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Stereological assessment of the reproductive status of female Atlantic northern bluefin tuna during migration to Mediterranean spawning grounds through the Strait of Gibraltar

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The ovarian mass and gonadosomatic index (I_G) of bluefin tuna *Thunnus thynnus*, caught in the Strait of Gibraltar (Barbate) during migration to Mediterranean spawning grounds, were several times lower than those found in bluefin tuna from Mediterranean spawning grounds (Balearic Islands). Some of the bluefin tuna from Barbate (8·3%) were classified as immature (the most advanced oocytes present in the ovaries were early vitellogenic), and the majority (the remaining 91·6%) as non-spawning mature; the ovary contained late vitellogenic oocytes, but there was no sign of spawning activity. Stereological estimation indicated that the ovaries of spawning bluefin tuna from the Balearic Islands contained five-fold more highly yolked oocytes than bluefin tuna from Barbate. When breeding bluefin tuna cross the Strait of Gibraltar the gonad is at an incipient stage of maturation. The average batch fecundity estimated from stereological quantification of stage 4 (migratory-nucleus) oocytes in the specimens collected from Balearic was 92·8 oocytes g^{-1} of body mass, and the spawning frequency in this area was calculated to be 1·2 days. In specimens from Barbate a relative batch fecundity of 96·3 oocytes g^{-1} was estimated using stage 3 (late vitellogenic) oocyte counts.

Key words: *Thunnus thynnus*; ovarian maturation; stereology; oocytes; vitellogenesis; atresia; postovulatory follicles.

INTRODUCTION

There are two sub-species of northern bluefin tuna *Thunnus thynnus* (L.), the Atlantic northern bluefin tuna *T. t. thynnus*, which occurs in the Atlantic Ocean and Mediterranean, and the Pacific northern bluefin tuna *T. t. orientalis*, which is found in the north-west Pacific Ocean (Ward, 1995). Two distinct stocks of Atlantic northern bluefin tuna are recognized primarily on the basis of the location of their spawning sites (Sissenwine *et al.*, 1998; Nemerson *et al.*, 2000). The eastern stock distribution extends from Norway southwards to the Canary Islands, into the Mediterranean and further south to the coast off South Africa. This bluefin tuna stock spawns in the Mediterranean, with two main spawning grounds located around the Balearic Islands and south of the Tyrrhenian Sea, between Sicily and Sardinia (Dicenta, 1977). The tuna fishermen of southern

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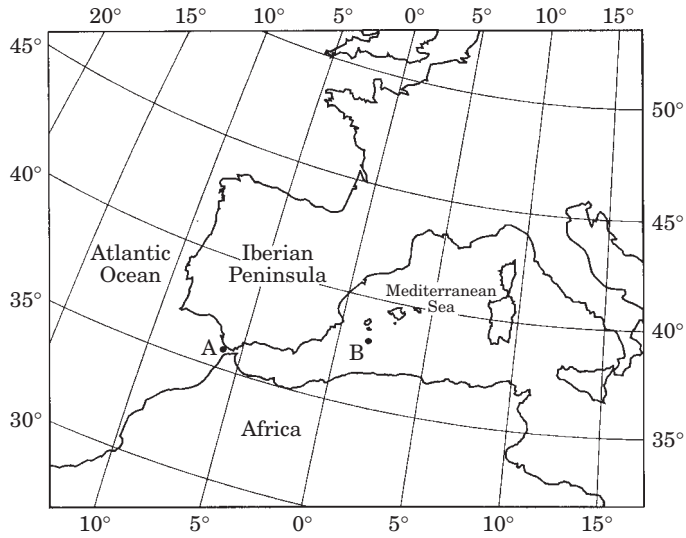

FIG. 1. Geographical location of the sampling stations. A, Barbate; B, Balearic Islands.

Spain take advantage of the reproductive migratory behaviour of bluefin tuna by using a fishing technique (' almadraba ') which is based on traps into which the bluefin tuna are driven as they move through the Strait of Gibraltar. Much of the current knowledge on the biology of the species during its passage through the Strait of Gibraltar is due to the work of Rodríguez-Roda (1957, 1964, 1967), who studied specimens caught in traps set along the southern Spanish coast from the late 1950s to 1960s.

Recently high fishing pressure has caused a reduction in the biomass of bluefin tuna populations (Sissenwine *et al.*, 1998; Forés *et al.*, 2000; SCRS, 2001). The effective management of these fish requires a thorough knowledge of their biology, particularly, their reproduction. Reproductive biology has been studied both in eastern Atlantic bluefin tuna (Rodríguez-Roda, 1964, 1967) and western Atlantic bluefin tuna (Baglin, 1982). Considerable progress has now been made in understanding the reproductive physiology of bluefin tuna from fish sampled in the central Mediterranean (Bridges *et al.*, 2000; Susca *et al.*, 2000, 2001*a*,*b*). In this paper the reproductive status of bluefin tuna off southern Spain during their migration to spawning grounds in the Mediterranean was investigated.

MATERIALS AND METHODS

SAMPLE COLLECTION

Over two consecutive reproductive seasons (1999 and 2000), 60 adult female bluefin tuna weighing between 85 and 269 kg (mean 157·37 kg) were caught by trap off Barbate de Franco (Cádiz, southern Spain) (Fig. 1) as they entered the Mediterranean to spawn. The sample of each season consisted of five (1999) and four (2000) sub-samples that extended from late April to early June, thus covering most of the period of migration towards Mediterranean spawning grounds. A total of 24 spawning bluefin tuna weighing between 30 and 217 kg (mean 143·83 kg) was fished by purse seine around the Balearic Islands (Fig. 1) on 26 June 1999, 26 June 2000 and 2 July 2000. The bluefin tuna were weighed (W) to the nearest kg and the ovaries dissected out, stripped of the associated fat

body, and weighed (W_G) to the nearest g. The total ovarian volume was calculated to the nearest 10 ml by the volume of water displaced from a container following immersion of the ovaries. The gonadosomatic index (I_G, %) was calculated from $I_G = 100\ W_G\ W^{-1}$. Measurements are expressed as the mean ± S.D. throughout.

HISTOLOGY

The fragments of gonad used for histology and stereology consisted of cross-sections (0·5 cm thick) removed from the broadest part of one of the ovaries of each animal (situated at about one third of its length from the rostral end). The tissue samples, which comprised the whole ovarian wall, were fixed for 48–96 h in 4% formaldehyde (10% formalin) in phosphate buffer, 0·1 M, pH 7·2. After dehydration in ascending concentrations of ethanol and clearing in xylene, the fragments were embedded in paraffin wax. Serial 6 µm sections were cut with a microtome Leica Jung RM2025, stained with haematoxylin-eosin or haematoxylin-VOF (Gutiérrez, 1967), and viewed and photographed with a light microscope Leitz DMR BE equipped with a Wild MPS 48/52 photomicrographic system. A minimum of eight different fields were photographed using the × 10 objective. A calibration scale with subdivisions of 10 µm was simultaneously photographed at the same magnification in order to calculate accurately the final magnification of the paper prints. The size of the micrographs was *c*. 15 × 23 cm and the final magnification × 200, whereby 1 cm on the printed micrographs was equivalent to 50 µm, and 1 cm^2 to 2500 μm^2.

CLASSIFICATION OF OOCYTES AND OVARIES

Based on previous classifications (Tyler & Sumpter, 1996; Coward & Bromage, 1998; Susca *et al*., 2001*a*), four distinct oocyte developmental stages were distinguished in the ovaries (stages 1–4). Furthermore, when present, atretic oocytes and postovulatory follicles were identified and their fractional volume estimated. Depending on the most advanced group of oocytes encountered in the gonad, each of the ovaries studied was classified into one of three maturation stages (Fig. 2): immature (where only oocytes at stage 1 and 2 were present), non-spawning mature (stage 3 oocytes were the most advanced group of oocytes, and there was no evidence of spawning activity), and spawning (stage 3 and 4 oocytes were present, as well as postovulatory follicles in most cases).

STEREOLOGY

For estimation of the number of each oocyte type contained in the gonads, the stereological method of Weibel & Gómez (1962) was applied. This technique has been previously used for counting fish oocytes with considerable success (Emerson *et al*., 1990; Greer Walker *et al*., 1994; Coward & Bromage, 1998). For this method to be reliable, the oocyte distribution throughout the ovary must be homogeneous, so that the gonad fragment used for histology actually represents the overall ovarian structure. June (1953) for yellowfin tuna *Thunnus albacares* (Bonnaterre), and Stéquert & Ramcharrun (1995) for skipjack tuna *Katsuwonus pelamis* (L.), found a homogeneous distribution of oocytes within and between the ovaries. Uneven distribution of hydrated oocytes, however, is likely to exist if females are sampled in the process of hydration, because hydration begins at the periphery and spreads to the central section of the ovary (Hunter *et al*., 1985). Studies on several tuna species have shown significant differences in the distribution of oocytes among central, peripheral and mid regions within the same section of the ovary (Yuen, 1955; Otsu & Uchida, 1959; Baglin, 1982). To avoid biased estimations due to heterogeneous distribution of oocytes throughout the ovarian wall, all the histological preparations in the present study contained the whole thickness of the ovarian wall, therefore oocytes were counted from the lumen to the periphery of the organ. A comparative analysis among anterior, mid and posterior parts of the ovary was not carried out, therefore a heterogeneous oocyte distribution cannot be ruled out. The sampling was valid for comparison among specimens from the Strait of Gibraltar and

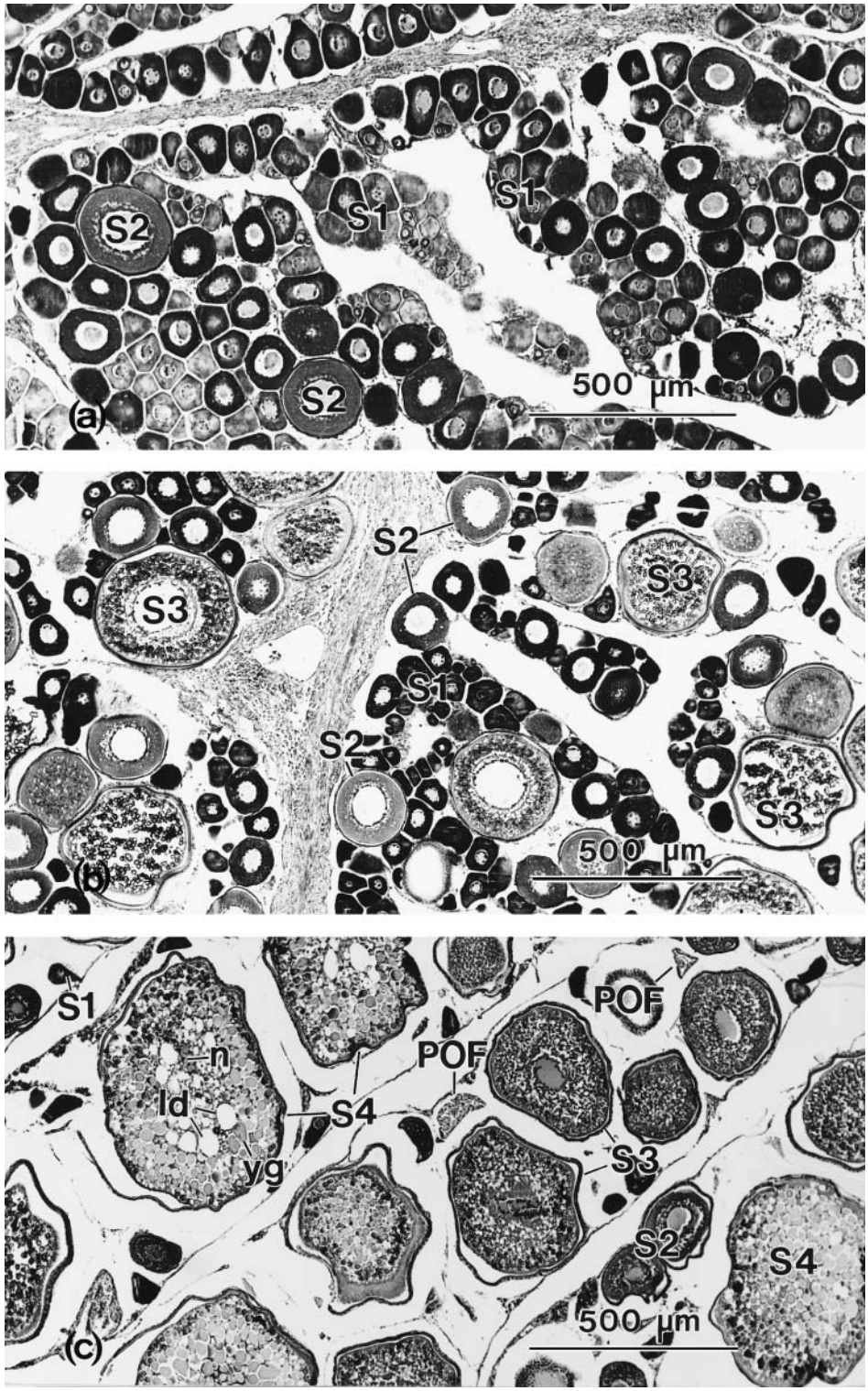

FIG. 2. Micrographs of immature (a), non-spawning mature (b) and spawning (c) ovaries showing the different stages of oocyte maturation. ld, Lipid droplets; n, nuclei; POF, postovulatory follicles; S1, stage 1 oocyte; S2, stage 2 oocyte; S3, stage 3 oocyte; S4, stage 4 oocyte; yg, yolk globules.

between these and individuals from the Balearic Islands, however, because in all specimens the gonad fragments were taken from the same region of the ovary.

From micrographs of histological sections, the numerical density (N_V, number of oocytes per unit volume), was calculated for each oocyte developmental stage according to the formula developed by Weibel & Gómez (1962) as further modified by Weibel *et al.* (1966):

$$N_V = (K N_A^{1.5})\ (\beta V_V^{0.5})^{-1}$$

where β and K are coefficients related to shape and size distribution of oocytes, respectively. For each oocyte type, β was determined approximately as a function of the axial ratio (calculated previously from multiple sections of the samples) using the function provided by Weibel (1969) for ellipsoidal particles. The estimated value of β ranged from 1·4 in stage 3 and 4 oocytes to 1·52 in the more irregularly shaped stage 1 oocytes. K is defined as:

$$K = (D_3 D_1^{-1})^{1.5}$$

where D_1 and D_3 are the first and third moment of the size distribution (Weibel, 1969; Williams, 1977). In the present samples K varied between 1·01 in stage 4 oocytes and 1·19 in stage 1 oocytes. 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 (transections that are cut by the left and upper margins are counted, whereas those cut by the right and lower margins are rejected). V_V is the volume fraction occupied by oocytes of a given category (volume density). It was calculated by the superimposition on to the micrographs of a test system consisting of a 14 × 22 cm square lattice (Weibel *et al.*, 1966) in which the unit area was 1 cm^2, representing an actual area of 2500 μm^2 in the sample; then, the volume density was estimated counting the number of points (cross-points of the lines) that overlaid the profiles of the oocyte type considered and dividing by the total number of points of the lattice (Weibel & Gómez, 1962; Weibel *et al.*, 1966; Weibel, 1969; Williams, 1977). The total number of oocytes of each category contained in the gonad of each individual was then readily obtained multiplying N_V by the entire ovarian volume. From this estimate, the number of oocytes g^{-1} of body mass was calculated for all specimens whose mass was available. This parameter is particularly useful for comparison among related species that have a markedly different size.

STATISTICAL ANALYSIS

To test differences in the degree of maturation of the ovaries between the two reproductive seasons in samples from Barbate, the values of I_G, V_V, N_V, number of oocytes g^{-1} of body mass, and total number of oocytes per individual were compared by the Student's t-test with $\alpha = 0{\cdot}05$. I_G and V_v values were arcsine transformed prior to statistical analysis. In case of heteroscedasticity (checked by the F-test), the nonparametric Mann-Whitney test was used (Zar, 1996). The same statistical analyses were applied to test spatio-temporal differences in the reproductive parameters between bluefin tuna caught in the Strait of Gibraltar and in the Balearic spawning grounds. Possible variations in the degree of maturation among the different sub-samples taken in Barbate throughout the migratory period in 1999 and 2000 were tested by single-factor analysis of variance or Kruskal-Wallis test in the case of heteroscedasticity (Zar, 1996).

RESULTS

BIOMETRY AND BIOLOGICAL INDICES

The average body mass of bluefin tuna collected from both sampling areas was similar (t-test: $P = 0{\cdot}31$), with mean values of $157{\cdot}4 \pm 51{\cdot}6$ kg for Barbate and $143{\cdot}8 \pm 48{\cdot}6$ kg for the Balearic Islands. Bluefin tuna caught off Barbate had

TABLE I. Biometric and stereological data from bluefin tuna caught by trap off Barbate during the spring fishing seasons of 1999 and 2000. The values are expressed as mean ± S.D.; n is the number of individuals examined. Differences between the two groups of means were analysed by a Student's t-test and were significant ($P<0{\cdot}05$) where indicated (*)

	Barbate (1999)	Barbate (2000)
I_G	1·23 ± 0·55 (n=21)	1·22 ± 0·59 (n=17)
Stage 1 oocytes		
V_V	0·21 ± 0·10 (n=32)	0·18 ± 0·08 (n=28)
N_V (ml^{-1}) (× 10^3)	723·50 ± 387·47 (n=32)	620·38 ± 229·11 (n=28)
No. per individual (× 10^9)	1·29 ± 0·47 (n=31)	0·84 ± 0·49* (n=28)
No. g^{-1} body mass (× 10^3)	7·14 ± 2·13 (n=20)	5·56 ± 1·45 (n=17)
Stage 2 oocytes		
V_V	0·20 ± 0·07 (n=32)	0·17 ± 0·08 (n=28)
N_V (ml^{-1}) (× 10^3)	57·43 ± 24·38 (n=32)	57·60 ± 18·46 (n=28)
No. per individual (× 10^6)	114·23 ± 51·43 (n=31)	85·33 ± 60·32* (n=28)
No. g^{-1} body mass	616·19 ± 202·47 (n=20)	533·81 ± 167·53 (n=17)
Stage 3 oocytes		
V_V	0·15 ± 0·12 (n=32)	0·17 ± 0·11 (n=28)
N_V (ml^{-1}) (× 10^3)	5·80 ± 4·65 (n=32)	7·63 ± 4·87 (n=28)
No. per individual (× 10^6)	14·96 ± 14·60 (n=31)	14·68 ± 17·98 (n=28)
No. g^{-1} body mass	77·35 ± 80·31 (n=20)	118·57 ± 118·79 (n=17)
Atretic oocytes		
V_V	0·02 ± 0·02 (n=32)	0·04 ± 0·04* (n=28)

relatively small ovaries, averaging 1·98 kg (range 0·55–5·06 kg). In contrast, spawning specimens fished around the Balearic Islands possessed much heavier gonads, with an average mass of 6·15 kg (range 1·24–14·28 kg).

The I_G calculated from migrant bluefin tuna caught off Barbate were almost identical in 1999 and 2000 (Table I). No significant differences were found in I_G among sub-samples from Barbate throughout any of the two reproductive seasons studied. When the pooled data from Barbate were compared with those from the Balearic Islands, a significant difference in I_G was found (Table II). Assuming that all sampled specimens belong to the same population of bluefin tuna, it is inferred that on average the I_G undergoes an almost four-fold increase

TABLE II. Comparison between biometric and stereological data from bluefin tuna caught off Barbate and around the Balearic Islands. The values are expressed as mean ± S.D.; n is the number of individuals examined. Differences between the two groups of means were analysed by a Student's t-test and were significant ($P<0{\cdot}05$) where indicated (*)

	Barbate (1999+2000)	Balearic Islands (1999+2000)
I_G	1·23 ± 0·56 (n=38)	4·19 ± 1·65* (n=24)
Stage 1 oocytes		
V_V	0·20 ± 0·09 (n=60)	0·04 ± 0·02* (n=24)
N_V (ml^{-1}) (× 10^3)	615·38 ± 324·95 (n=60)	187·29 ± 79·87* (n=24)
No. per individual (× 10^9)	1·08 ± 0·52 (n=59)	0.97 ± 0·57 (n=24)
No. g^{-1} body mass (× 10^3)	6·41 ± 1·99 (n=37)	6·47 ± 2·50 (n=24)
Stage 2 oocytes		
V_V	0·18 ± 0·07 (n=60)	0·06 ± 0·02* (n=24)
N_V (ml^{-1}) (× 10^3)	57·51 ± 21·64 (n=60)	23·30 ± 8·96* (n=24)
No. per individual (× 10^6)	100·51 ± 57·21 (n=59)	126·98 ± 91·01 (n=24)
No. g^{-1} body mass	578·34 ± 189·32 (n=37)	821·47 ± 376·94* (n=24)
Stage 3 oocytes		
V_V	0·16 ± 0·12 (n=60)	0·29 ± 0·08* (n=24)
N_V (ml^{-1}) (× 10^3)	6·65 ± 4·80 (n=60)	10·78 ± 2·86* (n=24)
No. per individual (× 10^6)	14·83 ± 16·15 (n=59)	66·38 ± 50·30* (n=24)
No. g^{-1} body mass	96·29 ± 100·54 (n=37)	442·27 ± 243·84* (n=24)
Stage 4 oocytes		
V_V	0 (n=60)	0·17 ± 0·08* (n=24)
N_V (ml^{-1}) (× 10^3)	0 (n=60)	2·39 ± 1·43* (n=24)
No. per individual (× 10^6)	0 (n=59)	12·60 ± 9·18* (n=24)
No. g^{-1} body mass	0 (n=37)	92·75 ± 60·09* (n=24)
Atretic oocytes		
V_V	0·03 ± 0·03 (n=60)	0·02 ± 0·02 (n=24)

from when the fish cross the Strait of Gibraltar until they reach the spawning area, reflecting an evident difference in the degree of sexual maturity between the two fishing areas.

HISTOLOGY

Histologically, the ovary of bluefin tuna corresponds to the asynchronous type, which is characterized by the presence of a heterogeneous array of maturing oocytes at distinct developmental stages (Fig. 2). For convenience, only four major stages have been distinguished throughout oocyte development, as excessive fragmentation of the process makes it difficult to establish clear border lines and renders the stereological analysis unnecessarily complicated. For each category, the oocyte size was calculated from the means of the measurements of the long and short axes of over 50 oocytes.

Stage 1 oocytes (perinucleolar stage oocytes; Tyler & Sumpter, 1996) are small polyhedric cells (73·0 ± 20·5 μm) which show a high nucleus-cytoplasm ratio and a homogeneous basophilic cytoplasm [Fig. 2(a)–(c)]. Stage 2 oocytes are easily distinguished by their larger size (143·5 ± 39·2 μm) and the appearance in the cytoplasm of lipid droplets first and tiny yolk vesicles afterwards [Fig. 2(a)–(c)]; this phase corresponds to the cortical alveolus stage (Tyler & Sumpter, 1996; Coward & Bromage, 1998) and is equivalent to the lipid stage described by Susca *et al.* (2001*a*) for *T. thynnus* ovaries. Stage 3 (vitellogenic) oocytes (382·7 ± 46·3 μm) contain yolk globules and conspicuous lipid droplets occupying a large part of the cytoplasm [Fig. 2(b),(c)]. Stage 4 oocytes (504·5 ± 38·2 μm) are characterized by progressive coalescence of the lipid droplets into a single globule, movement of the nucleus to the animal pole, and the onset of hydration [Fig. 2(c)]; this late phase of oogenesis corresponds to the maturation stage (Tyler & Sumpter, 1996) and comprises stages 6 and 7 of Coward & Bromage (1998). This is the most advanced stage of the oocyte development observed in this study, since fully hydrated oocytes were not found in any of the samples.

Atretic oocytes (oocytes in process of resorption showing irregular shape, yolk degradation and phagocytosis of granulosa) were present in all sampling groups. The incidence of atresia was 73·3% in the samples of pre-spawning fish from Barbate (atresia occurred in 44 out of 60 individuals), whereas the ovaries of all the specimens from the Balearic Islands apart from two (91·6%) contained atretic oocytes. Postovulatory follicles [Fig. 2(c)] appear as empty follicles resulting from expulsion of the oocyte, and consist of the surrounding thecal and inner follicle cells at different stages of degeneration. Postovulatory follicles were present in as many as 20 of the 24 bluefin tuna sampled from the Balearic fishing area, among them a 38 kg specimen. This proportion represents 83·3% of the sample and, in terms of spawning frequency, 1/0·83 (=1·2) days, if it is assumed that postovulatory follicles in this species do not persist more than 24 h before resorption is completed. No postovulatory follicles were encountered in the samples from Barbate.

Histological analysis of the ovaries indicated that 8·3% of the female bluefin tuna caught off Barbate were still immature (the most advanced group of oocytes found in the ovaries were at developmental stage 2) and 91·7% were classified as non-spawning mature (the ovaries contained stage 3 oocytes). All bluefin tuna from the Balearic Islands, including two small-sized individuals of 30 and 38 kg, were identified as spawning (they had all ovaries with stage 4 oocytes, most of them showing postovulatory follicles as well).

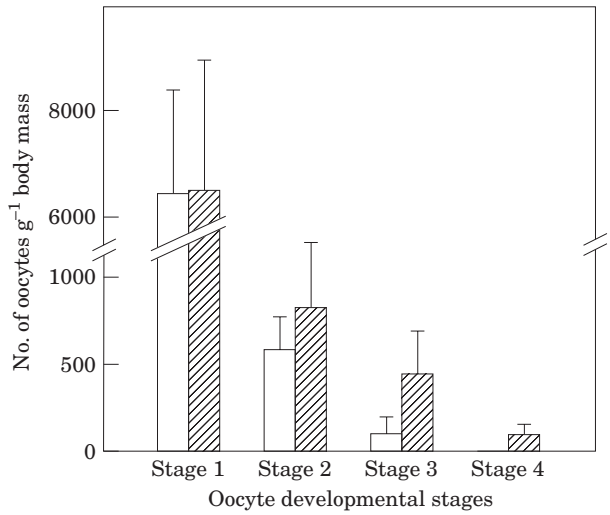

FIG. 3. Mean number (± S.D.) of the different oocyte categories per g of body mass of bluefin tuna from □, Barbate and ▨, Balearic Islands.

STEREOLOGY

After the stereological study of ovaries from migrant bluefin tuna caught off Barbate in 1999 and 2000, statistical analysis did not indicate significant differences in the number of the various oocyte categories and in the volume fraction of atretic oocytes among the sub-samples taken throughout both migratory seasons (Table I).

Pooled data of bluefin tuna collected off Barbate were compared with those from the Balearic fishing area (Table II). V_V and N_V of stage 1 oocytes were significantly higher in samples from Barbate as compared to those from the Balearic Islands. The estimated values for the number of stage 1 oocytes in bluefin tuna from the two sampling stations, however, were almost identical. Both the V_V and N_V of stage 2 oocytes from Barbate specimens were significantly higher than those estimated for bluefin tuna from around the Balearic Islands, but when these data were raised to numerical values it was observed that the spawning bluefin tuna had a higher number of stage 2 oocytes in comparison with those from Barbate. The number of highly yolked oocytes (stage 3 plus stage 4 oocytes) yielded by bluefin tuna from the Balearic Islands was as much as five-fold that produced by fish from Barbate, either expressed as number per individual or as number g^{-1} body mass. It is to be noted that this holds true even if the counts of stage 4 oocytes (which were not present in the samples from Barbate) are removed from this group (Fig. 3).

Although a statistical difference was found in the fractional volume of atretic oocytes of bluefin tuna from Barbate between 1999 and 2000, no significant difference was found in the proportion of the ovarian volume occupied by atretic oocytes when the grouped data from Barbate were compared with those from the Balearic Islands. An accurate quantification of degenerative oocytes in the ovary is important to determine the number of developing oocytes that will eventually form eggs (Tyler & Sumpter, 1996). A reliable stereological calculation of N_V,

and hence of its derived numerical estimates, however, was not possible for either atretic oocytes and postovulatory follicles, because of their highly variable shape and size.

DISCUSSION

The relatively low ovarian mass and I_G recorded in *T. thynnus* from the Strait of Gibraltar as they move into the Mediterranean indicate that at this point of the migratory route female bluefin tuna are still far from attaining optimum spawning condition. This is corroborated by histological and stereological studies: most of the bluefin tuna caught off Barbate were identified as non-spawning mature and some were categorized as immature, according to the classification of Farley & Davies (1998). When migrant tuna arrive at the spawning area around the Balearic Islands, however, their gonad mass and I_G have undergone a four-fold increase, and the ovarian histology reveals a spawning condition. Spawning ovaries were observed even in the two smallest bluefin tuna (30 and 38 kg) captured in Balearic waters. These body masses correspond to 116 and 126 cm in fork length (L_F), respectively, according to the equation provided by Rodríguez-Roda (1964) for the years 1956 to 1959. This observation supports the assumption of Rodríguez-Roda (1967) that nearly all bluefin tuna reach maturity between 110 and 120 cm L_F (sizes that are equivalent to an age of 3 years). Susca *et al.* (2001*a*, 2001*b*) arrived at the same conclusion from immunohistochemical studies carried out on Atlantic bluefin from the eastern stock, since oocytes containing vitellogenin-like material were found only in ovaries of specimens $\geq$110 cm L_F. The eastern Atlantic bluefin tuna, therefore, appears to attain maturity earlier than the western bluefin tuna, which is thought to mature at age 6 years (Baglin, 1982).

In spawning specimens, all oocyte developmental stages were present in the ovary, which indicates that the bluefin tuna has asynchronous oocyte development. Fishes with asynchronous ovaries are referred to as partial or multiple spawners (West, 1990). The simultaneous occurrence of stage 4 (migratory-nucleus) oocytes and postovulatory follicles in the ovaries of many bluefin tuna fished in Balearic waters indicate that they can ovulate several batches of oocytes. Thus, the northern bluefin tuna (Baglin, 1982), like other tuna species (June, 1953; Yuen, 1955; Buñag, 1956; Otsu & Uchida, 1959; McPherson, 1991; Stéquert & Ramcharrun, 1995; Schaefer, 1996; Farley & Davies, 1998), is a multiple spawner.

The increased size of the ovaries occurring between sampling off Barbate and the Balearic Islands is mainly accounted for by an intense production of highly yolked oocytes (oocytes at stages 3 and 4) from the stock of stage 1 oocytes, the amount of which remains more or less constant around 6500 units g^{-1} body mass. Stereology has revealed in *Tilapia zillii* (Gervais) that the volume fraction of vitellogenic oocytes increases significantly with I_G, whereas the proportion of previtellogenic oocytes is relatively consistent throughout the reproductive cycle (Coward & Bromage, 1998).

Spawning bluefin tuna from the Balearic area had five-fold more highly yolked oocytes than bluefin tuna from Barbate. This indicates the occurrence of clear spatio-temporal differences in the reproductive activity between the two

sampling stations. From the stereological study carried out on the 24 spawning bluefin tuna, the mean relative batch fecundity was calculated to be *c.* 93 oocytes g^{-1} body mass, an estimate that was obtained from the N_V of stage 4 oocytes, and is close to the average of 110 eggs g^{-1} reported by Stéquert & Ramcharrun (1995) for *K. pelamis*. This value is notably higher than that calculated by Farley & Davis (1998) (57 g^{-1}) for southern bluefin tuna *Thunnus maccoyii* Castelnau from counts of hydrated oocytes, and is also greater than the estimate reported by Schaefer (1996) (68 g^{-1}) for *T. albacares* which was calculated by quantification of both late vitellogenic and hydrated oocytes.

Rodríguez-Roda (1967) calculated the absolute fecundity of ten bluefin tuna caught off the south Atlantic coast off Spain in 1958, 1961, 1963 and 1964, as the total number of oocytes >333 μm contained in the gonads (this oocyte class would be the approximate equivalent of the stage 3 oocyte category). His estimates are comparable to the present observations on bluefin tuna from Barbate fished in 1999 and 2000 on similar dates, in which the fecundity was calculated from counts of stage 3 oocytes. Using the data of Rodríguez-Roda (1967), a relative fecundity of $126{\cdot}8 \pm 43{\cdot}3$ oocytes g^{-1} body mass can be obtained. Such a value is slightly higher than the present estimate of 96·3 g^{-1} calculated from counts of stage 3 oocytes. A possible reason for the difference between the present batch fecundity estimate and that of Rodríguez-Roda (1969) is that he may have counted some of the largest stage 2 oocytes within his most advanced oocyte size-class. Furthermore, this difference is not unexpected, since both these estimates are separated by *c.* 40 years, and variations in batch fecundity of a species may occur depending on several factors, such as the year, fish size, spawning area and time in the spawning cycle when samples are collected (Hunter *et al.*, 1985; Báez-Hidalgo & Bécquer, 1994; Stéquert & Ramcharrun, 1995; Farley & Davis, 1998). For instance, Baglin (1982) estimated that the western stock of Atlantic bluefin tuna is more fecund than the eastern stock.

Postovulatory follicles were absent in all specimens collected from Barbate between late April and early June. It appears evident from this and earlier observations (Rodríguez-Roda, 1964, 1967) that migrant eastern Atlantic bluefin tuna do not spawn until they have reached specific areas in the Mediterranean, the spawning taking place mainly during June and July, somewhat later than in the western Atlantic bluefin (Baglin, 1982). In agreement with this, Susca *et al.* (2000, 2001*a*) have reported that bluefin tuna from the central Mediterranean do not start to spawn until the middle of June.

Unlike specimens from Barbate, a high proportion of female bluefin tuna sampled in Balearic waters (20 out of 24) contained postovulatory follicles in the ovaries as a sign of recent egg release. If postovulatory follicles do not persist more than 24 h after ovulation, such as is thought to occur in *T. albacares* (McPherson, 1991; Schaefer, 1996), the spawning frequency of *T. thynnus* around the Balearic Islands would be 1·2 days, estimated according to the postovulatory follicle method (Hunter & Macewicz, 1985). This value should be considered with caution due to the low number of spawners studied, though it coincides with the estimates given for female *T. maccoyii* in ‘ prime spawning condition ’ in the south-east Indian Ocean (1·1 days) (Farley & Davis, 1998), and for *T. albacares* in the eastern Pacific Ocean (1·14 days) (Schaefer, 1996).

McPherson (1991) calculated a somewhat lower frequency (1·54 days) for *T. albacares* spawning in the western Coral Sea.

The absence of fully hydrated oocytes in the histological samples of spawning *T. thynnus* is probably due to the time of the day when the catch occurred. The process of hydration is known to occur in a few hours, so that hydrated oocytes are short-lived and the likelihood of encountering them is reduced unless the capture occurs just prior to spawning (Farley & Davis, 1998).

It has been reported that during their journey to Mediterranean spawning grounds the northern bluefin tuna do not feed significantly (Rodríguez-Roda, 1964), so that the nutrients required to produce mature eggs must be mobilized from reserves accumulated prior to the start of the migration. Rodríguez-Roda (1964) pointed out that towards the end of June the variation of the hepatosomatic index (I_H) and I_G in bluefin tuna caught off Barbate were antagonistic, suggesting that reserves accumulated in the liver are utilized in gametogenesis. In the present study I_G and I_H (unpubl. data) increased as spawning approached, so that gonad growth should take place at the expense of stores (mainly lipids) accumulated elsewhere and further processed and channelled to the ovaries through the liver. This is supported by the observation that resorption of the fat body associated with the ovaries appears to occur as the gonad grows (unpubl. data). The fat tissue is, in fact, more than two-fold more abundant in pre-spawning bluefin tuna from the Strait of Gibraltar than in spawning bluefin tuna collected around the Balearic Islands (pers. data). Similarly in male *Thunnus alalunga* (Bonnaterre) Ratty *et al.* (1990) observed that the fat body was proportionately larger in immature fish than in fish with gonads actively producing sperm, and suggested that the fat storage provides an energy source for spermatogenesis. G. Mourente, C. Megian & E. Diaz-Salvago (pers. comm.) found that the increase in lipid content observed during maturation of *T. thynnus* ovaries is concomitant with reduction of the fat body, whereas the lipid content of the liver remains unchanged. A plausible interpretation to this observation was the utilization of the fat body reserves for gonadal development.

The considerable gonad growth observed between fish in the Strait of Gibraltar and at the spawning grounds occurs in a relatively short period of time, with no apparent factor other than water temperature being involved in the process. A possible reason for the poor ovarian development when bluefin tuna cross the Strait of Gibraltar may be the low maximum surface temperatures (*c.* 18° C) recorded in May to early June (Establier & Margalef, 1964). There is some evidence in teleosts that later stages of gametogenesis, stimulation of maturation and spawning are influenced by temperature (Bye, 1989, 1990; Stacey, 1989; Ciereszko *et al.*, 1997). Natural spawning of *T. thynnus* in net cages has been achieved with surface water temperatures ranging between 21·6 and 25·6° C (Lioka *et al.*, 2000), however, this level is not reached in waters off Barbate until mid July to mid August (Establier & Margalef, 1964), sometime after the spawning season.

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