Ovarian cycle of the Mediterranean bluefin tuna (*Thunnus thynnus* L.)

D. Zubani*, A. Corriero*, S. Desantis, F. Acone***, M. de la Serna****, J. Eicker*****, C.R. Bridges*****, P. Megalofonou****** and G. De Metrio***

*Department of Animal Health and Welfare, University of Bari, Str. Prov. Casamassima km 3, Valenzano, 70010 Bari, Italy

**Department of Zoology and Comparative Anatomy, University of Bari, Via E. Orabona 4, 70125 Bari, Italy

***Department of Animal Biology, University of Sassari, Via Vienna 2, 07100 Sassari, Italy

****Centro Oceanográfico de Málaga, Instituto Español de Oceanografía (IEO), Puerto Pesquero s/n, Apdo. 285, 29640 Fuengirola, Spain

*****Institut für Zoophysiologie, Lehrstuhl für Stoffwechselphysiol, Heinrich-Heine Universität, D-40225 Düseldorf, Germany

******Department of Zoology and Marine Biology, Faculty of Biology, University of Athens, Panepistimioupolis, GR-157 84 Athens, Greece

SUMMARY – The results obtained by the histological analysis of Mediterranean bluefin tuna ovaries collected over a six-month period (March-August) are reported. Five phases of ovarian cycle were characterised: recrudescence (March-early May); ripening (middle May); pre-spawning (late May-June); spawning (late June-early July); and spent (late July-August). 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.

Key words: Bluefin tuna, reproductive cycle, ovary, histology, Mediterranean Sea.

RESUME – *"Cycle ovarien du thon rouge méditerranéen (*Thunnus thynnus *L.)". Les résultats obtenus par analyse histologique d'ovaires de thon rouge méditerranéen prélevés sur une période de six mois (mars-août) sont présentés. Cinq phases du cycle ovarien ont été caractérisées : reprise (mars-début mai) ; maturité (mi-mai) ; pré-ponte (fin mai-juin) ; ponte (fin juin-début juillet) ; et dépassement (fin juillet-août). Les ovaires de cinq spécimens pris pendant la période de ponte dans le nord de la mer Ionienne et dans le sud de la mer Adriatique ont montré une atrésie vitellogénique extensive. Chez les femelles qui étaient en conditions environnementales défavorables pendant la période de ponte, l'atrésie folliculaire pourrait représenter une façon de réabsorber des réserves de vitellus fortement énergétiques.*

Mots-clés : *Thon rouge, cycle reproductif, ovaire, histologie, mer Méditerranée.*

Introduction

The knowledge of Mediterranean bluefin tuna (BFT) reproductive cycle is limited to research based on seasonal changes of the gonadosomatic index (de la Serna and Alot, 1992), and on macroscopic classification of the gonads (Rodríguez-Roda, 1964, 1967). Susca *et al*. (2001) carried out the first attempt to correlate vitellogenin (VTG) and sex steroid plasma levels with ovarian cycle. Here a histological description of the changes occurring in BFT ovary throughout the reproductive cycle is reported.

Materials and methods

Ovary samples were obtained from 101 adult (fork length ≥ 120 cm) bluefin tuna. The samples were collected 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

The ovary consists of a thick 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 the five periods during the reproductive cycle:

(i) *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 (Fig. 1). Oocytes at lipid stage (diameter 110-220 µm) exhibited a weak ooplasm basophily and were characterised by small lipid droplets (Fig. 1A).

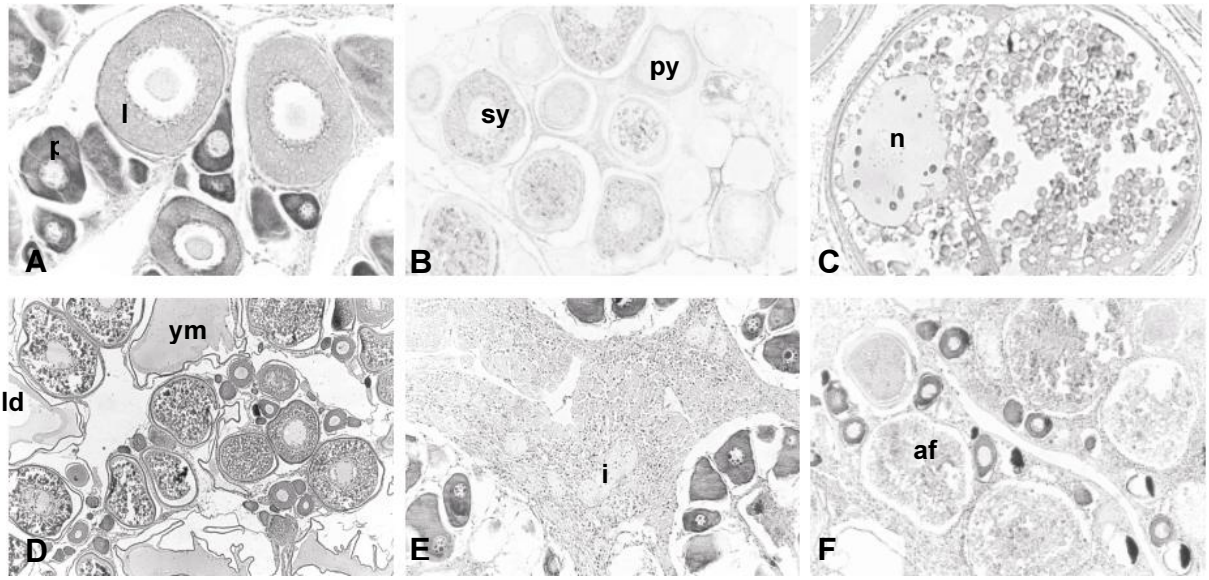

Fig. 1. A - Photomicrograph of the ovary from a BFT specimen caught in April showing oocytes at perinucleolus and lipid stage. Magnification, x 100. B – Section of the ovary from a BFT specimen caught in May immunostained with abBFT-VTG. VTG immunoreactive staining was detected in ooplasm of vitellogenic 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.

(ii) *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 (Fig. 1B).

(iii) *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 (Fig. 1C).

(iv) *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 (Fig. 1D).

(v) *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 (Fig. 1E). The ovaries of five specimens caught during the spawning period in the North Ionian Sea and South Adriatic Sea displayed extensive vitellogenic atresia (Fig. 1F).

Conclusions

Maturity development of BFT ovaries starts in early spring when the 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. Spawning occurs in late June-early July when pre-mature and mature oocytes were found in the ovaries of specimens caught in the Balearic Sea. 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. Therefore, it could be supposed that adult females, which find themselves in unfavourable environmental conditions during the spawning period, are unable to complete the maturation process and re-absorb their developing oocytes.

Acknowledgement

Financial support provided by EU grant CFP – BFTMED-97/0029.

References

de la Serna, J.M. and Alot, E. (1992). Análisis del sex-ratio por clase de talla y otros datos sobre la madurez sexual del atún rojo (*Thunnus thynnus*) en el área del Mediterráneo Occidental durante el periodo 1988-1991. *ICCAT Collective Volume of Scientific Papers,* XXXIX: 704-709.

Rodríguez-Roda, J. (1964). Biología del atún, *Thunnus thynnus* (L.), de la costa sudatlántica de España. *Investigaciones Pesqueras,* 25: 33-164.

Rodríguez-Roda, J. (1967). Fecundidad del atún, *Thunnus thynnus* (L.), de la costa sudatlántica de España. *Investigaciones Pesqueras,* 31(1): 33-52.

Susca, V., Corriero, A., Bridges, C.R. and De Metrio, G. (2001). Study of the sexual maturity of female bluefin tuna (*Thunnus thynnus*): Purification and partial characterization of vitellogenin and its use in an enzyme-linked immunosorbent assay. *J. Fish Biol.,* 58: 815-831.