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Notes on the reproductive biology of southern bluefin tuna *Thunnus maccoyii* in the southwestern Indian Ocean

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Gonad samples of 47 bluefin tuna (SBT), *Thunnus maccoyii,* were collected on board two Taiwanese longline vessels by fisheries observers in areas A (36°–39°S, 30°–42°E) and B (30°–35°S, 67°–73°E) of the southwestern Indian Ocean, from November 12, 2007 to January 15, 2008 and from June 13, 2008 to September 2, 2008, respectively. Morphological and histological observations of the gonad samples were used to determine the sexual maturity of each fish specimen. Ten mature fish (7 females and 3 males) were found. One lean female (fork length = 175 cm; dressed weight = 74 kg), caught at 31°S and 70°E on September 2, 2008, was the only mature fish to possess spent bloodshot flaccid ovaries. It was found in area B which is at least 5,000 km away from the well known SBT spawning ground. The remaining nine mature fish constituted one quarter of the SBT caught in area A. They were non-spawning mature fish with the smallest fork length of 135 cm for the females and 130 cm for the males.

Keywords: atretic oocyte, oogenesis, spent female, size at first maturity, spawning season.

Introduction

The southern bluefin tuna (SBT), *Thunnus maccoyii*, is a highly migratory species and an important exploited resource in the Indian and Pacific Oceans. Its well-known spawning ground is located in the northeastern Indian Ocean between the Sunda Islands and Australia[1,2]. The spawning of SBT mainly occurs from September to March[1,3] and may extend to April[4]. The recent stock assessment by the Extended Scientific Committee (ESC) of the commission for the Conservation of Southern Bluefin Tuna (CCSBT) indicates that the spawning stock of SBT in 2009 remained at a low level of roughly 5%[4]. The ESC suggested that the current, total allowable catch, TAC, should be reduced to rebuild the spawning stock and to lessen the risk of further poor recruitments[4]. Knowledge of the reproductive biology of this species can bolster efforts to assess and manage it.

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Several studies have been conducted on the reproductive biology of SBT. Thorogood[2] investigated the gonad development of female SBT and reported the size at first maturity for this species to be within the fork length (L_F) range of 110–125 cm. Farley and Davis[3] studied the spawning dynamics of SBT and suggested that females of a size (L_F) of less than 140 cm have no or minor gonad development. They found a low number of postspawning females in the spawning ground, and suggested that SBT quickly move off the spawning ground after they have finished spawning. They also found that postspawning fish existed in most months in the Southern Ocean, but they did not provide detailed information on the sampling areas of postspawning fish.

The southwestern Indian Ocean is postulated as the feeding ground for SBT[3], but only limited information is available on the reproductive characteristics of SBT in this area[3]. Using histological techniques and macroscopic examination, we determined the sexual maturity of SBT specimens collected from this area by scientific observers on board two longline vessels. By doing so, the reproductive condition of the SBT around the sampling area in the southwestern Indian Ocean will be understood, and it will also contribute some missing linking information to the previous reproductive biological studies of the species.

Materials and methods

Gonad samples of 47 SBT (25 females and 22 males) were collected from the two areas in the southwestern Indian Ocean by two scientific observers on board Taiwanese longline vessels. The samples from area A (36°–39°S, 30°–42°E) were collected from November 12, 2007 to January 15, 2008, and those from area B (30°–35°S, 67°–73°E) were collected from June 13, 2008 to September 2, 2008 (Fig. 1). The observers recorded the fork length (L_F, in centimeters), dressed (gilled and gutted) body mass (M_D, in kilograms), sex, and the fishing-operation location of each SBT specimen. All gonad samples of SBT specimens were frozen on board and transported back to the mother fishing port, Chiangcheng, Kaohsiung, Taiwan. In the laboratory, the gonad samples of each SBT specimen were weighed to the nearest gram, and several small pieces (< 1 cm^3) of gonad tissue were sub-sampled for histological investigations. Each SBT specimen with an intact paired gonad was used to calculate a gonado-somatic index[5] which can be expressed as

$$I_G = (M_G / L_F^{\,3}) \times 10^4,$$

where I_G is the gonado-somatic index and M_G is the gonad mass of the SBT specimen. Five fish that had incomplete gonads were excluded for calculating I_G.

The preparation of histological sections for the collected gonads followed the previous established method[5]. For each fish, each gonad subsample was first fixed with 10% neural buffered formalin. The formalin-fixed gonad subsample was then dehydrated with a graded series of ethyl alcohol solutions, cleared with xylene, and embedded with paraplast. Using a microtome, each embedded gonad subsample was sectioned to obtain thin slices (6–8 μm). These slices were then stained with Mayer's haematoxylin and eosin. Lastly, all stained slices were mounted on microscope slides using balsam. The histological slices were observed under a light microscope, and images of the slices were taken using a digital camera attached to a microscope. Because the gonad samples had been frozen for some time on board, the histological sections exhibited some degree of oocyte degeneration. In most sections, however, the oocyte development could be staged regardless of this degeneration.

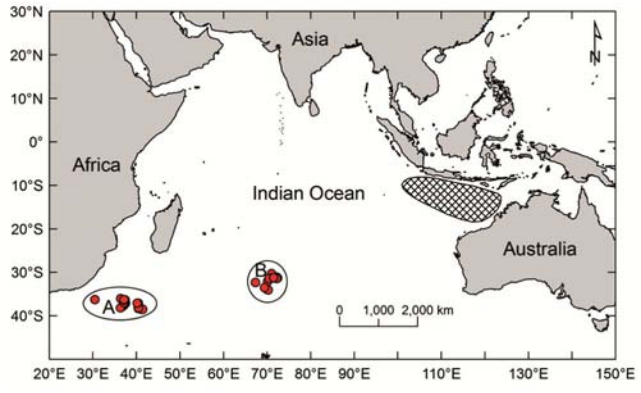

Fig. 1—Locations (solid circles) for collecting gonad samples of southern bluefin tuna, *Thunnus maccoyii*, in area A (36°–39°S, 30°–42°E) from November 12, 2007 to January 15, 2008 and in area B (30°–35°S, 67°–73°E) from June 13, 2008 to September 2, 2008 in the southwestern Indian Ocean. The crosshatching area indicates the spawning ground of southern bluefin tuna, modified from Thorogood (1986).

Follicle (oocyte) development was staged using criteria modified from Sarasquete *et al.*[6], Corriero *et al.*[7], Chen et al.[8], and McMillan[9]. On the basis of the histological examination, six stages were used in this study to describe the follicle development of SBT as follows.

(a) *Primary growth stage*: In this oocyte stage, the oocyte size is small (generally, < 120 μm in diameter); no lipid droplets, cortical alveoli, or zona pellucida exist in the cytoplasm as observed by light microscope.
(b) *Lipid stage*: Lipid droplets and cortical alveoli can be observed in the cytoplasm of the oocyte; no yolk granule is present.
(c) *Early yolked (vitellogenesis) stage*: Small yolk granules are found in the cortical cytoplasm of the oocyte; zona pellucida can be clearly seen.
(d) *Late yolked stage*: Large yolk granules are wholly distributed in the cytoplasm.
(e) *Migratory nucleus stage*: Migration of the nucleus toward the animal pole occurs; lipid droplets fuse to form large droplets.
(f) *Hydrated stage*: A single large yolk plate is present; the nucleus is not seen; one large oil droplet exists.

We determined the sexual maturity of each SBT specimen based on histological or macroscopic examination. In the classification criteria for mature SBT, mature females were those fish possessing atretic yolked oocytes[10] or those that were macroscopically observed to have distinguished spent ovaries. In addition, males were determined as mature fish if they possessed a large volume of whitish lobules.

Table 1—Sample code, sampling date, area, sex (F= female; M= male), size(L_F = fork length in cm; M_D = dressed body mass in kg), gonad mass (M_G in g; n.d.= not determined due to an incomplete goand, gonado-somatic index (I_G), and the method (M= Macroscopic, H = Histological) used for determining sexual maturity for 10 mature southern bluefin tuna (*Thunnus maccoyii*) collected from the areas A and B, southwestern Indian Ocean.

Code	Sampling date	Area	Sex	L_F (cm)	M_D (kg)	M_G (g)	I_G	Method
I-45	Nov. 15, 2007	A	F	150	55	n.d.	n.d.	H
I-42	Dec. 3, 2007	A	F	150	46	281	0.83	H
I-16	Dec. 20, 2007	A	F	141	43	156	0.56	H
I-26	Dec. 31, 2007	A	M	145	46	95	0.31	M
I-47	Dec. 31, 2007	A	M	141	43	161	0.58	M
I-32	Jan. 7, 2008	A	F	138	40	156	0.59	H
I-35	Jan. 7, 2008	A	M	130	30	53	0.24	M
I-38	Jan. 10, 2008	A	F	135	33	92	0.37	H
I-33	Jan. 17, 2008	A	F	152	51	n.d.	n.d.	H
I-09	Sep. 2, 2008	B	F	175	74	373	0.70	M & H

Results

The average size (L_F = 121 ± 18 cm, n = 34) of SBT specimens collected from area A was greater than that (L_F = 113 ± 20 cm, n = 13) of the specimens from area B. Seven females (L_F: 135, 138, 141, 150, 150, 152, and 175 cm) and 3 males (L_F: 130, 141, and 145 cm) were found to be mature fish (Table 1; Fig. 2). No spawning fish possessing hydrated oocytes or postovulatory follicles were found. Nine mature fish (6 females and 3 males) were sampled from area A during the main SBT spawning season, and only one large mature female fish (I-09) with L_F = 175 cm and M_D = 74 kg was sampled from area B (Table 1). From the size frequency distribution for the 47 southern bluefin tuna, the smallest size of maturity were 130 and 135 cm, and 30 and 33 kg for male and female, respectively (Table 2; Fig. 3). More large females were collected than males (Table 2; Fig. 3). The SBT caught in area B of the mid-central Indian Ocean were all young SBT smaller than 122 cm L_F, except for I-09. I-09 was caught at 31°S and 70°E on September 2, 2008 (Table 2). It possessed a pair of flaccid bloodshot spent ovaries (Fig. 2). The macroscopic and histological examinations of I-09's ovaries (Fig. 2) indicated that this fish had finished its spawning activity shortly before it was caught.

Discussion

The size range of mature SBT determined in this study is similar to that of SBT caught in the spawning ground[11-12]. However, it is slightly smaller than that (>140 cm) reported by Farley and Davis[3], and much smaller than Gunn *et al.*'s[13] suggestion that the mean age-at-maturity of SBT is approximately 11 years with a mean L_F of 163 cm from a growth-curve calculation. Nonetheless, the smallest L_F estimates of mature SBT determined in this study are greater than that (110 to 125 cm) suggested by Thorogood[2]. Inconsistent size-at-maturity estimates exist among the previous studies of SBT. Thus, here we provide more information regarding the smallest size of maturity, which can be used in the management of the species.

The fact that mature, non-spawning (reproductively inactive) SBT inhabit the southwestern Indian Ocean during the main spawning season suggests that the fish would migrate to spawn in the later three to four months of the season. This would require an average daily swim speed of around 80 km to reach the traditional spawning ground by April, which historical records indicate to be the end of the spawning season[3]. Patterson *et al.*[14] estimated that a tagged SBT would migrate at an average rate of approximately 80 km d^{-1}, traveling roughly 9,000 km from the western Tasman Sea to its spawning ground in 113 days.

One quarter of the SBT caught in area A were mature fish with potential spawning ability in the coming spawning season. The range of L_F was from 90 to152 cm, with an average L_f of 121 cm, echoing Shiao *et al.*[15] who stated that young SBT with a L_F of around 120 cm were the typical size caught by Taiwanese longline vessels operating in the area. This is the first report in the literature of such a high percentage of mature fish, and is important reproductive information for the management of the resource.

The large spent female (I-09) caught in area B is thought to be a lean fish[12]. In Farley and Davis's paper[3], they used three sub-criteria to determine post-spawning fish. Because only limited information is available on the duration of SBT possessing spent ovaries, we could not ascertain in which month the last spawning activity of I-09 occurred. Furthermore, the sexual maturity of I-09 was determined by macroscopic examination of the gonad because the

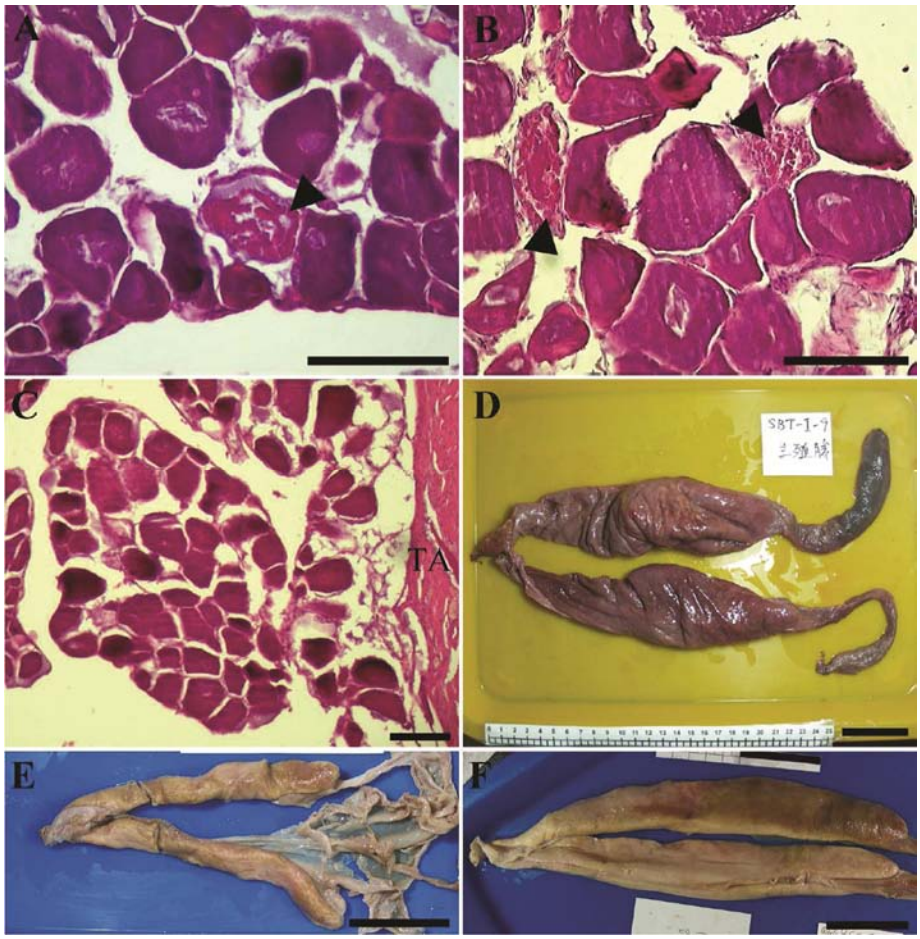

Fig. 2—Images of histological sections and whole gonads for five mature southern bluefin tuna (*Thunnus maccoyii*) in the southwestern Indian Ocean. (A) mature female, Fish code = I-38. Bar = 100 µm. (B) mature female, Fish code = I-16. The arrows point to the atretic yolked oocyte. Bar = 100 µm. (C) spent female (Fish code = I-09) showing that the most advanced group of oocytes underwent the primary growth stage. TA means tunica albuginea (Corriero et al., 2003). Bar = 100 µm. (D) morphological image of the spent ovaries of I-09 (gonad mass = 373 g). Bar = 10 cm. (E) whole testes from mature male, Fish code = I-35. Bar = 5 cm (F) whole testes from mature male Fish, code = I-47. Bar = 5 cm.

Table 2—Size frequency distribution of fork length (L_F) for the 47 southern bluefin tuna (Thunnus maccoyii) sorting by the areas A and B, sex (F=female; M=male) and maturity (I=immature; M=mature) in the southwestern Indian Ocean.

Size interval (L_F in cm)	A-FI	A-FM	A-MI	A-MM	B-FI	B-FM	B-MI	B-MM
<94.9	0	0	5	0	0	0	0	0
95.0-99.9	0	0	0	0	0	0	0	0
100.0-104.9	0	0	0	0	4	0	3	0
105.0-109.9	4	0	2	0	1	0	0	0
110.0-114.9	1	0	0	0	1	0	1	0
115.0-119.9	0	0	1	0	1	0	0	0
120.0-124.9	3	0	2	0	1	0	0	0
125.0-129.9	2	0	2	0	0	0	0	0
130.0-134.9	2	0	1	1	0	0	0	0
135.0-139.9	0	2	0	0	0	0	0	0
140.0-144.9	0	1	0	1	0	0	0	0
145.0-149.9	0	0	0	1	0	0	0	0
150.0-154.9	0	3	0	0	0	0	0	0
>155.0	0	0	0	0	0	1	0	0

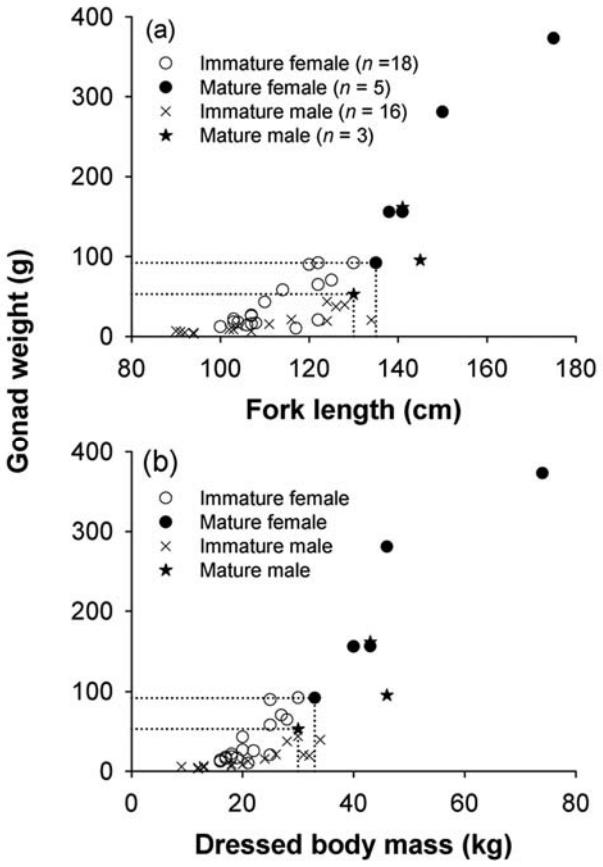

Fig. 3—Gonad weight versus fish dressed body mass for 42 southern bluefin tuna caught in the southwestern Indian Ocean between November 2007 and September 2008. For both males and females, dotted lines indicate the size of the smallest mature fish and the gonad weight.

histological sections provided little evidence of identifiable atretic yolked oocytes (Fig. 2). Possible explanations for this effect are that I-09 had reabsorbed the yolked oocytes, and therefore identifiable atresia of yolked oocytes was not found in the histological sections. This perhaps supports Corriero *et al*.'s[7] finding that northern bluefin tuna *Thunnus thynnus* in the Mediterranean Sea possess no atretic yolked oocytes in February and March, even though they are large enough to be considered as mature fish.

The fact that a large lean spent female (I-09) was caught in area B on 2 September 2008 may imply the extension of the spawning season or the spawning area of the SBT. This occurrence of a spent SBT in the southwestern Indian Ocean is two months earlier than the previous report for spent SBT found in the Tasman Sea[12]. The straight-line distance from the traditional spawning ground to the site of catching I-09 exceeds 5,000 km. This would thus suggest the unlikely situation that spent SBT move off the spawning ground to the feeding ground at a faster swimming speed than has ever been previously reported.

Secondly, if I-09 did not have the ability to swim above the highest speed ever recorded for SBT (i.e., at the unrealistic speed of 100 km d^{-1}), this would suggest that it could not transfer to the site where it was caught after it laid its eggs. Lutcavage *et al.*[16] reported that tagged adult Atlantic bluefin tuna exhibit net horizontal movements of up to 76 km d^{-1} in the Gulf of Maine, northwestern Atlantic. Therefore, it is very unlikely that I-09 would have left the traditional spawning site within *ca.* two months, suggesting that a nearby outer area of the traditional spawning ground, or somewhere else near the site it was caught, may be the spawning site for I-09. However, many publications have suggested that SBT have only a single spawning ground in the Indian Ocean[1-2].

With very limited information, at this point we still cannot draw a firm conclusion to support either of the above hypotheses. However, conclusively, the reproductive characteristics of SBT in the southwestern Indian Ocean were identified as follows: (1) the smallest L_F of mature SBT was determined to be 135 cm for females and 130 cm for males, (2) mature SBT comprised one quarter of the SBT caught in the 36°–39°S, 30°–42°E, southwestern Indian Ocean, from November to January, which is during the main spawning season in the Eastern Indian Ocean, (3) a lean spent SBT found in the mid-nonspawning season in the central Indian Ocean implies a variation of spawning season *or* spawning site for SBT, (4) the SBT caught in the mid-central Indian Ocean in the non-spawning season were all young SBT smaller than 122 cm L_F, except for the one spent female.

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