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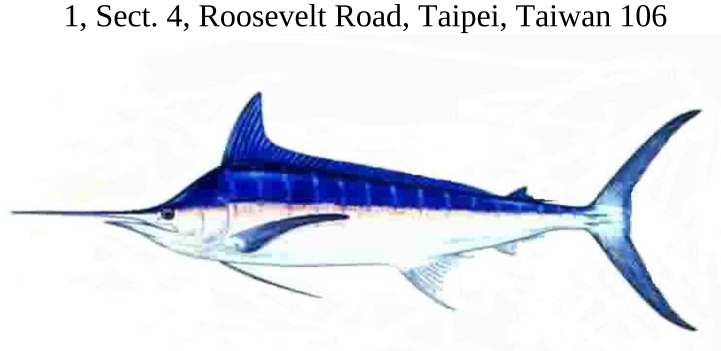
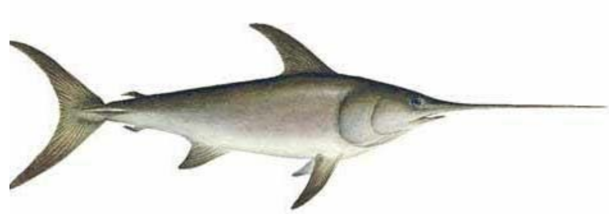
Chi-Lu Sun
Siao-Yun Chang
Yi-Jay Chang
Su-Zan Yeh
National Taiwan University
Insitute of Oceanography
1, Sect. 4, Roosevelt Road, Taipei, Taiwan 106

Wei-Chuang Chiang
Eastern Marine Biology Research Center, Fisheries Research Institute
Council of Agriculture, Executive Yuan, Taitung, Taiwan

Nan-Jay Su
National Taiwan University
Insitute of Oceanography
1, Sect. 4, Roosevelt Road, Taipei, Taiwan 106



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# Reproductive biology of female striped marlin, *Kajikia audax*, in the waters off Taiwan (preliminary)*

Chi-Lu Sun[1], Siao-Yun Chang[1], Yi-Jay Chang[1], Su-Zan Yeh[1],
Nan-Jay Su[1], and Wei-Chuan Chiang[2]

1. Institute of Oceanography, National Taiwan University
2. Eastern Marine Biology Research Center, Fisheries Research institute

## Abstract

Length-weight data of 1,219 striped marlin and gonad samples were collected at the fish markets in Tungkang, Shinkang, and Nanfangao during November 2004 to September 2010. Reproductive activity was assessed using histology and gonadosomatic index, indicated that the spawning season occurs from April to August. The estimated size-at-50%-maturity for females was 178.98 cm EFL. Based on the proportion of mature females with postovulatory follicles, the spawning fraction (0.29) implies that the striped marlin spawned once every 3.4 days on average. The averaged batch fecundity was 4.38 million oocytes and the average relative fecundity was 53.61 oocytes per gram of body weight.

## Introduction

Striped marlin have a wide latitudinal distribution occurring from 45°N to 45°S latitude mainly in tropical or subtropical waters in the Indo-Pacific Ocean. In the waters of Taiwan, striped marlin are mainly harvested as a bycatch of the longline fisheries; a few are taken by harpoon, gill net, and set net. For the past 10 years, the annual landings of striped marlin from coastal and offshore fisheries in Taiwan are fluctuated: landing was 279 metric tons (t) in 2002 and decreased to 84 t in 2005, then increased to 276 t in 2008.

The size and age at sexual maturity and the sex ratios are fundamental biological parameters used in stock assessments. Estimates of body size or age at sexual maturity are necessary parameters for age- and size-structured models (Quinn and Deriso, 1999). Kume and Joseph (1969) estimated the gonadosomatic index for the spawning groups of striped marlin in the northern and southern Pacific. Merrett

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(1970; 1971) identified the gonadal development and estimated the size-at-first maturity in the western Indian Ocean based on histological technique. Eldridge and Wares (1974) described the gonad and oocytes diameter in the eastern Pacific. Armas *et al.* (2006) determined the sex ratio and spawning season in the southern Gulf of California, Mexico. Kopf *et al.* (2009) provided the parameters of size-at-maturity, batch fecundity, and spawning frequency in the southwest Pacific Ocean.

The objectives of this study were to determine the sex ratio and reproductive season, to identify the ovary maturation stages by using histological techniques, to estimate the size-at-maturity, and to estimate spawning frequency and batch fecundity. The results of this study can be used as biological input parameters for further evaluation of the striped marlin stock in the western Pacific Ocean.

## Materials and methods

Length-weight data and gonad samples of striped marlin were collected at the Tunkang, Shinkang, and Nanfangao fish markets from July 2004 to September 2010. All samples were caught by offshore longline, gillnet, and harpoon fisheries operating off Taiwan waters (**Fig. 1**). Sex, eye-to-fork length (EFL), lower jaw-fork length (LJFL), and round weight (RW) were recorded for each fish. EFL and LJFL were measured to the nearest 1 cm, and RW was weighed to the nearest 0.1 kg. Samples of ovarian tissue were fixed in 10% neutral buffered formalin (de Sylva and Breder, 1997) for histological analysis. Three fresh ovary pairs were collected to evaluate the synchronicity of oocyte development within, and between, ovary pairs. These ovaries were each divided into left and right lobes, and each lobe further divided into anterior, middle, and posterior portion. The number of oocytes and the mean oocyte diameter of each 0.05-g subsample were calculated with the Image-Pro Plus software package in combination with a dissecting microscope (Leica MZ6) equipped with a CCD camera (Leica DFC420) and a high-resolution computer monitor (Friedland *et al.*, 2005). An ANOVA test found no significant difference in the mean oocyte diameter either between ovary lobes ($P > 0.05$) or among portions within ovary ($P > 0.05$), although there were differences in the mean oocyte diameter between the ovaries ($P < 0.05$). Similar results were found in the analysis of the number of oocytes.

Sex ratio was calculated as the proportion of the number of females to the total numbers of females and males. The gonadosomatic index (GSI) was determined as follows (Kume and Joseph, 1969; Eldridge and Wares, 1974; Gonzalez-Armas *et al.*, 2006):

$$GSI = \frac{GW}{EFL^3} \times 10^4$$

where $GW$ = gonad weight (g)

Gonadal developmental stages were categorized based on the criteria of Arocha (2002), Arocha and Bárrio (2009), and Kopf *et al.* (2009). Individuals were designated as mature if the most advanced oocytes were indicative of ≥ stage 4. Changes over time in the mean gonadosomatic index and the composition of ovarian development stages were evaluated to determine the spawning season. The probability that the *i* th fish was mature ($P_i$) was modeled with a logistic curve (Norman and Stevens, 2007; Sun et al., 2009):

$$P_i = 1 / \left\{ 1 + \exp[-\ln(19) \frac{EFL_i - EFL_{50}}{EFL_{95} - EFL_{50}}] \right\}$$

where $EFL_i$ = the EFL of fish *i*; and $EFL_{50}$ and $EFL_{95}$ = the EFLs at which 50% and 95% of the assemblage reached maturity. $EFL_{50}$ and $EFL_{95}$ were estimated by maximizing a log-likelihood function and by assuming a binomial error distribution with AD Model Builder (Fournier, 2000).

In this study, spawning frequency was determined by two approaches: (1) the hydrated oocyte method and (2) the postovulatory follicles (POF) method (Hunter and Macewicz, 1985). Spawning fraction was calculated by the proportion of females with POF (and hydrated oocytes) to the total number of females, mature females, and reproductively active females. The spawning frequency was estimated by the inverse of the spawning fraction. Batch fecundity was estimated by the hydrated oocyte method (Hunter, 1985). Three subsamples of 0.05-g were taken from the ovaries that contained hydrated oocytes without postovulatory follicles (Schaefer, 1996). The gravimetric method was employed to estimate the batch fecundity as follows (Hunter *et al.*, 1992):

$$BF = \frac{GW}{w} \times n$$

where $BF$ = batch fecundity; $n$ = the number of oocytes in the subsample, and $w$ = the weight of subsample of gonad (0.05 g). Relative fecundity (RF) was defined as batch fecundity divided by the round weight of the female (Hunter *et al.*, 1992).

**Results**

The relationship between LJFL and EFL was LJFL = 7.061 + 1.123 EFL ($n$=569, $r^2$=0.98). Of total 1,219 sampled fish, 536 were females (ranged 95-220 cm EFL), and 683 were males (ranged 92-220.5 cm EFL) (**Fig. 2**), with a sex ratio of 0.44 which significantly differed (Chi-squares value = 17.73, $P < 0.01$) from the expected 0.5. The sex ratio fluctuated from 0.35 to 0.5 without a significant pattern at a EFL of less than 130 cm. However, the sex ratio increased for EFL greater than 130 cm. The relationship between the sex ratio and EFL over the range from 130 to 220 cm was given by (**Fig. 3**)

$Sex\ ratio = 4 \times 10^{-9}\ EFL^{3.59}$ ($r^2$=0.97; $n$ = 10)

The monthly variation of mean gonadosomatic index (GSI) of females was shown in **Fig. 4**. Monthly mean GSI (years pooled) was relatively high in April-August and reached a maximum in June. GSI decreased from 2.77 in August to 0.21 in September, and remained less than 1.0 from September to March, then rapidly increased from 0.33 in March to 2.77 in April. Females with high GSI (> 1.9) were frequently observed from April to August. Five stages were identified for female striped marlin based on the histological examination: (1) immature, (2) developing, (3) mature, (4) spawning, and (5) resting. **Fig. 5** shows the monthly variation of the percentage of the ovarian stages, indicating that the reproductively active (mature and spawning stage, with migratory nucleus oocytes or hydrated oocytes) females only occurred in April-August, and most samples were immature in other months. The information in monthly changes of mean GSI values and percentage of vitellogenic ovaries together implied that the major spawning season for striped marlin in waters off Taiwan likely to be from April to August with a peak in May and June.

The relationship between the mature female proportion ($P_i$) and $EFL$ can be described by the logistic function (**Fig. 6**):

$$P_i = 1 / \left\{ 1 + \exp[-\ln(19) \frac{EFL_i - 178.98}{235.96 - 178.98}] \right\}$$

The $EFL_{50}$ and $EFL_{95}$ for female striped marlin were estimated to be 178.98 cm and 235.96 cm, respectively. The smallest female with mature ovary was 150.4 cm EFL. According to the age and growth study of striped marlin of Hsu (2010), the age-at-50% maturity ($A_{50}$) was estimated as 2.3 years for females. Spawning frequency estimated from the presence of postovulatory follicles and hydrated oocytes for all females, mature females, and reproductively active females during the spawning season (April to August) was shown in **Table 1** and **2**. The spawning intervals estimated with the hydrated oocyte method were greater than those by the postovulatory follicles method. Batch fecundity was preliminary estimated for 3 females as 2.39-6.43 million oocytes, with an average of 4.38 million oocytes. The estimated relative fecundity ranged 30.28-78.34 oocytes per gram of body weight, with an average of 53.61 oocytes per gram of body weight.

**Discussion**

In this study, sex ratio of striped marlin increased gradually with EFL between 130-220 cm, which was similar to the observation in the southwest Pacific Ocean (Kopf *et al.*, 2009). Kume and Joseph (1969) also showed similar results for striped marlin in the eastern Pacific. Total sex ratio was 0.4 in this study. Similar results were also suggested for striped marlin in the southern Gulf of California (Armas *et al.*, 2006) and in the eastern Pacific (Eldridge and Wares, 1974), although Merrett (1971) suggested that females were dominant than males in the western Indian Ocean. Furthermore, Kume and Joseph (1969) indicated that the predominance of males has been observed for striped marlin in the spawning ground.

The major spawning season of striped marlin seems to be from April to August in the waters off Taiwan based on the GSI values and staging of ovaries. Similar results were suggested for the northern Pacific (May-June) (Kume and Joseph, 1969), the eastern Pacific (June-July) (Eldridge and Wares, 1974), and southern Gulf of California, Mexico (July-August) (Armas *et al.*, 2006). However, spawning season was suggested from November to January in the southwestern Pacific (Kopf *et al.*, 2009). These implied that striped marlin is reproductively active during summer, perhaps because of higher temperatures at that time.

The size-at-maturity was estimated as 140-180 cm EFL for the female striped marlin in the northern Pacific and 160-220 cm EFL in the southern Pacific based on the GSI values (Kume and Joseph, 1969). Merrett (1971) estimated the size-at-first maturity as 140-160 cm EFL for the female striped marlin in the western Indian Ocean. The size-at-50% maturity ($EFL_{50}$) reported by Kopf *et al.*(2009) for female striped marlin was 202.6 cm LJFL, and the age-at-50% maturity ($A_{50}$) was 1.9 years, which were close to the estimates ($EFL_{50}$ = 178.98 cm and $A_{50}$=2.3 years) of present study. In this study, $EFL_{50}$ for females was based on large sample sizes and a broad size range of fish collected throughout the spawning season, and maturity was determined by histology. Thus, the results of this study should provide an accurate representation of the size-at-maturity of striped marlin in the western Pacific Ocean.

The estimated spawning frequency based on the postovulatory follicles method was equivalent to an average spawning interval of 3.4 day and which was less than the estimate value 2.2 (days) reported in the southwestern Pacific Ocean (Kopf *et al.*, 2009). The spawning frequency determined by postovulatory follicles method seems to less than the estimate value by the hydrated oocyte method. However, Chiang *et al.* (2006) indicated that the hydrated oocyte method is likely to underestimate the spawning frequency. The batch fecundity estimated in this study was 2.39-6.43 million oocytes. Similar batch fecundity of 3.1 million oocytes was estimated in the southwestern Pacific (Kopf *et al.*, 2009), but higher values were reported in the eastern Pacific (11.3-28.6 million oocytes; Kume and Joseph, 1969) and in the western Indian Ocean (12 million oocytes; Merrett, 1971).

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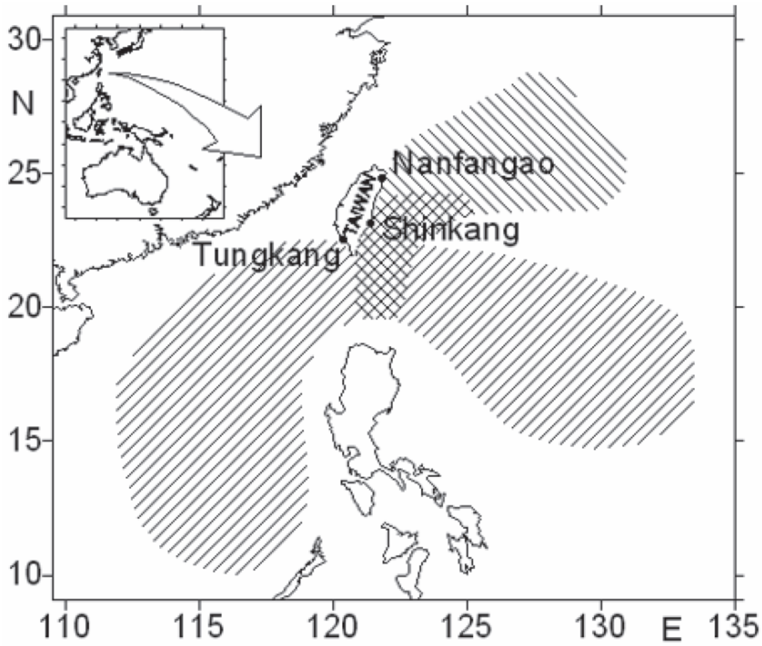


Figure 1. Three fishing ports in Taiwan where the length-weight data and gonad samples of striped marlin were collected (right diagonal, left diagonal, and mesh represent the fishing area for the fleets based at Tungkang, Nanfanao and Shinkang, respectively) (modified from Wang *et al.*, 2006)

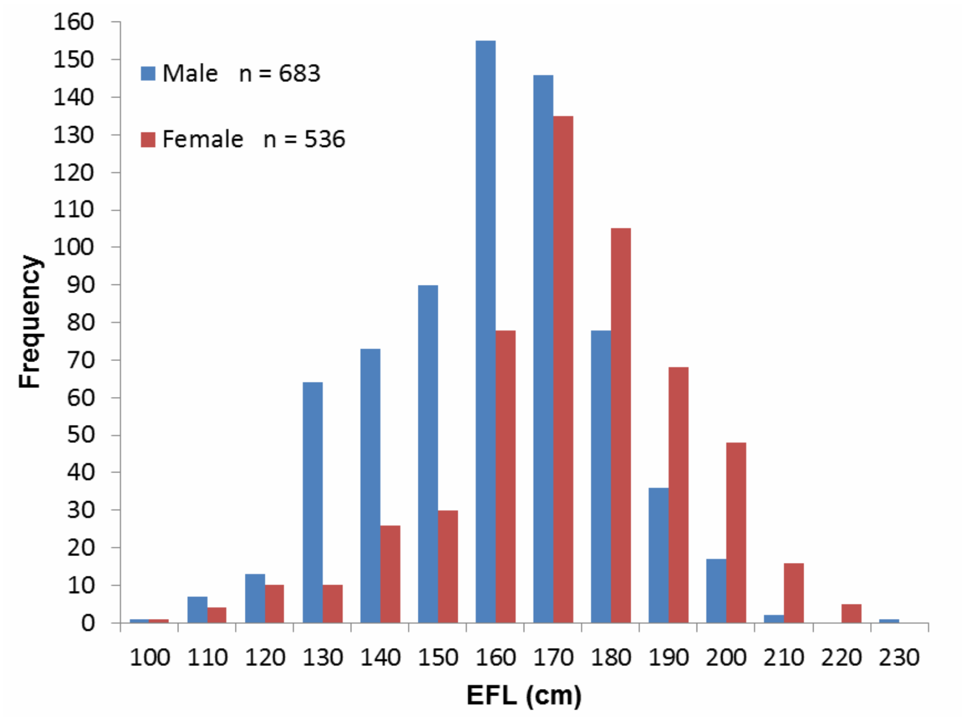


Figure 2. The length-frequency distribution of striped marlin sampled at the Tungkang, Shinkang, and Nanfangao fish markets.

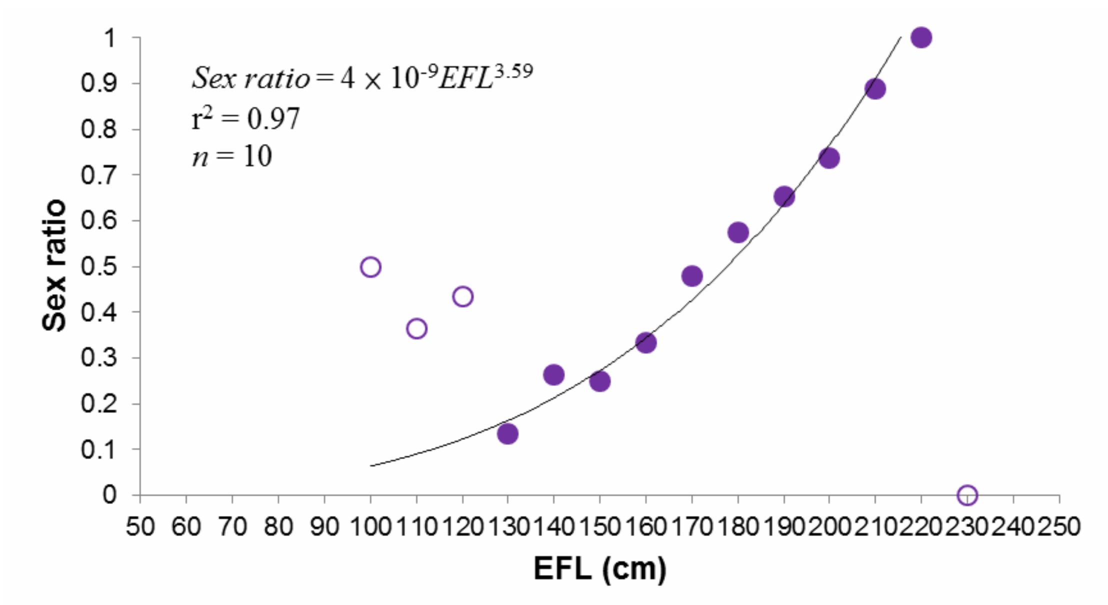


Figure 3. Relationship between sex ratio and eye-to-fork length (EFL, 5-cm classes) for the striped marlin in the waters off Taiwan. (the power function ranged in 130-220 cm EFL)

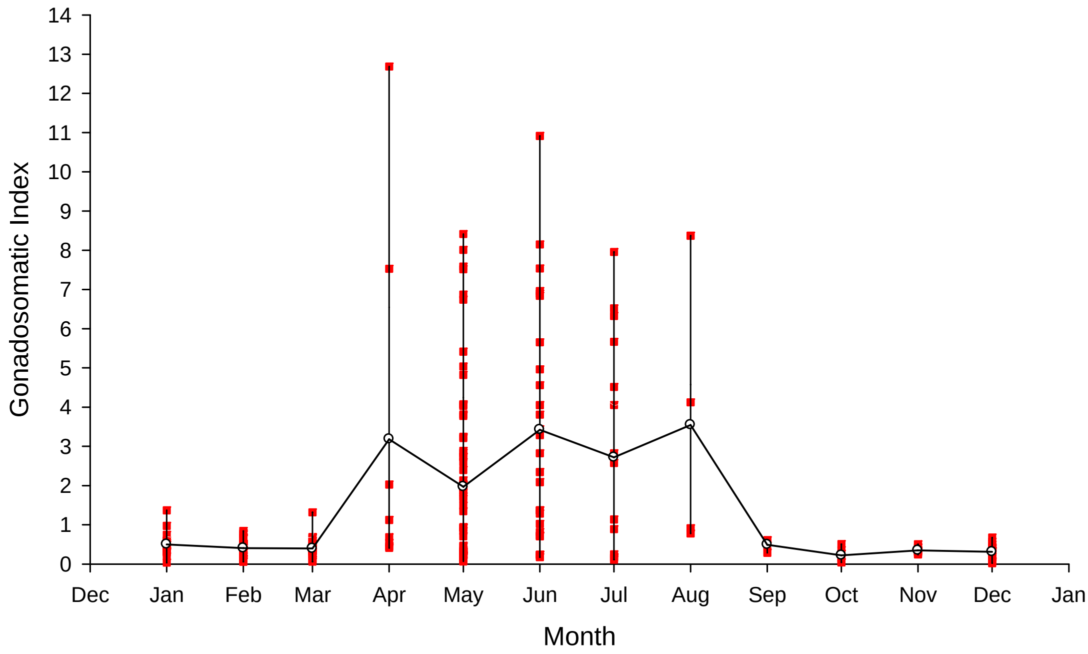


Figure 4. Monthly (years pooled) variation in mean gonadosomatic index of female striped marlin in the waters off Taiwan. (vertical bars, ranges).

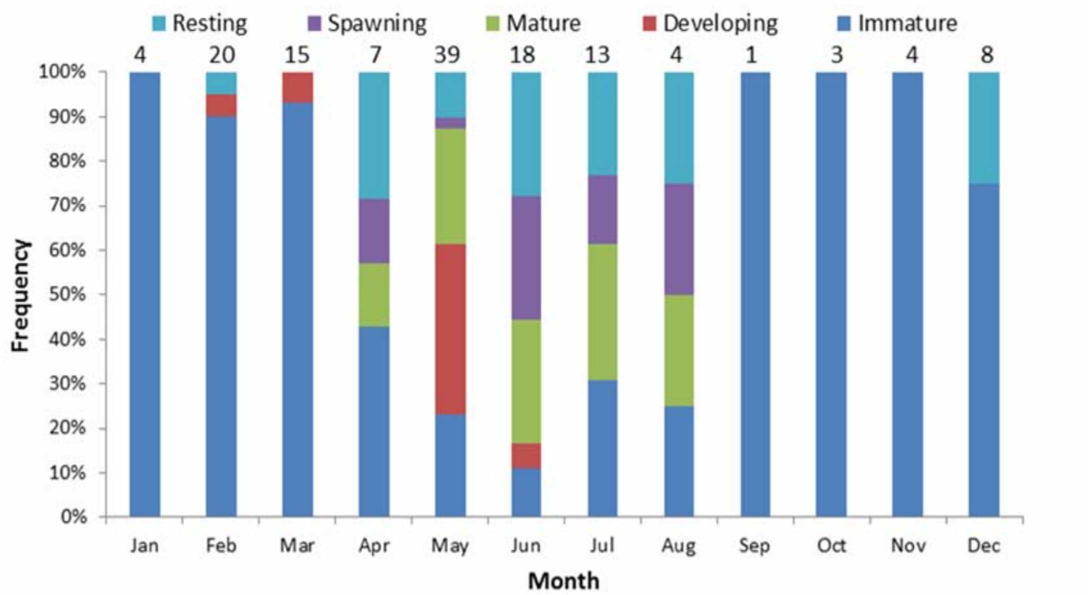


Figure 5. Monthly variation in the percentage of ovarian stages of female striped marlin in the waters off Taiwan. (numbers above vertical bars, sample size)

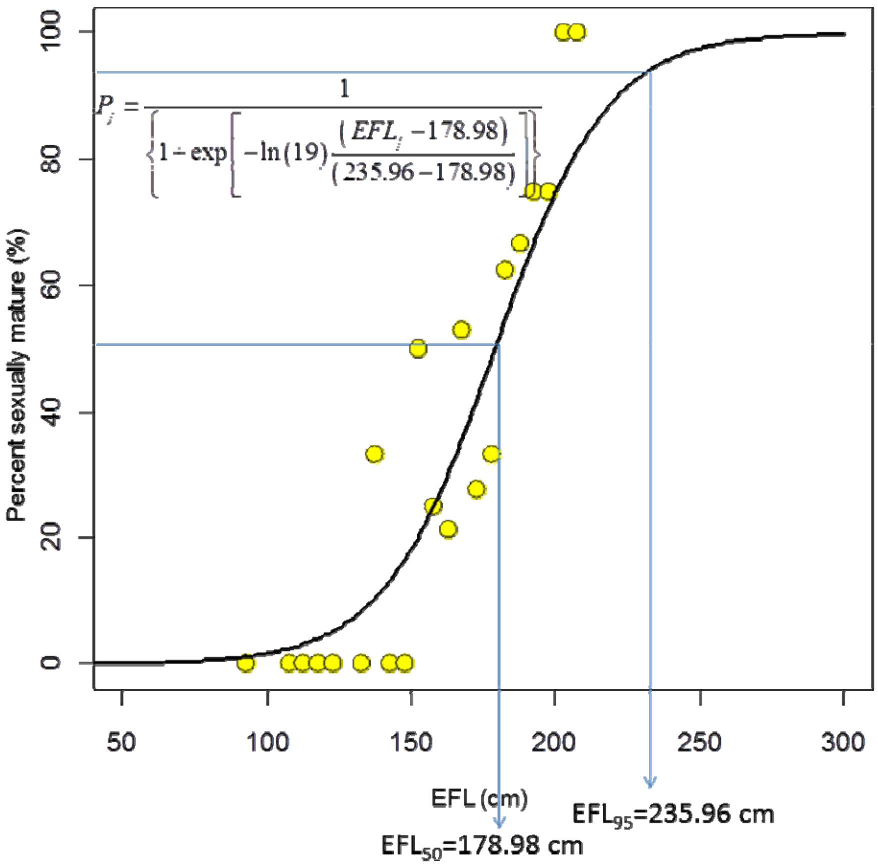


Figure 6. Maturity-at-length (eye-to-fork length, EFL) relationship for female striped marlin in the waters off Taiwan.

Table 1. Spawning fraction by postovulatory follicles method of striped marlin by month (years pooled) for all females, mature females, and females in spawning condition in the waters off Taiwan. (POF, postovulatory follicles; *n*, number of fish in sample).

| **Month** | **All females** | | | **Mature females** | | | **Females in spawning condition** | | |
|---|---|---|---|---|---|---|---|---|---|
| | ***n*** | ***n* with POF's** | **Spawning fraction** | ***n*** | ***n* with POF's** | **Spawning fraction** | ***n*** | ***n* with POF's** | **Spawning fraction** |
| **Jan** | 4 | 0 | 0.00 | 0 | 0 | 0.00 | 0 | 0 | 0.00 |
| **Feb** | 20 | 0 | 0.00 | 1 | 0 | 0.00 | 0 | 0 | 0.00 |
| **Mar** | 15 | 0 | 0.00 | 0 | 0 | 0.00 | 0 | 0 | 0.00 |
| **Apr** | 7 | 0 | 0.00 | 4 | 0 | 0.00 | 2 | 0 | 0.00 |
| **May** | 39 | 3 | 0.08 | 15 | 3 | 0.20 | 11 | 3 | 0.27 |
| **Jun** | 18 | 3 | 0.17 | 15 | 3 | 0.20 | 10 | 3 | 0.30 |
| **Jul** | 13 | 3 | 0.23 | 9 | 3 | 0.33 | 6 | 3 | 0.50 |
| **Aug** | 4 | 0 | 0.00 | 3 | 0 | 0.00 | 2 | 0 | 0.00 |
| **Sep** | 0 | 0 | 0.00 | 0 | 0 | 0.00 | 0 | 0 | 0.00 |
| **Oct** | 3 | 0 | 0.00 | 0 | 0 | 0.00 | 0 | 0 | 0.00 |
| **Nov** | 4 | 0 | 0.00 | 0 | 0 | 0.00 | 0 | 0 | 0.00 |
| **Dec** | 8 | 0 | 0.00 | 2 | 0 | 0.00 | 0 | 0 | 0.00 |
| **Total** | 135 | 9 | 0.07 | 49 | 9 | 0.18 | 31 | 9 | 0.29 |
| **Spawning season** | 81 | 9 | 0.11 | 46 | 9 | 0.20 | 31 | 9 | 0.29 |

Table 2. Spawning fraction by hydrated oocyte method of striped marlin by month (years pooled) for all females, mature females, and females in spawning condition in the waters off Taiwan. (HY, hydrated oocyte; *n*, number of fish in sample).

| Month | All females | | | Mature females | | | Females in spawning condition | | |
|---|---|---|---|---|---|---|---|---|---|
| | ***n*** | ***n* with HY's** | **Spawning fraction** | ***n*** | ***n* with HY's** | **Spawning fraction** | ***n*** | ***n* with HY's** | **Spawning fraction** |
| **Jan** | 4 | 0 | 0.00 | 0 | 0 | 0.00 | 0 | 0 | 0.00 |
| **Feb** | 20 | 0 | 0.00 | 1 | 0 | 0.00 | 0 | 0 | 0.00 |
| **Mar** | 15 | 0 | 0.00 | 0 | 0 | 0.00 | 0 | 0 | 0.00 |
| **Apr** | 7 | 1 | 0.14 | 4 | 1 | 0.25 | 2 | 1 | 0.50 |
| **May** | 39 | 1 | 0.03 | 15 | 1 | 0.07 | 11 | 1 | 0.09 |
| **Jun** | 18 | 2 | 0.11 | 15 | 2 | 0.13 | 10 | 2 | 0.20 |
| **Jul** | 13 | 0 | 0.00 | 9 | 0 | 0.00 | 6 | 0 | 0.00 |
| **Aug** | 4 | 1 | 0.25 | 3 | 1 | 0.33 | 2 | 1 | 0.50 |
| **Sep** | 0 | 0 | 0.00 | 0 | 0 | 0.00 | 0 | 0 | 0.00 |
| **Oct** | 3 | 0 | 0.00 | 0 | 0 | 0.00 | 0 | 0 | 0.00 |
| **Nov** | 4 | 0 | 0.00 | 0 | 0 | 0.00 | 0 | 0 | 0.00 |
| **Dec** | 8 | 0 | 0.00 | 2 | 0 | 0.00 | 0 | 0 | 0.00 |
| **Total** | 135 | 5 | 0.04 | 49 | 5 | 0.10 | 31 | 5 | 0.16 |
| **Spawning season** | 81 | 5 | 0.06 | 46 | 5 | 0.11 | 31 | 5 | 0.16 |