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Comparative gonadogenesis and hormonal induction of spawning of cultured and wild mediterranean amberjack (*Seriola dumerili*, Risso 1810)*

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SUMMARY: The histological characteristics of wild and cultured Mediterranean amberjack gonads were studied during the first four years of their life cycle. No differences were found in the gonad development of both wild and captive males and females during the first and second year. In the third year, wild females showed more advanced oocyte development than captive females. In the fourth year, vitellogenic oocytes were noted for captive females. Fertilised eggs were obtained after a HCG hormone treatment of four- to nine-year old wild specimens. A smaller quantity of mature and fertilised eggs was also obtained through the spawning of hormone-treated four-year old captive broodstock specimens. Eggs in the stage of gastrulation died within eight hours following fertilisation.

Key words: *Seriola dumerili*, gonadogenesis, hormonal treatment, eggs, wild.

INTRODUCTION

The Mediterranean amberjack *Seriola dumerili* (Carangidae), a pelagic migratory fish of warmer seas, is abundant throughout the Mediterranean (Bini, 1968). Due to its high growth rate and good adaptability to culture conditions (Lazzari and Barbera, 1988; Cavaliere *et al*., 1989; Porrello *et al*., 1993; Skaramuca *et al*., 1998), the amberjack has been selected as a potential species for fish aquaculture in the Mediterranean Sea (Abelan and Basurco, 1999).

A primary requirement for the artificial cultivation of a new fish species is the ability to fully control sexual maturation and spawning (Bromage, 1995). One of the problems in establishing this control is the lack of knowledge concerning sexual maturation under natural and culture conditions. The sexual maturation of wild amberjack in Italian waters (Pelagie Islands) was described by Marino *et al*. (1995) and in Spanish waters by Grau *et al*. (1996). In the research carried out, no important differences were noted in the sexual maturation process. Although significant research efforts have already been put into the study of amberjack reproduction, artificial spawning is still an unsolved problem. According to Manganaro *et al*. (1993), the artificial spawning of wild specimens is difficult as females with oocytes in the final phase of maturation are rarely caught. García and Díaz (1995) are of the opinion that vitellogenic oocytes do not develop under rearing conditions. Micale *et al*. (1998) also described problems with the oocyte maturation of older captive amberjacks and with the unsuccessful attempt at controlled spawning of amberjack using

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the LHRH hormone. Final vitellogenesis and oocyte maturation were inhibited under captive conditions (Micale *et al.*, 1999).

This study presents data on hormonal treatment results and on the sexual maturation of captive broodstock and mature wild specimens captured during the spawning season.

MATERIAL AND METHODS

Gametogenesis was observed during a four-year period (1995 to 1999) in 40 broodstock amberjacks. Three to four-month old wild fingerlings were captured in southeastern Adriatic coastal waters from August to September of 1995 and were then reared in cages (Mali Ston Bay- Fig. 1a). The fish were mainly fed with frozen sardines (*Sardina pilchardus*). Gonad samplings from 20-25 fish were taken every three months. Gonad samplings were taken every month prior to and during the spawning season. Gonad samples were obtained by biopsy, through the insertion of a 1.2-mm inner diameter plastic cannula. A microscope was used for the examination of fresh gonad samples. Measurements of oocyte size were taken and the percentages of pre-vitellogenic and vitellogenic oocytes were determined. Gonad maturation was defined according to the description offered by Marino *et al.* (1995). Nine developmental stages of oocytes and four stages of spermatocytes were determined. The developmental oocyte stages are: oogonia, chromatin nucleolus, perinucleolus, lipid vesicles A, lipid vesicles B, yolk granules A, yolk granules B, mature and post-ovulatory follicles. The developmental spermatocyte stages are: spermatogones, Type I and Type II spermatocytes, spermatids and spermatozoa. At the beginning of May, all captive males were examined to see whether any sperm would be released following the application of pressure. An analysis of spermatozoid motility was made for sperm obtained in this manner by using a microscope, after the addition of sea water. Motility was determined by estimating the number of motile and immotile spermatozoids using an arbitrary motility scale of 1-10. This analysis was performed three times using three drops of sperm for each fish during the spawning season. A certain quantity of sperm was left at room temperature in order to determine spermatozoid motility after the addition of sea water.

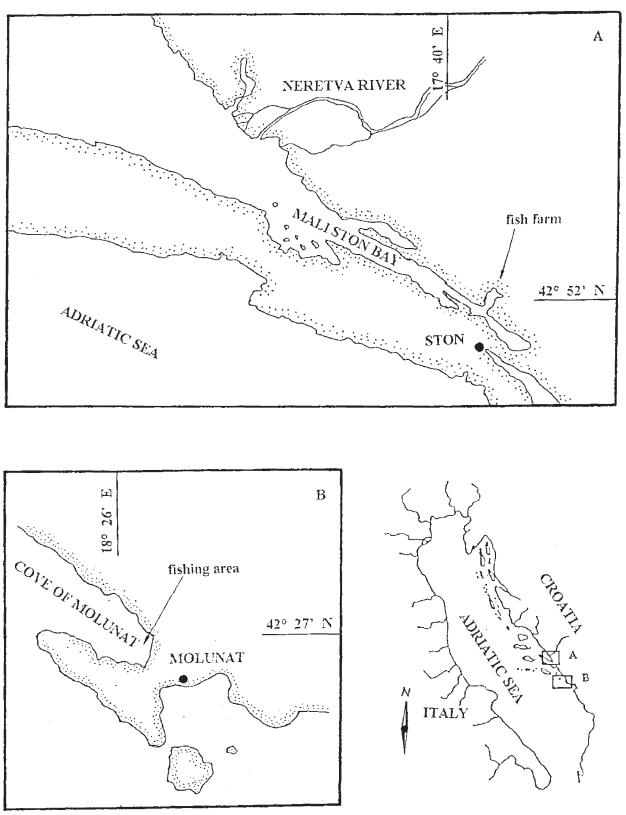

FIG. 1. – Study area: a) Rearing site in the Bay of Mali Ston, b) Donji Molunat Bay, traditional fishing grounds of the Mediterranean amberjack

Temperature, oxygen and salinity were measured every 15 days in the fishing area and rearing site using a multiprobe "Hydrolab Surveyor 3". In the Mali Ston Bay, the average annual sea temperature was 17.8±4.3°C. The average annual salinity was 35±3.0‰, and the average annual oxygen quantity was 5.69±1.28 mg/l for the same period.

The gonad specimens and biometric measurements of fish (n=206, ranging from 0.5 to 9 kg) captured by fishermen in the Donji Molunat Bay from 1997 to 1998 (Fig. 1b) were used to compare the maturation rates of captive and wild fish. Wild fish were caught during spawning seasons and their ages were determined from scale readings. In the Donji Molunat Bay, the average annual sea temperature was 18.2±3.7°C, the average annual salinity was 37±0.8‰, and the average annual oxygen quantity was 7.50±0.70 mg/l.

Spawning was arranged in a 70 m^3 square tank. The size and number of fish chosen for induced spawning was as follows. In 1998 - five wild specimens, four to ten years old, total body length from 98 - 141 cm, and body weight from 11.50 - 26.50 kg, when oocyte diameters were 550-750 μm (Fig. 2f). In 1999 - six captive specimens, four years old,

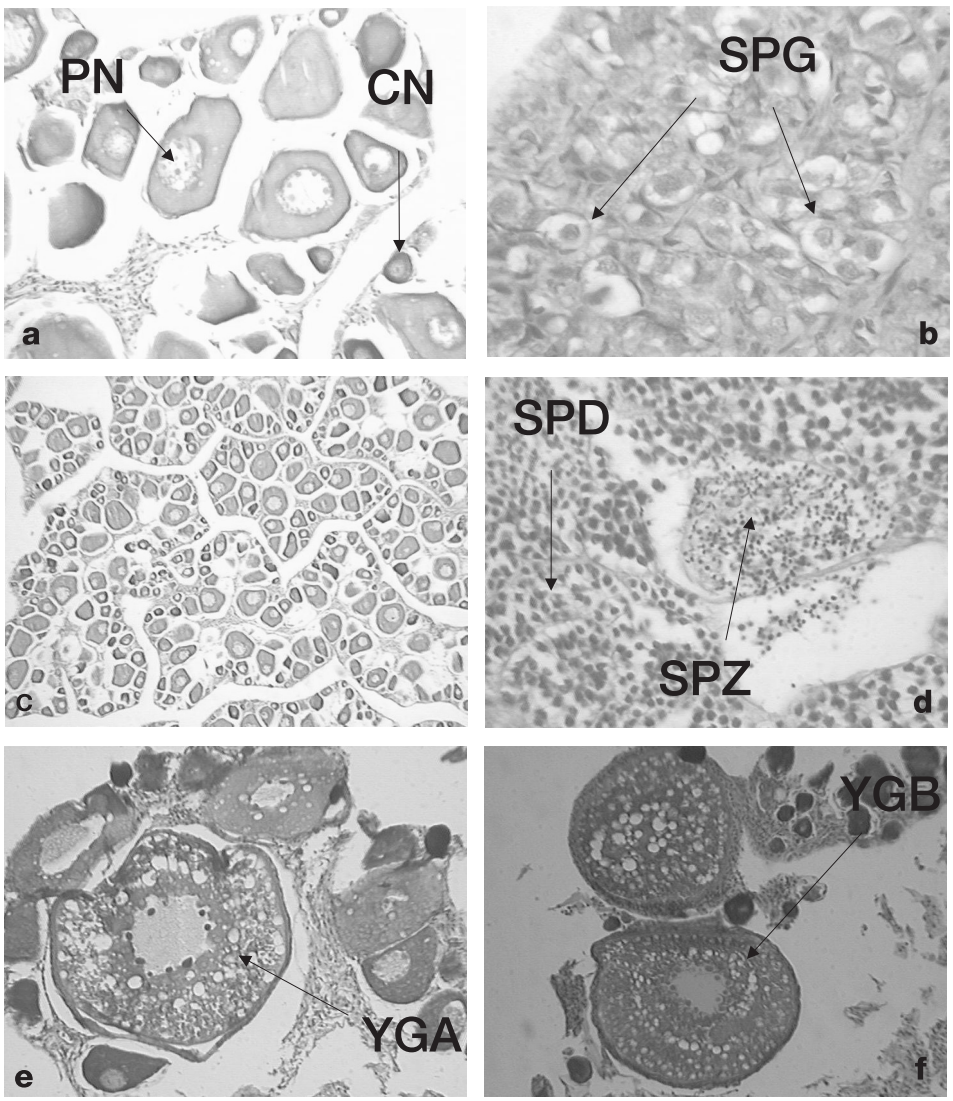

FIG. 2. – Ovaries and testis of captive and wild Mediterranean amberjack, *Seriola dumerili*. 2a) oocytes in a chromatin nucleolus stage (CN) and perinucleolus stage (PN) (x100); 2b) spermatogonias (SPG) of an immature male (x1000); 2c) gonad sample of the three year old captive female, oocyte sizes up to 89 μm (x200); 2d) spermatides (SPD) and spermatozoa (SPZ) in testis of male (x 400); 2e) oocytes in the yolk granule A (YGA) of wild amberjack captured during the spawning season (x 400); and 2f) vitellogenic oocytes in the yolk granule B stage (YGB), sized up to 750 μm in four-year old captive fish (x 200).

total body length from 103-107 cm, and body weight from 9.90-11.70 kg. Hormonal treatments were applied when oocytes were sized 550-600 μm (Fig. 2f). The male/female ratio was 1:1.5 in 1998 and 1:1 in 1999. The fish were anaesthetised with benzocaine. An injection of 1000 I.U./kg of human chorionic gonadotropin (HCG) was given intramusculary at the base of the dorsal fin. Both fish groups were given hormonal treatments on the 7th and 18th of June 1998 and on the 14th and 20th of June 1999. Eggs were obtained by natural spawning (1998) and by stripping (1999). They were incubated in 300 l tanks supplied with ambient seawater (19.2°C, 37.2‰), and with gentle aeration from the bottom.

After the first hormonal treatment in 1999, the eggs and sperm were obtained by stripping. Eggs were mixed with sperm for 3 minutes, following which sterilized sea water was added. After 15 minutes, the egg-sperm mixture was rinsed in seawater and put in a graduated beaker for the separation of fertilized and unfertilized eggs.

RESULTS

Oogonia of 8-14 μm were prevalent in the ovaries of newly collected 3 to 4-month old fish, as well as a lesser number of primary oocytes in a chromatin-nucleolus stage (13-24 μm) (Fig. 2a). We

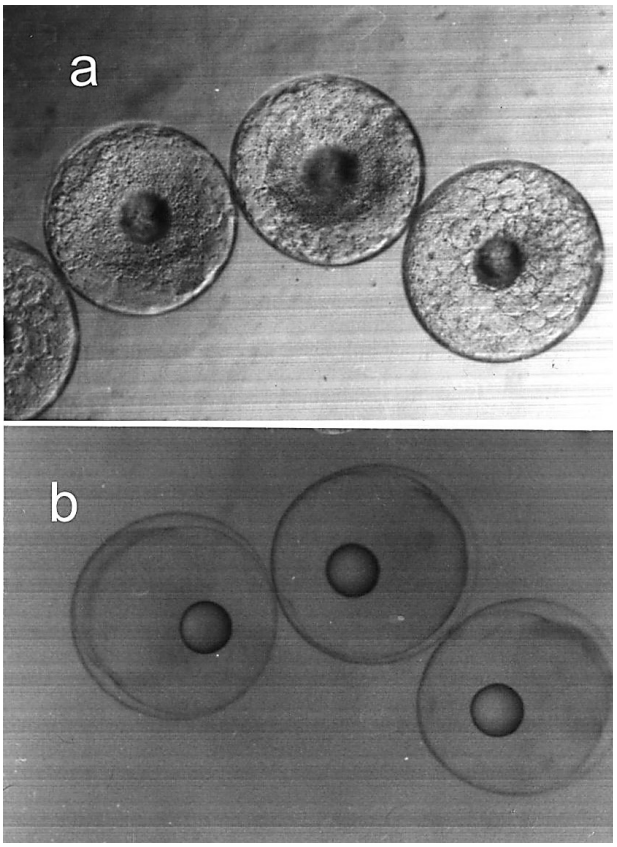

FIG. 3. – Pictures of amberjack eggs after fertilization (a) and in the stage of gastrulation (b).

found spermatogonia of 10-16 μm (Fig. 2b) in the peripheral region of the testis.

In the second year of life, no increase in oocyte size was observed for either wild or captive females. An increase in the quantity of spermatozoa in the testis of males was detected (Fig. 2d).

In the third year of cage-rearing, female oocyte sizes were up to 89 μm (Fig. 2c). Samples of equally-aged wild amberjack captured during the spawning season had oocytes in the yolk granule A stage, sized up to 260 μm (Fig. 2e). Three-year old wild males showed a completely developed testis. In four-year old broodstock fish, we noted vitellogenic oocytes in the yolk granule B stage, sized up to 750 μm (Fig. 2f).

HCG hormonal treatments of wild fish were applied on June 07, 1998. A natural spawning occurred after 66 hours following the first hormonal injection, whereby 750 grams of unfertilised eggs were collected. A sperm motility of 50-90% was noted in samples taken from males chosen for spawning. Another spawning followed 90 hours after a hormonal treatment. This time, 1250 g of eggs was obtained, of which 200 grams (16%) were fertilised (Fig. 3a). No spawning occurred with the same group of fish following a second hormonal treatment on June 18, 1998.

Mature eggs were also obtained following a treatment of four-year old captive fish on June 14th and on June 20th of 1999. In this case, fertile eggs were obtained from only one female. Approximately 46 hours after the hormonal treatment, we proceeded with the stripping of the female and obtained approximately 20 grams of eggs, of which up to 10 grams (50%) were fertilised.

In both the 1998 and 1999 experiments, a large number of unequal divisions were noticed with the fertilised eggs. A smaller quantity of eggs died during the first division**,** and all the eggs died by the time of the gastrulation phase (Fig. 3b).

Amberjack eggs are pelagic and have one oil globule. The surface of the yolk sac is hexagonally-grooved. This appearance is not obvious at first glance, but observing the egg under a magnification of 50x, slightly darkened, shows a kind of "mosaic" (Fig. 3a). There were no other visible characteristics, such as colour or pigmentation of a particular area, which could differentiate the amberjack eggs from the list of known and well-described pelagic eggs of various similar and far-related species.

The average egg diameter was 1.14±0.047 mm (1.03-1.26 mm) in 1998, while the average oil globule diameter was 0.27±0.037 mm (0.21-0.35 mm). In 1999, the average egg diameter was 1.12±0.051 mm (1.00-1.27 mm), while the average oil globule diameter was 0.27±0.039 mm (0.19-0.36 mm).

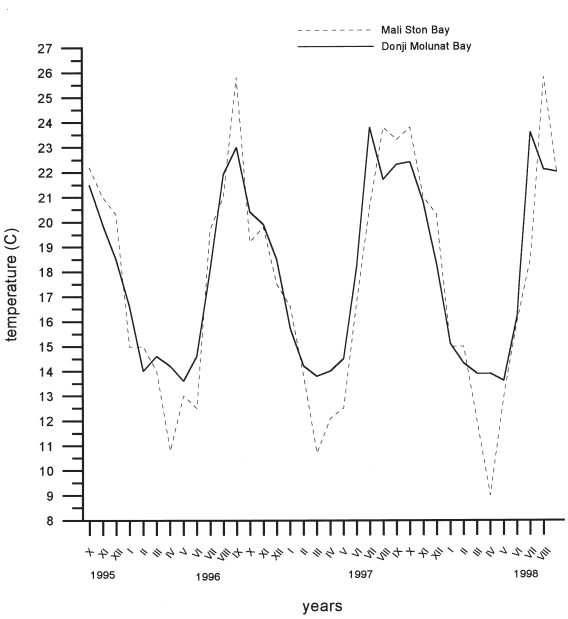

FIG. 4. – Seawater temperatures of Molunat and Mali Ston Bay waters during the experimental period.

DISCUSSION

There is a clear difference between male and female gonads of 3 to 4-month old fingerlings. However, as younger specimens were not caught, we can only speak of the first sexual differentiation for the above-mentioned specimens. Oogonia and spermatogonia sizes conform to those presented by Marino *et al*. (1995) and Grau *et al*. (1996).

The sexual maturation and growth of captive fish are similar to wild specimens during the first two years of growth. This identical gonad development and similar growth rate could be explained by the fact that amberjack fingerlings live in coastal waters during this period. Sexual maturation begins after the second year, when amberjack migrate to warmer open waters. The captive fish were exposed to lower winter temperatures as they were kept in a coastal environment throughout the year.

This was confirmed by the sea temperature measurements taken throughout the four-year period. The average sea temperature during the winter was 12.2±2.5°C at the cage site. In southern Adriatic waters around the Donji Molunat Bay, for the same period, it was 13.6±1.5°C. It seems that temperature is one of the factors that influence the sexual maturation of captive amberjack in southeastern Adriatic waters, where winter temperatures are as low as 10°C (Fig. 4). We suggest that low temperatures are the reason behind delayed gonad development in captive fish. Our research on the sexual maturation of amberjack did not disclose any histological differences except in the maturation time, which varies from the data given by other scientists. Cage-reared female amberjack reach their first sexual maturity one year earlier, and one year later than wild females as compared to other research work. However, cage-rearing conditions slow down the process of sexual maturation. Thus, three-year old fish show no signs of sexual maturity and the oocytes are only over 90 μm. In comparison, a smaller number of wild females are sexually mature and have oocytes in the developmental yolk granule A stage sized 200-400 μm. It is only in the fourth year of cage-rearing that females have vitellogenic oocytes in a yolk granule B stage sized 600-700 μm. Slower sexual maturation has been noted in other research as well (Marino *et al*., 1995). Data exists that confirms the blockage of sexual maturation under controlled rearing conditions (Garcia and Diaz, 1995; Micale *et al*., 1999). Alongside the fact that sexual maturation is slowed down under conditions of captivity, problems exist with the formation of a broodstock of sexually mature female specimens that undergo damage and stress due to netting and transport methods. The fish find it difficult to adapt to tank feeding as a result of the stress, which probably also affects the spawning results.

Problems in establishing a successful protocol for the artificial spawning of captive amberjack in Italian coastal waters were reported by Micale *et al*. (1998). They also noted vitellogenic oocytes in five-year old captive amberjack, which is one year later when compared to our investigations.

The hormone-treated fish spawned in the early morning hours during the controlled tank-spawning carried out in 1998. In a repeat experiment in 1999, fertilised eggs were retrieved after stripping the females and mixing the eggs with sperm. The spontaneous tank-spawning in 1998 can probably be attributed to the fact that the experiment used older specimens that had already spontaneously spawned during earlier seasons. The specimens used in 1999 were in their first season of sexual maturity and were cage-reared from the fingerling stage.

In spite of all the research that has been done up until now in the Mediterranean, a great deal still remains unknown with regards to the spawning of the amberjack. Further research and compilation will enable the successful rearing of this species, which is of high interest to mariculture.

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