years-old) develop the testicular tissue and the ovaries degenerate before sexual maturity ("primary males" type). In the second way, the development of the gonads is completed with maturation of the ovarian zone, and the fish function as females. In the last way, after a single, or possibly repeated spawnings, females change sex and function as males ("secondary males" type). Fishes mature for the first time at the age of 3-years (11.2% of 3-years old females and 77% of 3-years old males) but more than 50% of individuals were mature at the age of 4-years. They have an annual reproductive cycle. Gametogenesis starts during the autumn period when photoperiod and temperature decrease and spawning occurs during spring (March to May) with temperature ranging from 15-19°C. The evolution of 17β-estradiol, oestrone and vitellogenin in females plasma and of testosterone and 11-ketotestosterone in male plasma have been followed.

Key words: Sparid, *Pagrus pagrus*, reproduction, hermaphroditism, protogynous, vitellogenin, sexual steroids.

RESUME – *"Progrès récents concernant les aspects de reproduction de* Pagrus pagrus*". La plupart des connaissances acquises sur la biologie du pagre commun,* Pagrus pagrus*, sont issues du milieu naturel. Ici, le travail expérimental a été réalisé dans des conditions d'élevage à l'Institut de Biologie Marine de Crète (IMBC, Iraklion, Grèce). Les poissons ont été pêchés sur des aires de nurseries du golfe d'Iraklion, à un âge d'environ 6 mois (poids corporel moyen = 15,8 ± 4,2 g) puis élevés pendant 6 ans dans les installations de l'IMBC.* Pagrus pagrus *est un hermaphrodite protérogyne. Le développement des gonades peut se faire selon trois possibilités. Dans le premier cas, des poissons immatures (moins de 3 ans) développent un tissu testiculaire et l'ovaire dégénère avant la maturation sexuelle (type "mâle primaire"). Dans le second cas les gonades se développent en ovaire jusqu'à la maturation complète (femelle fonctionnelle). Dans le dernier cas, après une ou plusieurs pontes, la femelle change de sexe pour donner un mâle fonctionnel (de type "secondaire"). La première maturation sexuelle du pagre apparaît à 3 ans (pour 11,2% des femelles et 77% des mâles de cet âge), mais plus de la moitié des individus sont matures à 4 ans. Ils présentent un cycle annuel de reproduction. La gamétogenèse commence en automne quand la photopériode et la température décroissent, et la ponte survient au printemps (mars à mai) avec des températures de 15-19°C. Les évolutions de l'oestradiol, de l'oestrone et de la vitellogénine plasmatiques chez les femelles, de la testostérone et de la 11-cétotestostérone chez les mâles, ont été suivies.*

Mots-clés : *Sparidé,* Pagrus pagrus*, reproduction, hermaphrodisme, protérogyne, vitellogénine, stéroïdes sexuels.*

Introduction

A new sparid aquaculture could be based on the common sea bream, *Pagrus pagrus* (Linnaeus, 1758), which has a large geographical distribution and a large market, especially for big fish which are more rarely caught by fishing (Walker, 1950; Manooch, 1976; Manooch and Hassler, 1978; Alekseev, 1982; Vassilopoulou and Papaconstantinou, 1992; Vaughan *et al.*, 1992). The common sea bream participates significantly in commercial fisheries and also contributes to the recreational harvest of reef

fishes. This demersal fish living at depths from 18 m to 280 m was suspected to have extreme temperature tolerance values of 8-29°C, with an optimum between 13°C and 26°C, has the ability to grow fast, and shows adaptability to heavy stocking (Divanach *et al.,* 1993; Kentouri *et al*., 1994). A close species from the same genus, the read sea bream *Pagrus major*, has been successfully farmed in Japan (Foscarini, 1988) where it is one of the most valuable farmed marine fish.

Until now, *Pagrus pagrus* reproductive biology have been studied in the wild and it was suspected to be a protogynous hermaphrodite, with spawning occurring in Spring (Manooch, 1976). Some attempts of rearing this fish in farm conditions were successful. However, at this time, mainly juveniles caught in the wild were used for growing and aquaculture could not rely on such catches since some natural stocks have already experienced landing declines (Vaughan *et al*., 1992; Harris and McGovern, 1997).

The main objective of this study was to describe the reproductive features of this species when maintained in fishfarming conditions. The work involved morphological, histological and endocrine studies of gametogenesis in female and male and of the inversion process by analysing monthly during one year fish sampled from six groups of increasing age (about 6 to 66 months old at the beginning of the experiment).

Materials and methods

Fish

Fish were caught from the wild at age of 0+ in Heraklion Bay (Crete, Greece) and were acclimatized to rearing conditions. They were maintained until aged 3+ to 6+ years in outdoor tanks of 10 m^3 each, under natural photoperiod (Lat.: 35.20N, Long.: 25.09E) and water temperature (15-25°C) at the experimental facilities of the IMBC. They were fed *ad libitum* with artificial food (Aqualim) (Kokokiris *et al*., 1998).

Sampling

In total, 1142 fish (1002 females, 140 males) were used for this experiment, sampled on a monthly basis: 20 fish each month for 1, 2 and 3 years old fish; 7 to 20 fish each month for 4 and 5 years old fish; and 5 to 10 fish each month for 6 years old fish. Fish were anaesthetized by immersion in seawater containing 2-phenoxyethanol (0.3 ml/l). Blood was collected in 670 fish (3 to 6 years old fish) for vitellogenin and steroids measurement. After bleeding from the caudal vein into a syringe, blood was kept on ice before centrifugation (3000 g, 10 min, 4°C), and serum was stored at –20°C until analysis. Body (BW), visceral (VS), and gonad (GW) weight were measured for GSI calculation [GSI = GW/(BW – GW – VS)].

Gonads histology

Tissue samples gonad were fixed in Bouin's solution and prepared using routine histological procedures (Gabe, 1968). In preliminary studies, no differences were detected in gonadal development between sections of the right and left gonad, or sections of the anterior, middle and posterior parts of ovaries or testes. Therefore, only tissue samples from the middle of the right gonad of each fish were used for histological examination, and 4-6 µm sections were stained with Harris haematoxylin and eosin and examined under light microscope. Gonadal stages were determined according to the presence of the most advanced type of female and male germ cells (Table 1). Besides, the highest oocyte diameter was determined as the mean diameter of the 30 largest oocytes (MOD) within one histological cross-section of the ovary.

Plasma steroids assays

Plasma samples (0.5-1.0 ml) were extracted twice with 5 volumes of cyclohexane/ethyl acetate

(50:50). Testosterone (T), 11-ketotestosterone (11KT), 17β-oestradiol (E2), and oestrone (E1) levels were measured by radioimmunoassay according to Fostier *et al*. (1982) and Jalabert and Fostier (1984).

Table 1. Maturity stages of the gonads

Maturity stage	Histological features
Females	
F1: Previtellogenesis	Only previtellogenic oocytes with densely stained cytoplasm are present
F2: Beginning of vitellogenesis	Oocytes with lipid globules in their cytoplasm are present
F3a: Early large oocyte growth	Less than 50% of the histological section is occupied by oocytes in which ooplasm contains small, spherical yolk globules
F3b: Late large oocyte growth	More than 50% of the section is occupied by vitellogenic oocytes. Ooplasm is occupied by large populations of yolk and lipid globules
F4: Final oocyte maturation	Presence of hydrated mature oocytes
Males	
M1: Testis gonia stage	Well defined lobular organisation of the testes. Only spermatogonia present
M2: Spermatogenesis	Appearance of spermatocytes and spermatids. Predominance of spermatocytes
M3: Spermiogenesis	Large parts of the testis filled with spermatids and spermatozoa
M4: Spermiation	Sperm hydration. Presence of sperm in the vas deferens
M5: Post-spawning	Lobules devoid of spermatozoa. Residual sperm present

Vitellogenin assay

Vitellogenin (Vg) has been measured in plasma using a homologous enzyme-linked immunosorbent assay (ELISA). Vg was purified by low pressure chromatography from plasma of fish treated with estradiol benzoate (Mananos *et al*., 1994). ELISA has been settled according to Nunez *et al*. (1989).

Statistical analysis

Statistical errors are expressed as the standard error of the mean (SEM). Coefficient of correlation was considered significant at the 5% significance level. One-way analysis of variance (ANOVA) and a Sheffe's multiple range test was applied to compare mean levels at different stages. Bartlett's test was used to verify the homogeneity of variances. When necessary (non-homogeneity of variances), data were log transformed before subjecting them to ANOVA.

Results and discussion

Sexuality

Evolution of the gonadal structure

Gonads of 6-month-old fish had a thread like appearance and were either undifferentiated or ovaries with oogonial nests or developed oocytes. Undifferentiated gonads consisted of a pair of laterally compressed tubules occupied by connective tissue, each having a large central cavity. In ovaries, colonization with oocytes started at the caudal part of the gonad and was directed to the anterior. Oocytes were at the perinuclear stage.

Gonads of 12-months-old fish were either ovaries or bisexual, possessing ovarian and testicular tissue. In the ovaries both their size but also the size and the number of oocytes were increased in comparison to those ones of younger fish. Testicular tissue of the bisexual gonads appeared as separated islets on the gonadal surface and occupied the ventral part of the gonad. The first nests with spermatogonia appeared at the caudal part.

Gonads of 2-years-old (12-24th month) fish were either ovaries or bisexual. Ovaries were larger in size and contained more perinuclear oocytes than those of 12-month-old fish. Bisexual gonads were well formed ovaries, with a ventrally developed testicular zone. Spermatogonial divisions were apparent.

Gonads of 3-year-old fish (25-36th month) were ovarian, bisexual or testicular in form. Ovaries contained previtellogenic or vitellogenic oocytes. Bisexual gonads were histologically similar to those described in the previous stage except of some fish with bisexual gonads in which the ovarian zone was occupied by oocytes at F2 stage. No oocytes at F3 stage were present. Testicular zone on bisexual gonads showed active signs of spermatogenesis, with well formed lobules and spermatocytes present in the lobular lamina. The size of testicular zone was smaller or had the same size than the ovarian zone. No signs of degeneration were present in the ovarian tissue. Testes in fish of 3-years-old were rare but they showed active spermatogenesis and were characterized by complete anatomical differentiation with a well formed vas deferens, located in the ventrolatterally part of the gonad, in proximity to the ovarian cavity.

Gonads of 4 to 6.5-years-old fish (37-78th month), were ovarian, bisexual or testicular in form. Ovaries were large in size. In some of them, a few spermatogonial nests limited in size and randomly distributed along the ventral part, could be recognized. Bisexual gonads had the same characteristic than those described in previous ages and although they were less frequently encountered, they still existed at least until the age of 70-months-old. Ovarian part could be at stage F2. Testes showed active spermatogenesis (M2 stage and over). In most of the testes of 5 and 6-years-old fish either the perinuclear oocytes in the ovarian zone were degenerating, surrounded by eosinophilic granulocytes, and covered by fatty tissue, or the ovarian tissue was completely absorbed with a remnant central cavity in the middle of the well-developed testis.

Sex ratio

1002 of the 1142 fish sampled were females having ovaries at different stages of maturity with no traces of developed testicular tissue. 52 fish had bisexual gonads, in which, the testes were not anatomically differentiated although testicular zone presented signs of spermatogenesis. 88 fish were functional males, with a complete anatomical differentiation (presence of a well formed vas deferens) of the testes at various stages of sexual maturation.

The sexual structure of age groups is summarized in Table 2. At the age of 12-months all fish were females. Individuals with bisexual gonads (hermaphrodites) appeared first at the age of 24 months and they were found up to the age of 70 months (6-years-old fish) in a relatively constant frequency 15-20%. Functional males did not appeared until the age of 21 months (2-years-old fish), then increased in number.

Sexual maturity

Females were considered as mature if their ovaries were either at active vitellogenesis (F3a, F3b) or final maturation (F4) stage. Females were immature until the age of 2 years. Mature females were observed for the first time at the age of 3-4 years (35-39th month) but their percentage was low. Mature females were observed from January to March. A higher percentage of females were mature (more than 50%) at age 4, 5 and 6 years.

Males were considered as mature if their testis were either at spermatogenesis (M2) or spermiogenesis (M3) and spermiation stage (M4). First mature males were observed at the age of 3 years (34-36th month). A higher frequency of mature males (more than 50% of males sampled) was observed at the age of 4-years and over.

Table 2. Frequencies (%) of the various sexual types according to the age

Age (years)	Sexual types		
	Females†	Potential males††	Males†††
1	100 (140)	–	–
2	98.9 (257)	0.01(3)	–
3	87.9 (225)	8.2 (21)	3.9 (10)
4	84.4 (178)	6.1 (13)	9.5 (20)
5	77.3 (102)	4.5 (6)	18.2 (24)
6	69.9 (100)	6.3 (9)	23.8 (34)
Total	87.7 (1002)	4.6 (52)	7.7 (88)

†Ovary without testicular zone.
††Ovary with a testicular zone showing spermatogenesis.
†††Functional testis.
(n) = Number of fish.

Discussion

The histological features and chronological appearance of ovaries, bisexual gonads and testes in relation to the age and sexual maturity strongly suggested the following sexual pattern and nature of protogynous hermaphroditism. The gonads differentiate as a bisexual organ like in *Pagrus major* (Matsuyama *et al.*, 1988b) with two isolated heterosexual zones, ovarian and testicular, which develop sequentially. This development takes place asynchronously so that all the fish, before the first sexual maturity, pass through a stage at which the ovarian zone is differentiated into an ovary.

Pagrus pagrus could be considered as a species with delayed sexual maturity (4-years-old). The maintenance of fish under high temperature and unlimited food availability although resulting in a higher growth rate in rearing conditions (3 times higher than the wild populations, Machias *et al.*, 1998) had no effect on the age of sexual maturity since no precocious maturation was recorded in our study, compared to the wild populations. It seems that temperature and food availability are not determinative factors of the age at sexual maturity. These findings make us to point out that sexual maturity might be linked to the age or to particular phenomena related to behavioural or ecological aspects of the species life cycle.

The secondary phase of gonads differentiation and thus the choice of the primary or secondary developmental pathway in diandric species, it is not exclusively genetically determined. In many hermaphrodites, individuals change sex, not only because they reached a particular size or age but also due to behavioural or/and social (demographic) changes in their social system (Shapiro, 1992). Captivity could result in an intensification of behavioural signals by artificially creating such social groups. Sex change and sexual structure of populations could be also affected by environmental factors (Chan and Yeung, 1983). It has been shown in sea bream *Sparus aurata*, a protandrous hermaphrodite species that special conditions of captivity could influence sex inversion (Zohar *et al.*, 1984). The presence of old females decreased the proportion of sex inversion of young males and, the presence of young males increased the sex proportion of old males that changed sex. Maintenance of fish under high temperature and the unlimited availability of food could be involved to an acceleration of the development of gonads and the precocious sex change (Zohar *et al.*, 1978). Higher percentages of hermaphrodite individuals were recorded in reared populations of the juvenile bisexual *Pagrus major* comparing to the wild populations and were mainly attributed to high temperature and food availability of the rearing conditions. In the same way, in captivity reared populations of the grouper *Epinephelus microdon*, deviations in sex ratio could lead to individuals changing sex from female to male and even from male to female, producing an adjustment at approximately a 1:1 sex ratio (Debas, 1989; Debas *et al.*, 1990). In *Pagrus pagrus* the large zone of coexistence of the two sexes in the age frequency distribution, but also the realisation that sex change does not occur to all females at least until the age of 6.5-years-old, enforce the hypothesis that other factors except of age

or size could be implicated to sex change. It remains to be explained which are the environmental and demographic (social) factors could influence the sex change and also the timing at which those factors intervene.

The high dispersion of individuals with bisexual gonads in all age groups indicate that sex change is not related to one particular age or size. Development of the testicular zone seems to occur progressively during the female reproductive cycle and is invariably combined with ovarian tissue at different phases of degeneration. Sex change in *Pagrus* might be a discontinuous and cyclic process that is not necessarily completed during one reproductive cycle. Similar observations have been done in other sparids like *Sparus aurata* (Bruslé-Sicard and Fourcault, 1997), and *Lithognathus mormyrus* (Besseau and Bruslé-Sicard, 1991).

Seasonal reproductive cycle

Females reproductive cycle

From June to October, the ovaries were in the resting phase and mainly filled with previtellogenic oocytes (stage F1). Females at F2 stage occurred at the beginning of November. At this time, the percentage of maturing females was about 10% which increased significantly over the following months to reach a high percentage in early January (54%). Stage F2 was associated with a significant increase of MOD up to 390 μm in January and also a significant increase ($p < 0.05$) of the mean concentration of plasma Vg (69.5 μg/ml) and GSI values. During this period, the water temperature and daylength were still decreasing. Daylength reached its minimum in January.

Active vitellogenesis started during January and February and a high percentage of stage F3a females was found (25%). Exogenous vitellogenesis reached its highest level of activity during March (stage F3b) when MOD (560 μm), plasma levels of Vg (405.5 μg/ml), and GSI (3.1%) reached their highest values.

The spawning period started during March-April and lasted until the beginning of May. Females at the final maturation stage (stage F4) could then be found. During this period, GSI and MOD gradually decreased and Vg concentrations declined. Vitellogenic activity continued until the beginning of May when Vg levels, although decreasing were still higher than the basal levels of the previtellogenic period. This period (January-March) was accompanied by a further decrease of temperature to its minimum (15°C in March). Both temperature and day length increased from March to May. During this period, only a low percentage of mature females at stage F4 and no female with empty follicles could be found, suggesting that no spontaneous spawning occurred. High atretic rates were recorded in the mature ovaries during the initiation of the active vitellogenesis period (February). Vitellogenic oocytes interrupted their growth and became atretic. Atretic rates were high during March reaching values around 18% and remained at high values (although decreasing) until the beginning of April, then reached a basal level in May (2%).

The average concentration of E2 was lower than 2 ng/ml. E2 values first increased significantly at stage F2 and again during stages F3. They remained high during oocyte maturation (stage F4). Concentration remained at the same levels from early exogenous vitellogenesis to final maturation stage. The pattern of E2 changes was similar to that of Vg and their correlation was statistically significant although it was low [$R(E2/Vg) = 0.34$, $p < 0.05$, $n = 588$].

E1 mean levels were lower than E2 (less than 300 pg/ml). The concentration increased significantly later than E2, i.e. only at the beginning of exogenous vitellogenesis (stage F3a) and remained in comparable levels during the next stages. E1 and E2 levels were significantly and positively correlated [$R(E2/E1) = 0.62$, $n = 66$] and E1 was better correlated to GSI than E2 [$R(E2/GSI) = 0.19$, $n = 588$; $R(E1/GSI) = 0.44$, $n = 66$]. Correlation of Vg with E1 [$R(Vg/E1) = 0.37$, $n = 66$] was similar to that of E2 (Table 3).

Fecundity

The total fecundity increased with the size of females and changes were well described by body weight (BW; $r = 0.54$, $p < 0.05$, $n = 59$) or standard length (SL; $r = 0.53$, $p < 0.05$, $n = 59$). Fecundity

ranged from 1200 (for a female of 1.3 kg) to 336,000 oocytes (for a female of 2.1 kg). By interpreting the linear regression between total fecundity and weight or length, the smallest fish potentially fecund weighed more than 700 g with a length exceeding 25.5 cm. Above this size total fecundity increases by 105 oocytes per g of BW or 11,880 oocytes per cm of SL.

Table 3. Characteristics of female maturity stages. Means and (SEM)

Stages	GSI (%)	E2 (ng/ml)	Vg (µg/ml)
F1	0.49 (0.01)	0.29 (0.02)	0.58 (0.17)
F2	0.77 (0.05)	0.51 (0.07)	8.89 (3.32)
F3a	1.46 (0.09)	1.21 (0.26)	159.1 (34.2)
F3b	3.80 (0.22)	1.40 (0.18)	533.0 (77.2)
F4	8.10 (0.62)	1.86 (0.44)	1037 (192)

Male reproductive cycle

The testis gonia stage was present from September to November (stage M1). The highest proportion of spermatogenesis stage (M2) was found in November (50% of males were in this stage). Spermiogenesis stages (M3) occurred in high proportion (more than 20%) from December to April although they could be detected during the whole period from October to May. Active spermiation (M4) extended from February to May. During this period GSI of males reached its highest values (April 2.4%). The first post-spawning males (M5) were found in May (less than 20%) and in higher proportion during summer (June to August) but there were still a few males exhibiting recovery in September and October (around 20%).

T levels in males increased significantly during spermiogenesis and spermiation stages to reach around 3 ng/ml, then they decreased to basal levels after spawning. T and 11KT values presented a similar pattern of changes during spermatogenesis and they were significantly and positively correlated [R(T/11KT) = 0.66, n = 66]. However, 11KT levels stayed at about half those of T. T and 11KT levels were both significantly and positively correlated to GSI [R(T/GSI) = 0.59, n = 85; R(11KT/GSI) = 0.68, n = 66] (Table 4).

Table 4. Characteristics of male maturity stages. Means and (SEM)

Stages	GSI (%)	T (ng/ml)	11KT (ng/ml)
M1	0.29 (0.13)	0.71 (0.17)	0.17 (0.08)
M2	0.21 (0.05)	0.38 (0.22)	0.15 (0.05)
M3	1.02 (0.63)	2.85 (0.58)	2.05 (0.53)
M4	2.12 (0.45)	2.89 (0.73)	1.60 (0.36)
M5	0.16 (0.02)	0.51 (0.17)	0.13 (0.02)

Discussion

The present study provides accurate information on seasonal changes of gonadal development during the annual reproductive cycle, based on the microscopic examination of gonads. Gonadal histology and frequency distribution of gonad stages of maturity in relation to time, indicated the separation of the female and male reproductive cycle into three main periods. The autumn period when gametogenesis begins (October-November), the period of exogenous vitellogenesis (January-March) or spermiation (December-March) and a primarily spring reproductive season (March to May).

The initiation of gametogenesis that was characterized by the presence of females at the endogenous vitellogenesis stage and males at the spermatogenesis stage, occurred mainly during

November. During this period, water temperature and daylength were decreasing whereas both parameters were increasing during the reproductive period. The reproductive season was characterized by a high GSI, the presence of fish with mature gonads at exogenous vitellogenesis and spermiation stages, high MOD and Vg values also. The significant increase in both GSI and Vg values indicated the beginning of the vitellogenesis period at January. Only a small proportion of females at stage of final maturation (F4) was found and in almost all mature females, high atresia rates were detected. Rates of atresia reached maximum levels during the main vitellogenic activity (18% in March) and decreased to basal levels only at the end of the reproductive period (2% in May). The reproductive period of males was almost one month longer than for females. A high percentage of males were already in active spermiation in February. GSI reached its maximum values in February and remained at high values until May.

Despite its wide geographic distribution, the timing of the reproductive season does not show any important variation between different areas. Spawning generally takes place during late winter-spring. It peaks from March to April in Western (Manooch, 1976) and Eastern Atlantic (Pajuelo and Lorenzo, 1996) and from late February to May in Eastern Mediterranean Sea (Vassilopoulou and Papaconstantinou, 1992). As was revealed by this study, rearing of fish under culture conditions has no effect on this timing although water temperature in rearing tanks was higher than the deep waters of Heraklion Bay in Crete where the species normally lives (Machias *et al*., 1998).

Males mature earlier than females. The same observation has been made for other species also under rearing conditions. Microscopic examination of male gonads indicated the appearance of spermatozoa even in the early stage of testicular development but also revealed the asynchronous type of testis development, with germinal cell types present in each stage. Such an early appearance, already observed in other species such as the closely related *Pagrus major* (Matsuura *et al*., 1987) is not an indication that fish are in spawning condition or they will spawn soon. Pressure of the abdomen revealed that although appreciable amounts of spermatozoa are found in tubules, release of sperm does not occur. Thus, mature testes are only those with vas deferens filled with spermatozoa.

Although *Pagrus* populations have been studied in the wild, no data was concerning the relationships between gonadal development (GSI), gametogenesis, and its endocrine control. Vitellogenin levels were very low before the beginning of exogenous vitellogenesis, then they increased and stayed high during the final stages of gametogenesis. The maintenance of a high level of vitellogenin during these last stages may be related to the oogenesis pattern of this multiple spawner species. Vitellogenesis is still active during the spawning season, as could be also concluded from the evolution of plasma E2. After a spawn, a new batch of oocytes achieves vitellogenesis in order to be ready for another spawn. Such a pattern has been reported in other sparids like *Sparus aurata* (Kadmon *et al.*, 1985; Zohar *et al*., 1988).

At the stage of endogenous vitellogenesis, plasma concentration of E2 was increased significantly. This is in agreement with findings in other teleosts (Pankhurst and Conroy, 1987; Berlinsky and Specker, 1991; Rinchard *et al*., 1993). Such an increase preceded the appearance of yolk globules in the oocytes and is consistent with the role of estrogens in promoting hepatic synthesis of the yolk precursor (vitellogenin). E2 concentrations were significantly correlated to plasma Vg levels. E2 levels increased during exogenous vitellogenesis and remained high until final maturation. Similar patterns have been reported in other multiple spawner fish with asynchronous ovarian development, such as *Carassius auratus* (Kagawa *et al*., 1983), *Gobio gobio* (Rinchard *et al*., 1993) *Sparus aurata* (Kadmon *et al*., 1985*), Dicentrarchus labrax* (Prat *et al*., 1990) and *Pagrus major* (Matsuyama *et al*., 1988a; Ouchi *et al*., 1988) or *Pagrus auratus* (Carragher and Pankhurst, 1993; Scott *et al*., 1993). In these fish, after ovulation there are still oocytes at the stage of exogenous vitellogenesis in the ovary. These remaining vitellogenic oocytes are able to produce E2 after ovulation contributing in this way to high plasma levels (Kagawa *et al*., 1983). Levels of E2 are within the same levels as other marine species with asynchronous ovarian development. Although E1 showed a similar pattern to E2, E1 levels were about five times lower. E1 is considered as less active than E2 on hepatic synthesis of Vg (Ng and Idler, 1983), and it could be a precursor of E2, via androstenedione, since a high correlation was observed between E1 and E2 ($r = 0.62$).

In males a large increase of T serum levels was observed at the spermiogenesis stage. Such an increase coincided with the rapid increase of the GSI and the appearance of spermatids in the testes. A similar increase in serum T level has been reported at this stage in other species and was thought to regulate male germ cell differentiation (Billard *et al*., 1978). The serum 11KT levels were significantly

lower than the corresponding levels of T, however the two steroids exhibited the same pattern in plasma. In contrast, 11KT levels were higher than T levels in many other fish species including Pacific and Atlantic salmon (Idler *et al*., 1971), rainbow trout (Scott *et al*., 1980), brook trout (Sangalang and Freeman, 1974), common carp (Barry *et al*., 1990), walleye, *S. vitreum*, (Malison *et al*., 1994), and even *Pagrus auratus* (Carragher and Pankhurst, 1993). The increase of T and 11KT during male gametogenesis is similar to patterns described in other male teleosts (Pankhurst and Carragher, 1991) and supports the hypothesis that androgens are involved in initiating and maintaining testicular development (Carragher and Pankhurst, 1993). The 11KT profile in *Pagrus pagrus* is compatible with the hypothesis that this steroid regulates the process of spermiogenesis and spermiation (review by Billard *et al*., 1982; Fostier *et al*., 1983, 1987; Nagahama, 1987; Borg, 1994). 11KT levels are elevated when spermatids and mature spermatozoa almost completely populate the testis. Levels remain high during sperm hydration and spawning. It is possible that 11KT has an important role in maintaining not only spermatozoa viability and sperm storage (Malison *et al*., 1994), but also spermiation and spermiogenesis. The same pattern was observed in another marine teleost the sea bass, *Dicentrarchus labrax* (Prat *et al*., 1990).

Conclusion

Histological evidences of protogynous sex change of *Pagrus pagrus* is based on the presence of ovotestes after first female maturity (3-years old) in which testicular tissue present signs of spermatogenesis while ovaries enter a process of degeneration. Gonadal development proceeds through three different ways. In the first way, immature fish (less than 3 years) develop the testicular tissue and the ovaries degenerate before sexual maturity. These fish function as males throughout their life, omitting the functional female phase (equivalent to the primary males of true diandric species). In the second way, the development of the gonads is completed with maturation of the ovarian zone, and the fish function as females (functional females). After a single, or possibly repeated spawnings, females change sex and function as males (equivalent to secondary males of true diandric species). In the last way, fish remain females without changing sex. Rudiments of the testicular tissue remain in their ovaries, but they do not show noticeable structural and functional changes (persistent females).

Histological analysis of the common sea bream gonads revealed that under rearing conditions, this species mature for the first time at the age of 3-years (11.2% of 3-years old females and 77% of 3-years old males) but more than 50% of individuals were mature at the age of 4-years. Our results concerning sexual maturity differ than the 50% frequency of mature females at age 3 and 100% at age 4 reported for the wild populations of the species at different geographic area (Manooch, 1976; Vassilopoulou and Papaconstantinou, 1992; Pajuelo and Lorenzo, 1996).

Pagrus pagrus has an annual reproductive cycle. Gametogenesis starts during the autumn period when photoperiod and temperature decrease and spawning occurs during spring (March to May) with temperature ranging from 15-19°C. Spontaneous spawning under culture conditions could not be excluded under favourable conditions of management of a genitors stock. *P. pagrus* is a multiple spawner species with fecundity of the indeterminate type. It presents similar reproductive characteristics with other already reared marine fish species like sparids (*Sparus aurata*, *Pagrus* spp.) or the sea bass, *Dicentrarchus labrax*.

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