GONADAL CYCLE OF ATLANTIC BLUEFIN TUNA (*Thunnus thynnus*) IN THE MEDITERRANEAN SEA

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ABSTRACT

The results obtained by the histological analysis of Atlantic bluefin tuna gonads collected in the Mediterranean Sea over a six-month period (March-August) in Italian and Spanish seas are reported. Maturity development of BFT gonads starts in early spring; exogenous vitellogenesis takes place in the ovaries from May throughout June; spawning occurs in late June-early July. The ovaries of five specimens caught during the spawning period in the North Ionian Sea and South Adriatic Sea displayed extensive vitellogenic atresia. In females which find themselves in unfavourable environmental condition during the spawning period, follicular atresia could represent a way to re-absorb highly energetic yolk reserve.

INTRODUCTION

The Atlantic bluefin tuna (*Thunnus thynnus* L.) is one the most important commercial species among the large pelagic fish living in the Atlantic Ocean and Mediterranean Sea (SUSCA *et al.*, 2001). The knowledge of the biology of reproduction is extremely important for allowing a management of the species aimed to its conservation, as well as to enhance the chance of success of the domestication attempts which have been unfruitful until now (DOUMENGE, 1996; LIOKA *et al.*, 2000). Since recent reports on the reproductive biology of the eastern Atlantic bluefin tuna (SUSCA *et al.*, 2001; MEDINA *et al.*, 2002; SARASQUETE *et al.*, 2002), did not provide exhaustive details on the reproductive cycle, the aim of this paper was to provide a histological description of the changes occurring in BFT gonads throughout the reproductive cycle.

MATERIALS AND METHODS

Ovary and testis samples (No = 101 and 81, respectively) were obtained from adult (fork length ≥ 120 cm) bluefin tuna. The samples were collected from March to September on board of professional vessels in Italian and Spanish seas. Fragments of the gonads were fixed in Bouin's solution or neutral 10% formaline, dehydrated in ethanol and embedded in paraffin wax. Sections (5 μm thick) were stained with haematoxylin-eosin. To identify vitellogenic oocytes, certain sections were immunostained with rabbit anti BFT vitellogenin serum (abBFT-VTG). The immunohistochemical reaction was visualised by means of the avidin-biotin peroxidase complex (ABC) procedure.

RESULTS AND DISCUSSION

Ovary

The ovary consists of a tick muscle wall and numerous follicles in different stages of development (asynchronous ovary) embedded in a mass of connective tissue. Each follicle consists of an oocyte rounded by a single layer of follicular cells.

The activity of the ovaries showed seasonal changes allowing the characterisation of five periods during the reproductive cycle:

Recrudescence period (March-early May) – The specimens caught during the recrudescence period showed oocytes at perinucleolus and lipid stage. Perinucleolus stage (diameter 10 -110 μm) was characterised by intense ooplasm basophily and numerous small nucleoli adjoining the nuclear envelope (Figure 1A). Oocytes at lipid stage (diameter 110-220 μm) exhibited a weak ooplasm basophily and were characterised by small lipid droplets (Figure 1A).

Ripening period (middle May) – All the specimens analysed showed the presence both of previtellogenic and vitellogenic oocytes. Vitellogenic oocytes (diameter 220-500 μm) were immunopositive with the anti Vtg serum (Figure 1B).

Pre-spawning period (late May-June) – In the ovaries of the specimens caught in this period, migratory nucleus stage oocytes (diameter ranging from 500 to 600 μm) could be observed, together with the previous stages (Figure 1C);

Spawning period (late June-early July) – All the females caught in this period showed pre-mature (diameter 600-700 μm) or mature (diameter 700-850 μm) oocytes (Figure 1D).

Spent period (late July-August) – In this period, only perinucleolus stage oocytes were found. Irregular cell masses containing pigmented inclusions and large lipid droplets, likely residue of the re-absorbing process could be observed (Figure 1E). The ovaries of five specimens caught during the spawning period in the North Ionian Sea and South Adriatic Sea displayed extensive vitellogenic atresia (Figure 1F).

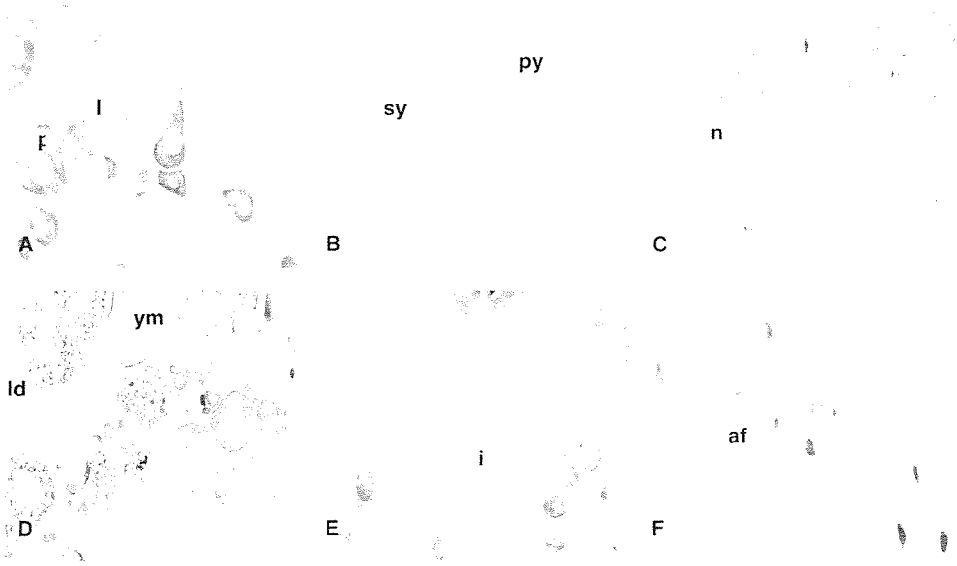

Figure 1. A – Photomicrograph of the ovary from a BFT specimen caught in April showing oocytes at perinucleolus and lipid stage. Magnification, x 100. B – Section of the ovary from a BFT specimen caught in May immunostained with abBFT-VTG. Vtg immunoreactive staining was detected in ooplasm of vitellogenic oocytes. Magnification, x 32. C – Section of the ovary from a BFT specimen caught in June showing a migratory nucleus stage oocyte. Magnification, x 88. D – Section of the ovary from a BFT specimen caught at the beginning of July showing mature oocytes. Magnification, x 36. E – Section of the ovary from a BFT specimen caught in August. Only perinucleolus stage oocyte can be observed. Irregular cell masses, likely residue of the re-absorbing process, are present in the connective tissue. Magnification, x 48. F – Photomicrograph of the ovary from a BFT specimen caught in July in the South Adriatic Sea showing extensive atresia. Magnification, x 48. Haematoxylin-Eosin staining. af, atretic follicle; i, irregular cell mass; l, lipid stage; ld, lipid droplet, n, nucleus; p, perinucleolus stage; py, primary yolk stage; sy, secondary yolk stage; ym, yolk mass.

Testis

Bluefin tuna testis (Figure 2A) is constituted by seminipherous tubules radiating from the longitudinal main sperm duct toward the testicular periphery. Testicular structure is cystic: each cyst contains germinal cells in the same development stage, branched by the cytoplasm of somatic cells (Sertoli cells).

The activity of the testes showed seasonal changes allowing the characterisation of five periods during the reproductive cycle:

Quiescence (March) - Seminipherous tubules showed germinal cysts containing spermatogonia and spermatocysts. Rare spermatidic cysts and few spermatozoa in the lumina were also observed (Figure 2B).

Early spermatogenesis (April-early May) – Testes showed germ cells at all stages of spermatogenesis and there was an increase in the number of spermatocytes and spermatids. Only few spermatozoa were observed in tubule lumina (Figure 2C).

Late spermatogenesis (middle May) – Active spermatogenesis took place in testes. The wall of seminipherous tublues was lined with meiotic and spermatidic cysts. Spermatozoa were more abundant in the lumen of seminipherous tubules, efferent ducts and main sperm duct than in previous stage (Figure 2D).

Spawning (late May-early July) – The lumen of seminipherous tubules, efferents and main sperm duct were filled with spermatozoa. Residual meiotic and spermatidic cysts were still present along the tubule wall (Figure 2E).

Regression (late July-August) – Lumina of seminipherous tubules and efferent ducts were almost devoid of spermatozoa, whereas residual spermatozoa could be observed in efferent ducts and in the main sperm duct (Figure 2F).

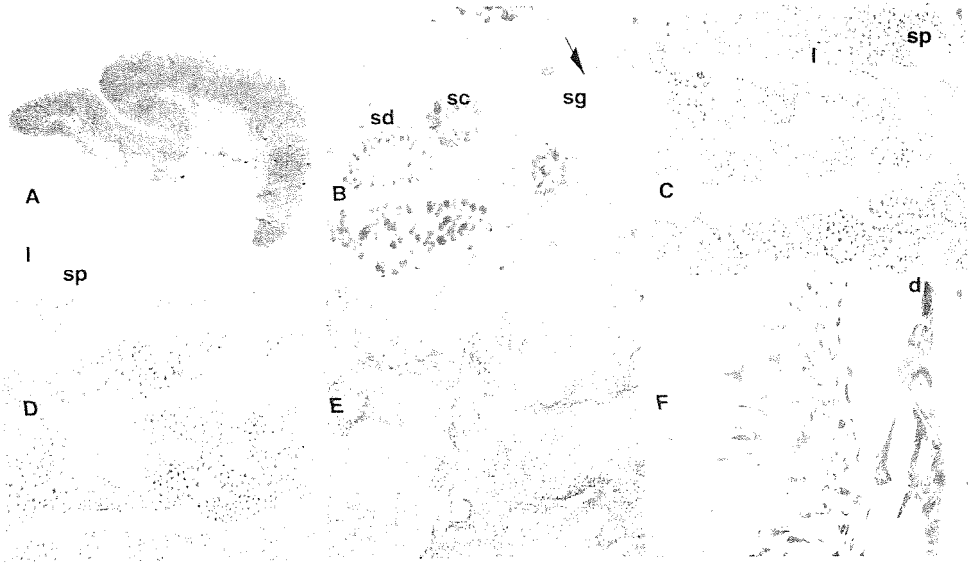

Figure 2. A – Photomicrographs of the testis from a BFT specimen caught in March. Magnification, x 9. B – Higher magnification of part of Figure 2A showing a seminipherous tubule. Magnification, x 500. C – Photomicrographs of the testis from a BFT specimen caught in April. Note the presence of rare spermatozoa in the lumen. Magnification, x 250. D – Photomicrographs of the testis from a BFT specimen caught in May. Note the abundance of sperm cysts and spermatozoa in the lumen of seminipherous tubule. Magnification, x 250. E – Photomicrographs of the testis from a BFT specimen caught in June. Seminipherous tubules are filled with spermatozoa. Magnification, x 123. F – Photomicrographs of the testis from a BFT specimen caught in August showing residual spermatozoa in the efferent ducts and in the main sperm duct. Magnification, x 30. Haematoxylin-Eosin staining. Arrow, Sertoli cell nucleus; d: main sperm duct; l, lumen of seminipherous tubule; sg, spermatogonium; sc, spermatocytic cyst; sd, spermatidic cyst; sp, spermic cysts.

CONCLUSIONS

Maturity development of BFT gonads starts in early spring when the testicular spermatogenic activity recommences and oocytes enter endogenous vitellogenesis. From May throughout June, exogenous vitellogenesis takes place in the ovaries. Vitellogenin uptake starts in oocytes having a minimum diameter of 220 μm, as revealed by immunohistochemical staining with abBFT-VTG. Testes are full mature from late May to the end of July when seminipherous tubules, efferent ducts and main sperm duct are filled with spermatozoa, while hydrated oocytes, sign of imminent spawning were found only in the Balearic Sea in late June-early July. At the end of July gametogenic activity is arrested: only residual spermatozoa can be observed in the testes and the ovaries shows only perinucleolar stage oocyte.

In the ovaries of the specimens caught during the spawning period in the North Ionian Sea and South Adriatic Sea no sign of recent spawning was observed and most of unyolked and yolked oocytes were atretic In teleosts, a high incidence of atretic oocytes has been interpreted as a sign of cessation of the spawning activity (HUNTER *et al.*, 1986; SCHAEFER, 1998) or failure in attainment of final oocyte maturation (MYLONAS *et al.*, 1997). The presence of spawning areas in South Adriatic and North Ionian Sea has never been reported in the literature. If the South Adriatic and the North Ionian seas are not spawning areas, it could be proposed that mature females, inhabiting non-spawning areas during the spawning season, reabsorb their yolk reserve and do not spawn. The finding of non-spawning specimens in non-spawning areas could account for an answer to the question: do all adult bluefin tuna migrate towards spawning areas when reproductive period approaches? LUTCAVAGE et al. (1999) and BLOCK *et al.* (2001) reported that some bluefin tuna large enough to be considered mature, tagged with electronic devices, showed no residence in any of the known spawning areas during the spawning period, thus raising questions on the existence of non-reported spawning areas (LUTCAVAGE *et al.*, 1999; BLOCK *et al.*, 2001).

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