Aspects of reproductive biology of Mediterranean amberjack (Seriola dumerilii Risso, 1810): Gonadal development

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Aspects of reproductive biology of Mediterranean amberjack (*Seriola dumerilii* Risso, 1810): Gonadal development

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SUMMARY- Wild *Seriola dumerilii* juveniles (77.3 ± 23 g total body weight, 17.4 ± 1.8 cm standard length; 2-3 months of age), were caught in the Eolian Island and reared in floating cages for a 14 months period. Gonads were collected routineously and submitted to histological and histochemical analysis to study the type and timing of gonadal differentiation and development. The histological examination shows that sex differentiation is clearly expressed in juveniles 23-25.5 cm long, 4-5 months old. A "differentiated" development was detected, as sexes early differentiate into female or male. In both sexes, anatomical and cytological differentiation proceed in parallel. Gonadal differentiation is completely achieved by the end of the first year of age. Within this period also cytological differentiation was completed in reared males, whereas females do not show the secondary growth phase oocytes. These findings agree with the previous data on wild *S. dumerilii* caught in natural environment. Comparing data reported by other Authors, gonadal development seems to be linked to fish size more than to age. Gonadal development in cage-reared fish was continuous and no pathological or degenerative signs were histologically observed. From an aquaculture perspective cage rearing conditions, being similar to the natural ones, seem to be suitable to correctly develop and manage broodstock in captivity.

Key words: *Seriola dumerilii*, gonadal differentiation, gonadal development, cage culture

RESUME- Des alevins sauvages de *Seriola dumerilii* (77.3 ± 23 gr poids corporel; 17.4 ± 1.8 cm longeur standard; de 2-3 mois d'âge) ont été capturés dans l'Archipel des Eolies et élevés dans des cages flottantes pendant 14 mois. Des gonades ont été collectées cycliquement et ont été soumises à des analyses histologiques et histochimiques aufin d'étudier la modalité et les temps de différentiation et de développement des alevins élevés. L'analyse histologique montre que la différentiation sexuelle dans le alevins ayant une longeure standard de 23-25.5 cm et 4-5 mois d'age est évidente. On a remarqué qu'il y avait eu un développement "différencié" car le sexes des mâles et des femelles se différenciaient très tôt. Dans les deux sexes, la différentiation cytologique et anatomique procedait de façon parallele. La différentiation des gonades est définitivement accomplie vers la fin du premier an de vie. Dans la même période, la différentiation cytologique des mâles était complètement accomplie tandis que pour les femelles la phase de croissance secondaire des oocytes n'est pas encore évidente. Ces résultats s'accordent avec les données précedents sur la *S. dumerilii* capturée en environnement naturel. Des données de comparaison recueillis par Micale *et al.* (1993) sur la *S. dumerilii* sauvage élevée en bassins montrent que le développement des gonades semble etre lié aux dimensions du poisson plus qu'à son âge. Le développement des gonades dans les poissons élevés en cages était continu et aucun signe ni patologique ni dégéneratif a été observé. Du point de vue de l'aquaculture, les conditions d'élevage en cage, constituant le type d'élevage le plus naturel, semblent etre appropriées pour le développement et la gestion des alevins en captivité.

Mots-clés: *Seriola dumerilii,* différentiation, développement, gonades, cages flottantes.

INTRODUCTION

The selection of new marine fish species for Mediterranean aquaculture diversification is performed on market analysis and on the potential production of the selected species depending on juveniles availability and biological performance in captivity. *Seriola dumerilii* is considered a high price demand, high adaptability to culture conditions, high growth and survival rates fish (Benovic, 1980; Giovanardi *et al.*1984; Navarro and Belmonte, 1987; Lazzari and Barbera, 1988; Cavaliere *et al.* 1989; Porrello *et al.* 1993). Despite this, *S. dumerilii* culture is performed only on an experimental scale in Spain and Italy. The major bottleneck for further intensification of *S. dumerilii* culture consists in the availability of fingerlings as the supply of juveniles from natural resource is inadequate and artificial reproduction techniques are not set up yet.

With the view of utilizing wild juveniles for *S. dumerilii* culture, suitable catch methods using circular nets and attractive systems were set up by Greco *et al.* (1991). Improved transport techniques were also achieved by Greco *et al.* (1991) and by Porrello *et al.* (1993). Some recruiting areas were identified in the South Thyrrenian Sea by Caridi *et al.* (1992) and juveniles availability was assessed by Andaloro (1993). Catches of about two million juveniles/per year (less 200 gr total body weight) were recorded in the northern and eastern coasts of Sicily, although

most of juveniles were caught by sport fishermen and rarely devoted for aquaculture purposes.

There is very little knowledge on the reproductive biology of *S. dumerilii*. Some "spotted" data on wild populations captured in the Sicilian Channel and in the Eolian Island were recorded during the spawning season . Sex ratio was determined at 1:1 by Lazzari and Barbera (1989), Andaloro (1993) and Micale *et al.* (1993). Several Authors determined the spawning period between mid of May and mid of July, even though according to recent studies (Marino *et al.*, 1994) the spawning season seems to be longer, especially for younger age groups. Monitoring cycle over six years to determine spawning temperature showed that it is in the range of around 21 ± 0.5 °C (Andaloro,1993), as also indicated by Lazzari and Barbera (1989). Total body weight at first maturity was roughly estimated >20 Kg for females and >10 Kg for males by Manganaro *et al.* (1993) and in the range of 10-12 Kg in both sexes by Lazzari and Barbera (1989). Sexual maturity in wild fish caught in Pelagie Islands was firstly observed at four-five years of age and median standard length (50%) at maturity was recorded at 109 and 113 cm in males and females (Marino *et al.*, 1994).

In the Mediterranean area artificial reproduction techniques were not yet set up. The spontaneous release of sexual products has never been achieved in sexual mature females, and only occasionally in five years old males (pers. comm.). Furthermore, gonadal structure disorganization and germ cells degenerations were detected by histological analysis in already architecturally differentiated ovary and testis of 17 months old *S. dumerilii* reared in tanks (Micale *et al.*, 1993). The effects of captivity condition on gonadal development and on sexual maturity in *S. dumerilii* are fully unknown and further research work is necessary to properly develop and manage broodstock in captivity.

This study is aimed at verifying if type and timing of gonadal development in wild *S. dumerilii* juveniles reared in cages can be affected by culture conditions.

MATERIALS AND METHODS

700 wild *S. dumerilii* juveniles (77.3 ± 23 g total body weight, BW; 17.4 ± 1.8 cm standard length, SL), were caught in August 1990 under artificial wreckages in the Eolian Island. According to the typical spawning period in the area, juveniles were estimated to be two-three months old from hatching. Fish were transferred in 300mc floating cages at an initial density of 1.66 Kg/mc and were reared for a 14 months period (Porrello *et al.* 1993). Gonads were routineously sampled and immediately fixed in Bouin's fluid or glutaraldehyde 2.5% in Na cacodilate buffer 0.1M, pH 7.2 and embedded in paraffin or resin (Kulzer 7100). Gonad diameters were microscopically measured. In order to test the heterogeneity of germ cells distribution, apical, central and caudal portions of the gonads were analysed. Sections 2µm thickness were submitted to routine histological and histochemical stainings in order to test gonad differentiation and development. The number and size of ovigerous folds were also determined.

RESULTS

Males

In juveniles 23-25.5 cm long, testis is prevailingly constituted by connective tissue and a wide blood capillaries network. Only at the periphery of the testis, one or two discontinuous strings of cysts contain well differentiated spermatogonia (Fig. 1). These are round cells (12-15µm) with a large basophilic nucleus (8-10µm) and a high nucleus/cytoplasm ratio (0.7). In the medial-central part of the testis the deferens duct is already visible as a very thin and convoluted structure lined by unilayered cubic cells.

From the 4th (up to 32 cm SL) to the 9th month of age (up to 42 cm SL) testis development is characterized by an evident increase in the number of spermatogonia, already well organized in lobules, even though limited to 2-3 complete strings at the periphery of the testis (Fig. 2).

Less numerous and thinner connective fibers are still detactable between lobules. In this stage Sertoli cells are first observed. Lobular organization is better defined from the apical to the caudal portion of the testis, even if no morphological differences seem to be present in spermatogonia feature. Deferens duct still presents a convoluted and thin wall.

By the end of 1st year of life reared *S. dumerilii* show testis fully organized in all directions. Lobules are surrounded by a thin layer of connective tissue and are distributed along the whole testis. Within the lobule wall, spermatogonia, spermatocytes I (7µm) and II (5µm) and spermatids (3µm) in cysts are present (Fig. 3). At this stage free spermatozoa start to be found in the lumen of the lobule. Lobules development differs from the central to the caudal portion of testis, as the number of free spermatozoa (1.5µm) in the lumen increases. Thus, the maturation of germ cells appears to have a central peripheric direction.

13-14 months old fish, about 48-51 cm long, had completely organized and intensively vascularized testis. Lobules increase in diameter and show a stretched wall. Germ cells are present within the wall at all maturity stages: rare spermatogonia, cysts of spermatocytes and spermatids and numerous spermatozoa (Fig. 4). Free spermatozoa are now recognizable both in the lumen of the lobules and in the deferens duct. In these samples deferens has a thicker and less convoluted wall.

By the 16-17 months of life, (54-57 cm SL samples) testis presents spermatogonia at the periphery, few cysts of spermatocytes and spermatids and residual spermatozoa in the central portion testifying the regressive phase of sex cycle.

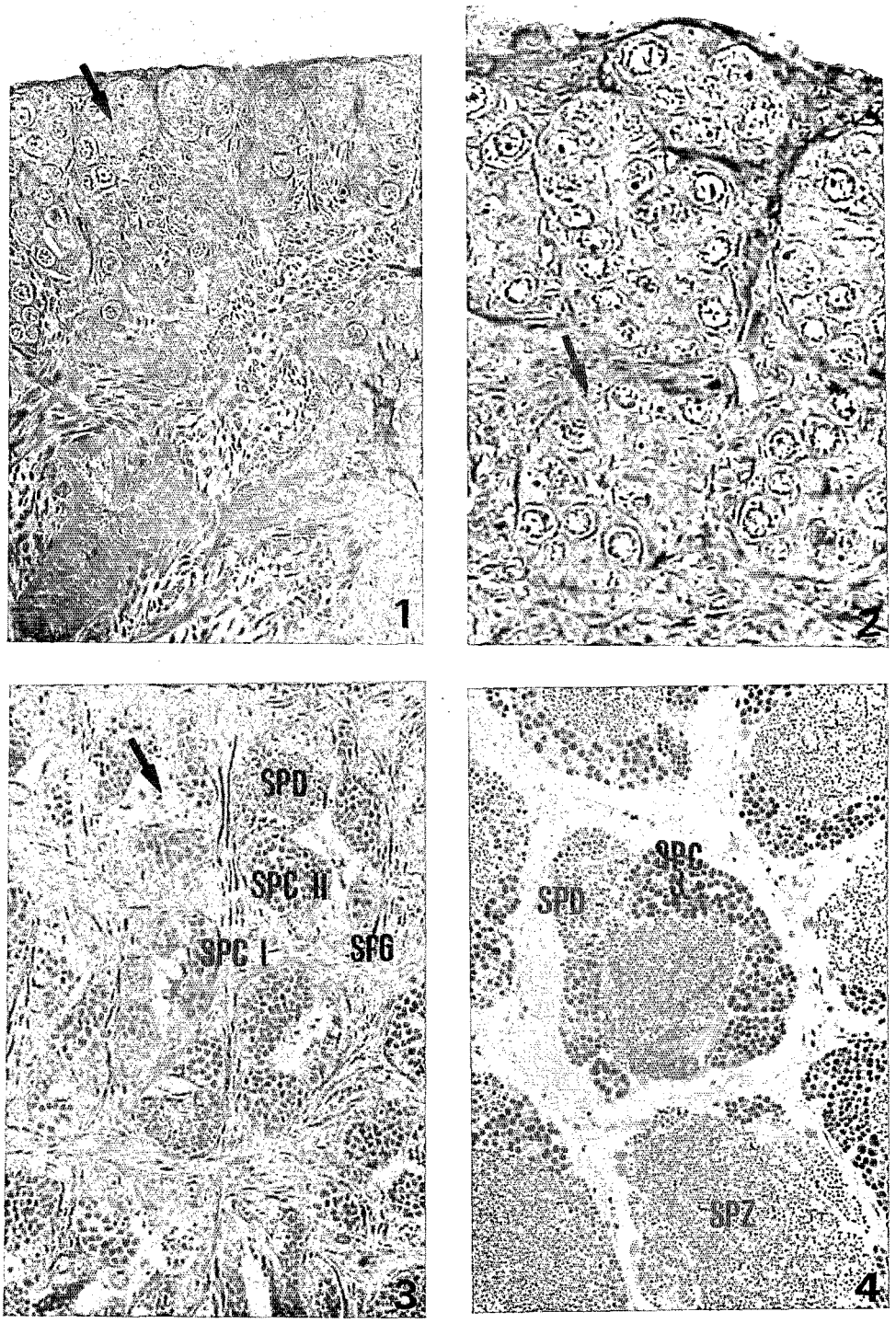


Fig. 1. Transversal section of the testis of a 4 months old male. Only one or two strings of cysts containing spermatozoa are organized at the periphery of the testis (arrow). Gentian violet - x750.

Fig. 2. Nine months old male testis; three strings of cysts containing spermatogonia are organized at the periphery. Sertoli cells are indicated (arrow).Gentian violet - x1500.

Fig. 3. Spermatogonia (SPG), spermatocytes I and II (SPCI, SPCII) and spermatids (SPD) are present in the lobule wall of an 11 months old male. Few free spermatozoa are first noticed (arrow). Hematoxilin-eosin - x1000.

Fig. 4. Free spermatozoa (SPZ) are in the lumen of the lobules in the testis of a young male reared for 12 months in floating cages. Cysts of spermatocytes (SPC) and spermatids (SPD) are also present.Hematoxilin-eosin - x750.

Females

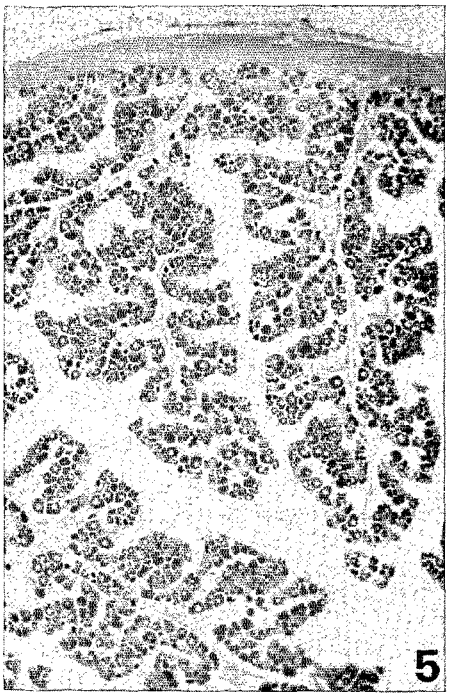
In 23-26 cm SL juveniles the ovary (1.1 X 1.02 mm) consists of 10-11 ovigerous folds (240-360 μm) transversally arranged along the longitudinal axis. Oogonia (10μm), immersed in an abundant connective stroma, are already detectable at this stage.

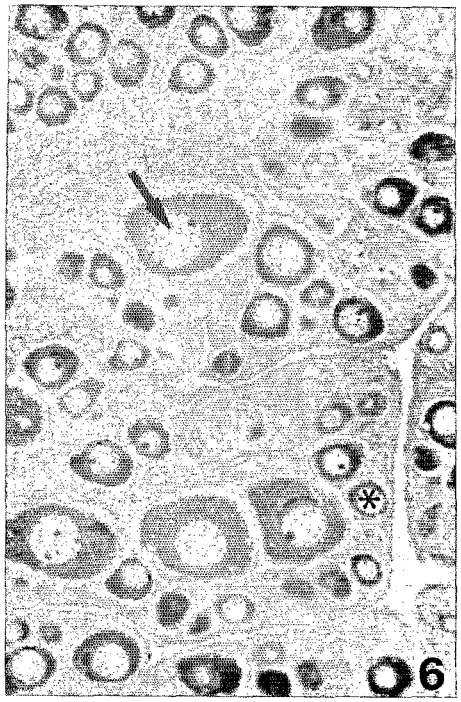
Juveniles, 28-32 cm long and 4-5 months old, show ovaries (2.4x1.9 mm) organised in ovigerous folds (10-14) containing oogonia and few primary oocytes at chromatin nucleolus stage (stage 2; 12-30μm).

In females 37-40 cm long, 9-10 months old, ovaries show more numerous ovigerous folds (11-20) only partially filling the ovarian lumen (Fig. 5). Within the connective tissue, nets of oogonia and numerous primary oocytes at stage 2 are visible. Very few primary oocytes at perinucleolus stage (stage 3; 30-60μm) start to be present (Fig. 6).

The ovaries of 11-12 months old females present most significant differences related to the dimension of the ovigerous folds (1.5-3 mm) starting to totally fill the ovarian cavity. Numerous primary oocytes at the perinucleolus stage are evident (Fig. 7).

14 -16 months old females (up to 58 cm SL) do not show significant differences in the ovary morphology increasing both in size (8.4 X 8.1 mm) and in number of ovigerous folds. Even though the anatomical differentiation of the ovary is completed within the first year of age, no secondary growth phase oocytes were observed during the second year of life.





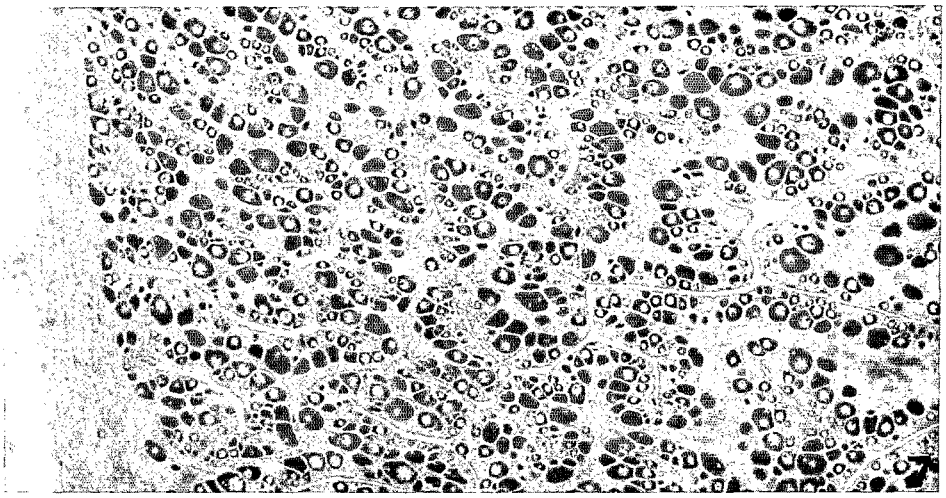


Fig. 5. Cross section of the ovary of a young female with few ovigerous folds containing oogonia and previtellogenic oocytes. Hematoxilin-eosin - x75.
Fig. 6. Many chromatin nucleous stage (*) and few perinucleolus stage (arrow) oocytes within the ovigerous folds in the ovary of a 9 months old female.
Gentian violet - x750.
Fig. 7. Ovigerous folds with previtellogenic oocytes completely fill the ovarian cavity of a female 11 months old. Gentian violet - x150.

DISCUSSION

Sex differentiation is clearly expressed in *S. dumerilii* juveniles 24-25.5 cm long, four-five months of age. The sexual indifferent period of gonad development, lasting more than one year in other teleost (Moiseeva *et al.* 1988) was not recorded in *S. dumerilii*. Although sexual maturity is not attained before 4-5 years of age (Marino *et al.* 1994) sexual differentiation is early detectable (4-5 months from hatching). *Seriola dumerilii* can be considered a "differentiated gonochoristic" species (Yamamoto, 1969), as sexes early differentiate into male or female. Two types of gonocytes, oogonia and spermatogonia, are early found one containing a single nucleolus the other two or three. Thus, germ cells occur as two equivalent but morphologically distinct varieties. Furthermore, both in females and males, anatomical differentiation and cytological sex differentiation proceed in parallel as structurally organizing gonad already contain differentiated gonocytes. The same chronological sequence of development stages is also characteristic of other teleost (Mezhnin, 1978; Mogil'naya and Moiseeva, 1985).

In our study, ovary differentiation was considered as completed when ovigerous folds had completely filled the ovarian cavity and oocytes in meiotic prophase were present. Testis were deemed as differentiated when lobules appeared well organised in all directions and all stages of germ cells were present.

S. dumerilii juveniles rearing in floating cage completely attained gonad differentiation by the accomplishment of the first year of life. Cytological differentiation was also completed in reared male fish, whereas females do not show the secondary growth phase oocytes up to the second year of age. These findings agree with our previous data (Marino *et al.*, 1994) on wild *S. dumerilii* caught in natural environment showing vitellogenic oocytes not before the 4th year of age and differentiated male germ cells during the 2nd year of life. Conversely, our data differ from those reported by Micale *et al.*(1993) on *S. dumerilii* reared in tanks. In this study the persistence of undifferentiated gonad is reported until the 11th month of life and complete gonad differentiation is attained by the end of the 2 year of age.

Differences in timing of sex determination may reflect differing criteria by which observers distinguish sex or can be expected when fish are reared at different temperatures or, generally, in different culture conditions (Foyle, 1993). Since gonad differentiation and development is not comparable in *S. dumerilii* having comparable age (this study vs Micale *et al.* 1993), differences may be related to different fish size, taking into account differences in growth rates of *S. dumerilii* reared in tanks (Cavaliere *et al.* 1989; Garcia Gomez, 1993) and in floating cages (Porrello *et al.*, 1993). From our data gonad differentiation and development seem to be size-dependent. Ovary size measured 1.1X1.02 mm in 23-26 cm SL fish and increased up to 8.4X8.1 mm in fish about 58 cm long.

With the view of controlling *S. dumerilii* reproduction in captivity, fish size might be considered a reliable parameter of gonad development. Furthermore, in fish reared in cages, gonad development was continuous and no anatomical or cytological degeneration were histologically recorded. Cage rearing conditions, being similar to the natural ones, seem to be suitable to properly develop and manage broodstock in captivity.

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