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BLUEFIN TUNA MATURITY IN TUNISIAN WATERS: A PRELIMINARY APPROACH

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SUMMARY

The aim of this paper is to increase our knowledge of the reproductive biology of the Mediterranean bluefin tuna. Particularly about sexual maturity, reproductive season and spawning area in Tunisian waters as an important tool for determining a correct policy for the management of fish stock. The histological analysis indicates that all the 3-age tunas were immature ones. The remaining bluefin females studied were mature independently of their age or FL. The activity stages found in the Tunisian tunas suggest us that these tunas are going to spawn in a short period, possibly in the central Mediterranean or Balearic island, along May and June. In conclusion, our data indicates that all 4-age Tunisian tunas are mature and are going to do effective spawning. At present, ICCAT use in the evaluation process the 5-age class like the first maturity one, if our data are confirmed by ulterior studies this age could change.

INTRODUCTION

Fishing for tuna and similar species has been undertaken for a long time, particularly by traps. In fact, traces of tuna fishing in the Tunisian coasts can be reported from many years back. Fishing was practised by means of harpoons until it slowly progressed to the rods, gillnets and encircling nets. Besides, the chroniclers of the old time have extensively described this fishing, which provides us information about how important fishing was. Lookouts installed in towers informed fishers about the coastal movements of tuna; the fish were immediately circled. Records of bluefin tuna caught by fixed trap-nets goes back to 1820. However, in recent years new methods were introduced to catch tuna species along Tunisian coast namely the encircling nets introduced in 1976. Currently, around 70 seiner tuna fishing boats are dedicated to the tuna fishing along the Tunisian coasts.

In Tunisian coast, the family of Scomrbridae is without any doubt the most important one. We mention particularly bluefin tuna (*Thunnus thynnus*), little tuna (*Euthynnus alletteratus*) which provide a local industry, as a great amount of this fish is sold for canning. Bluefin tuna has become one of the most important species for Tunisian fisherman. It is a preferential species for the export market. This product is mainly addressed to Japan market.

In spite of its economic importance, the actual knowledge of reproductive biology of bluefin tuna is limited to some research such as those based on the distribution of eggs and larvae (Piccinetti *et al*., 1977, 1996; Cavallaro *et al*., 1996; Nishida *et al*., 1997), or based on seasonal variation of the gonadosomatic index (De la Serna et A lot, 1992) and on macroscopic classification of gonad maturity stage (Rodriguez-Roda, 1964, 1967). An histological study of Western Atlantic bluefin tuna ovaries has been carried out by Baglin (1982). Recently a study was carried out correlating plasma concentrations of the steroid hormones with gonad histological maturity stage (Susca et al., 2001).

On the basis of these documents, SCRS assumed that BFT first successfully spawn at age 8 in the west Atlantic compared to ages 4 to 5 in the Mediterranean. However, according to reports from

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COPEMED, 1999, 2000 it was pointed out that the size of migrated BFT to Mediterranean ranges between 101 to 260 cm and weight from 18 to 450 kilograms. Taking into consideration that identifying the age at maturity and spawning areas of highly exploitable fish species like BFT is critical for effective management (Huppell and Sullivan 2000, SCRS 2001), extensive investigations to study the biology and reproduction of BFT in the Tunisian waters within the COPEMED program is progressing.

The aim of this paper is to increase our knowledge of the reproductive biology of the Mediterranean bluefin tuna. Particularity about sexual maturity, reproductive season and spawning area in Tunisian waters as an important tool for determining a correct policy for the management of fish stock.

MATERIALS AND METHODS

Specimen collection

Body weight, sex and fork length measurements were obtained from 64 bluefin individuals caught by Tunisian fisheries (purse seine) operating mainly in the south coast of the country. Data on catch, fishing areas and the biological sampling collection (gonad sampling, first spine and muscles) was accomplished in the months of March to June 2000 and 2001 within the frame of FAO-COPEMED project aiming to study " research on fishing biology of bluefin tuna (*Thunnus thynnus*) and swordfish (*Xiphias gladius*) in the Mediterranean sea". In addition the gonad weight of the female samples was obtained with the purpose of knowing the Gonad-somatic index (Kume and Josep, 1969) indicating the levels of sexual maturity of the tuna fish. A portion of a central slide of one of the ovaries is obtained and the pieces are placed in a labelled container with, at least, the triple of fixative solution volume that the one of the weight (fixative solution: formalin 4% in sea water).

Histologic preparation of the sample studied.

Samples of the central portion of the ovarium from 0,5 cm of thickness were washed, dehydrated in a increasing ethanol series, n-butilic alcohol and embedded in paraffin.

Three separated samples were used to obtain sections of 10 µm in thickness. The sections were stretched in a bath with distilled water to (40°C) and gathered with the glass properly labelled and dealt with an adherent solution (Poly-l-lysine, 0,01% in distilled water) and dried during 24 hours in a 37°C stove to 1 hour in stove at 60 °C.

Sections were stained with a Mallory's trichrome stain for a general assesment of the histological components of the ovary.

Histological classification

To estimate reproductive condition of bluefin tuna, two different histological classification systems were used: one for estimating the sexual maturity and the other for assesing the activity stage of mature females. Each ovary was histologically classified according to both systems (Hunter and Goldberg, 1980; Hunter and Macewicz, 1980, 1985a, b; Hunter, Macevicz and Sibert, 1986).

Sexual maturity

It is considered that a female is a mature one when has the capability to reproduce in a determinate spawning season. Histological signs of maturity are the presence in the ovary of yolked oocytes, hidrated oocytes or postovulatory follicles. The inmature females have not reached the sexual maturity and are unable to reproduce in a determinate season.

Sexual activity

Four different stages of activity have been taken into consideration:

Inactive females: the histological analysis indicates that the ovary contain no yolked oocytes and no atretic structures.

Prespawning females: Those females showing signs of an imminent spawning like hidrated or in nuclear migration phase oocytes but not postovulatory follicles or extended atresia. High oocyte density in the ovary.
Spawning females: The histological analysis shows signs of past spawning (postovulatory follicles) and enough vitellogenic oocytes to complete more spawning.
Postspawning females: Those females showing signs of past spawning (postovulatory follicles) but have not enough vitellogenic oocytes to complete more spawning. Extended atresia in vitellogenic oocytes. Low oocytes density in the ovary.

The gonadosomatic index was calculated according to Kume and Joseph (1969)

The age of the tunas was calculated according to Rodriguez-Roda (1964) and Hattour (1984).

RESULTS

All the GSI by age class are over 6. The highest GSI was shown by the 6 years old age class. The GSI reach a stable value, near to 7, in the 8-age and older age class(figure 1 E).

The histological analysis indicates that all the 3-age tunas (TBFT 22, 33 and 34 with fork-length =101 and 105cm) were immature ones (figure 1 A and B). These females show their ovaries containing no yolked oocytes and no atretic structures. No signs of future spawning could be observed. The remaining bluefin females studied were mature independently of their age or FL. Hundred per cent of the 4 years old females (n = 14) were mature.

Regarding to the activity stages, all the studied mature bluefin tuna ovaries could be classified into the prespawning developmental stage (see table II). These females showed a mature ovary with many fully yolked oocytes, few partially yolked and unyolked oocytes, high oocyte density and only incidental atresia. Any nuclear migration stage oocytes, hidrated oocytes or postovulatory follicles could be observed. All these signs indicates us a prespawning stage in all the Tunisian tunas caught along May and June (figure 1. C and D). There are no histological differences between the 4-age class ovaries and the older age class ones.

The activity stages found in the Tunisian tunas suggest us that these tunas are going to spawn in a short period, possibly in the central Mediterranean or Balearic island, along May and June.

DISCUSSION

Research on bluefin tuna in the eastern Atlantic and Mediterranean suggest that they reach the sexual maturity at ages 2-4 (lengths of 75-125cm) (Mather *et al.*, 1995). Several authors have studied this questions but their results were confused and sometimes contradictory (Frade and Manacas, 1933; Arena, 1964; Sará, 1973; Rodriguez-Roda, 1969; Frade and Vilela, 1962). So Sella (1929) noted that the bluefin usually spawned first at age-3 although few 2-age tunas might also spawn, unlike Scaccini et al. (1975) reported the smallest mature bluefin tuna observed at 4-age class. In a general view Mather *et al*. (1995) conclude that the first spawning of eastern bluefin tuna occurs at age 3, in exceptional cases, and usually at age 4. By age 6 all of the tunas are mature.

Our data agree with the previous literature and indicates that the first maturity occurs between ages 3-4. Because the 100 % of 4-age bluefin tuna caught in Tunisian waters were mature (n= 14) and the 3-age ones were immature, the age at first maturity (age at 50%) must occur at 3-age class. The three years old tunas used in this study were caught along March, early in the spring, and soon for the

spawning season. This circumstance could induce errors in the histological classification of these 3-age tunas. Indeed, this tuna could be mature inactive females and not immature ones.
On the other hand, our data indicates that in Tunisian waters all of 4-age tunas were mature unlike the Mather *et al*. (1995) conclusions. This conclusion agree with those from a similar study in Libyan waters (Tawill *et al*.; 2001, personal communication).

Another question is: is the fraction of tuna sampled representative of the whole age class?. Does all the 4-age tuna accomplish the reproductive migration? Or, only mature tunas do it? If the 3-age tunas studied were effectively immature then some immature fish could do the reproductive migration or constitute a part of a local Mediterranean stock. More extensive studies are needed to answer these questions.

In conclusion, our data indicates that all 4-age Tunisian tunas are mature and are going to do effective spawning. Actually, ICCAT use in the evaluation process the 5-age class like the first maturity one, if our data are confirmed by ulterior studies this age could change.

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Table I. Summary of information on *Thunnus thynnus* specimens used in the study (FL = Fork length; GW = Gonadal weight).

ID code	Date	Gear	FL(cm)	Age (Years)	Sex	GW (gr)
TBFT-22	09/03/00	Purse seine	101	3	F	
TBFT-23	24/06/00	Purse seine	198	10	F	6050
TBFT-24	26/06/00	Purse seine	140	6	F	5250
TBFT-25	13/06/00	Purse seine	268	10	F	10400
TBFT-26	23/06/00	Purse seine	240	10	F	10600
TBFT-27	23/06/00	Purse seine	210	10	F	13500
TBFT-28	23/06/00	Purse seine	215	10	F	6000
TBFT-29	20/05/00	Purse seine	172	9	F	
TBFT-30	23/06/00	Purse seine	270	10	F	13100
TBFT-31	20/05/00	Purse seine	170	8	F	
TBFT-32	20/05/00	Purse seine	158	7	F	
TBFT-33	09/03/00	Purse seine	105	3	F	
TBFT-34	09/03/00	Purse seine	105	3	F	
TBFT-35	20/05/00	Purse seine	118	4	F	
TBFT-36	23/06/00	Purse seine	229	10	F	4000
TBFT-37	20/05/00	Purse seine	123	4	F	
TBFT-38	24/06/00	Purse seine	193	10	F	4500
TBFT-39	20/05/00	Purse seine	125	4	F	
TBFT-40	23/06/00	Purse seine	230	10	F	6100
TR-06 m	17/06/01	Purse seine	110	4	F	1200
TR-12 b	29/05/01	Purse seine	231	10	F	8700
TR-14 n	17/06/01	Purse seine	135	5	F	2800
TR-16	29/05/01	Purse seine	128	5	F	900
TR-18	29/05/01	Purse seine	246	10	F	10600
TR-21	17/06/01	Purse seine	141	5	F	1100
TR-22 e	29/05/01	Purse seine	243	10	F	6700
TR-23	29/05/01	Purse seine	251	10	F	12300
TR-24	17/06/01	Purse seine	143	5	F	2000
TR-25 c	29/05/01	Purse seine	218	10	F	4800
TR-27	29/05/01	Purse seine	247	10	F	8700
TR-28	17/06/01	Purse seine	121	4	F	2000
TR-30	29/05/01	Purse seine	198	10	F	4500
TR-31	29/05/01	Purse seine	238	10	F	9700
TR-32 a	29/05/01	Purse seine	246	10	F	8600
TR-32 l	17/06/01	Purse seine	120	4	F	1500
TR-33	29/05/01	Purse seine	221	10	F	6100
TR-33 j	31/05/01	Purse seine	146	6	F	4000
TR-34 i	31/05/01	Purse seine	122	4	F	1300
TR-35	31/05/01	Purse seine	145	6	F	2400
TR-35 d	29/05/01	Purse seine	235	10	F	9600
TR-37	29/05/01	Purse seine	206	10	F	5500
TR-39	29/05/01	Purse seine	250	10	F	14200
TR-41	29/05/01	Purse seine	207	10	F	6700
TR-45	31/05/01	Purse seine	161	7	F	2500
TR-46	31/05/01	Purse seine	133	5	F	1900
TR-47 f	31/05/01	Purse seine	130	5	F	900
TR-49	31/05/01	Purse seine	143	6	F	1600
TR-50	17/06/01	Purse seine	121	4	F	1500
TR-51	31/05/01	Purse seine	140	5	F	2100
TR-52	31/05/01	Purse seine	131	5	F	2000
TR-53 k	17/06/01	Purse seine	139	5	F	1700
TR-54	31/05/01	Purse seine	112	4	F	1000
TR-55 g	31/05/01	Purse seine	114	4	F	1100
TR-56	31/05/01	Purse seine	133	5	F	2000
TR-58	31/05/01	Purse seine	113	4	F	600
TR-59 h	31/05/01	Purse seine	132	5	F	1300
TR-61	31/05/01	Purse seine	140	5	F	1400
TR-62	31/05/01	Purse seine	138	5	F	2800
TR-65	17/06/01	Purse seine	119	4	F	1500
TR-70	17/06/01	Purse seine	167	8	F	3300
TR-73	17/06/01	Purse seine	129	5	F	1300
TR-75	17/06/01	Purse seine	124	4	F	1900
TR-77	17/06/01	Purse seine	139	5	F	3400
TR-78 o	17/06/01	Purse seine	124	4	F	2200

Table II. Maturity and activity results of the Tunisian bluefin sample.

ID code	Age (Years)	GSI	Maturity	Activity
TBFT-22	3		immature	inactive
TBFT-23	10	7,8	mature	prespawning
TBFT-24	6	19,1	mature	prespawning
TBFT-25	10	5,4	mature	prespawning
TBFT-26	10	7,7	mature	prespawning
TBFT-27	10	14,6	mature	prespawning
TBFT-28	10	6,0	mature	prespawning
TBFT-29	9		mature	prespawning
TBFT-30	10	6,7	mature	prespawning
TBFT-31	8		mature	prespawning
TBFT-32	7		mature	prespawning
TBFT-33	3		immature	inactive
TBFT-34	3		immature	inactive
TBFT-35	4		mature	prespawning
TBFT-36	10	3,3	mature	prespawning
TBFT-37	4		mature	prespawning
TBFT-38	10	6,3	mature	prespawning
TBFT-39	4		mature	prespawning
TBFT-40	10	5,0	mature	prespawning
TR-06 m	4	9,0	mature	prespawning
TR-12 b	10	7,1	mature	prespawning
TR-14 n	5	11,4	mature	prespawning
TR-16	5	4,3	mature	prespawning
TR-18	10	7,1	mature	prespawning
TR-21	5	3,9	mature	prespawning
TR-22 e	10	4,7	mature	prespawning
TR-23	10	7,8	mature	prespawning
TR-24	5	6,8	mature	prespawning
TR-25 c	10	4,6	mature	prespawning
TR-27	10	5,8	mature	prespawning
TR-28	4	11,3	mature	prespawning
TR-30	10	5,8	mature	prespawning
TR-31	10	7,2	mature	prespawning
TR-32 a	10	5,8	mature	prespawning
TR-32 l	4	8,7	mature	prespawning
TR-33	10	5,7	mature	prespawning
TR-33 j	6	12,9	mature	prespawning
TR-34 i	4	7,2	mature	prespawning
TR-35	6	7,9	mature	prespawning
TR-35 d	10	7,4	mature	prespawning
TR-37	10	6,3	mature	prespawning
TR-39	10	9,1	mature	prespawning
TR-41	10	7,6	mature	prespawning
TR-45	7	6,0	mature	prespawning
TR-46	5	8,1	mature	prespawning
TR-47 f	5	4,1	mature	prespawning
TR-49	6	5,5	mature	prespawning
TR-50	4	8,5	mature	prespawning
TR-51	5	7,7	mature	prespawning
TR-52	5	8,9	mature	prespawning
TR-53 k	5	6,3	mature	prespawning
TR-54	4	7,1	mature	prespawning
TR-55 g	4	7,4	mature	prespawning
TR-56	5	8,5	mature	prespawning
TR-58	4	4,2	mature	prespawning
TR-59 h	5	5,7	mature	prespawning
TR-61	5	5,1	mature	prespawning
TR-62	5	10,7	mature	prespawning
TR-65	4	8,9	mature	prespawning
TR-70	8	7,1	mature	prespawning
TR-73	5	6,1	mature	prespawning
TR-75	4	10,0	mature	prespawning
TR-77	5	12,7	mature	prespawning
TR-78 o	4	11,5	mature	prespawning

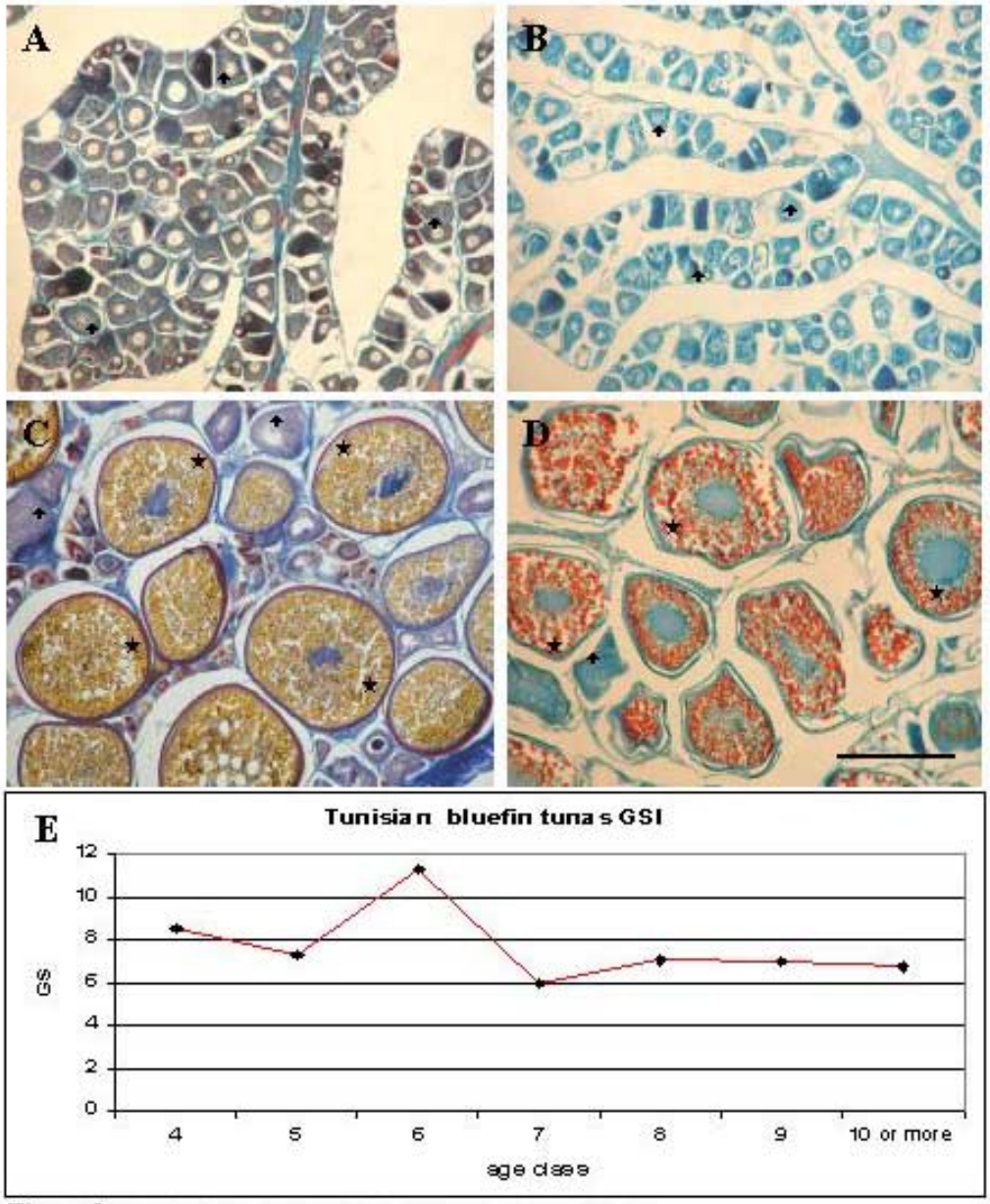

Figure 1. Maturity stages in the Tunisian bluefin tunas (bar = 400 µm)

A and **B** images show two immature three year old ovaries that show a lot of unyolked oocytes (cross).

C. A five year old mature ovary that shows maturity signs: yolked oocytes (stars).

D. This image shows a four year old mature ovary with many yolked oocytes (stars) and some unyolked ones (cross).

E. The graphic shows the gonadosomatic index by age class of the tunas used in this study.