SEX RATIOS, SIZE AT SEXUAL MATURITY, AND SPAWNING SEASONALITY OF SAILFISH *ISTIOPHORUS PLATYPTERUS* FROM EASTERN TAIWAN

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ABSTRACT

Lower jaw fork length (*LJFL*), round weight (*RW*) data, and 246 female gonads from 6279 sailfish ranging in size from 78 to 239 cm *LJFL* (or 1–60 kg *RW*) were randomly sampled at the Shinkang Fishing Port in southeastern Taiwan from July 1998 to December 2002. Sixty-nine percent of these sailfish were males. The sex ratio defined as the proportion of females in the sample increased as the *LJFL* increased beyond 145 cm and reached 98.6% at sizes > 227 cm *LJFL* (or 46 kg) (sex ratio = 3.07×10^{-7} $LJFL^{3.9782}$). Female gonads were classified into seven stages of maturity based on histological structures. Sexually mature individuals were defined as females with ripe ovaries or advanced oocytes. Estimated mean *LJFL* at sexual maturity (L_{50}) was 166 cm for females, and the smallest mature female was 162 cm *LJFL*. Monthly variations in gonadosomatic indices of 313 mature sailfish peaked during April–September, which is coincident with the histological findings that females were in spawning and recently-spawned stages during this time, indicating this to be the spawning period off eastern Taiwan.

Sailfish, *Istiophorus platypterus* (Shaw in Shaw and Nodder, 1792), are widely distributed in tropical and temperate surface waters of the world's oceans (Nakamura, 1985). Off Taiwan's eastern coast, sailfish are of substantial economic importance and are seasonally abundant from April to October (peaking from May to July). This species is taken primarily by drift gill nets, although they are also caught by set nets, harpoons, and as incidental by-catch in inshore longline fisheries (Chiang, 2004). For the past 10 yrs, annual landings of sailfish off Taiwan have fluctuated from 500 to 1000 metric tons (mt), of which > 50% are from waters off Taitung (eastern Taiwan).

The size and age at sexual maturity and sex ratios are fundamental biological parameters used in stock assessments (Wang et al., 2003). Estimates of body size, or age at sexual maturity, are necessary parameters for calculating spawning stock biomass in size- and age-structured models (Deriso et al., 1985; Gabriel et al., 1989; Quin II et al., 1990; Foale and Day, 1997). They are also required in estimating biological reference points used in determining the status of fish populations (Hilborn and Walters, 1992).

Several studies on the reproductive characteristics of sailfish have been published for the eastern Pacific Ocean. Kume and Joseph (1969a), Shingu et al. (1974), Miyabe and Bayliff (1987), Nakano and Bayliff (1992), and Uosaki and Bayliff (1999) used gonad index data to estimate the body size at sexual maturity for female sailfish and described the geographic distribution of mature sailfish in the eastern Pacific. Eldridge and Wares (1974) used gonad index data and the number of modes in the oocyte diameter distribution to infer the sexual maturity and spawning seasonality for female sailfish. Using histology, Hernández-Herrera and Ramírez-Rodriguez (1998) estimated the spawning seasonality and length at maturity of sailfish caught by the

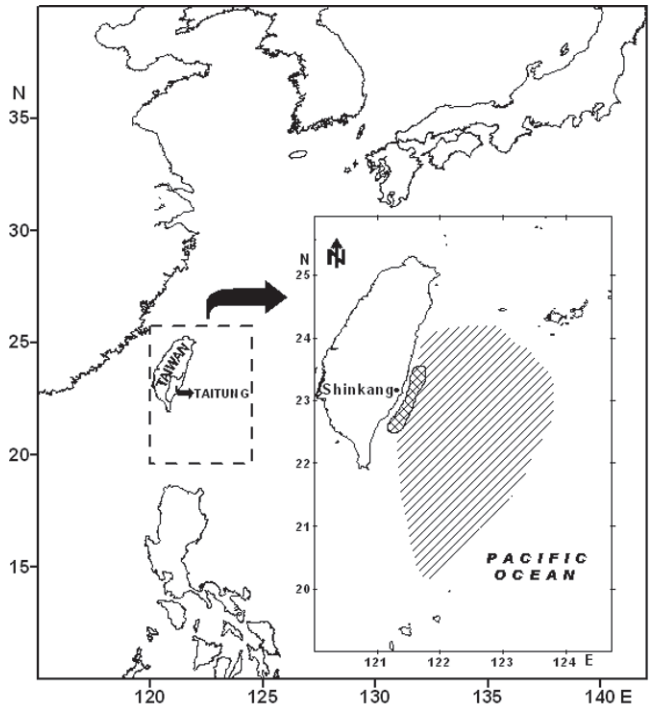

Figure 1. The fishing port in Taitung (eastern Taiwan) where gonad samples of sailfish (*Istiophorus platypterus*) and measurements for the sex ratio analysis were collected. Oblique and cross line areas indicated the fishing grounds of longline and gillnet fisheries based at the Shinkang fishing port.

Mexico's recreational fishery. In contrast, there has been no histological analysis of the reproductive biology of sailfish in the western Pacific.

The objective of this study was to estimate sex ratios and length at sexual maturity for sailfish in the waters off eastern Taiwan. To evaluate maturity, gonadal development was determined through histological examination in addition to the measurement of the most advanced group of oocytes and estimates of a gonad index. The results of this study will be used as biological input parameters for stock assessment of the sailfish population in the northwestern Pacific Ocean.

Materials and Methods

Collection of Samples and General Biological Data.—Sailfish biological data were collected monthly at Shinkang fishing port (Fig. 1) from July 1998 to December 2002. Specimens were selected at random from landings, and length/weight data and gonad samples were collected. The sex of each specimen was identified based on the appearance of the gonads. Specimens were measured to the nearest centimeter for length (EFL = eye fork length, *LJFL* = lower jaw to fork length), to the nearest kilogram for weight (*RW*) and to the nearest 0.1 g for ovarian weight (*OW*). Gonadosomatic index (*GSI*) was calculated as in Uchiyama and Shomura (1974). Sex ratio was expressed as proportion of females to total numbers of females and males by length class (5-cm length intervals) and by quarter intervals (3 mo).

Preparation for Histological Examination.—Gonads were preserved in 10% buffered formalin for histological examination. The developmental stages of oocytes were categorized following several authors (Hunter et al., 1992; Arocha, 2002). Microscopic slides were

examined with a Leica DM LS compound microscope at a magnification of 40–1000×. Histological classification of gonadal developmental stages was based on the criteria of DeMartini et al. (2000). Stages 4–6 are reproductively active stages, and stages 1–3 and 7 are reproductively inactive stages (Wang et al., 2003).

Oocyte Measurement.—Oocyte size was obtained by measuring the diameter of oocytes on histological slides using the Image-Pro Image analysis software package (Media Cybernetics, Silver Spring MD, 1997) in combination with a dissecting microscope (model: Leica-MZ6) equipped with a charged coupled device (CCD) camera (model: Toshiba IK-630) and a computer with high-resolution monitor. However, histological sectioning deforms the oocyte from its sphere-like shape and three different measurements were made following Arocha (2002): (1) early developed oocytes were measured using the major axis crossing the nucleus, (2) maturing oocytes were measured across the nucleus from well-formed spheres, and (3) fully mature oocytes diameter (D) was calculated from $D = P\,\pi^{-1}$, where P is the circumference of the oocyte. Samples of 200–350 oocytes were measured at various stages of maturity.

The relationship between oocyte size and the probability of reproductive activity was represented by a logistic model (DeMartini et al., 2000; Wang et al., 2003) described as:

$$\ln\left(\frac{p}{1-p}\right) = a + b \times OD$$

where OD = whole oocyte diameter (μm); and p = probability of an egg of size OD being defined as in the reproductive activity stage 4–6.

Sexual Maturity.—Sexually mature individuals were defined as females with ovaries in the ripening or more advanced stages. The length at which 50% of all individuals were sexually mature (L_{50}) was estimated from a logistic model (King, 1995) described as:

$$P = \frac{1}{1 + \exp[-r \times (L - L_{50})]}$$

where P = the proportion of mature individuals within length class L (5 cm length interval); r = the slope of the curve describing the rate of changes in P from 0 to 1; and L_{50} = the length (*LJFL*) at 50% sexual maturity. L_{50} and r were estimated using the nonlinear least square procedure (Gauss-Newton method, NLIN of SAS Institute, 1990).

Results

Size Distribution and Sex Ratio.—The *LJFL* of 1927 females and 4352 males was measured for the size-specific sex ratio analysis. The range of the *LJFL* was 80–239 cm for females and 78–227 cm for males, and the range of *RW* was 2–60 kg for females and 1–46 kg for males (Fig. 2).

The estimated sex ratio for all samples was 0.31 which differed significantly (χ^2 = 936.55; P < 0.01) from the expected value of 0.5 or 1:1. The proportion of males was higher than the proportion of females in each quarter and year. Sex ratios differed significantly from the expected value of 0.5 during the following periods: 1stQ 2000, 3rdQ to 4thQ 2000, 4thQ 2001, and 1stQ 2002 (Table 1). However, it should be noted that the sample size in a quarter was small.

The sex ratio fluctuated without significant pattern for fish with a *LJFL* of < 145 cm. The sex ratio increased for specimens with a *LJFL* > 145 cm, and all specimens with a *LJFL* > 230 cm were females. The relationship between the sex ratio and *LJFL* over the range from 145 to 230 cm (Fig. 3) was given by

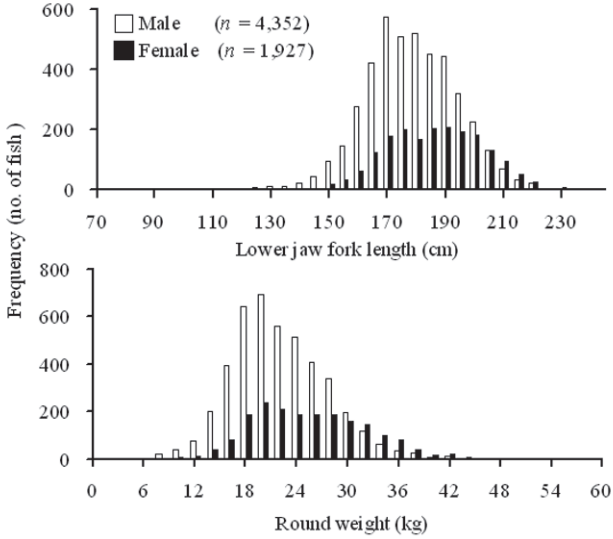

Figure 2. The size-frequency distributions by (A) 5-cm intervals and (B) 2-kg intervals for male and female sailfish (*Istiophorus platypterus*) collected from the waters off eastern Taiwan.

$$Sex\ ratio = 3.07 \times 10^{-10} LJFL^{3.9782}$$

($r^2 = 0.933$; n = 18; 5-cm classes; $P < 0.05$)

Sexual Maturity.—Size at maturity was estimated based on the ovarian examination of 246 females (98–225 *LJFL*) randomly collected during January 1999–September 2000. Of these 246 ovarian samples, 183 (74%) were designated as mature (developmental stage ≥ stage 4) (Fig. 4; Table 2). The minimum size at maturity was 162 cm *LJFL*.

The proportion of mature females for each length class (5-cm intervals) was fitted to the logistic curve to estimate the L_{50}:

$$P = \frac{1}{1 + \exp[0.2277 \times (LJFL - 166.376)]}$$

($r^2 = 0.987$; n = 22; 5-cm classes).

The 95% confidence interval (C.I.) for L_{50} was 166.4 ± 1.3 cm (Fig. 5), corresponding to an age at maturity of about 5 yrs (Chiang et al., 2004).

Oocyte Composition During Ovarian Maturation.—As the largest oocytes reached the tertiary yolk stage, a clutch consisting of oocytes ~500 μm in size was found. In the spawning stage ovary, the oocytes formed an advanced batch, which was distinct from adjacent groups of smaller oocyte cohorts. In the mature ovary, only the oocytes in the advanced batch increased in size, while those in the less advanced oocyte cohorts remained < 500 μm.

The logistic regression equation of reproductive activity on the diameter of oocytes (OD) was estimated as:

Table 1. Numbers of male and female sailfish (*Istiophorus platypterus*) grouped by annual quarter intervals with χ^2 values assuming a 1:1 sex ratio in each interval.

Year	Quarter	Female	Male	Sex ratio	χ^2 value	P value	DF
1998	3rd	94	393	0.19	183.58	< 0.00001**	1
1998	4th	37	132	0.22	53.40	< 0.00001**	1
1999	1st	13	43	0.23	16.07	0.00006**	1
1999	2nd	295	694	0.30	160.97	< 0.00001**	1
1999	3rd	362	1154	0.24	413.76	< 0.00001**	1
1999	4th	32	52	0.38	4.76	0.02910*	1
2000	1st	19	27	0.41	1.39	0.23819	1
2000	2nd	230	379	0.38	36.45	< 0.00001**	1
2000	3rd	107	131	0.45	2.42	0.11978	1
2000	4th	30	38	0.44	0.94	0.33198	1
2001	1st	16	54	0.23	20.63	0.00001**	1
2001	2nd	174	275	0.39	22.72	< 0.00001**	1
2001	3rd	142	365	0.28	98.08	< 0.00001**	1
2001	4th	26	35	0.43	1.33	0.24918	1
2002	1st	3	8	0.27	2.27	0.13167	1
2002	2nd	47	80	0.37	8.57	0.00341**	1
2002	3rd	250	415	0.38	40.94	< 0.00001**	1
2002	4th	50	77	0.39	5.74	0.01658*	1
subotal (1998)		131	525	0.20	236.64	< 0.00001**	3
subotal (1999)		702	1943	0.27	582.26	< 0.00001**	3
subotal (2000)		386	575	0.40	37.17	< 0.00001**	3
subotal (2001)		348	729	0.32	134.78	< 0.00001**	3
subtotal (2002)		350	580	0.38	444.90	< 0.00001**	3
χ^2 among intervals (1998)					1.67	0.19585	1
χ^2 among intervals (1999)					17.06	0.00069**	3
χ^2 among intervals (2000)					4.20	0.24074	3
χ^2 among intervals (2001)					18.26	0.00039**	3
χ^2 among intervals (2002)					0.69	0.87605	3
χ^2 among years (1998~2002)					161.59	< 0.00001**	17

* Significant at 5% level.
** Significant at 1% level.

$$\ln\left(\frac{p}{1-p}\right) = -5.8829 + 0.0157 \times OD$$

($r^2 = 0.921$; n = 24; 50-μm classes).

Accordingly, 50% of oocytes were active when the oocyte diameter was about 348 μm, 90% of oocytes were active when the oocyte diameter was 515 μm, and 99% of oocytes were active when the oocyte diameter was 667 μm.

Spawning Seasonality.—Females with maturing stage ovaries occurred from March to August. Females with spawning stage or recently spawned stage ovaries occurred from April to September. The ovary samples were all in spent or resting stage between October and December (Fig. 6). The mean *GSI* began to increase in April and remained high until August, then decreased gradually in September and remained low from November to March (Fig. 7). All these coincident findings indicated that the spawning season of sailfish off eastern Taiwan was from April to September.

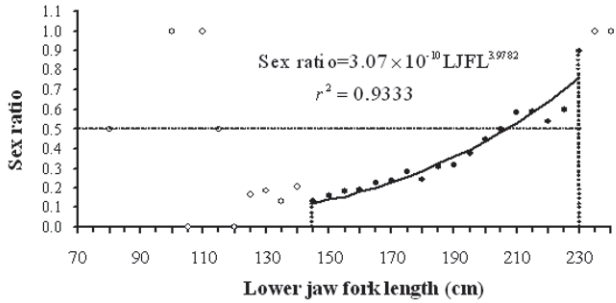

Figure 3. Relationship between proportion of females and lower jaw fork length (*LJFL*, 5-cm classes) for the sailfish (*Istiophorus platypterus*) collected from the waters off eastern Taiwan. Closed circles, values used for estimating the relationship; open circles, values not used.

Discussion

The proportion of female sailfish off eastern Taiwan increased gradually with fish length at 145–205 cm *LJFL*. As fish length increased beyond 205 cm *LJFL*, the proportion of males declined sharply, and no males > 230 cm *LJFL* were caught. The sex ratio remained at < 0.5 in every season, in contrast to that found in the eastern Pacific (Hernández-Herrera and Ramírez-Rodriguez, 1998) where sex ratios tended to be larger than 0.5 during spawning season, but did not differ from 1:1 at other times. Off eastern Taiwan waters, the sex ratio followed an increasing power function between 145 and 230 cm *LJFL*. Our results are consistent with other observations of sex ratios in blue marlin (Tzeng, 2002) and swordfish (Wang et al., 2003) in the same study area. Billfishes have a clear sexual dimorphism, which may result from growth rate differences between males and females (Sun et al., 2002, Chiang et al., 2004) or from sex-specific fishing and natural mortality rates (Sun et al., 2005). The relationship between the sex ratio and body size can provide effective information to reconstruct the sex composition from catch data.

Ovaries were examined for reproductive activity using three different methods: histology, largest oocyte size, or *GSI* (West, 1990). All three techniques can be used as proxies for each other (Young et al., 2003). Histological examination of the de-

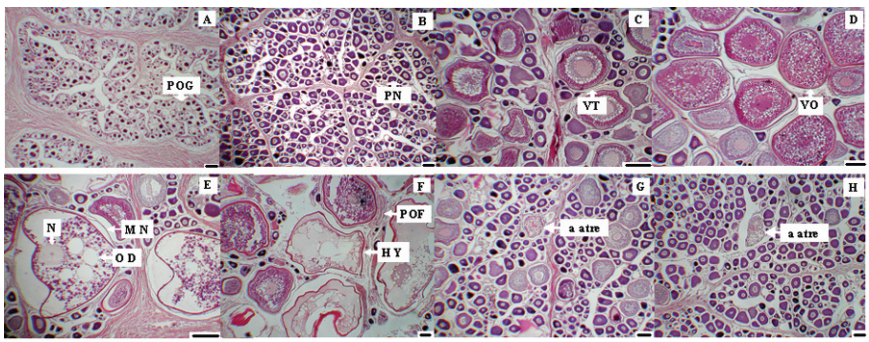

Figure 4. Photomicrographs of ovaries at different maturity stages in sailfish (*Istiophorus platypterus*): (A) immature; (B) developing; (C) maturing; (D) ripening; (E), spawning; (F) recently spawned; (G) spent; and (H) resting. POG: primitive oogonia; PN: perinucleolar oocytes; VT: early vitellogenic oocytes; VO: vitellogenic oocytes; MN: migratory nucleus oocyte; OD: oil droplet; HY: hydrated oocyte; N: nucleus; POF: post ovulatory follicle; α atre: α atretic oocytes. Scale bar = 100 μm.

Table 2. Histological criteria for classification of gonadal developmental stages and maturation in female sailfish (*Istiophorus platypterus*) collected from the waters off eastern Taiwan. Maturity stages were based on the criteria of DeMartini et al. (2000). Gonadosomatic index were shown for each stage.

Stage	Maturity classification	Histological appearance of most advanced gamete stage present in gonads	*GSI* mean ± SD (sample size)
1	Immature	Only primitive oogonia present and no vitellogenesis basophilic cytoplasm, darkly stained with Hematoxylin. (Fig. 4A)	0.39 ± 0.08 (2)
2	Developing	Chromatin nucleolar and early perinucleolar stage oocytes present. (Fig. 4B)	0.46 ± 0.15 (26)
3	Maturing	Late perinucleolar, previtellogenic oocytes of different sizes and small vitellogenic oocytes present, red staining yolk granules and globules noticeable. (Fig. 4C)	1.61 ± 1.12 (35)
4	Ripening	Active stage of vitellogenic oocytes and perinucleolar and small vitellogenic oocytes present. (Fig. 4D)	2.46 ± 0.99 (36)
5	Spawning	Migratory nucleus and hydrated oocytes present; hydrated oocytes appeared translucent, advanced vitellogenic oocytes were opaque. (Fig. 4E)	5.01 ± 2.86 (27)
6	Recently spawned	Post ovulatory follicles present, previtellogenic oocytes become more visible and all size of oocytes obvious. (Fig. 4F)	5.28 ± 2.10 (56)
7	Spent or resting	Only perinucleolar stage oocytes present, advanced atresia of oocytes evident. (Fig. 4G and H)	0.79 ± 0.46 (64)

velopmental stages of oocytes is the most accurate method for determining sexual maturity, but the preparation of histological sections is expensive and time-consuming. *GSI* could be affected by the size of fish and may not be an accurate indicator to separate between mature but reproductively inactive females (stage 7) and immature females (stage 1–3) during nonspawning seasons (Mejuto and Garcia, 1997; Hernández-Herrera and Ramírez-Rodriguez, 1998; Wang et al., 2003). Generally, maximum oocyte diameter, calibrated by histology, is a more accurate indicator of the temporal and geographical extent of spawning in scombrids (Schaefer, 2001). In order to

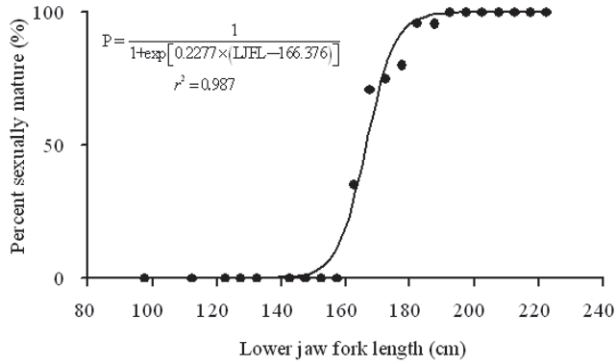

Figure 5. Relationship between percentage of mature female sailfish (*Istiophorus platypterus*) and lower jaw fork length (5 cm class) in the waters off eastern Taiwan. 50% maturity is attained at 166.37 cm *LJFL*.

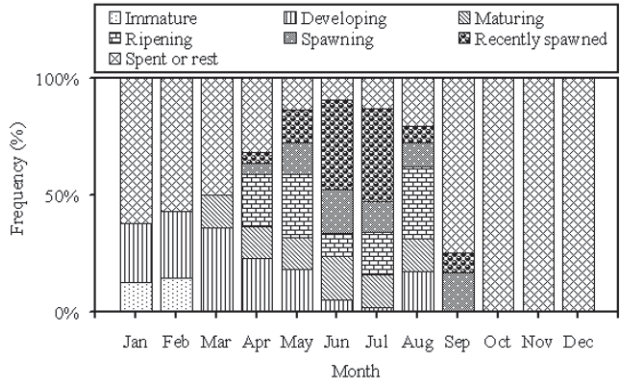

Figure 6. Monthly changes in the frequency of occurrence of various maturity stages of ovaries of sailfish (*Istiophorus platypterus*) in the waters off eastern Taiwan.

determine reproductive activity, female samples were classified using the criterion of an oocyte diameter of > 348 μm (i.e., the probability of sexual maturity, P > 50%). Similar results were obtained using histological observation and the criterion of oocyte diameter. Only two of the 56 ripening samples were determined to be inactive. Therefore, the "reproductively active oocyte diameter" predicted in this study should be more accurate than the reproductively active diameter estimated by Eldridge and Wares (1974). They used the number of modes in the oocyte diameter distribution (0.3 mm) to infer the sexual maturity and spawning seasonality for female sailfish in the eastern Pacific Ocean.

Nakano and Bayliff (1992) and Uosaki and Bayliff (1999) assumed that eastern Pacific female sailfish were about to spawn when the gonad index was ≥ 3, and the body length was about 121–140 cm EFL. In this study, a L_{50} of 146 cm EFL (or 167 cm *LJFL*, according to the relationship between *LJFL* and EFL; Chiang et al., 2004) was estimated based on histological examination for female sailfish caught in the eastern waters of Taiwan. Hernández-Herrera and Ramírez-Rodriguez (1998) estimated L_{50} values to be 175 cm EFL for female sailfish caught by the Mexico's recreational fishery based on histological examination. The difference between the eastern and western Pacific might be a result of geographical isolation, stock structuring, different environmental conditions, or sampling errors resulting from sample size and timing. The different fisheries are also a source of variation. Further studies are needed to evaluate and identify the factors causing differences between the eastern and western Pacific populations.

Sailfish are distributed from the coast of Ecuador to Mexico in the eastern Pacific Ocean and their migration pattern is related to the 28 °C surface isotherm (Ovchinnikov, 1966; Kume and Joseph, 1969b). Hernández-Herrera and Ramírez-Rodriguez (1998) found that the sailfish spawning season in the eastern Pacific is protracted during summer and autumn when the surface isotherm was 27–30 °C. Similarly, we found that sailfish spawned from April to September in the waters off eastern Taiwan when the surface isotherm was 26–29 °C (IGOSS, 1999). Beardsley et al. (1975) inferred that spawning of sailfish might occur throughout warm tropical waters. We believe that, while sailfish are migrating, they reproduce wherever they can find optimal water temperatures and suitable oceanic conditions. It is clear that sailfish spawn extensively in eastern Taiwan waters. Additional research (including tagging

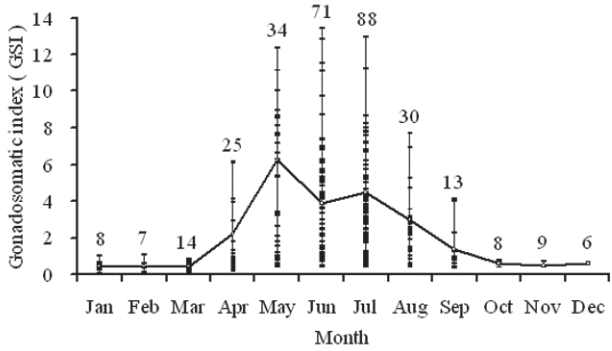

Figure 7. Monthly changes in mean gonadosomatic index (*GSI*) of female sailfish (*Istiophorus platypterus*) in the waters off eastern Taiwan (vertical bars, standard error; numbers above vertical bars, sample size).

experiments) is necessary to better understand the migration route after spawning. Furthermore, international cooperative research and management are necessary to maintain optimal harvest levels for this species.

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