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Recent progress on reproductive biology of skipjack tuna in the tropical region of the Western and Central Pacific Ocean

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Hiroshi Ashida [1], Toshiyuki Tanabe [2], Nobuhiro Suzuki [3]

[1] Graduate School of Bioscience, Tokai University, Orido, Shimizu, Shizuoka, Japan

[2] National Research Institute of Far Seas Fisheries, Fisheries Research Agency, Orido, Shimizu, Shizuoka, Japan

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[1] Graduate School of Bioscience, Tokai University, Orido, Shimizu, Shizuoka, Japan,
[2] National Research Institute of Far Seas Fisheries, Fisheries Research Agency, Orido, Shimizu, Shizuoka, Japan

Abstract

The reproductive biology of female skipjack tuna, *Katsuwonus pelamis*, in the tropical region of the Western and Central Pacific Ocean from July 2003 to April 2006 was examined. The 701 ovarian samples were collected by purse seine and pole and line fisheries (the number of operations was 152) and were fixed by 10% neutral buffered formalin on board. The ovarian maturity phases classified into five phases based on histological character. Mature individuals appeared at 40cm fork length (FL) and length at 50% maturity estimated 47.9cm FL. The appearance time of the final mature phase which was composed of the migratory nucleus stage or hydrated oocyte in the ovary appeared during the time from15:00 to 21:00. The estimated mean spawning frequency of individuals was estimated 0.50, indicating the spawning intervals of 1.99 days. Batch fecundity (BF) was estimated by counting the number of advanced oocytes with diameter≥0.5 mm at the final mature phase. The mean BF and relative BF were 615,000 oocytes and 147.8 oocytes per gram of somatic weight, respectively. Most gonad index classes included from two to four maturity phase. The mature fish and spawning fish were observed all year around and the seasonal change of maturity phase was not observed in the tropical region of Western and Central Pacific Ocean.

Introduction

The skipjack tuna *Katsuwonus pelamis* is known to multiple spawning fish and main spawning grounds in the western and central Pacific Ocean is the tropical area (Matsumoto et al. 1984). In the past studies of reproductive biology of skipjack tuna in the tropical region of western and central Pacific Ocean, the ovarian maturity phase was defined based on various method such as morphological observation (Marr 1948, Wade 1950), distribution of oocyte diameter (Brock 1954, Bũnag 1956, Yoshida 1964) and the gonad index (Naganuma 1979). But the ovarian maturity phase based on these methods was insufficient for grounds of histology or physiology.

In recent studies on reproductive biology of tunas, ovarian maturity phase was defined by using histological method (Hunter et al.1986, Schaefer 1987, 1996, 1998, Schaefer et al. 2005). The histological method is generally used for reproductive biology of many marine species and it is easy to assess the reproductive states of individuals correctly. However, if this technique would be applied, a large number of gonad samples which were fixed by formalin or Bouin's solution freshly were necessary. Frozen gonad samples should not be recommended applying for histological method, because it is difficult to judge postovulatory follicles and developmental stage of oocyte.

Although Asano and Tanaka (1971) was defined ovarian maturity phase based on the developmental stage of oocyte using histological observation in the tropical region of western Pacific Ocean, this maturity phase is not considered for the importance of histological character such as postovulatory follicles or atresia (atretic follicles). Hunter et al. (1986) observed degenerating process of postovulatory follicle and atretic oocyte, defined the ovarian maturity phase which was considered for postovulatory follicle and atresia and applied for 87 individuals which was caught in the South Pacific Ocean.

Since the study of Hunter et al. (1986), the reproductive biology using histological method of skipjack tuna in the Pacific Ocean was not found. Furthermore, batch fecundity or spawning frequency was not estimate. The objective of this study is to define the ovarian maturity phase based on histological observation and to provide information on the reproduction of female skipjack tuna in the tropical region of the western and central Pacific Ocean. The details were described in Ashida et al. (2007) and Ashida et al. (2008).

Materials and Methods

Sampling and collection

A total of 701 female skipjack tuna were colleted by purse seine and pole and line fisheries (the number of operations was 152) in the tropical region of the western and central Pacific Ocean during July 2003 through April 2006 (Figure 1). Specimens were measured fork length (FL, cm), body weight (BW, kg) and after the ovary (GW, g) were weighted, it was cut off central portion of left or right ovary randomly and was fixed by 10% neutral buffered formalin on board.

Histological method

Ovarian samples were cut into the small pieces and they were dehydrated by ethanol, embedded in paraffin wax, sectioned at 6-8μm, stained with Mayer's hematoxylin and 1% eosin (HE stains) and observed under a light microscope. Developmental stage of oocyte was defined as pre-nucleus, yolk vesicle, early yolked, late yolked, migratory nucleus, hydrated and postovulatory follicles followed to Hunter and Macewicz (1985), Hunter et al. (1986) and Schaefer (1998) (Figure 2). The degenerating process of atresia was defined as α atresia (degenerate yolked oocyte) and β atresia followed to Hunter and Macewicz (1985) and Hunter et al. (1986) (Figure 3).

Ovarian maturity phase

The ovarian maturity phase was defined as immature, active mature, final mature, post-ovulated, inactive mature (Table 1, Figure 4), which modified maturity stage of Hunter and Macewicz (1985), Hunter et al. (1986), Schaefer (1998).

Using this maturity phase, we evaluated the individual reproductive condition, length at first maturity and 50% maturity, spawning time, spawning frequency, batch fecundity, correspondence of GI to ovarian maturity phase and spawning season.

Result and Discussion

Length at maturity

Mature fish was defined as an individual which was categorized as active mature, final mature, post-ovulated and inactive mature phases. The proportion of mature fish in each 2 cm FL interval was calculated. The mature fish began to appear at 40–42cm FL class and proportion of mature fish increased with larger FL classes. Both the length at first maturity and spawning individuals were 40.0cm FL (ashida et al. unpublished data). Relationship between proportion of mature fish and fork length was described following logistic equation and length at 50% maturity estimated 47.9cm (Figure 5).

$$\mathrm{P(FL)} = \frac{1}{\left[1 + Exp\left(12.0117 - 0.25114 \times FL\right)\right]}$$

Thus, we concluded that the length at first maturity in the topical region of western and central Pacific Ocean was 40 cm FL.

Spawning time

The final mature phase (i.e. migratory nucleus oocyte or hydrated oocyte present in the ovary) disappeared during 6:00 through 15:00, while it appeared during 15:00 through 21:00 (Figure 6). Therefore, we concluded that the final maturation process of oocyte for skipjack tuna have a diurnal rhythm and spawn at night.

There is two hypothesis about the spawning time of skipjack tuna in the past studies (Hunter et al. 1986, Iverson et al. 1970, Matsumoto et al. 1984). The spawning behavior during the day was observed by some fisherman and researcher (Iverson et al. 1970, Matsumoto et al. 1984), while Hunter et al. (1986) reported that the migratory nucleus oocyte which indicates state of the imminent spawning appeared in 19:55. In the yellowfin tuna *Thunnus albacares* and bigeye tuna *Thunnus obesus,* the spawning occurred at mid night because of migratory nucleus stage and hydrated oocyte appeared from evening to mid night (Nikaido et al. 1991, Schaefer 1996, 1998). The diurnal rhythm and spawning time of skipjack tuna are similar to these tuna.

Spawning frequency

Spawning frequency is known as one of the indices assessing for potential of spawning fish and it is essential for understanding reproductive biology of multiple spawning fishes. In this study, mean spawning frequency and spawning interval were estimated 0.50 and 1.99 per days in consideration for duration time of postovulatory follicles.

Individuals which were categorized for final mature phase had postovulatory follicles in the ovary (Figure 7). Hunter et al. (1986) reported that the postovulatory follicles observed 24h after spawning at 24ºC. Ashida et al. (2008) indicated that postovulatory follicles might degenerate less than 24h after spawning based on histological observation of postovulatory follicles. Individuals of final mature phase with postovulatory follicles indicated that a part of spawning fish may spawn continuously 2 days at least.

Batch fecundity

Batch fecundity (BF) was estimated by individuals which were categorized final mature phase by counting number of oocytes at the most advanced oocyte diameter group (oocyte diameter≥ 0.5mm). The mean batch fecundity and relative batch fecundity of skipjack tuna in the tropical region of western and central Pacific Ocean estimated 615,000 oocytes and 147.8 oocytes / g. Relationship between batch fecundity and fork length or somatic weight were describe as following equations (Figure 8).

$$BF = 22304 \times FL - 661391 \quad (r = 0.67)$$

$$BF = 86.3 \times SW + 225940 \quad (r = 0.64)$$

$$SW = BW - GW$$

In the yellowfin tuna and black skipjack tuna *Euthynnus lineatus,* it was reported that estimated batch fecundity is different in each sampling area or period (Schaefer 1987, 1998). To examine differentiation of batch fecundity for skipjack tuna in the tropical region of western and central Pacific Ocean, the ovarian samples with final mature phase need to collect in various areas and periods.

Relationship between ovarian maturity phase and Gonad index

The mean gonad index ± standard division in immature, active mature, final mature, post-ovulated and inactive mature phases was 1.20 ± 0.62, 3.35 ± 1.33, 7.27 ± 1.84, 5.01 ± 1.53 and 2.34 ± 0.55, respectively. Although the gonad index increased with the developmental phase of ovaries, the indices between active mature and inactive mature had no significant difference (t-test $p>0.05$). In composition of ovarian maturity phase at gonad index class (one interval), two or more ovarian maturity phases were included in the same gonad index class (Figure 9).

Although gonad index used for evaluating reproductive condition of skipjack tuna in the western and central Pacific Ocean (Naganuma 1979), it was suggested that the ovarian maturity phase should not be distinguished by only gonad index in this study.

Spawning season

The spawning individuals with final mature and post-ovulated phases appeared all year around in the tropical region of western and central Pacific Ocean. It was not observed obvious seasonal change of ovarian maturity composition (Figure 10). Ueyanagi (1969) and Nishikawa et al. (1985) reported that the skipjack tuna larva appeared all year around in the tropical region of western and central Pacific Ocean. Therefore, it was concluded that skipjack tuna spawn all year around in the area.

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Table 1. Histological character of ovarian maturity phase of skipjack tuna in the tropical region of western and central Pacific Ocean. (From Ashida *et al.* 2008)

Ovarian maturity phase	Histological character
Immature	No yolked oocyte (pre-nucleus stage and yolk vesicle stage) present.
Active mature	Yolked oocyte (early or late yolked oocyte stage) present but no postovulatory follicles. If alpha atresia of yolked oocyte is present, less than 50% of the yolked oocyte are in alpha atresia of yolked oocyte.
Final mature	Migratory nucleus oocyte stage or hydrated oocyte stage present. Postovulatory follicles may be present.
Post-ovulated	Postovulatory follicles and yolked oocyte present.
Inactive mature	Yolked oocyte present but no postovulatory follicles. 50% or more of yolked oocyte are in the alpha atresia of yolked oocyte.

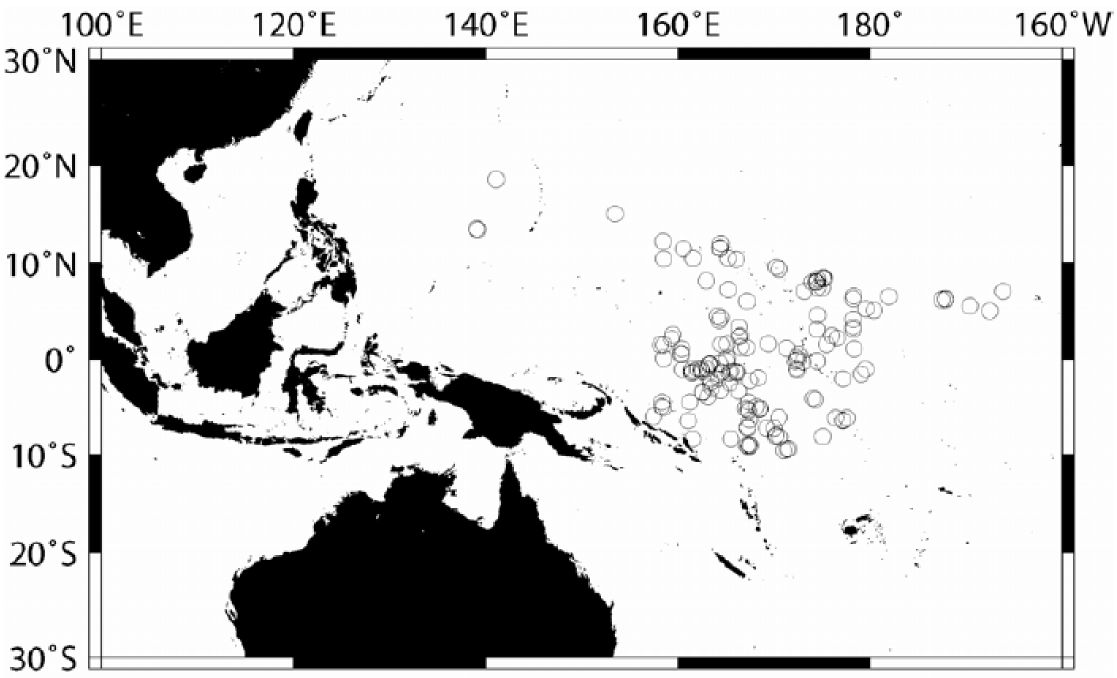

Figure 1. Sampling location (circles) of female skipjack tuna in the tropical region of western and central Pacific Ocean (edited from Ashida et al. 2008).

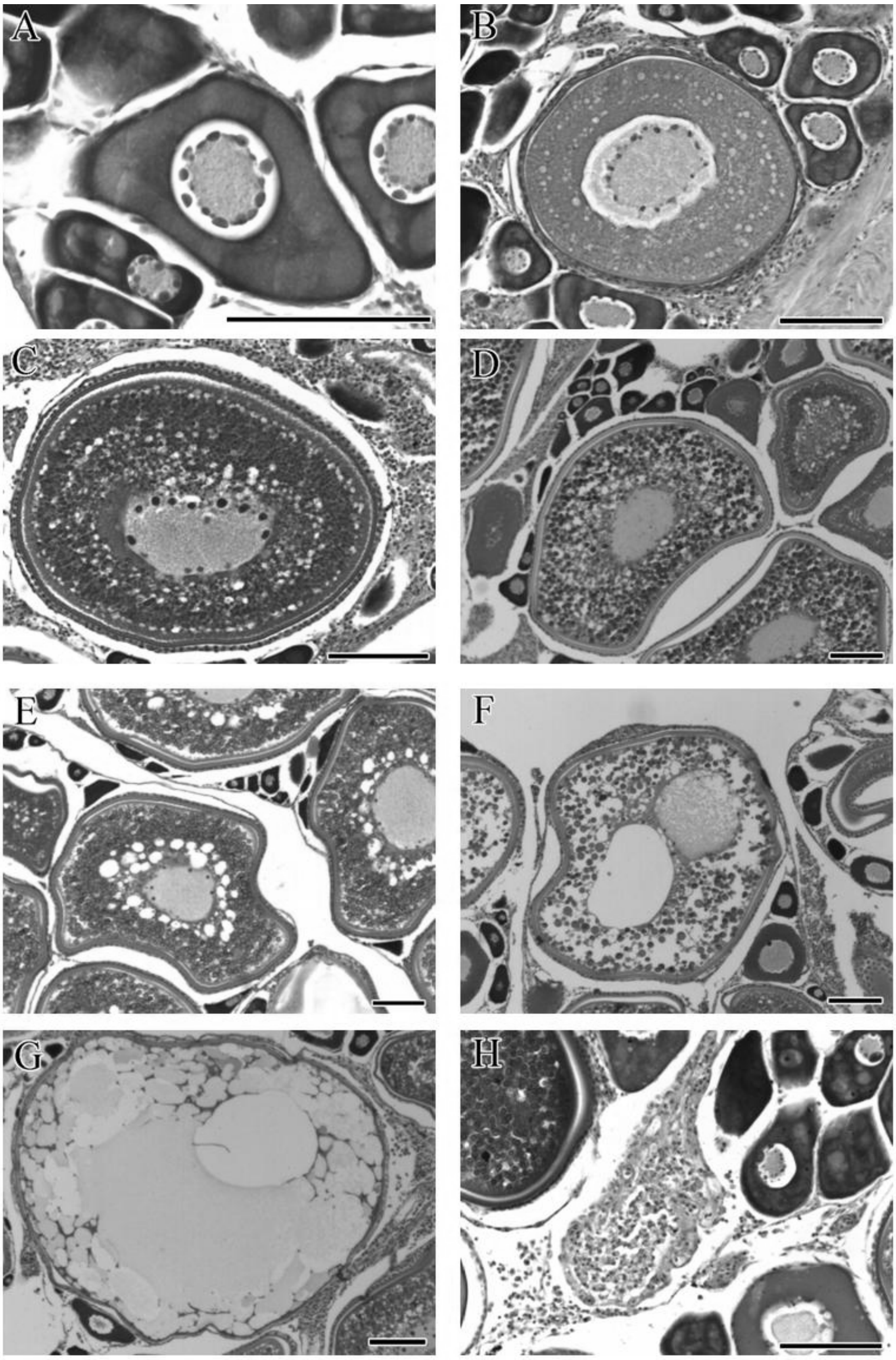

Figure 2. Developmental stage of oocyte in the skipjack tuna. Scale bars were 100µm. A, Prenucleus stage; B, Yolk vesicle stage; C, Early yolked oocyte stage; D,E, Late yolkd oocyte stage; F, Migratory nucleus stage, G; Hydrated oocyte, H; Postovulatory follicles

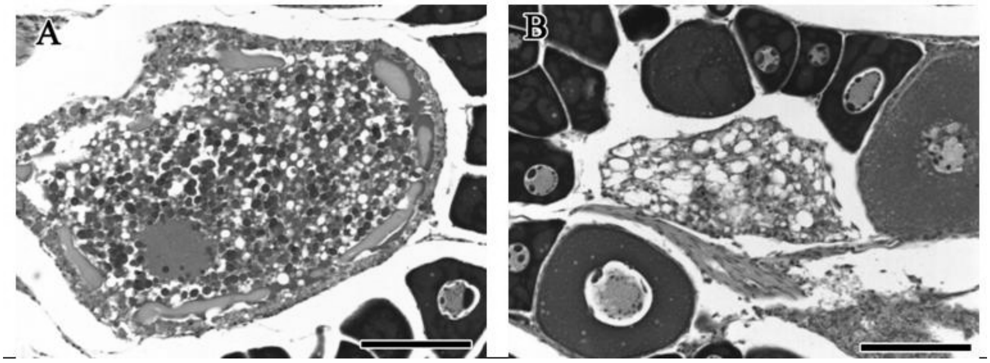

Figure 3. Degenerating stage of atresia in the skipjack tuna. Scale bars were 100μm.
A, α atresia; B, β atresia

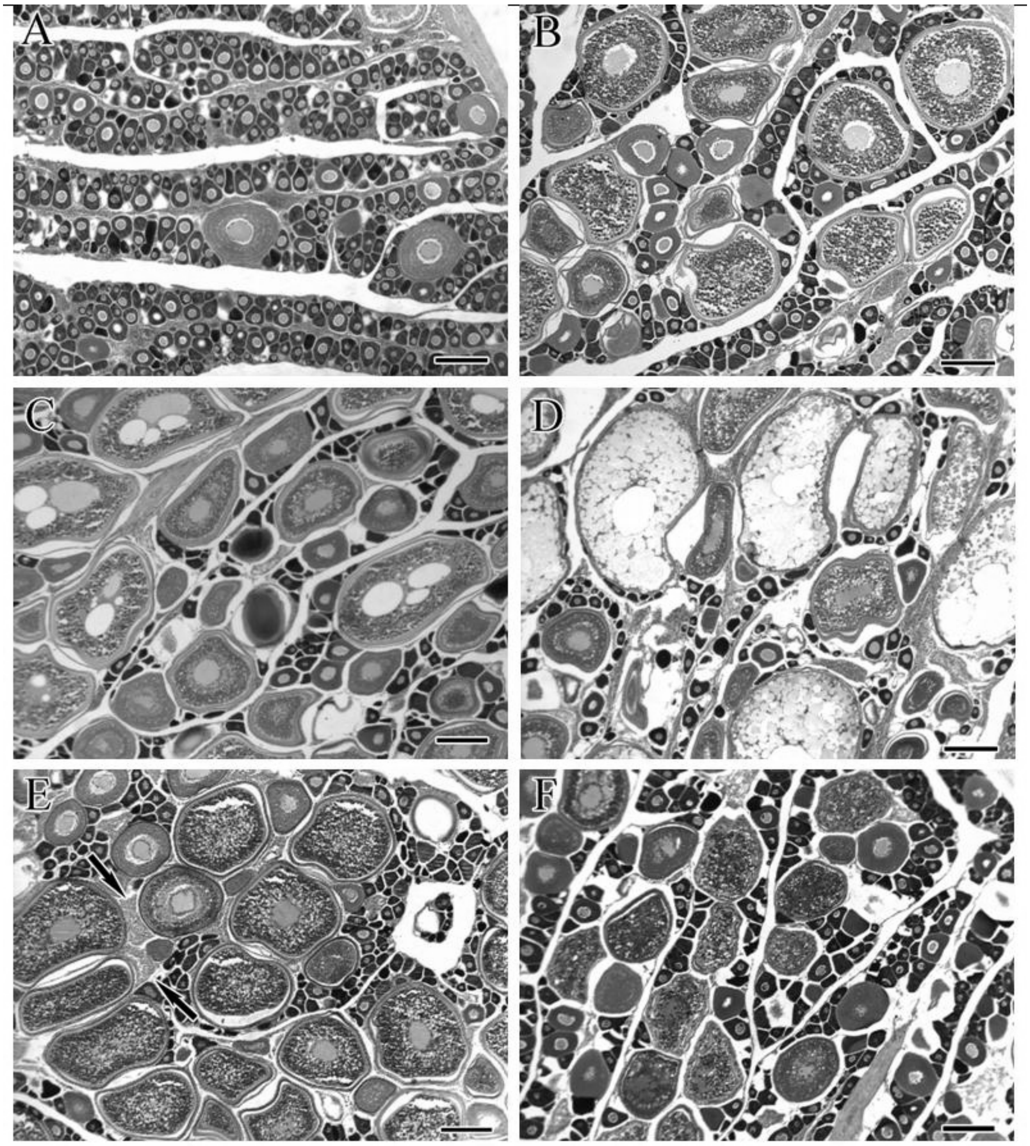

Figure 4. Photographs of the histological character in each ovarian maturity phase of skipjack tuna. Scale bars were 200μm. A, Immature phase; B, Active mature phase; C,D, Final mature phase; E, Post-ovulated phase; F, Inactive mature phase. Arrows indicate postovulatory follicles.

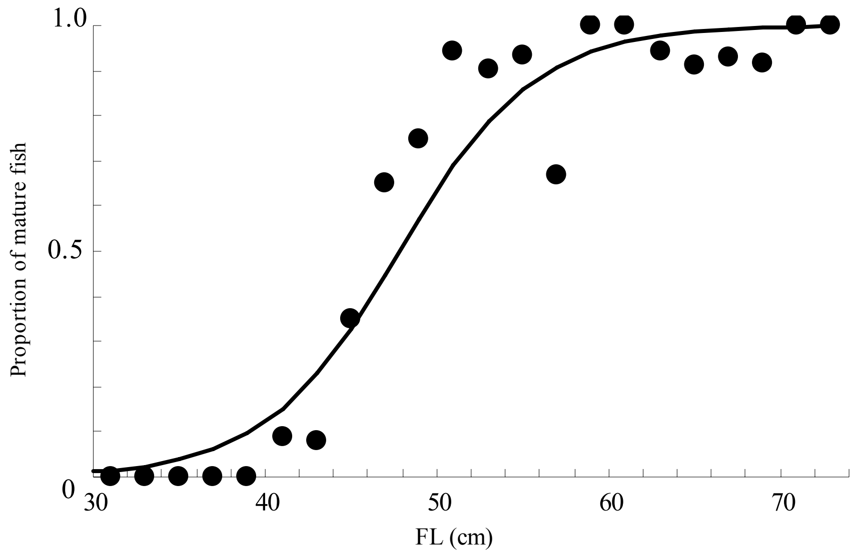

Figure 5. Relationship between fork length class (2cm intervals) and proportion of mature fish of skipjack tuna in the tropical region of western and central Pacific Ocean. (From Ashida *et al.* 2007)

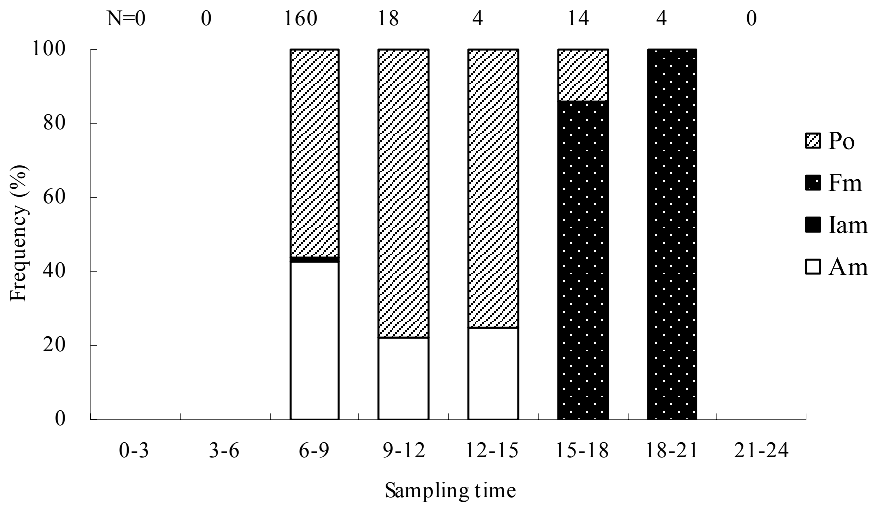

Figure 6. Daily change in ovarian maturity phase (excluding immature phase) of skipjack tuna in the tropical region of western and central Pacific Ocean (From Ashida *et al.* 2007). Am, Active mature phase; Fm, Final mature phase; Iam, Inactive mature phase; Po, Post-ovulated phase

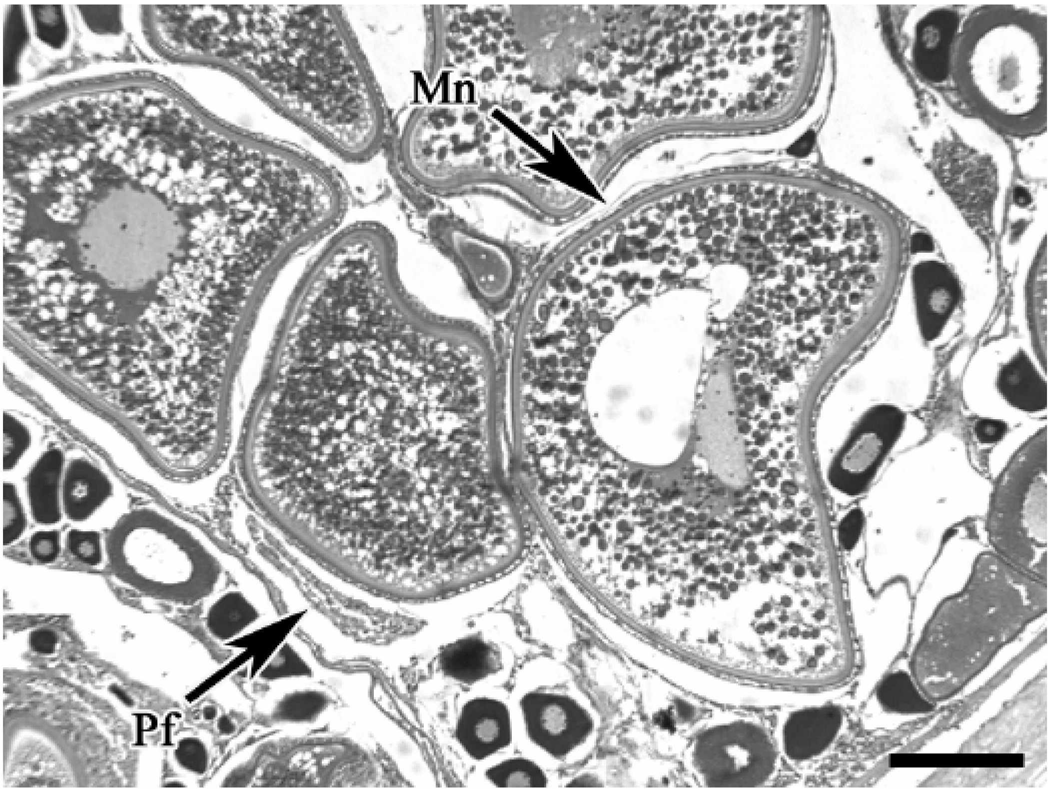

Figure 7. Photograph of the final mature phase of skipjack tuna ovary. Arrows show postovulatory follicles in the ovary of a skipjack tuna. Scale bar was100μm (From Ashida *et al.* 2008). Mn, migratory nucleus-stage oocyte; Pf, postovulatory follicles.

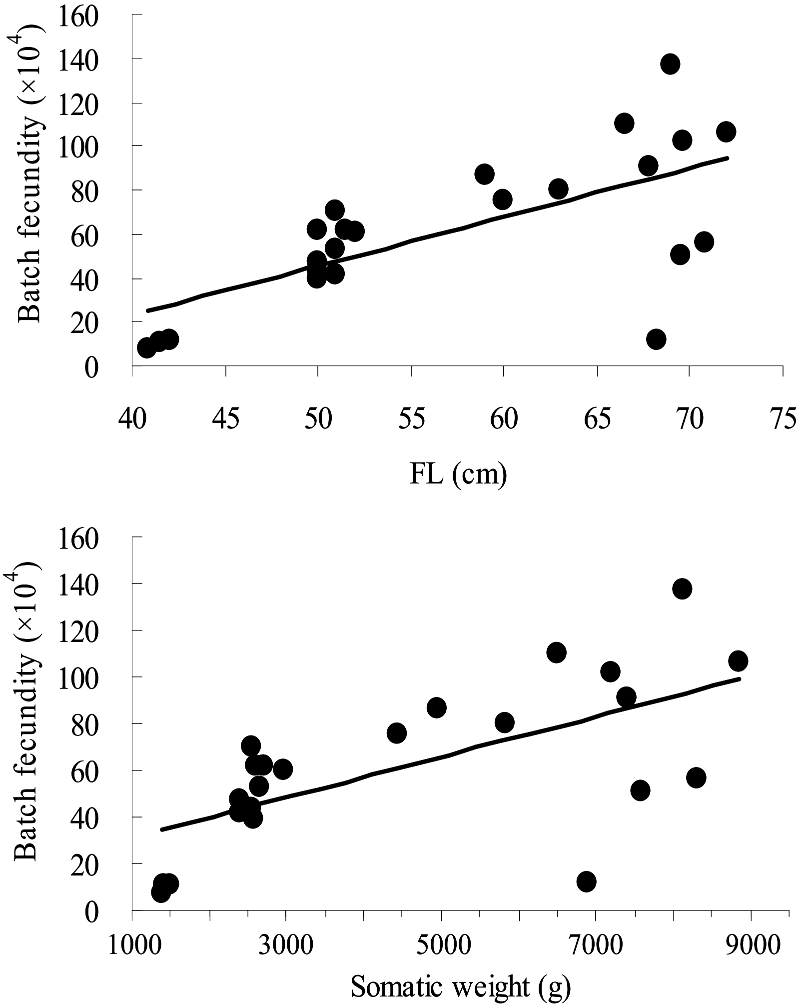

Figure 8. Relationship between batch fecundity and fork length (upper), somatic weight (lower) of skipjack tuna (From Ashida *et al.* 2008). Somatic weight indicates body weight which excludes ovarian weight.

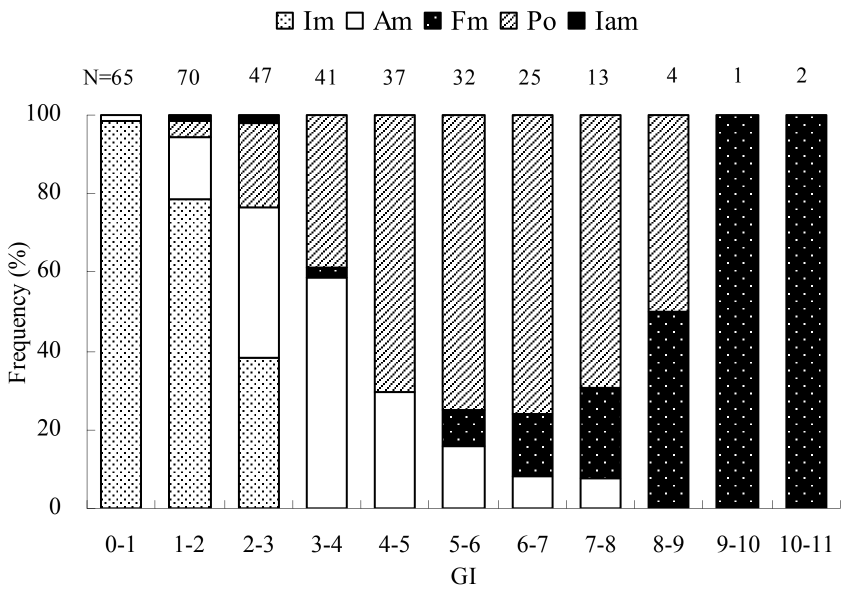

Figure 9. Relationship between gonad index (GI) class and ovarian maturity phase of skipjack tuna in the tropical region of Western and Central Pacific Ocean (From Ashida et al. 2007). Am, Active mature phase; Fm, Final mature phase; Iam, Inactive mature phase; Im, Immature phase; Po, Post-ovulated phase.

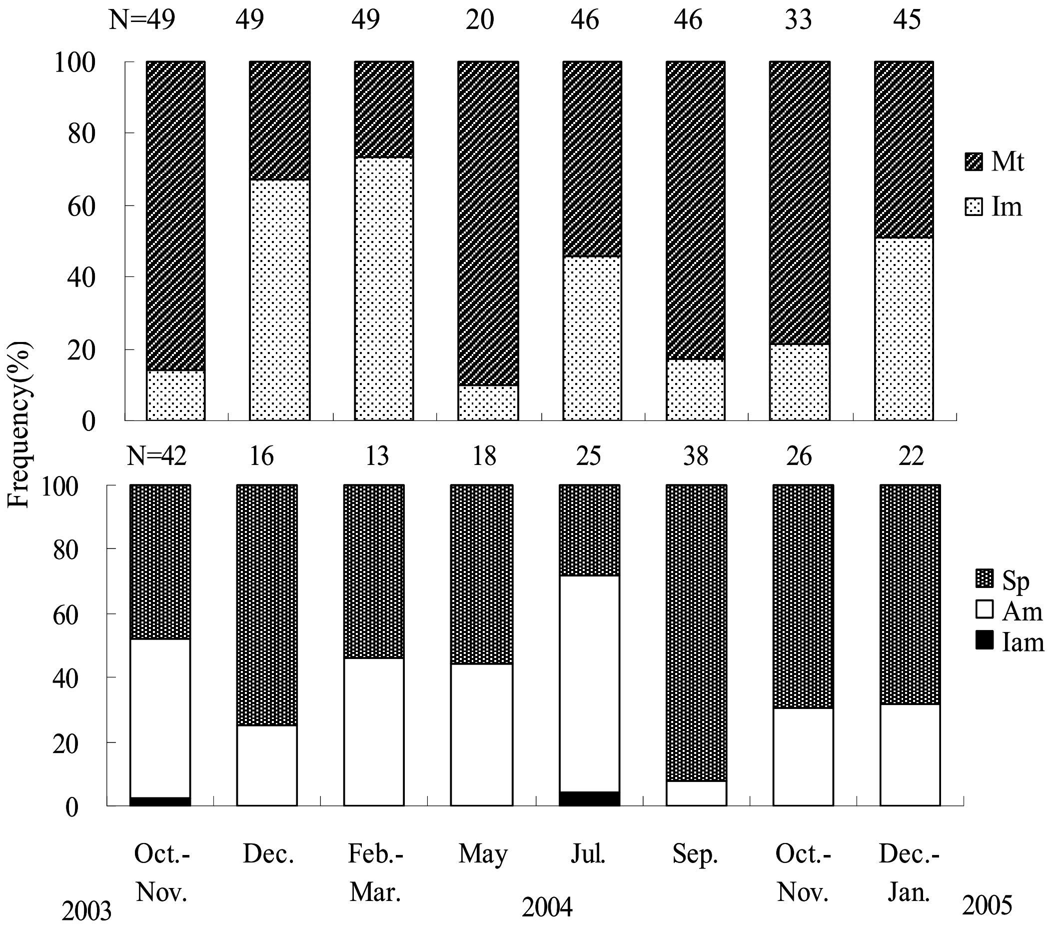

Figure 10. Monthly change in frequency of occurrence of mature (upper) and spawning phase (lower) of female skipjack tuna caught in the tropical region of the Western Central Pacific Ocean in 2003－2005 (edited by Ashida et al. 2007). Am, Active mature phase; Fm, Final mature phase; Iam, Inactive mature phase; Im, Immature phase; Mt, Mature fish (combined Am, Fm, Po and Iam); Po, Post-ovulated phase; Sp, Spawning fish (combined Fm and Po).