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Reproductive status of bluefin tuna during migration to Mediterranean spawning grounds through the Straits of Gibraltar

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SUMMARY – The sexual maturation stage of adult bluefin tuna caught at the Strait of Gibraltar (Barbate, southern Spain) during migration to Mediterranean spawning grounds was assessed by histological and stereological studies of the gonads. The gonad weight and gonadosomatic indices (GSI) of these migrant tunas were several times lower than those found in tuna from Mediterranean spawning grounds (Balearic Islands). The testes of migrant male tuna contained gametes at all stages of spermatogenesis; there were no marked histological differences between them and male spawners from Baleares, though the gonad size and GSI were significantly higher in the latter. A small proportion of female bluefin tuna caught off Barbate (~8%) was immature, whereas most of them (~92%) were non-spawning mature. The ovaries of spawning tuna from the Balearic Islands contained 5-fold more vitellogenic oocytes than did tuna from Barbate. The average fecundity per spawning estimated from stereological quantification of migratory-nucleus oocytes in the specimens collected from Balearic waters was some 13 million eggs (93 oocytes per g of body weight), and the inter-spawning interval in this area was calculated to be 1.2 days. In specimens from Barbate a fecundity per spawning of around 13 million eggs (relative batch fecundity: 96 per g) was estimated using counts of late vitellogenic oocytes. These estimates are close to those calculated for other tuna species.

Key words: Reproduction, sexual maturation, bluefin tuna, *Thunnus thynnus*, gonads, stereology.

RESUME – *"Situation reproductive du thon rouge pendant la migration aux zones de reproduction de la Méditerranée à travers le détroit de Gibraltar". Le stade de maturation sexuelle de thons rouges adultes capturés dans le détroit de Gibraltar (Barbate, sud de l'Espagne) pendant la migration aux zones de reproduction de la Méditerranée a été évalué par des études histologiques et stéréologiques des gonades. Le poids des gonades ainsi que les indices gonadosomatiques (GSI) de ces thons migrants étaient plusieurs fois inférieurs à ceux trouvés chez des thons des zones de reproduction de la Méditerranée (Iles Baléares). Les testicules des thons migrants contenaient des gamètes à tous les stades de la spermatogenèse ; il n'y avait pas de différences histologiques marquées entre ceux-ci et les reproducteurs mâles des Iles Baléares, bien que la taille des gonades et le GSI aient été significativement supérieurs chez ces derniers. Une petite proportion de thons rouges femelles pris au large de Barbate (environ 8%) étaient immatures, tandis que la plupart d'entre eux (environ 92%) étaient matures mais sans ponte. Les ovaires des thons en reproduction des Iles Baléares contenaient 5 fois plus d'ovocytes vitellogéniques que les thons de Barbate. La fécondité moyenne par ponte estimée à partir de la quantification stéréologique d'ovocytes à noyau migratoire chez les spéciments collectés dans les eaux Baléares était de 13 millions d'oeufs (93 ovocytes par g de poids corporel) et l'intervalle calculé entre pontes dans cette zone était de 1,2 jours. Chez les spécimens de Barbate, on a estimé une fécondité par ponte d'environ 13 millions d'oeufs (fécondité fractionnée relative : 96 par g) en utilisant les comptages d'ovocytes vitellogéniques tardifs. Ces estimations sont proches de celles que l'on calcule pour d'autres espèces de thonidés.*

Mots-clés : *Reproduction, maturation sexuelle, thon rouge,* Thunnus thynnus*, gonades, stéréologie.*

Introduction

Thunnus thynnus (the northern bluefin tuna) and the closely related *Thunnus maccoyii* (the southern bluefin tuna) are unique among the tuna species in that they live mainly in cold waters and move into warmer waters to spawn (Lee, 1998). The eastern stock of the Atlantic northern bluefin tuna (BFT) spawns in the Mediterranean Sea, the two main spawning grounds being located around the Balearic Islands and south of the Tyrrhenian Sea, between Sicily and Sardinia (Dicenta, 1977). Therefore, every spring BFT from the eastern stock migrate from different locations in the Atlantic to the Mediterranean to spawn in waters where conditions are optimal for the offspring survival. Migrant BFT have then to pass through the Strait of Gibraltar, where fishermen can catch them by trap.

A number of reasons exist to recommend the development of technologies specific to the culture of *Thunnus thynnus*. One such reason is the high fishing pressure to which this species is being subjected in recent years, which has provoked a worrying reduction in the biomass of natural populations (Sissenwine *et al.*, 1998; Forés *et al.*, 1999). To support the sustainability of the resource an appropriate management of tuna fisheries should be accompanied by the development of effective aquaculture technologies for stock enhancement. The high commercial value and impressive growth rate of the bluefin and other large tunas point to these species as ideal candidates for a profitable and ubiquitous aquaculture industry.

Prior to de development of the Atlantic BFT aquaculture technology it is essential to understand the reproductive biology of the species, and more particularly to know the reproductive potential of the stock that can be used as broodfish. In this paper we aim to contribute to the understanding of the reproductive biology of BFT from the coasts of southern and eastern Spain with a view to improving the knowledge on the biology of the species thus establishing the basis for further development of the aquaculture of *Thunnus thynnus*.

Materials and methods

Animals

Adult pre-spawning BFT were caught by trap off Barbate de Franco (Cádiz, southern Spain) from late April to early June, 1999, 2000 and 2001. Spawners were fished by purse seine around the Balearic Islands in June. At the moment of sampling, the total body weight, gonad weight and gonad volume of each specimen were recorded, and the gonadosomatic index calculated according to the equation: GSI (%) = (gonad weight / total body weight) × 100.

Histology (light and electron microscopy)

Samples of gonad tissue were fixed for 48-96 h in 4% formaldehyde in phosphate buffer, 0.1 M, pH 7.2. After dehydration in ascending concentrations of ethyl alcohol and clearing in xylene, they were embedded in paraffin wax. 6-μm sections were stained with haematoxylin-eosin for examination on the light microscope (Medina *et al.*, 2002).

Small tissue samples (~1 mm^3) were fixed for 3-4 h in 2.5% glutaraldehyde buffered with 0.1 M sodium cacodylate buffer (pH 7.2). Following two 30-min washes in cacodylate buffer for 1 h, they were postfixed for 1 h at 4ºC in cacodylate-buffered osmium tetroxide, rinsed several times in the buffer, dehydrated in acetones, and embedded in Spurr's resin (Abascal *et al.*, 2002). Thin sections (~80 nm thick) were picked up on copper grids, doubly stained with uranyl acetate and lead citrate and examined in a Jeol JEM 1200 EX electron microscope.

Stereology

For estimation of the number of the different oocyte types contained in the gonads, the stereological method of Weibel and Gómez (1962) was applied to paraffin sections of the ovaries. N_V (number of oocytes per unit volume), was calculated for each oocyte developmental stage according to the formula (Weibel and Gómez, 1962; Weibel *et al.*, 1966):

$$N_V = \frac{K}{\beta} \frac{N_A^{3/2}}{V_V^{1/2}},$$

where β and K are coefficients related to shape and size distribution, respectively (Weibel, 1969; Williams, 1977). In our samples, the estimated value of β ranged from 1.4 to 1.52, whereas K varied between 1.01 and 1.19 (Medina *et al.*, 2002). N_A is the number of transections of oocytes per unit section area, and was calculated as the number of oocyte profiles lying within the stereological test system divided by the test area. V_V (the volume fraction occupied by oocytes of a given category) was calculated by the superimposition on to the micrographs of a test system (Weibel and Gómez, 1962;

Weibel *et al*., 1966; Weibel, 1969; Williams, 1977) consisting of a 14 × 22 cm square lattice in which the unit area was 1 cm^2, representing an actual area of 2500 μm^2 in the histological samples.

Results and discussion

Males

The histological study did not reveal significant qualitative differences between males from the Strait of Gibraltar (Barbate) and spawning grounds in the Mediterranean (Balearic Islands). An intense spermatogenetic activity was observed in both cases, with large masses of spermatozoa accumulating in the seminiferous tubules. Nevertheless, as seen in Table 1, there was an evident quantitative difference in the gonad development, with an average GSI in male tuna from the Balearic Islands that was more than 4-fold higher than the GSI in Barbate specimens. Hence, the spawners produced a volume of sperm several times larger than the BFT captured during migration to the spawning grounds. Consequently, the specimens sampled in Barbate were not usually fluent and milt could not be obtained by conventional methods. When suspended in seawater, sperm removed from the testes had a low motility rate (around 30%) in comparison with that observed in spermatozoa obtained by cannulation from tuna reared in cages in Mazarrón (Murcia), which showed over 70% motility rate (pers. observ.). These observations reveal a marked spatio-temporal difference in the degree of male gonad maturation between the Strait of Gibraltar and the Mediterranean spawning grounds, and suggest a relatively quick development of the testis in the reproductive season.

Table 1. A comparison of the gonadosomatic indices (GSI) between male BFT from Barbate and the Balearic Islands. The values are expressed as mean ± SD; N is the number of individuals examined

Sampling locations	Years	GSI	N	Total GSI	N
Barbate	1999	1.2	17	1.1	45
	2000	1.3	10		
	2001	1.0	18		
Balearic Islands	1999	5.6	5	4.7	35
	2000	3.6	10		
	2001	5.0	20		

The mature sperm (Fig. 1) of *Thunnus thynnus* have the typical ultrastructure of the perciform type II spermatozoon, which is characterised by the asymmetrical insertion of the flagellum and the centriolar complex located outside of the nuclear fossa (Mattei, 1970). These and other morphological features, such as: (i) the shallow nuclear fossa forming a groove over the proximal segment of the axoneme; (ii) centrioles located perpendicularly to each other; (iii) deep and narrow cytoplasmic canal; and (iv) flagellum lacking lateral fins, determine a consistent homogeneity in the sperm ultrastructure within the family Scombridae (Mattei, 1991; Hara and Okiyama, 1998; Abascal *et al.*, 2002).

Females

Histology

Most female BFT caught off Barbate (92%) had maturing ovaries in which the most advanced group of oocytes (MAGOs) were vitellogenic (stage 3) oocytes (averaging 380 micrometers in diameter) that contained yolk globules and lipid droplets. Only 8% were immature: all the oocytes were previtellogenic oocytes at the perinucleolar stage (stage 1) or at lipid stage (stage 2). The mean GSI in specimens from the Strait of Gibraltar was 1.23. In contrast, all BFT from the Balearic Islands had spawning ovaries in which the MAGOs were migratory-nucleus oocytes (stage 4) measuring over 500 micrometers in diameter, the GSI being as high as 4.19. In 83% of the histological samples examined postovulatory follicles were found, which indicates an inter-spawning interval of 1.2 days according to the method of Hunter and Macewicz (1985).

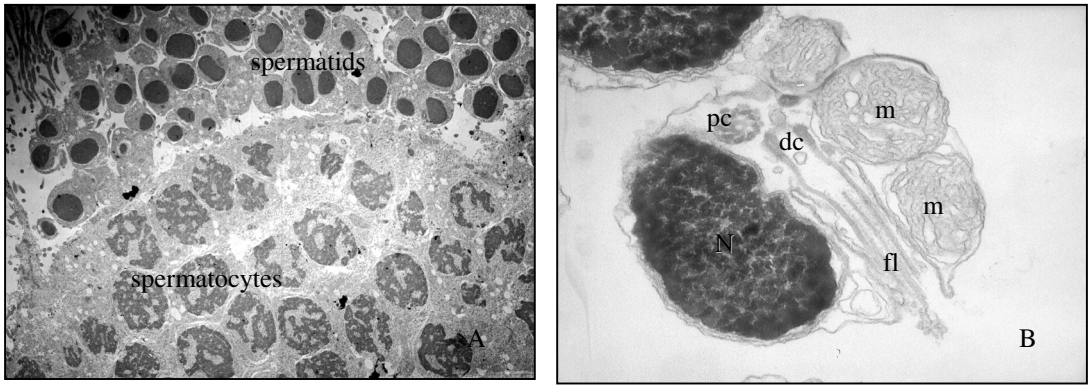

Fig. 1. Transmission electron micrographs of tuna testes from Barbate. (A) partial view of a tubule showing spermatocytes and spermatids; (B) sagittal section of spermatozoon; dc: distal centriole, fl: flagellum, m: mitochondria, N: nucleus, pc: proximal centriole.

BFT fished off Barbate in May 2000 were reared in seacages for 6 weeks and sampled in spawning time. After this short confinement, vitellogenic oocyte resorption had occurred concomitant with a significant decline of the GSI to 0.99. The experience was repeated in 2001, but in this case GnRHa implants of the sustained-release delivery system type (see review by Zohar and Mylonas, 2001) were injected to many of these fish. The histological structure of the gonad and the GSI (2.27) were now close to or even higher than that observed in wild tuna from Barbate. These results appear to confirm the applicability of GnRHa implant treatments in the BFT aquaculture, though they should be taken with caution as the number of experimental specimens used was low and the maturation pattern of the species could vary between years.

The cytoplasm of previtellogenic oocytes at the perinucleolar stage is poor in organelles, microvilli are short and scarce, and the vitelline envelope is thin. No sign of endocytosis is evident (Fig. 2A). At the lipid stage there are no significant ultrastructural changes except that lipid droplets become apparent in the cytoplasm. Vitellogenic oocytes (Fig. 2B) show a dense brush border embedded in the multilayered vitelline envelope. The well-developed microvilli as well as the presence of abundant coated pits and vesicles in the cortical cytoplasm of vitellogenic oocytes suggest a selective uptake of extracellular material, which, as shown by Susca *et al.* (2001), most probably consists of vitellogenin. The inner ooplasm of vitellogenic oocytes displays lipid droplets and yolk granules whose size increases gradually towards the centre of the oocyte. In migratory-nucleus (stage 4) oocytes the lipid droplets coalesce to form the single globule, the nucleus move to the oocyte animal pole, and hydration begins. The absence of fully hydrated oocytes in our samples of spawning ovaries must be due to the time of the catch and to the fast hydration process, which is known to occur very shortly before spawning (Farley and Davis, 1998).

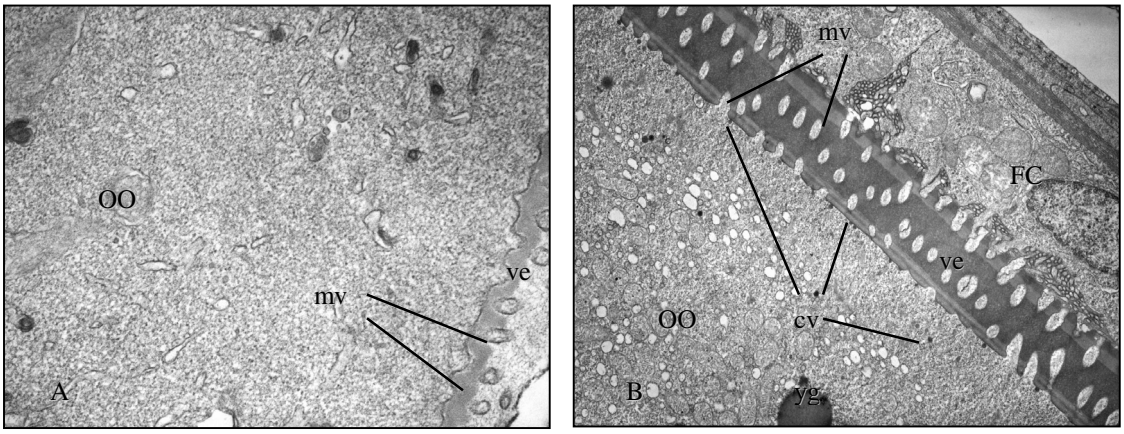

Fig. 2. Transmission electron micrographs of BFT oocytes. (A) partial view of a previtellogenic oocyte (stage 1); (B) cortical region of vitellogenic oocyte (stage 3); cv: coated pits and vesicles, FC: follicle cells, mv: microvilli, OO: oocyte, ve: vitelline envelope, yg: yolk granule.

Stereology

The application of the stereological method to histological sections allowed us to estimate for each oocyte category the volume density, numerical density, total no. of oocytes per individual, and no. of oocytes per gram of body weight. These values did not differ significantly in BFT from Barbate either throughout the migratory season within a year or between years 1999 and 2000, but significant differences did become evident when these values were compared with those from BFT caught off the Balearic Islands (Table 2). For instance, though the estimated number of previtellogenic oocytes (stages 1 and 2) was similar in both cases, BFT from the Balearic fishing area produced five-fold more vitellogenic oocytes than did tuna sampled off Barbate. Therefore, the considerable growth undergone by BFT ovaries in an apparently short period of time (between the Strait of Gibraltar and Balearic samplings) is largely accounted for by an active production of yolky (vitellogenic and migratory-nucleus) oocytes from the previtellogenic oocyte stock, which appears to remain constant throughout the reproductive cycle (Medina *et al.*, 2002). As commented in the histological description, migratory-nucleus oocytes were present only in the Balearic specimens.

From the stereological data presented in Table 2 the average fecundity per spawning estimated from counts of migratory-nucleus oocytes is around 13 million eggs (equivalent to some 90 eggs per gram of total body weight).

Table 2. Comparison of stereological data (V_v and N_v not shown) from tuna caught off Barbate and around the Balearic Islands during the reproductive season in 1999 and 2000. The values are expressed as mean ± SD; *n* is the number of individuals examined in each case (modified from Medina *et al.*, 2002)

	Barbate	Balearic Islands
Stage 1 oocytes		
No. per individual ($\times 10^6$)	1080 (n = 59)	970 (n = 24)
No. per g of BW (g^{-1})	6410 (n = 37)	6470 (n = 24)
Stage 2 oocytes		
No. per individual ($\times 10^6$)	101 (n = 59)	127 (n = 24)
No. per g of BW (g^{-1})	578 (n = 37)	821 (n = 24)
Stage 3 oocytes		
No. per individual ($\times 10^6$)	15 (n = 59)	66 (n = 24)
No. per g of BW (g^{-1})	96 (n = 37)	442 (n = 24)
Stage 4 oocytes		
No. per individual ($\times 10^6$)	0 (n = 59)	13 (n = 24)
No. per g of BW (g^{-1})	0 (n = 37)	93 (n = 24)

Conclusions

BFT caught in the Strait of Gibraltar during their migration towards spawning grounds in the Mediterranean are at a relatively early stage of sexual maturation. Fully mature gametes cannot be obtained from these fish, whereby artificial fertilisation is probably unviable or at least difficult.

The BFT is a multiple, highly fecund spawner in which the average fecundity per spawning around the Balearic Islands was estimated in ~13 million eggs (~90 eggs/g total weight). The inter-spawning interval has been estimated in about 1.2 days.

BFT reared for several weeks in seacages off Barbate undergo resorption of vitellogenic oocytes. The use of GnRHa implants could be of help to stimulate sexual maturation, especially in adverse environmental conditions.

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