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Abstract

Spawning activity of male skipjack tuna *Katsuwonus pelamis* was examined from May 2005 to December 2007 in the tropical western and central Pacific Ocean. Testis maturity was classified into five stages (i.e., immature, early maturation, mid maturation, late maturation, and regression) on the basis of histological observation. The estimated minimum size at first maturity was 35.5cm FL and the size at 0.5 maturity (FL₅₀) was 40.7 cm in FL. It is difficult to clearly differentiate the testis maturity stage based on the gonad index (GI) class only, because each GI class contained mature fish. Monthly changes in GI did not follow a seasonal cycle. Mature individuals dominated throughout the year. These results imply that male skipjack tuna in this area showed evidence of spawning activity throughout the year without a clear seasonal pattern.

Introduction

The skipjack tuna *Katsuwonus pelamis* is known as a highly migratory fish and is distributed from tropical to subtropical waters (Collette and Nauen 1983). In the western and central Pacific Ocean, some populations seasonally migrate to the temperate zone (Matsumoto et al. 1984). Skipjack tuna spawn multiple times in areas where the sea surface temperature is higher than 24°C (Matsumoto et al. 1984). In the tropical region of the Pacific Ocean, larval skipjack tuna are distributed more abundantly in the western and central regions than in the eastern region (Matsumoto et al. 1984, Ueyanagi 1969). Although skipjack tuna in the tropical western and central Pacific Ocean are estimated to spawn throughout the year on the basis of histological analysis of ovaries (Ashida et al. 2007, 2008) and larval occurrence (Nishikawa et al. 1985, Ueyanagi 1969), basic reproductive information on male skipjack tuna in this region (such as minimum size at sexual maturity) is still unknown. To resolve the reproductive information of male is need to interpret the reproduction of skipjack tuna in the tropical western and central Pacific Ocean.

Previous studies on the reproductive biology of male skipjack tuna based on morphological observations of testis or gonad index (GI) criteria (Marr 1948, Batts 1972, Naganuma

1979) are inadequate for evaluating the reproductive status of males. Furthermore, the histological observation was used for reproductive biology in other tunas as this method provides precision for reproductive status (Schaefer 1996, 1998, 2001, Abascal et al. 2004).

The objectives of this study were (A) to define the classification of the testis maturity in male skipjack tuna on the basis of histological observation; (B) to compare between defined testis maturity and gonad index and (C) to clarify the reproductive information such as the length at maturity, reproductive cycle of male skipjack tuna in the tropical western and central Pacific Ocean.

Materials and methods

Testes sampling

A total of 316 male skipjack tuna were collected by purse seine, pole and line, and trolling in the tropical western and central Pacific Ocean (21°57'N–6°39'S, 136°50'E–176°42'E) from May 2005 to December 2007 (Fig. 1, 2). The fork length (FL, cm) and body weight (BW, kg) of specimens were measured on board. The gonad weight (GW, g) was weighed to the nearest 1g and a portion from the middle of the left or right testis was fixed in 10% neutral buffered formalin.

Histological analysis

The testis samples, including the main sperm duct and the peripheral tunica just under the main sperm duct, were cut into small pieces. These pieces were dehydrated in ethanol, embedded in paraffin wax, sectioned at 6–8µm thickness, stained with Mayer's hematoxylin and 1% eosin and observed under a light microscope.

Testis structure and classifications of the testis maturity

The testis structure was judged according to Grier (1993). The developmental stages of germ cells were distinct as spermatogonia, spermatocytes, spermatids, and sperm as detailed in Abascal et al. (2004) and Schaefer (2001). The testis maturity stages in this study were categorized on the basis of a modified classification of Grier (1993), Grier and Taylor (1998) and Taylor et al. (1998) as immature, early maturation, mid maturation, late maturation, and regression (see Result section).

Length at maturity

Mature fish were defined as individuals in the mid maturation, late maturation, or regression stages, according to Brown-Peterson et al. (2002) and Taylor et al. (1998). The proportion of mature fish in each category of 2-cm FL interval was calculated. The following logistic equation was fitted using a nonlinear regression method and parameters a and b were estimated from a Kaleida graph (ver. 3.50J, Synergy Software, PA):

$$P(FL) = 1 / [1 + \text{Exp}(a - b \times FL)].$$

Gonad index

The gonad index (GI) was calculated using the following equation from Naganuma (1979):

$$GI = GW / FL^3 \times 10^4.$$

Results

Testis structure

The testis of the skipjack tuna is bilobed and the main sperm duct is located in the hollow of the testis. Lobules were observed radiating from the main sperm duct to the peripheral tunica and the presence of spermatogonia was unrestricted in the testis. In these characteristics, the skipjack tuna testis structure was classified as the lobular testis type (Grier 1993).

Testis maturity stage

The immature stage was defined by spermatogonial renewal and spermatogonial proliferation in the testis. Only spermatogonia were present and the lobules were lined with a continuous germinal epithelium. Spermatocysts of spermatocytes, spermatids and sperm were absent.

The early maturation stage was characterized by the occurrence of spermatogenesis throughout the entire lobules. As a result of meiosis, spermatocysts of spermatocytes and spermatids were formed in the testis. The germinal epithelium was continuous throughout the entire lobules. Thirty-five of 37 the specimens that were categorized as being in the early maturation stage were observed to have sperm in the lobular lumen or main sperm duct.

The mid maturation stage was characterized by morphological changes in the germinal epithelium. The continuous germinal epithelium changed to be discontinuous in the proximal part of the lobules near the main sperm duct as a result of developing sperm in spermatocysts and release of mature sperm in the lobular lumen. At the periphery of the testis, lobules were still lined with continuous germinal epithelium. When the germinal epithelium changed from continuous to discontinuous, they were fused with neighboring lobules and formed an anastomosing network. Sperm were present in the lobular lumen and main sperm duct.

The late maturation stage was characterized by active production of sperm in the entire lobules. The germinal epithelium was discontinuous in the entire lobules. The lobule became enlarged and the fusion of lobule became more complex. The main sperm duct and lobular lumen were filled with sperm.

The regression stage was characterized by the cessation of sperm production and only spermatogonia being present in the lobules as germ cells. The regression stage in skipjack tuna was

also represented by a thick lobule compartment, and tunica, and by the occurrence of residual sperm in the lobular lumen and main sperm duct.

Length at maturity

In total, 260 of 316 specimens were judged to be mature. Mature specimens first appeared in the category of 34–36 cm FL, and the proportion of mature fish tended to increase with increasing FL class. All specimens in size classes larger than 64–66 cm FL were mature. The relationship between the proportion of mature fish and FL was described by the following logistic equation:

$$P(\text{FL}) = 1/[1 + \text{Exp}(31.665 - 0.774 \times \text{FL})] \quad (R^2 = 0.945).$$

The minimum size at sexual maturity was 35.5 cm FL and the length at 0.5 maturity (FL_{50}) was estimated to be 40.7 cm FL (Fig. 3).

Relationship between gonad index and testicular maturity stage

Changes in the mean GI at each maturity stage are shown in Fig. 4. The mean GI increased as the testis maturity progressed, peaking in the mid maturation stage (Fig. 4, Scheffé's F -test, $P < 0.05$) and it decreased in the late maturation and regression stages respectively. The composition of maturity stages by GI class, the frequencies of occurrence of immature, early maturation, and regression stage were 19.4%, 26.5% and 6.1%, respectively, in the 0–1 GI class and these values tended to decrease with increasing of GI class (Fig. 5). The occurrence of the mid maturation stage was 22.4% in the 0–1 GI class and it tended to increase with increasing GI class (Fig. 5). All specimens in the 9–10 and 10–11 GI classes were mid maturation. The late maturation stage appeared in 25.5% of specimens in the 0–1 GI class, peaked value at 2–3 GI class (50.0%). The occurrence of late maturation in the 4–5 GI class or more tended to decrease with increasing GI class (Fig. 5).

Monthly changes in the gonad index, and maturity stage

Monthly changes of GI and maturity stage composition were analyzed using fish, which were the minimum size of maturity or larger and pooled for each year. Although the value of GI was highest in November and was lowest in March, the mean GI changed randomly throughout the year (Fig. 6). Although mid maturation and late maturation stages appeared at frequencies 12.5–64.0% and 18.2–63.2% throughout the year, respectively, the occurrence of immature, early maturation, regression stages was limited and did not show a clear pattern (Fig. 7).

Discussion

The minimum size at sexual maturity in this study was smaller than that reported in previous studies. In previous studies, the size at first maturity of male skipjack tuna was estimated as

43.5 cm FL in the waters off North Carolina of the Atlantic Ocean (Batts 1972), 40 cm FL in the western Pacific Ocean (Naganuma 1979), and 40–42 cm FL and 39 cm FL in the western Indian Ocean (Stéqueurt and Ramcharrun 1996, Timohina and Romanov 1996). The differences in size at first maturity estimates between this study and past studies can be explained by differences in the methods used to classify the maturity stage (i.e., histology, morphology, and description according to GI).

The minimum size at sexual maturity and the FL₅₀ of male skipjack tuna in the tropical western and central Pacific were smaller than the respective estimated sizes for females (size at first maturity, 40.0 cm FL; FL₅₀, 48.9 cm; Ashida et al. 2007). The estimated ages at FL₅₀ of both sexes of skipjack tuna were also younger than the 0.9 and 1.1 years, respectively, predicted using the growth curve of Uchiyama and Struhsaker (1981). In yellowfin tuna the FL₅₀ of males (reported as 69 cm) was smaller than females (reported as 92 cm) (Wild 1986), and the ages at these sizes were estimated at about 1.5 and 2.0 years, respectively, using the growth curve of Wild (1986). Furthermore, a study of captive Pacific bluefin tuna *Thunnus orientalis* reported that only males matured at an age of 2+ years with female maturing at an older age (Sawada et al. 2007). The results of the present study lead us to conclude that male skipjack tuna mature at a younger age, with a younger age at FL₅₀, compared with female skipjack tuna as found in other tuna species.

It was difficult to judge the maturity stage from the GI because many maturity stages that were differentiated on physiological criteria were grouped into low GI classes (Fig. 8). Although Naganuma (1979) defined testis maturity using a description of the GI that included “ripe fish” being defined as individuals with a GI of 8 or more, this classification of testis maturity had no physiological foundation. Furthermore, fish classified as mature on the basis of histological observations in this study were placed in low GI classes (e.g., 0–1 or 1–2). This phenomenon suggested that male with a low GI can be reproductively active.

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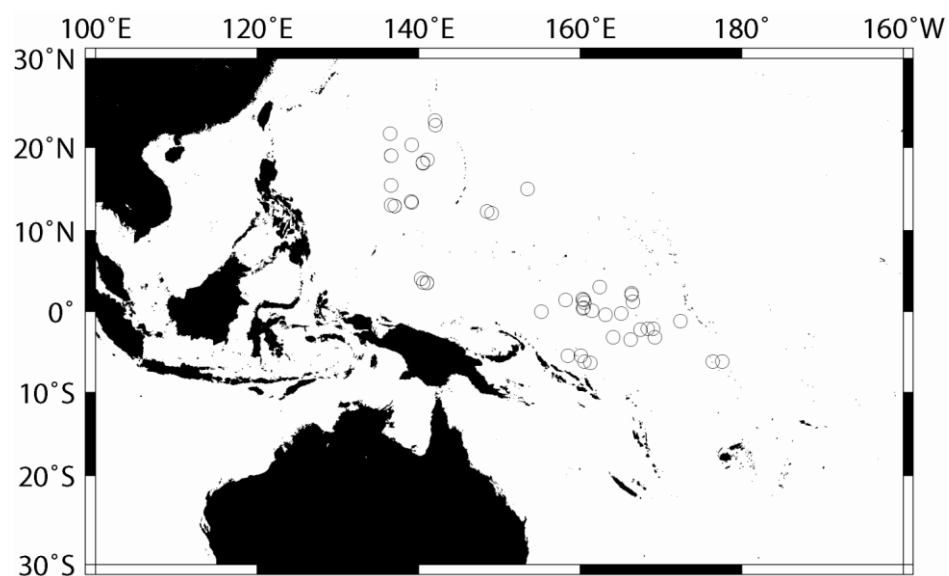


Fig. 1 Sampling locations of skipjack tuna in the tropical western and central Pacific Ocean between 2005 and 2007.

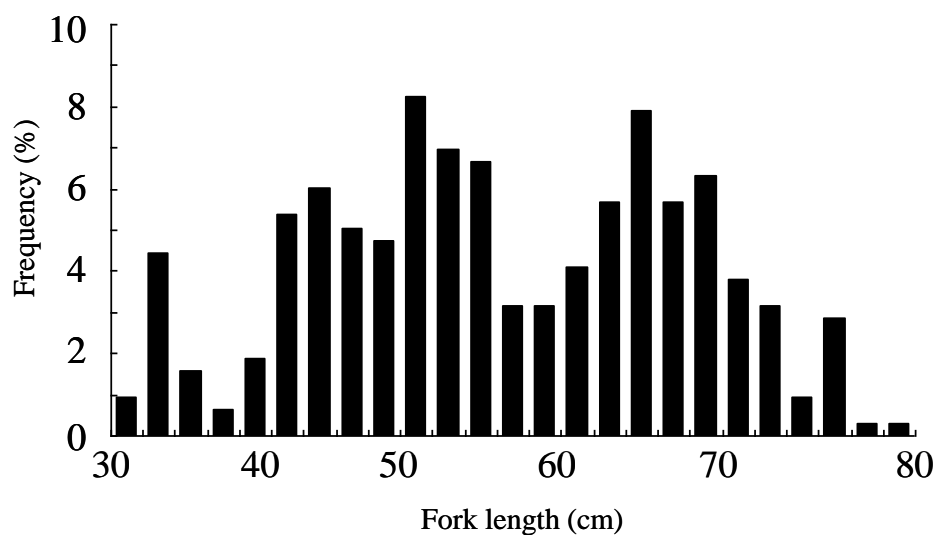


Fig. 2 Size frequency distribution of skipjack tuna collected in the tropical western and central Pacific Ocean.

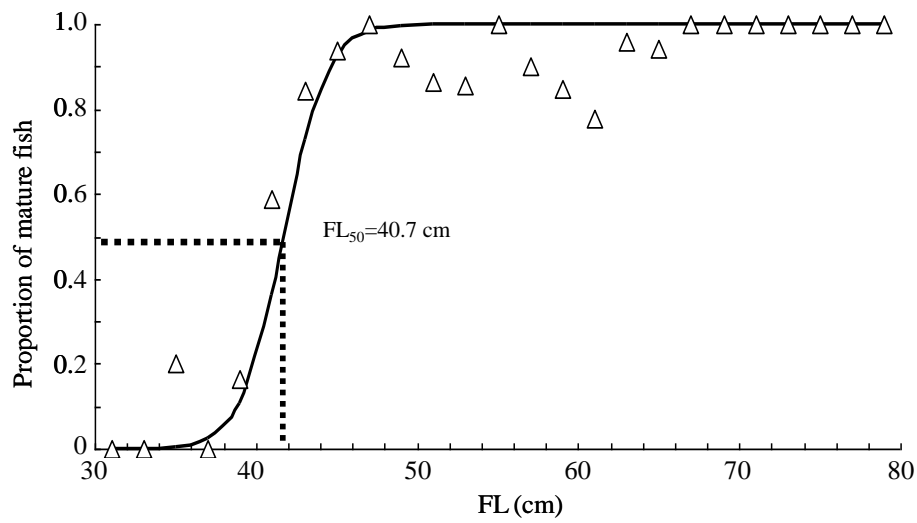


Fig. 3 Relationship between fork length and proportion of mature individuals from the tropical western and central Pacific Ocean.

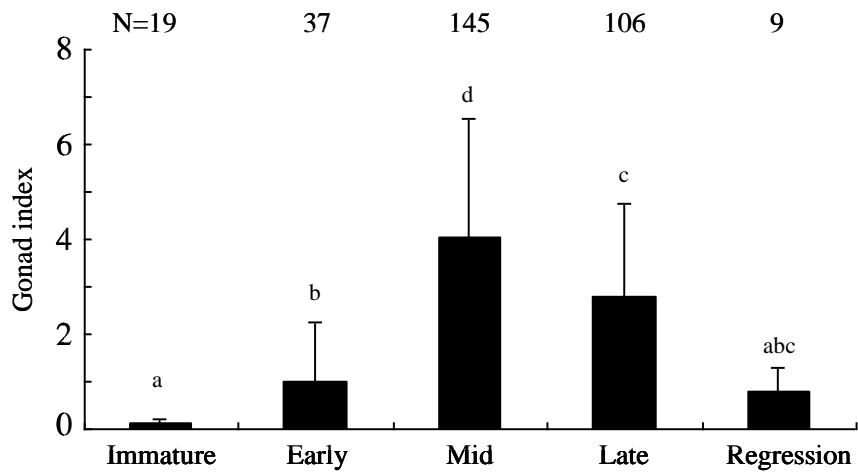


Fig. 4 Mean gonad index in each maturity stage of skipjack tuna in the tropical western and central Pacific Ocean.

Vertical bars indicate standard division. The same letter described no significant difference ($P > 0.05$). Early; Early maturation, Mid; Mid maturation, Late; Late maturation

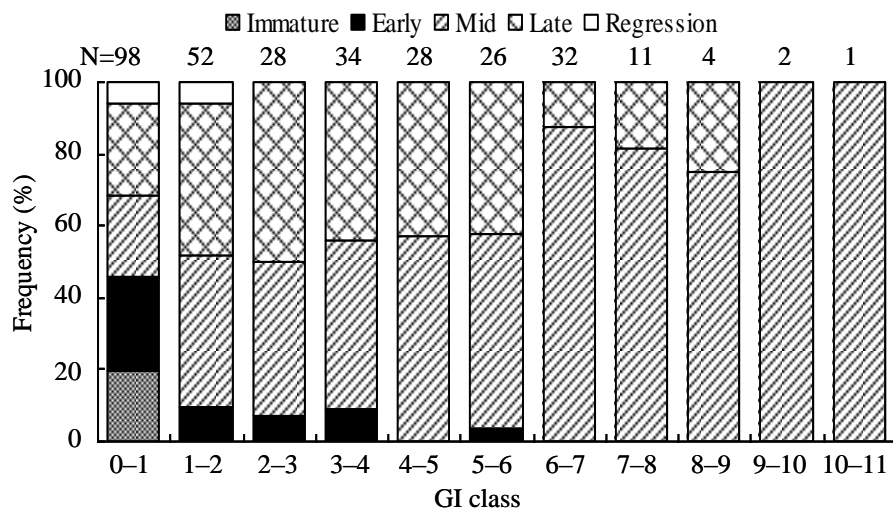


Fig. 5 Proportion of testis maturity stages for each gonad index class (GI) of male skipjack tuna in the tropical western and central Pacific Ocean.

Early; Early maturation, Mid; Mid maturation, Late; Late maturation

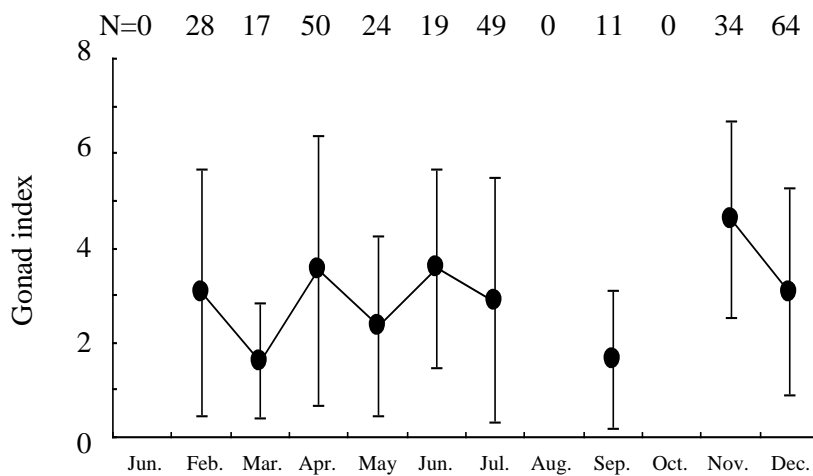


Fig. 6 Monthly changes gonad index of skipjack tuna in the tropical western and central Pacific Ocean.

The circles and vertical bars indicate mean values and standard deviations. Only specimens larger than the minimum size at sexual maturity ($FL \geq 35.5$ cm) were used for this analysis.

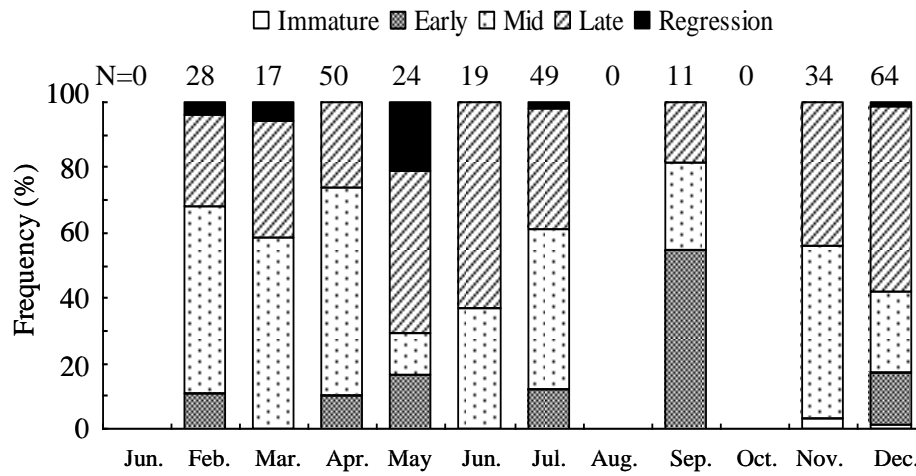


Fig. 7 Monthly changes in testis maturity stage of skipjack tuna in the tropical western and central Pacific Ocean.

Only specimens larger than the minimum size at sexual maturity ($FL \geq 35.5$ cm) were used in this analysis. Early; Early maturation, Mid; Mid maturation, Late; Late maturation