Journal of Fish Biology (2003) **63,** 108–119
doi:10.1046/j.1095-8649.2003.00132.x, available online at http://www.blackwell-synergy.com

Histological investigation on the ovarian cycle of the bluefin tuna in the western and central Mediterranean

A. Corriero*, S. Desantis*, M. Deflorio*, F. Acone†, C. R. Bridges‡, J. M. de la Serna§, P. Megalofonou¶ and G. De Metrio*,**

**Department of Animal Health and Welfare – University of Bari, S.P. per Casamassima km. 3, I-70010 Valenzano (BA), Italy, †Department of Animal Biology – University of Sassari, via Vienna 2, I-07100 Sassari, Italy, ‡Institut für Zoophysiologie, Lehrstuhl für Stoffwechselphysiologie, Heinrich Heine Universität, D-40225 Düsseldorf, Germany, §Centro Oceanografico de Malaga, Apartado 285, 29640 Fuengirola, 4 IEO, Malaga, Spain and ¶Department of Zoology, Marine Biology – University of Athens, Panepistimiopolis GR 15784 Athens, Greece*

(Received 6 August 2002, Accepted 24 April 2003)

The histological analysis of eastern Atlantic bluefin tuna *Thunnus thynnus* ovaries caught from February to September 1999–2000, made it possible to distinguish the presence of seven oocyte developmental stages and allowed the characterization of six time-dependent ovary maturity stages. The ovaries of mature (fork length, $L_F \geq 110$ cm) bluefin tuna were non-active from August (spent period) to March (quiescent period) when they contained only perinucleolar-stage oocytes. Ovary development started in April to early May (recrudescent period) with the appearance of oocytes at the lipid stage. Vitellogenesis appeared in mid-May (ripening period) and post-vitellogenesis occurred in late May to mid-June (pre-spawning period). In late June to early July, hydrated oocytes, a sign of imminent spawning, were found only in specimens caught in Balearic waters. Females ranging between 100 and 110 cm L_F, captured during the recrudescent and ripening periods, had the largest oocytes at the lipid stage, most of which were degenerating. An extensive vitellogenic atresia was observed in the ovaries of five females caught during the spawning period in non-spawning areas.

Key words: histology; oocytes; ovarian cycle; reproduction; *Thunnus thynnus*.

INTRODUCTION

The Atlantic bluefin tuna *Thunnus thynnus* (L.) is one of the most important commercial species among the large pelagic fishes living in the Atlantic Ocean and Mediterranean Sea (Susca *et al*., 2001).

The International Commission for the Conservation of Atlantic Tunas (ICCAT) recognizes two management units of Atlantic bluefin tuna: west and east Atlantic, the latter unit including the Mediterranean. In the last few years,

**Author to whom all correspondence should be addressed. Tel.: +39 080 5443907; fax: +39 080 5443908; email: g.demetrio@veterinaria.uniba.it

an increase in fishing pressure has resulted in a dramatic biomass reduction for the bluefin stock, thus raising serious concern for the survival of this resource (Sissenwine *et al.*, 1998; SCRS, 2002*a*). The presence of mixing between the units, although controversial (Lutcavage *et al.*, 1999; Nemerson *et al.*, 2000; Block *et al.*, 2001; De Metrio *et al.*, 2002), has provided an opportunity to redesign a management plan for the Atlantic bluefin tuna (SCRS, 2002*b*).

Knowledge on reproduction is extremely important for the management of the species particularly for its conservation, as well as enhancing the success of domestication, which has been 'unfruitful' until now (Doumenge, 1996; Iioka *et al.*, 2000). Because recent reports on the reproductive biology of the eastern Atlantic bluefin tuna (Susca *et al.*, 2001; Medina *et al.*, 2002; Sarasquete *et al.*, 2002) have not provided exhaustive details on the reproductive cycle, the aim of the present study was to classify, in greater detail, the oocyte and ovary development stages in bluefin tuna collected during the sexual cycle in different areas of the Mediterranean Sea.

MATERIALS AND METHODS

Ovaries were obtained from 131 bluefin tuna (fork length, L_F, ranging from 63 to 236 cm) captured during February to September 1999–2000 in seven different Mediterranean areas (Fig. 1 and Table I) by: (1) commercial vessels that made use of various fishing gears (long lines, drift nets and purse seines) and operated in the north Ionian Sea (Gulf of Taranto) ($n = 41$), south Adriatic Sea ($n = 8$), Ligurian Sea ($n = 5$) as well as around the Balearic Islands ($n = 28$), (2) traditional traps in Sardinia Channel ($n = 43$) and Barbate

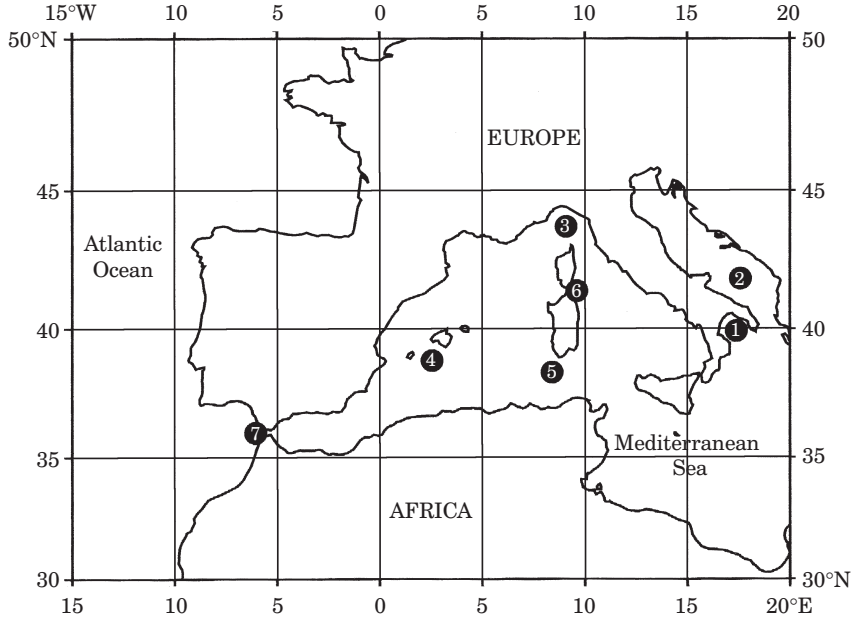

FIG. 1. Geographical location of sampling areas. 1, north Ionian Sea (Gulf of Taranto); 2, south Adriatic Sea; 3, Ligurian Sea; 4, Balearic Islands; 5, Sardinia Channel; 6, Bocche di Bonifacio; 7, Barbate.

TABLE I. Sampling period and location, individual size, most advanced oocyte stage and assigned state of sexual maturity for eastern Atlantic bluefin tuna

Month	Location	L_F (cm)	Most advanced oocyte stage	Assigned state of fish sexual maturity
February	1	90	Perinucleolar	n.d.
February	1	95	Perinucleolar	n.d.
February	1	125	Perinucleolar	n.d.
February	1	140	Perinucleolar	n.d.
February	1	142	Perinucleolar	n.d.
March	1	75	Perinucleolar	n.d.
March	1	78	Perinucleolar	n.d.
March	1	85	Perinucleolar	n.d.
March	1	94	Perinucleolar	n.d.
March	1	111	Perinucleolar	n.d.
March	1	145	Perinucleolar	n.d.
March	1	149	Perinucleolar	n.d.
March	1	157	Perinucleolar	n.d.
March	1	175	Perinucleolar	n.d.
March	1	175	Perinucleolar	n.d.
April	1	81	Perinucleolar	n.d.
April	1	85	Perinucleolar	n.d.
April	1	97	Perinucleolar	n.d.
April	1	98	Perinucleolar	n.d.
April	1	103	Lipid*	Immature
April	1	107	Lipid*	Immature
April	1	118	Lipid	n.d.
April	1	125	Lipid	n.d.
April	1	131	Lipid	n.d.
Early May	1	130	Lipid	n.d.
Early May	1	145	Lipid	n.d.
Early May	7	149	Lipid	n.d.
Early May	7	161	Lipid	n.d.
Early May	7	172	Lipid	n.d.
Mid-May	1	65	Perinucleolar	Immature
Mid-May	1	79	Perinucleolar	Immature
Mid-May	1	79	Perinucleolar	Immature
Mid-May	5	102	Lipid*	Immature
Mid-May	5	110	Late vitellogenesis	Mature
Mid-May	5	121	Late vitellogenesis	Mature
Mid-May	5	134	Late vitellogenesis	Mature
Mid-May	5	135	Late vitellogenesis	Mature
Mid-May	5	135	Late vitellogenesis	Mature
Mid-May	5	137	Late vitellogenesis	Mature
Mid-May	5	139	Late vitellogenesis	Mature
Mid-May	5	140	Late vitellogenesis	Mature
Mid-May	5	142	Late vitellogenesis	Mature
Mid-May	5	144	Late vitellogenesis	Mature
Mid-May	5	145	Late vitellogenesis	Mature
Mid-May	5	150	Late vitellogenesis	Mature
Mid-May	5	152	Late vitellogenesis	Mature

Mid-May	5	153	Late vitellogenesis	Mature
Mid-May	5	170	Late vitellogenesis	Mature
Mid-May	5	170	Late vitellogenesis	Mature
Mid-May	5	175	Late vitellogenesis	Mature
Mid-May	5	181	Late vitellogenesis	Mature
Mid-May	5	190	Late vitellogenesis	Mature
Mid-May	5	197	Late vitellogenesis	Mature
Mid-May	5	203	Late vitellogenesis	Mature
Mid-May	5	217	Late vitellogenesis	Mature
Mid-May	5	220	Late vitellogenesis	Mature
Mid-May	5	227	Late vitellogenesis	Mature
Mid-May	5	236	Late vitellogenesis	Mature
Late May	5	115	Late vitellogenesis	Mature
Late May	5	138	Late vitellogenesis	Mature
Late May	5	152	Migratory nucleus	Mature
Late May	5	171	Late vitellogenesis	Mature
Late May	5	222	Late vitellogenesis	Mature
Late May	5	230	Migratory nucleus	Mature
Early June	1	78	Perinucleolar	Immature
Early June	1	78	Perinucleolar	Immature
Early June	1	81	Perinucleolar	Immature
Early June	5	133	Late vitellogenesis	Mature
Early June	5	144	Late vitellogenesis	Mature
Early June	5	150	Migratory nucleus	Mature
Early June	5	153	Late vitellogenesis	Mature
Early June	5	170	Late vitellogenesis	Mature
Mid-June	5	175	Late vitellogenesis	Mature
Mid-June	5	175	Late vitellogenesis	Mature
Mid-June	5	187	Late vitellogenesis	Mature
Mid-June	5	195	Late vitellogenesis	Mature
Mid-June	5	210	Migratory nucleus	Mature
Mid-June	5	235	Late vitellogenesis	Mature
Late June	4	142	Late vitellogenesis	Mature
Late June	4	144	Hydrated	Mature
Late June	4	151	Late vitellogenesis	Mature
Late June	4	159	Late vitellogenesis	Mature
Late June	4	171	Late vitellogenesis	Mature
Early July	2	63	Perinucleolar	Immature
Early July	2	68	Perinucleolar	Immature
Early July	2	105	Perinucleolar	Immature
Early July	2	132	Late vitellogenesis**	Mature
Early July	2	135	Late vitellogenesis**	Mature
Early July	1	82	Perinucleolar	Immature
Early July	1	87	Perinucleolar	Immature
Early July	1	133	Late vitellogenesis**	Mature
Early July	1	134	Late vitellogenesis**	Mature
Early July	1	190	Late vitellogenesis**	Mature
Early July	4	127	Late vitellogenesis	Mature
Early July	4	133	Hydrated	Mature
Early July	4	140	Late vitellogenesis	Mature
Early July	4	142	Late vitellogenesis	Mature
Early July	4	142	Late vitellogenesis	Mature

Early July	4	147	Late vitellogenesis	Mature
Early July	4	147	Late vitellogenesis	Mature
Early July	4	149	Hydrated	Mature
Early July	4	149	Hydrated	Mature
Early July	4	149	Late vitellogenesis	Mature
Early July	4	150	Late vitellogenesis	Mature
Early July	4	151	Late vitellogenesis	Mature
Early July	4	151	Late vitellogenesis	Mature
Early July	4	151	Late vitellogenesis	Mature
Early July	4	152	Late vitellogenesis	Mature
Early July	4	152	Hydrated	Mature
Early July	4	155	Late vitellogenesis	Mature
Early July	4	157	Hydrated	Mature
Early July	4	158	Hydrated	Mature
Early July	4	159	Late vitellogenesis	Mature
Early July	4	159	Late vitellogenesis	Mature
Early July	4	159	Hydrated	Mature
Early July	4	179	Late vitellogenesis	Mature
August	3	95	Perinucleolar	Immature
August	3	105	Perinucleolar	Immature
August	3	107	Perinucleolar	Immature
August	3	123	Perinucleolar[#]	Mature
August	3	125	Perinucleolar[#]	Mature
August	2	75	Perinucleolar	Immature
August	2	81	Perinucleolar	Immature
August	2	138	Perinucleolar[#]	Mature
August	1	81	Perinucleolar	Immature
August	1	85	Perinucleolar	Immature
August	1	127	Perinucleolar[#]	Mature
August	1	130	Perinucleolar[#]	Mature
September	6	95	Perinucleolar	Immature
September	6	130	Perinucleolar[#]	Immature
September	6	145	Perinucleolar[#]	Mature

1, north Ionian Sea; 2, south Adriatic Sea; 3, Ligurian Sea; 4, Balearic waters; 5, Sardinia Channel; 6, Bocche di Bonifacio; 7, Barbate; [*], degenerating oocytes surrounded by eosinophilic granulocytes; [**], extensive atresia; [#], brownish granules and yellow-pigmented globules; n.d., not determined.

de Franco Islands (Cádiz, southern Spain) ($n = 3$) and (3) troll lines in the Bocche di Bonifacio (Corsica) ($n = 3$).

Gonad slices (1 cm thick), taken immediately after the fish had been caught, were fixed in Bouin's solution or neutral 10% formalin. The samples were dehydrated in increasing ethanol concentrations, clarified in Histolemon and embedded in paraffin wax. Sections (5 μm thick) were stained with haematoxylin–eosin, Mallory's trichrome and Periodic acid–Shiff (Pas) reaction.

Oocyte diameters were measured on histological slides using a Quantimet 500W (Leica, Cambridge, U.K.) image analyser.

The oocyte classification was made adapting the scheme used by Matsuyama *et al.* (1988), Schaefer (1998) and Arocha (2002), and the ovaries were considered mature when vitellogenic or post-vitellogenic oocytes (Lowerre-Barbieri *et al.*, 1996; Arocha, 2002; Medina *et al.*, 2002), as well as signs of previous spawning, were present.

RESULTS

OVARY STRUCTURE AND FOLLICLE DEVELOPMENT

The ovary parenchyma consisted of ovigerous lamellae containing numerous follicles at different stages of development [Fig. 2(a)], embedded in a mass of connective tissue. Each developing oocyte was surrounded by a single layer of follicular cells. On the basis of oocyte morphology, staining affinity and diameter, it was possible to define seven oocyte developmental stages.

Perinucleolar stage

The perinucleolar-stage oocytes (diameter 25–110 μm) were polyedric cells with an intense ooplasm basophily, numerous small nucleoli adjoining the nuclear envelope and a high nucleus : cytoplasm ratio [Fig. 2(a), (b), (d)].

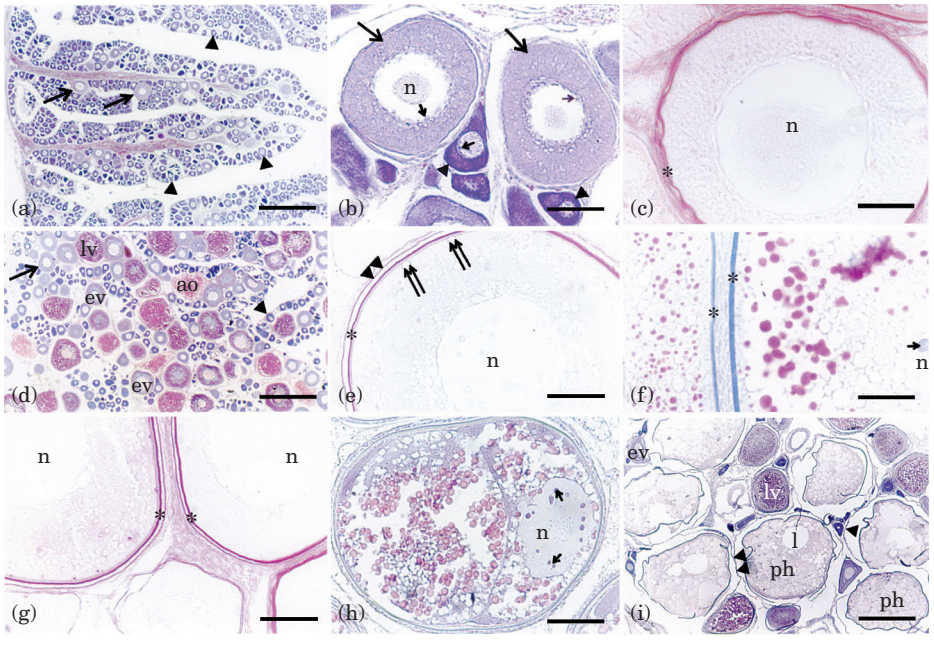

FIG. 2. Micrographs of the ovaries from different bluefin tuna specimens. (a) Ovary showing ovigerous lamellae (bar = 750 μm). (b) Oocytes at perinucleolar and lipid stage (bar = 80 μm). (c) Oocyte at lipid stage showing a thin Pas^+ *zona radiata* (bar = 40 μm). (d) Oocytes at perinucleolar, lipid, early vitellogenesis and late vitellogenesis stages and atretic oocytes (bar = 800 μm). (e) Oocyte at early vitellogenesis stage showing Pas^+ cortical *alveolus* (bar = 55 μm). (f) Increase of both *zona radiata* thickness and acidophilic yolk globules in oocytes at early and late vitellogenesis stages (bar = 40 μm). (g) Pas positivity of oocytes at lipid, early vitellogenesis and late vitellogenesis stages (bar = 85 μm). (h) Oocyte at migratory nucleus stage (bar = 120 μm). (i) Ovary showing pre-hydrated oocytes characterized by the coalescence of lipids and yolk globules and detachment of follicular cell layer (bar = 600 μm). (a), (b), (d), (h) and (i), haematoxylin–eosin staining; (c), (e) and (g), Pas reaction; (f), Mallory's trichrome staining. ▶, perinucleolar-stage oocyte; ⟶, lipid-stage oocyte; *, *zona radiata*; ⟩, follicular cell layer; ⇉, cortical *alveolus*; ➔, nucleolus; ao, atretic oocyte; ev, early vitellogenesis oocyte; l, lipid droplet; lv, late vitellogenesis oocyte; n, nucleus; ph, pre-hydrated oocyte.

Lipid stage

The lipid-stage oocytes (diameter 110–220 μm) exhibited a weak ooplasm basophily and were characterized by small lipid droplets [Figs 2(a), (b), (d) and 3(c)] as well as by the appearance of a thin Pas^+ *zona radiata* [Fig. 2(c), (g)].

Early vitellogenesis stage

The oocytes at the beginning of vitellogenesis (diameter 220–300 μm) were characterized by small spherical acidophilic granules (yolk globules) [Fig. 2(d), (f), (i)], Pas^+ cortical *alveoli* and a 3 μm thick *zona radiata* surrounded by cubic granulosa cells [Fig. 2(e), (g)].

Late vitellogenesis stage

The oocytes at an advanced stage of vitellogenesis (diameter 300–500 μm) displayed a remarkable increase in both size and number of yolk globules [Fig. 2(d), (f), (i)], as well as an emergence of Pas^+ granules in the peripheral ooplasm, and an increase in the thickness of the *zona radiata* (12 μm) [Fig. 2(g)].

Migratory nucleus stage

The oocytes at migratory nucleus stage (diameter 500–650 μm) showed progressive migration of the nucleus towards the animal pole and the beginning of lipid droplet and yolk globule coalescence [Fig. 2(h)].

Pre-hydrated stage

The pre-hydrated oocytes (diameter 650–750 μm) showed a coalescence of lipids and yolk globules, the nuclear envelope breakdown and the detachment of the follicular cell layer [Fig. 2(i)].

Hydrated stage

The hydrated oocytes (diameter 750–900 μm) were irregular in shape and contained a single yolk mass and a large oil droplet, derived by the complete coalescence of yolk granules and lipids [Fig. 3(a)].

Most of the ovaries in either vitellogenic or post-vitellogenic development contained a few atretic oocytes characterized by ooplasm and yolk degradation and *zona radiata* disappearance [Figs 2(d) and 3(b)].

The classification of all the ovaries, based on the most advanced oocyte stage observed, is given in Table I.

OVARIAN CYCLE

The ovaries of all fish ($n = 26$) <100 cm L_F had only perinucleolar-stage oocytes.

The three animals with $100 < L_F < 110$ cm caught in April to May exhibited oocytes at the lipid stage, most of which were degenerating and surrounded by eosinophilic granulocytes [Fig. 3(c)]. Only perinucleolar-stage oocytes were found in three fish of this size class caught in July to August.

The activity of the ovaries of fish ≥ 110 cm L_F ($n = 99$) showed monthly changes, thus allowing for definition of six periods in the reproductive cycle.

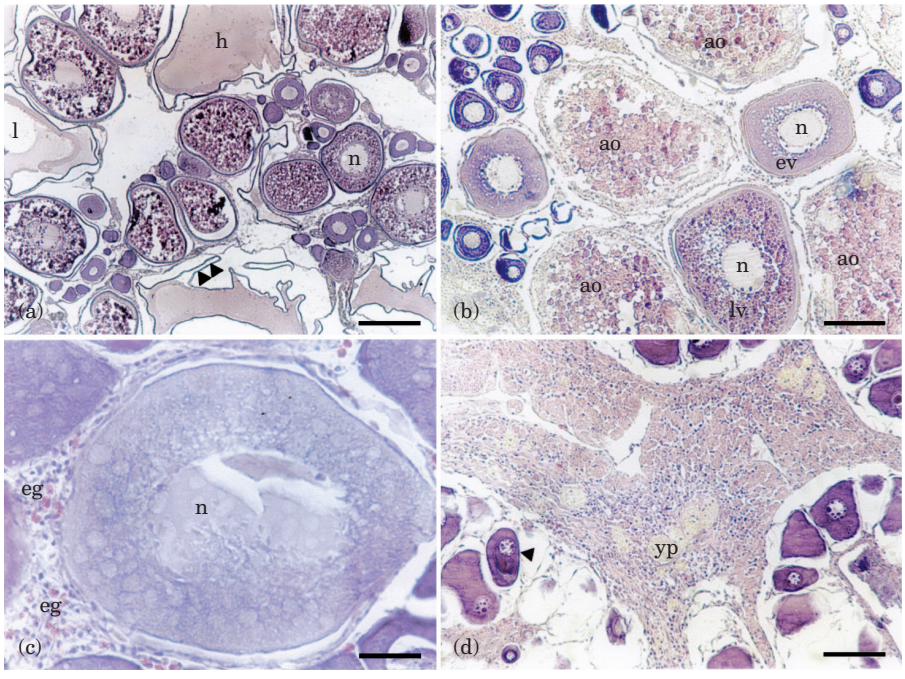

FIG. 3. Micrographs of the ovaries from different bluefin tuna specimens. (a) Ovary showing hydrated oocytes with a single yolk mass and a large oil droplet (bar = 400 μm). (b) Ovary showing a high density of atretic follicles (bar = 140 μm). (c) Degenerating lipid-stage oocyte, surrounded by eosinophilic granulocytes. (d) Post-ovulatory ovary with perinucleolar-stage oocytes and yellow-pigmented globules (bar = 140 μm). (a), (b), (c) and (d), Haematoxylin–eosin staining (bar = 35 μm). ▶, perinucleolar-stage oocyte; ▶, follicular cell layer; ao, atretic oocyte; eg, eosinophilic granulocytes; ev; early vitellogenesis oocyte; l, lipid droplet; lv, late vitellogenesis oocyte; n, nucleus; h, hydrated oocyte; yp, yellow-pigmented globules.

In the February to March period the ovaries of all of the nine specimens analysed, caught in the north Ionian Sea, displayed only oocytes at perinucleolar stage. In April to early May the ovaries of the specimens captured in the north Ionian Sea ($n = 5$) and in Barbate ($n = 3$), displayed oocytes at both perinucleolar and lipid stage. In mid-May the 25 specimens, caught in the Sardinia Channel, had ovaries containing oocytes at perinucleolar, lipid, primary and secondary yolk stages. In the late May to mid-June period the ovaries of all of the fish ($n = 17$) caught in the Sardinia Channel had yolked oocytes. The ovaries of four of them also contained oocytes at the migratory nucleus stage. In late June to early July the ovaries of the fish caught in Balearic waters ($n = 28$) showed yolked oocytes; eight of them also contained pre-hydrated and hydrated oocytes, while the samples of south Adriatic Sea ($n = 2$) and north Ionian Sea ($n = 3$) displayed an extensive vitellogenic atresia [Fig. 3(b)]. In August to September the ovaries of all the seven animals captured in the north Ionian, south Adriatic and Ligurian seas, and in the Bocche di Bonifacio contained only perinucleolar-stage oocytes. Amorphous brownish granules and yellow-pigmented globules were observed in the ovarian interstitial tissue [Fig. 3(d)].

DISCUSSION

This study represents the first attempt at a detailed histological identification of oocyte developmental stages, characterization of ovarian cycle and data on minimum body length at sexual maturity of the bluefin tuna.

In the ovaries from eastern Atlantic bluefin tuna analysed in this study, seven oocyte developmental stages were observed: perinucleolar, lipid, early vitellogenic, late vitellogenic, migratory nucleus, pre-hydrated and hydrated. In recent histological studies on this species (Medina *et al.*, 2002; Sarasquete *et al.*, 2002), four stages of oocyte development were described with no fully hydrated oocytes. Since hydration occurs immediately before spawning (Farley & Davis, 1998), the presence of hydrated oocytes is used to detect ready-to-spawn animals.

The simultaneous presence of all of the oocyte developmental stages in the spawning specimens indicates that bluefin tuna has an asynchronous oocyte development (Baglin, 1982; Medina *et al.*, 2002) and, like other tuna species (McPherson, 1991; Schaefer, 1996, 1998; Farley & Davis, 1998), it is a partial or multiple spawner.

In specimens $\geq$110 cm L_F, six time-dependent ovary maturity stages were distinguished on the basis of the oocyte stages present as well as on the general histological aspect of the ovaries. The ovaries were inactive from August (spent period) to March (end of quiescence). Recrudescence started in April to early May when lipid-stage oocytes were observed. Ripening occurred in mid-May when ovaries also contained vitellogenic oocytes. The pre-spawning period, characterized by the presence of migratory-nucleus stage oocytes, took place in late May until mid-June. Spawning occurred in late June to early July as indicated by the presence of hydrated oocytes. The present results are consistent with previous studies. Rodríguez-Roda (1967) reported a spawning period between May and July; Susca *et al.* (2001) stated that spawning occurs in the second half of June and Medina *et al.* (2002) found pre-hydrated oocytes at the end of June and in the first days of July.

No post-ovulatory follicle was found in the examined samples, probably because bluefin tuna swim into deep water soon after spawning (Block *et al.*, 2001) and these follicles can be detected for only a short time (<24 h) following spawning in a number of tuna species (Hunter *et al.*, 1986; McPherson, 1991; Schaefer, 1996).

Specimens ready to spawn were observed only in the waters around the Balearic Islands. Nishida *et al.* (1997) found a high occurrence (100–13 000 per 100 m^2) of bluefin tuna larvae, in addition to the Balearic waters, in the south Tyrrhenian Sea (north of Sicily) and Central Mediterranean (south of Malta). Medina *et al.* (2002) found signs of spawning activity in bluefin tuna from the Balearic Islands. The fish caught off Barbate were thought to be migrating through the Strait of Gibraltar towards Mediterranean spawning grounds (Medina *et al.*, 2002). The other sampling areas of the present study (north Ionian Sea, south Adriatic Sea, Ligurian Sea, Sardinia Channel and the Bocche di Bonifacio) are probably feeding and growing areas. De Metrio *et al.* (2002), in an electronic tagging experiment, reported a feeding area in the waters around Corsica for both pre- and post-spawning bluefin tuna.

Sparse atretic oocytes were observed in most of the maturing ovaries analysed. Five large fish caught in the first days of July in the south Adriatic and

north Ionian seas showed ovaries with no sign of previous spawning and a high level of vitellogenic atresia. In teleosts, a high incidence of atretic oocytes has been interpreted as a sign of cessation of spawning activity (Hunter *et al*., 1986; Schaefer, 1998) or failure in attainment of final oocyte maturation (Mylonas *et al*., 1997).

As far as is known, the presence of spawning areas in the south Adriatic and north Ionian Seas has not been reported. If the south Adriatic and north Ionian Seas are not spawning areas, it could be possible that mature females inhabiting non-spawning areas during the spawning season reabsorb their yolk reserve and do not spawn. Do all adult bluefin tuna migrate towards spawning areas when the reproductive period approaches? The finding of non-spawning specimens in non-spawning areas could answer this. 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. These findings have raised questions on the existence of non-reported spawning areas (Lutcavage *et al*., 1999; Block *et al*., 2001).

Considering that the presence of vitellogenic oocytes is a sign of sexual maturity (Lowerre-Barbieri *et al*., 1996; Arocha, 2002; Medina *et al*., 2002), all the fish $\geq$110 cm L_F, caught from mid-May to early July, could be considered mature (Table I). It is noteworthy that all the fish >110 cm L_F caught during August and September showed signs of previous spawning (Table I).

According to the criteria used by Schaefer (1998), mature inactive females can be distinguished from immature females because their ovaries contain yolked atretic oocytes. In the present study, no atretic oocyte was observed in any fish captured in February and March. Therefore, it was not possible to distinguish between inactive (quiescent) mature and immature animals.

The results of histological investigations seem to be consistent with the observations of Rodríguez-Roda (1967) who, using simple macroscopic analysis of the ovaries, reported that eastern Atlantic bluefin tuna reached sexual maturity between 110 and 120 cm. In addition, the smallest spawning female found in the Balearic waters by Medina *et al*. (2002) was 116 cm.

Specimens ranging from 100 to 110 cm L_F caught during both the recrudescent and the ripening periods had the largest oocytes in the lipid stage, most of which were degenerating and surrounded by eosinophilic granulocytes. The involvement of eosinophilic granulocytes in the phagocytic activity towards the female germ cells has been already described in other teleosts (Besseau & Faliex, 1989; Bruslè-Sicard & Fourcault, 1997; Kokokiris *et al*., 1999). Extensive atresia of lipid-stage oocytes has been observed in young female teleosts before the attainment of first sexual maturity (Hassin *et al*., 1997).

We wish to thank all of the fishermen who helped us in sample collection and A. Marinelli, V. Pesola, M. Cacucci and A. Di Sabato for their technical assistance. We are also grateful to two anonymous referees who helped to improve the manuscript considerably. This work was financially supported by contract number 97/029 from the European Commission.

References

Arocha, F. (2002). Oocyte development and maturity classification of swordfish from the north-western Atlantic. *Journal of Fish Biology* **60,** 13–27. doi: 10.1006/jfbi.2001.1805.

Baglin, R. E. & J. R. (1982). Reproductive biology of western Atlantic bluefin tuna. *Fishery Bulletin* **80,** 121–134.

Besseau, L. & Faliex, E. (1989). Presence of granulocytes and brown bodies in the ovotestis of *Lithognathus mormyrus* (L.) (Teleost, Sparidae). *Ichtyophysiologica Acta* **13,** 109–114.

Block, B. A., Dewar, H., Blackwell, S. A., Williams, D., Prince, E. D., Farwell, C. J., Boustany, A., Teo, S. L. O., Seitz, A., Walli, A. & Douglas, F. (2001). Migratory movements, depth preferences, and thermal biology of Atlantic bluefin tuna. *Science* **293,** 1310–1314.

Bruslè-Sicard, S. & Fourcault, B. (1997). Recognition of sex inverting protandric *Sparus aurata*: ultrastructural aspects. *Journal of Fish Biology* **50,** 1094–1103.

De Metrio, G., Arnold, G. P., Block, B. A., de la Serna, J. M., Deflorio, M., Cataldo, M., Yannopoulos, C., Megalofonou, P., Beemer, S., Farwell, C. & Seitz, A. (2002). Behaviour of post-spawning Atlantic bluefin tuna tagged with pop-up satellite tags in the Mediterranean and Eastern Atlantic. *ICCAT Collective Volume of Scientific Papers* **54,** 415–424.

Doumenge, F. (1996). Aquaculture of bluefin tuna. *Biologia Marina Mediterranea* **3,** 258–288.

Farley, J. H. & Davis, T. L. O. (1998). Reproductive dynamics of southern bluefin tuna, *Thunnus maccoyii*. *Fishery Bulletin* **96,** 223–236.

Hassin, S., de Mombrison, D., Hanin, Y., Elizur, A., Zohar, Y. & Popper, D. M. (1997). Domestication of white grouper, *Epinephelus aeneus*. 1. Growth and reproduction. *Aquaculture* **156,** 305–316.

Hunter, J. R., Macewicz, B. & Sibert, J. R. (1986). The spawning frequency of skipjack tuna, *Katsuwonus pelamis*, from the South Pacific. *Fishery Bulletin* **84,** 895–903.

Iioka, C., Kani, K. & Nhhala, H. (2000). Present status and prospects of technical development of tuna sea-farming. *Cahiers Options Mediterraneennes* **47,** 275–285.

Kokokiris, L., Bruslè, S., Kentouri, M. & Fostier, A. (1999). Sexual maturity and hermaphroditism of the red porgy *Pagrus pagrus* (Teleostei: Sparidae). *Marine Biology* **134,** 621–629.

Lowerre-Barbieri, S. K., Chittenden, M. E. Jr. & Barbieri, L. R. (1996). The multiple spawning pattern of weakfish in the Chesapeake Bay and Middle Atlantic Bight. *Journal of Fish Biology* **48,** 1139–1163.

Lutcavage, M. E., Brill, R. W., Skomal, G. B., Chase, B. C. & Howey, P. W. (1999). Results of pop-up satellite tagging of spawning size class fish in the Gulf of Maine: do North Atlantic bluefin tuna spawn in mid-Atlantic? *Canadian Journal of Fisheries and Aquatic Sciences* **56,** 173–177.

Matsuyama, M., Adachi, S., Nagahama, Y. & Matsuura, S. (1988). Diurnal rhythm of oocyte development and plasma steroid hormone levels in the female red sea bream, *Pagrus major*, during the spawning season. *Aquaculture* **73,** 357–372.

McPherson, G. R. (1991). Reproductive biology of yellowfin tuna in the eastern Atlantic fishing zone, with special reference to the north-western Coral Sea. *Australian Journal of Marine and Freshwater Research* **42,** 465–477.

Medina, A., Abascal, F. J., Megina, C. & García, A. (2002). Stereological assessment of the reproductive status of female Atlantic northern bluefin tuna during migration to Mediterranean spawning grounds through the Strait of Gibraltar. *Journal of Fish Biology* **60,** 203–217. doi: 10.10006/jfbi.2001.1835.

Mylonas, C. C., Woods, L. C. III & Zohar, Y. (1997). Cyto-histological examination of post-vitellogenesis and final oocyte maturation in captive-reared striped bass. *Journal of Fish Biology* **50,** 34–49.

Nemerson, D., Berkeley, S. & Safina, C. (2000). Spawning site fidelity in Atlantic bluefin tuna, *Thunnus thynnus*: the use of size-frequency analysis to test for the presence of migrant east Atlantic bluefin tuna on Gulf of Mexico spawning grounds. *Fishery Bulletin* **98,** 118–126.

Nishida, T., Tsuji, S. & Segawa, K. (1997). Spatial data analyses of Atlantic bluefin tuna larval surveys in the 1994 ICCAT BYP. *ICCAT Collective Volume of Scientific Papers* **48,** 107–110.

Rodríguez-Roda, J. (1967). Fecundidad del Atún, *Thunnus thynnus* (L), de la costa sudatlántica de Espana. *Investigaciones Pesqueras* **31,** 33–52.
Sarasquete, C., Cárdenas, S., Gonzáles de Canales, M. L. & Pascual, E. (2002). Oogenesis in the bluefin tuna, *Thunnus thynnus* L.: A histological and histochemical study. *Histology and Histopathology* **17,** 775–788.
Schaefer, K. M. (1996). Spawning time, frequency, and batch fecundity of yellowfin tuna, *Thunnus albacares*, near Clipperton Atoll in the eastern Pacific Ocean. *Fishery Bulletin* **94,** 98–112.
Schaefer, K. M. (1998). Reproductive biology of yellowfin tuna (*Thunnus albacares*) in the eastern Pacific Ocean. *Bulletin Inter-American Tropical Tuna Commission* **21,** 489–528.
SCRS (2002*b*). ICCAT Workshop on bluefin mixing. *Collective Volume of Scientific Papers* **54,** 261–352. http://www.iccat.es/documents/BFT_mix.pdf
Sissenwine, M. P., Mace, P. M., Powers, J. E. & Scott, G. P. (1998). A commentary on Western Atlantic bluefin tuna assessment. *Transactions of the American Fisheries Society* **127,** 838–855.
Susca, V., Corriero, A., Bridges, C. R. & De Metrio, G. (2001). Study of the sexual maturity of female bluefin tuna: purification and partial characterization of vitellogenin and its use in an enzyme-linked immunosorbent assay. *Journal of Fish Biology* **58,** 815–831. doi: 10.1006/jfbi.2000.1490.

Electronic Reference

SCRS (2002*a*). Report of the Standing Committee on Research and Statistics. In *ICCAT Report, 2002–2003. Executive Summary BFT*, pp. 53–67. http://www.iccat.es/documents/BFT.pdf/