Zoological Studies **50**(3): 296-308 (2011)

Zoological Studies

Reproductive Biology of the Needlefish *Tylosurus acus melanotus* in Waters around Hsiao-Liu-Chiu Island, Southwestern Taiwan

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(Accepted December 23, 2010)

Yih-Yia Liao and Yu-Hsin Chang (2011) Reproductive biology of the needlefish *Tylosurus acus melanotus* in waters around Hsiao-Liu-Chiu Island, southwestern Taiwan. *Zoological Studies* **50**(3): 296-308. In this study, we investigated the reproductive biology of the needlefish *Tylosurus acus melanotus* in waters of Hsiao-Liu-Chiu off southwestern Taiwan. In total, 658 specimens (329 females and 329 males) were collected monthly from driftnet catches in July 2008-Aug. 2009. Relationships between the eye fork length (EFL) and body weight (BW) did not significantly differ between sexes. Oocyte development was categorized into 8 stages based on histological examinations. The spawning season extended from Apr. to Aug. based on the macroscopic appearance of the gonads, a histological examination of the ovaries, estimations of the gonadosomatic index, and the frequency of oocyte diameters. Sizes at 50% maturity were respectively estimated to be 56.8 and 53.3 cm in EFL for females and males. The sex ratio did not significantly differ from 0.5 according to a Chi-squared test ($p > 0.05$). The mean fecundity of this species was estimated to be 25,629 ± 6902 eggs. The batch fecundity was estimated to be in the range of 1062-24,576 eggs with a mean of 5748 ± 4670 eggs.
http://zoolstud.sinica.edu.tw/Journals/50.3/296.pdf

Key words: Reproductive biology, *Tylosurus acus melanotus*, Hsiao-Liu-Chiu, Southwestern Taiwan.

The needlefish *Tylosurus acus melanotus* (Bleeker, 1850) is an epipelagic species that is widely distributed in tropical and subtropical waters of the Indo-Pacific region. In Taiwan, this species is abundant and can be found in waters around the main island except for the northeastern coast. Hsiao-Liu-Chiu I. is located off the southwestern coast of Taiwan, and is the only coral reef island in Taiwan. This species can be caught, mainly by gill nets, in waters of this island year round, and it is a commercially important species for the local fishery. The unit price of this species is relatively high (US$3/kg) in local fish markets. Unfortunately, catch statistics of this species are not available because it is not sold through regular fish markets. Hence, the exact amount of the catch and population dynamics of this species are still unknown.

Biological information for the family Belonidae is generally well documented. For example, Briggs (1970) and Cressey and Collette (1971) documented host-parasite relationships of *Ablennes anastomella*; Forster (1974) described the behavior, development, and early life history of *Xenentodon cancila*; Zhao and Jiang (1983) reported the habits and early morphogenesis for *A. anastomella*; James (2002) observed the swimming shape of *Strongylura marina*; and Banford et al. (2004) studied the taxonomy of the Belonidae. However, fishery biology information, which is essential for fishery management of this species, is still poorly known.

The aim of the present study was to provide the 1st information on the reproductive biology including oocyte and ovarian development, the spawning season, the sex ratio, the gonadosomatic index (GSI), the hepatosomatic index (HSI), the condition factor (CF), the size at sexual maturity,

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fecundity, and batch fecundity of the needlefish in southwestern Taiwan waters. All these parameters can be used as useful inputs for further assessments of this stock.

MATERIALS AND METHODS

Specimens of needlefish, caught by gill nets, were collected monthly from Hsiao-Liu-Chiu I., southwestern Taiwan from July 2008 to Aug. 2009 (Fig. 1, Table 1). Standard length (SL), fork length (FL), total length (TL), and eye fork length (EFL) were measured to the nearest 0.1 cm; body weight (BW) and gutted body weight to the nearest 0.1 g; and gonad weight to the nearest 0.01 g. The sex was identified after the fish were brought back to the laboratory.

The relationship between BW and EFL was expressed by $BW = a \times EFL^b$, where a and b are constants. The BW-EFL relationship, after being logarithmically transformed, between sexes was compared using an analysis of covariance (ANCOVA).

To facilitate comparisons with other work that reported their results in a measure other than EFL, a linear regression was used to convert between measurements. These regression lines were

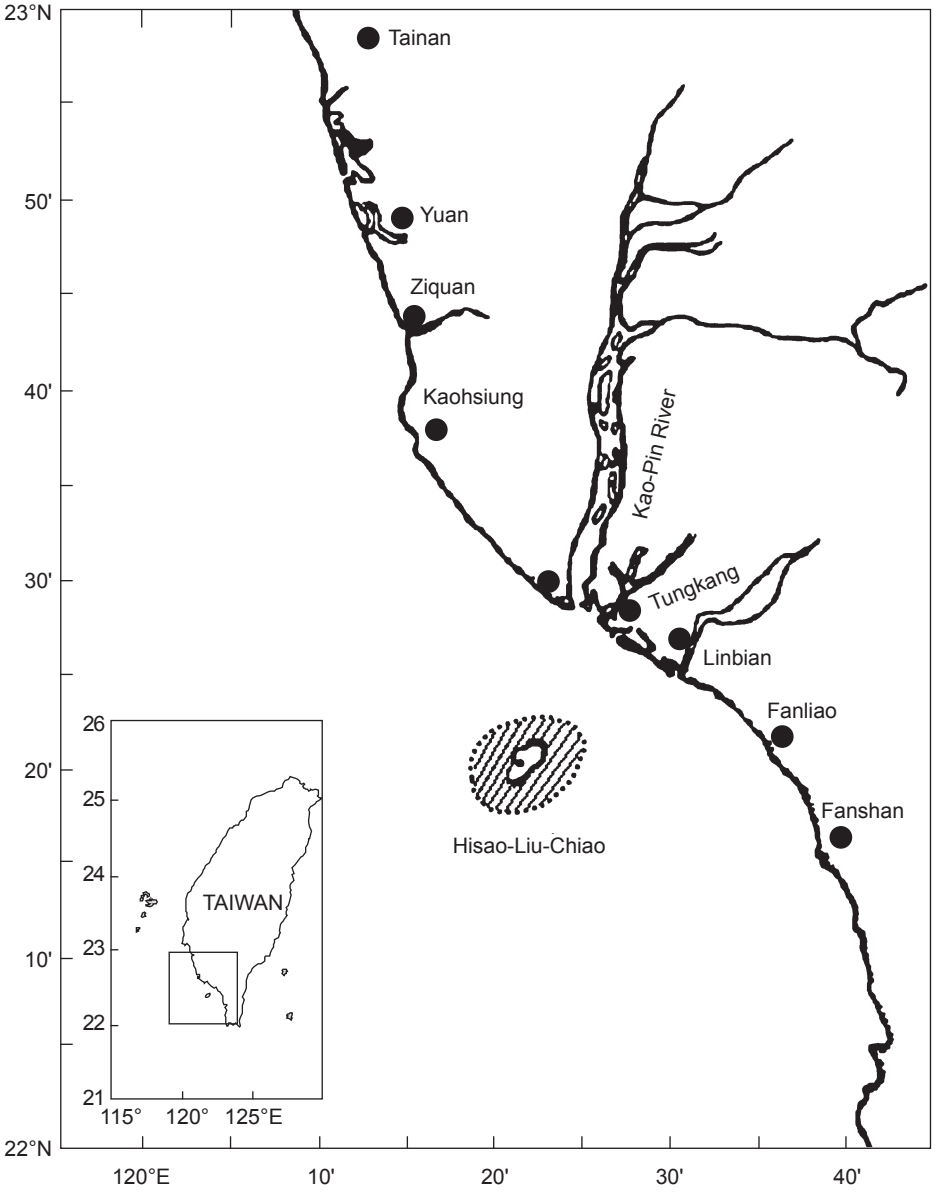

Fig. 1. Fishing grounds of the gill net fishery in waters around Hsiao-Liu-Chiu, Taiwan in this study.

compared between sexes using ANCOVA.

Three ovaries taken from different individuals at different development stages were used to examine the homogeneity of oocyte diameter and the number of oocytes. Each ovary was divided into 6 portions (the anterior, middle, and posterior portions of each lobe), and 0.5 g taken from each portion was placed on Petri dishes with a grid scale. All oocytes were measured and counted with a projector (25x) (Nikon V-12B, Tokyo, Japan). A two-way analysis of variance (ANOVA) showed that there was no significant difference in the number of oocytes among portions for an individual ($p > 0.05$, $n = 18$), but a significant difference was found for the same portion among individuals ($p < 0.05$). This suggests that oocyte diameters and the number of oocytes were homogeneous among portions. For consistency, the middle portion of the right lobe of each ovary was examined in this study.

Gonads were macroscopically examined and preserved in 10% Bouin's formalin for further analysis. The histological procedures used in this study followed Humason (1979) and Wu et al. (2008). In total, 280 gonads (183 females and 97 males) were used for the histological procedures in this study. Gonads were embedded in paraffin, sectioned at 5-10 μm in thickness, and routinely stained with hematoxylin and eosin. The development stages of oocytes were categorized according to Yamamoto (1956) after slight modification. The oocyte diameter at each stage of oocyte development was determined from measurements of 30 random oocytes. The size range was determined from the means of the smallest and largest oocytes for each stage of oocyte development.

Between 200 and 400 oocytes from each ovarian development phase were examined to determine the size-frequency distribution of oocytes within each maturity phase. All oocytes of > 0.1 mm were measured for all stages. The maturity phases were examined by histological observation. The ovary of the immature phase only contained small pre-vitellogenic oocytes with basophilic cytoplasm. The maturing phase contained oocytes in different stages of vitellogenesis. The mature phase contained the largest oocytes of the dissolving-nucleus stage and fusion of yolk globules.

The GSI was calculated as GSI = (gonad weight / gutted body weight) × 10^3. The HSI was calculated as HSI = (liver weight / gutted body weight) × 10^3. The CF was calculated as CF = (body weight/ eye fork length^3) × 10^3. All fish larger than the minimum size at sexual maturity (see "RESULTS") were used to examine seasonal changes in the GSI, HSI, and CF.

The sex ratio was expressed as the number of females/number of both sexes combined. A Chi-squared test was used to examine the homogeneity of the sex ratio.

Four techniques were used to determine the spawning season of needlefish in this study: (1) the macroscopic appearance of the ovaries, i.e., swollen, containing many eggs, and being yellowish-orange (Erickson et al. 1985, Iqbal et al. 2007); (2) histological examination of the ovaries, i.e., containing advanced vitellogenic oocytes (Yamada et al. 1998, Yoneda et al. 1998a, Moreno et al. 2005, Yamaguchi et al. 2006, Iqbal et al. 2007); (3) oocyte diameter measurements, i.e., the largest oocytes exceeding 1.6 mm, which belonged to the migratory-nucleus stage or above (Erickson et al. 1985); and (4) GSI, i.e., with values higher than those in other months (Hunter and Goldberg 1979, Erickson et al. 1985, Yamada et al. 1998, Funamoto et al. 2004, Iqba et al. 2007). The spawning season was determined by a judgment considering all information derived from the above 4 methods.

A sample was taken from the middle portion of the right lobe of each ovary to estimate either fecundity or batch fecundity. Ovarian

Table 1. Specimens of *Tylosurus acus melanotus* collected during the period from July 2008 to Aug. 2009 in this study

Year	Date	Numbers		Range of EFL (cm)	Sex ratio	χ^2
		Female	Male			
2008	July 29	10	24	43.8-102.8	0.29	5.76*
	Aug. 29	11	17	46.0-73.9	0.39	1.29
	Sept. 26	33	12	40.3-61.4	0.73	9.8*
	Oct. 31	38	22	35.9-65.0	0.63	4.27*
	Nov. 28	27	28	45.1-73.6	0.49	0.02
	Dec. 30	37	13	42.4-62.0	0.74	11.52*
2009	Jan. 20	30	20	40.9-59.6	0.6	2.0
	Feb. 25	24	24	40.6-65.0	0.5	0
	Mar. 26	24	28	45.2-67.3	0.46	0.31
	Apr. 27	19	33	40.5-84.0	0.37	3.77
	May 21	15	34	45.0-75.1	0.31	7.37*
	June 24	16	33	43.4-69.7	0.33	5.9*
	July 24	31	19	44.6-85.2	0.62	2.88
	Aug. 25	14	22	45.4-88.6	0.39	1.78
Total		329	329			

EFL, eye fork length. *Significant at the 5% level ($p < 0.05$).

tissue samples (0.5 g each), each containing approximately 150-350 oocytes, were placed on Petri dishes with a grid scale in water. Advanced oocytes were counted using a projector (25x). The fecundity (F) was estimated from the following equation: F = (number of oocytes of > 0.2 mm in diameter in 0.5 g of the ovary) × (weight of the ovary) / (0.5 g ovary). In each ovary, oocytes of > 0.2 mm in diameter corresponded to the primary yolk stage. Batch fecundity (BF) was estimated from specimens with ovaries containing oocytes in the migratory-nucleus or mature stage (Yoneda et al. 2002) during the spawning season. It was estimated by BF = (number of oocytes > 1.9 mm in diameter for 0.5 g of the ovary) × (weight of the ovary) / (0.5 g ovary). In total, 21 ovaries were used to estimate the F and BF.

The size at maturity estimates were based on observations of 116 females and 182 males collected from Apr. to Aug. (the spawning season). Sexually mature individuals were defined as individuals with gonads in the developing or more-advanced stages as determined by histological observations. The size at 50% maturity was estimated using a logistic function described as:

$$P = \frac{1}{(1 + e^{a + bEFL})};$$

where P is the proportion of mature fish in each length interval, EFL is the eye fork length, and a and b are parameters. The logistic function was fitted to the fraction of mature fish per 5-cm-length interval using a non-linear regression (Marquardt method). The size at 50% maturity was then obtained by substituting P = 0.5 in the above equation.

RESULTS

Length-weight relationship

In total, 658 specimens of needlefish (329 females and 329 males) were used in this study. The length and weight frequency distributions indicated that females were in the range of 35.9-102.8 cm in EFL with body weights of 208.4-3350 g, while males were 40.6-88.6 cm in EFL with body weights of 297.4-1396.4 g. The length-weight relationships were estimated as: $BW = 0.0139EFL^{2.72}$ ($p < 0.05$, n = 329) for females and $BW = 0.0065EFL^{2.91}$ ($p < 0.05$, n = 329) for males. Since no significant difference between sexes was found, the combined BW-EFL relationship of both sexes was expressed as: $BW = 0.012EFL^{2.76}$ ($p < 0.05$, n = 658) (Fig. 2).

EFL-SL, EFL-FL, EFL-TL relationships

Since there was no significant difference between sexes, combined relationships of the sexes were used to describe EFL-SL, EFL-FL, and EFL-TL:

EFL = -5.67 + 0.87SL ($p < 0.05$, n = 658),
EFL = -4.45 + 0.82FL ($p < 0.05$, n = 658), and
EFL = -6.44 + 0.82TL ($p < 0.05$, n = 658).

Sex ratio

The sex ratio of all specimens was 0.5, which was the same in numbers for both females and males (Table 1). Females did not outnumber males during the spawning season. However, the Chi-squared test indicated that females outnumbered males at ≥ 71 cm EFL (Fig. 3).

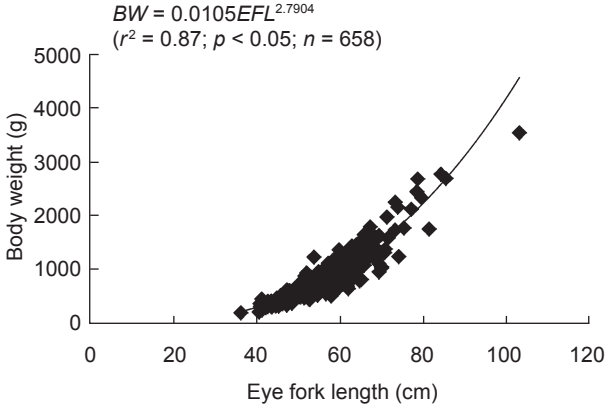

Fig. 2. Relationship between the body weight and eye fork length of *Tylosurus acus melanotus*.

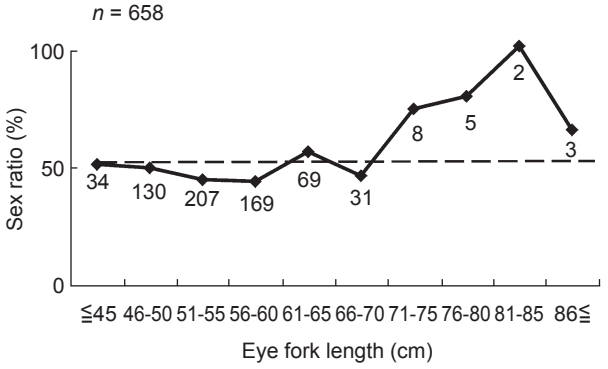

Fig. 3. Variation of the sex ratio in different size intervals (EFL, eye fork length) for *Tylosurus acus melanotus*.

Macroscopic structure of the gonads

Paired gonads of needlefish were easily distinguishable by sex from their appearances as they were not equal in shape or size. Male gonads were triangular or diamond-shaped, and female gonads were cylindrical. Ovaries of immature specimens were slender and small, and their color was pale yellowish-orange, but those of mature ones were very swollen containing many eggs,

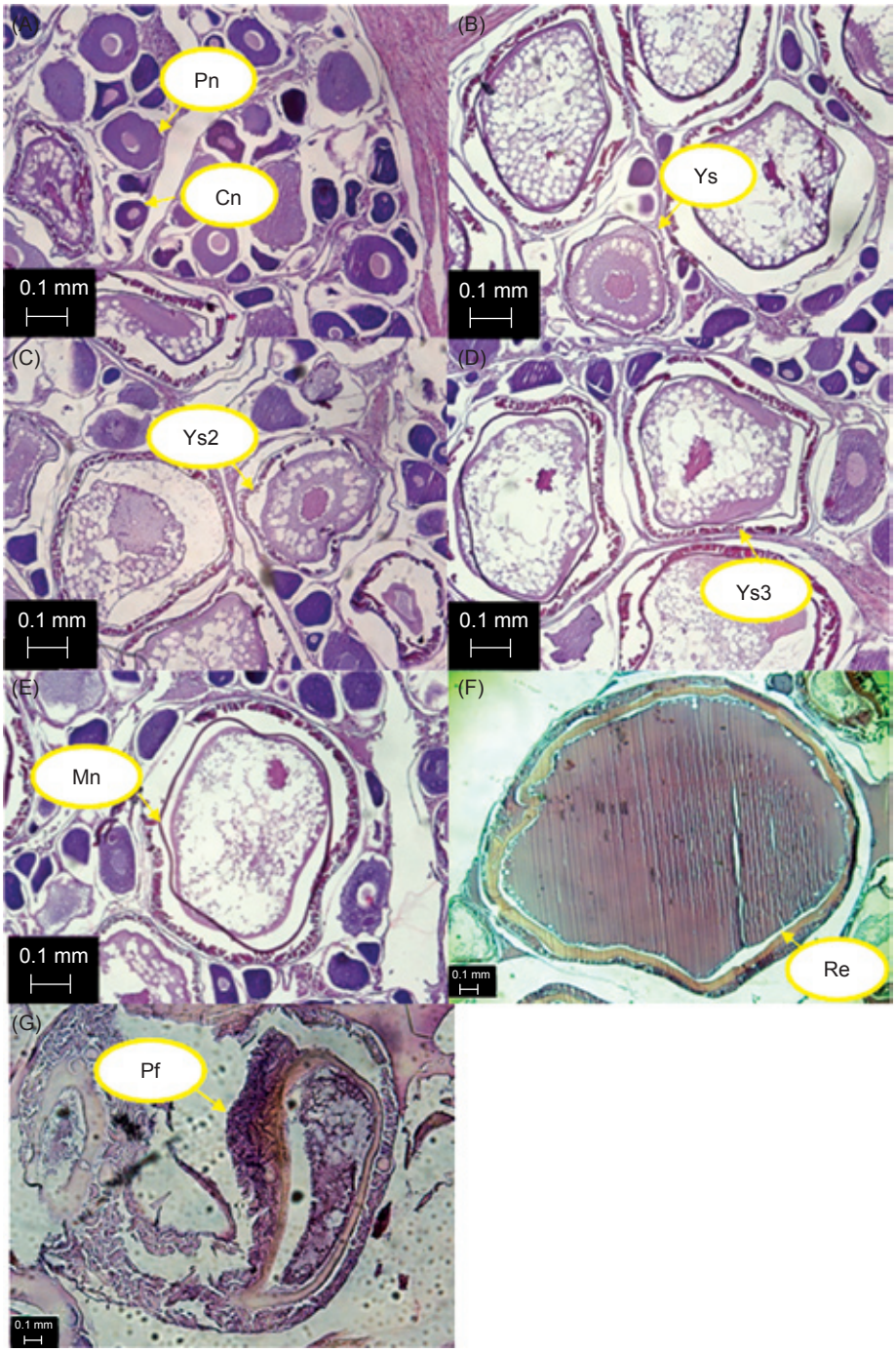

Fig. 4. Histological preparations of oocytes of *Tylosurus acus melanotus*: (A) chromatin (Cn) and the perinucleolus stage (Pn), (B) primary yolk globule (Ys1), (C) secondary yolk globule stage (Ys2), (D) tertiary yolk globule stage (Ys3), (E) migratory-nucleus stage (Mn), (F) mature stage (Re), (G) postovulatory follicle stage (Pof).

and their color was deep yellowish-orange. Testes of immature fish were thicker with a pale yellow color, but those of mature ones were white, and milt occurred in the spermatic duct.

Histological observation of ovaries

The development of oocytes of needlefish can be classified into 8 stages based on cytological characteristics of cells following Yamada et al. (1998), Maack and George (1999), Yamaguchi et al. (2006), and Iqbal et al. (2007) as described below.

(1) The chromatin-nucleolus stage was comprised of the youngest and smallest oocytes. The large nucleus was surrounded by cytoplasm. The oocytes remained strongly basophilic, and were deeply stained purple with hematoxylin. Oocyte diameters ranged 0.04-.21 mm (Fig. 4A).

(2) In the peri-nucleolus stage, the cytoplasm had become less basophilic, and stained pale with hematoxylin. At the end of this stage, a number of nucleoli of different sizes were situated in the periphery of the nucleus. Oocyte diameters ranged 0.1-0.25 mm (Fig. 4A).

(3) In the primary yolk stage, the size of oocytes had become larger, but they still stained with hematoxylin. Oil-droplets and yolk vesicles began to appear in the cytoplasm. Some yolk globules began to appear in the cytoplasm. Oocyte diameters ranged 0.22-0.4 mm (Fig. 4B).

(4) In the secondary yolk stage, the accumulation resulted in the rapid growth of oocytes. Yolk globules and oil-droplets rapidly increased in size and number. Oocyte diameters ranged 0.32-0.84 mm (Fig. 4C).

(5) In the tertiary yolk stage, yolk globules and oil-droplets continued to increase in size and number. The nucleus which is located at the center of the oocyte was spherical, and the nucleus was irregularly shaped. Oocyte diameters ranged 0.76-1.9 mm (Fig. 4D).

(6) In the migratory-nucleus stage, the nucleus had moved toward the animal pole of the egg, and a few larger oil droplets were found. The oil-droplets first migrated towards the centripetal nucleus. Oocyte diameters ranged 1.6-2.3 mm (Fig. 4E).

(7) In the mature stage after germinal vesicle breakdown, yolk globules were fused with each other in the peripheral cytoplasm. Oocyte diameters ranged 2.2-3.2 mm (Fig. 4F).

(8) In the postovulatory follicle stage, various types of postovulatory follicles with different morphological features were found. Postovulatory follicles were characterized by a large follicular lumen formerly occupied by the oocyte. It

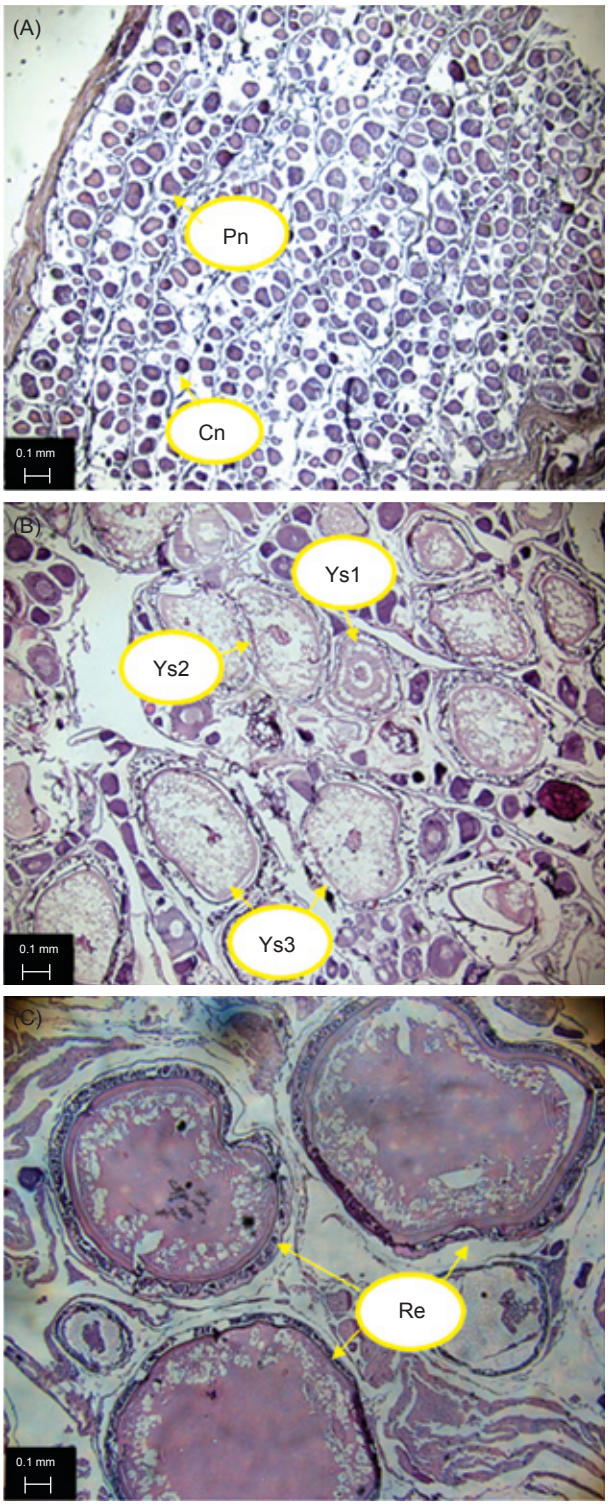

Fig. 5. Histological preparation of oocytes of *Tylosurus acus melanotus* in different developmental phases: (A) immature phase, (B) maturing phase, and (C) mature phase.

gradually lost its lumen and was invaded by follicular cells (Fig. 4G).

Ovarian development

Ovarian development based on ovarian color and a histological examination was divided into the following 3 phases (Erickson et al. 1985, Iqbal et al. 2007). In the immature phase, the ovaries were small, slender, and translucent. Only small unyolked oocytes were detected. Most oocytes were in the chromatin-nucleolus to peri-nucleolus stages (Fig. 5A), and the GSI was 1.2 ± 0.9 (n = 32). In the maturing phase, the size of the oocytes had become larger and swollen, and the color was yellowish-orange. Oocytes in different vitellogenic stages were present. Most oocytes were in the primary-yolk to tertiary-yolk stages (Fig. 5B), and the GSI was 40.2 ± 17.2 (n = 64). In the mature phase, ovaries were more swollen than in the maturing stage and were a deep yellowish-orange color. The hydrated ovary contained oocytes in all different developmental stages. Most advanced oocytes exceeded the migratory nucleus stage (Fig. 5C), and the GSI was 67.3 ± 21.6 (n = 21).

Distribution of oocyte sizes

Sizes of oocytes gradually increased with ovarian development (Fig. 6). Larger-sized groups of > 1.6 mm were mainly found during Apr. and Aug., and sizes decreased from Sept. The frequency distribution of oocyte diameters was highly correlated with the GSI. Oocyte diameters were always < 1.6 mm for individuals with a GSI < 28.7. As the oocytes reached 1.6 mm (in the late

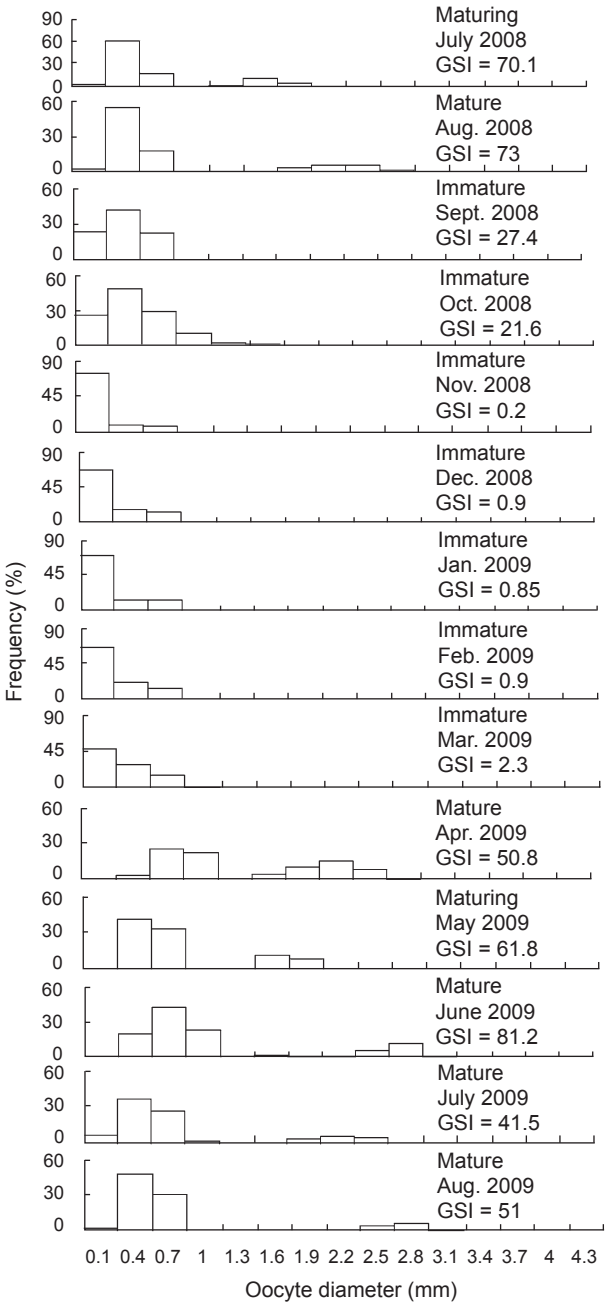

Fig. 6. Monthly frequency distribution of oocyte diameters of *Tylosurus acus melanotus*.

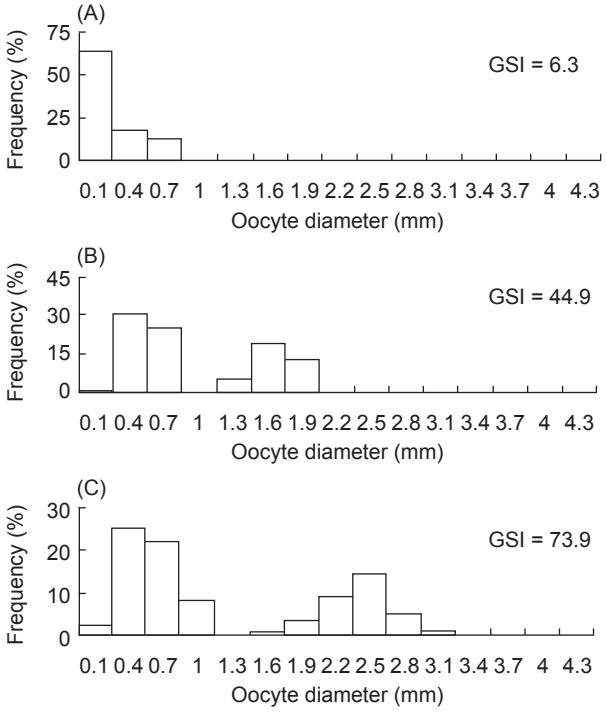

Fig. 7. Frequency distribution of oocyte diameters in different maturity phases in ovaries of *Tylosurus acus melanotus*. (A) Immature phase, (B) maturing phase, and (C) mature phase.

tertiary yolk stage), a clutch consisting of advanced oocytes was formed. Once oocytes grew larger than 2.2 mm in diameter, they became mature and formed an advance batch, which was distinct from adjacent groups of smaller oocyte cohorts with sizes up to 1.0 mm. Three specimens collected between Apr. and Aug. had postovulatory follicles. Postovulatory follicles only contained oocytes in the tertiary, migratory-nucleus, and mature stages.

To examine the primary maturity phases, 1 representative female of each phase was chosen to show the typical frequency distribution of oocyte sizes (Fig. 7). The vast increases in the size and volume of advanced oocytes were distinct. In a reproductively active ovary, there was a hiatus in the oocyte size distribution. This means that oocyte development was not continuous, and a hiatus appeared between pre-vitellogenic and vitellogenic oocytes in either the maturing or mature phases (Figs. 5, 7). Maturation of oocytes and the growing GSI were reflected in the increasing oocyte diameters of different ovaries. In the mature ovary, only the advanced group of oocytes formed the batch which increased in size and largely occupied the entire volume of the ovaries, while those in less-advanced oocyte

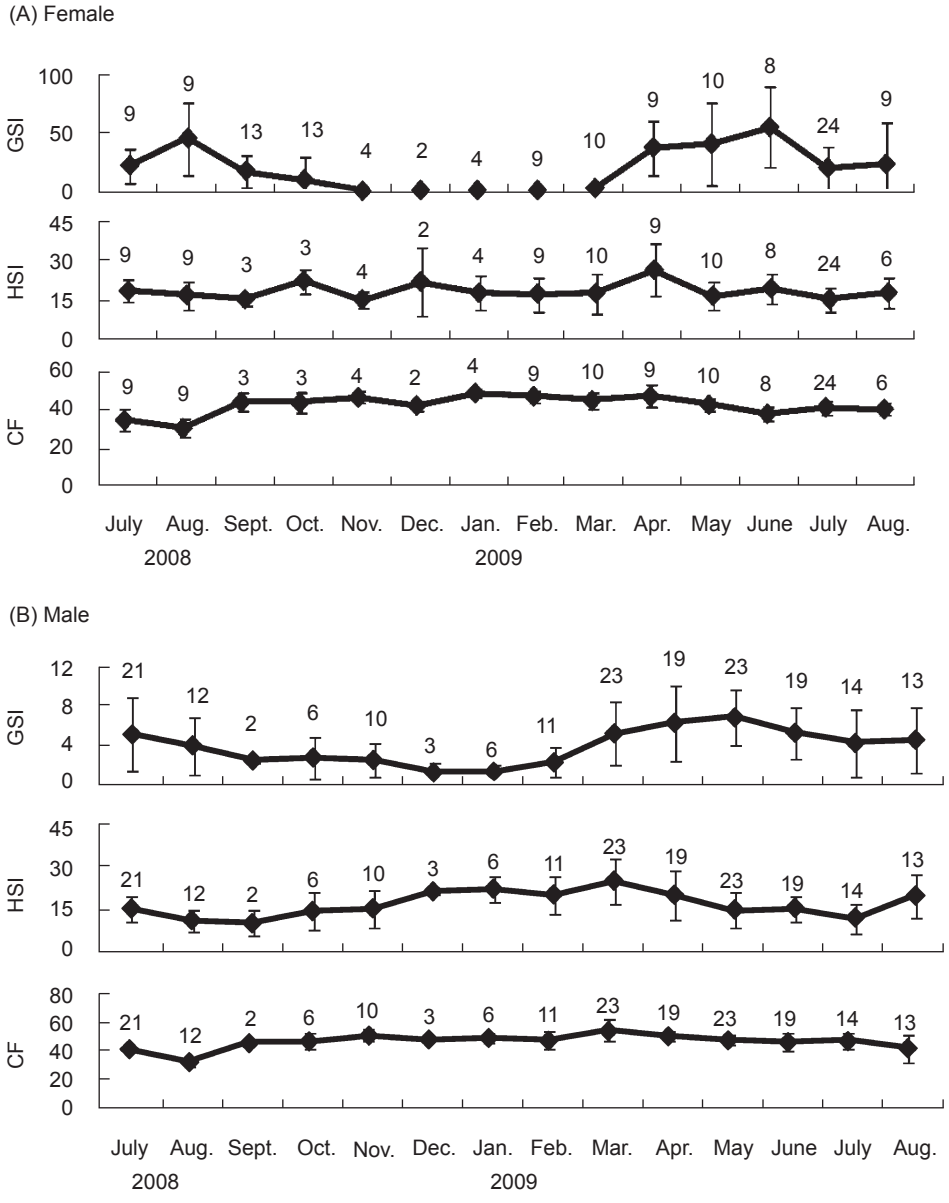

Fig. 8. Monthly variations in the gonadosomatic index (GSI), hepatosomatic index (HSI), and condition factor (CF) for *Tylosurus acus melanotus*. (A) Females and (B) males.

groups remained in the pre-vitellogenic stage and were smaller.

Testis development

Based on the color and a histological examination, testis development was divided into the following 3 stages. In the immature stage, the testes were thicker, and the color was pale-yellow. Most germ cells were in the spermatogonia stage to primary spermatocytes. In the maturing stage, the testes had rapidly increased in size. Most germ cells were in the spermatid stage. In the mature stage, the testes had become larger than in the maturing stage. Most germ cells were in the spermatozoa stage.

Monthly changes in the GSI, HSI, and CF

Monthly changes in the GSI of female needlefish are shown in figure 8A. The mean GSI of females declined in Sept. 2008, and dropped to the lowest (0.53-2.88) in Mar. 2009, then began to increase in Apr. and peaked in June (48.86). It gradually declined in July. The GSI of males began to decline from Sept. 2008, and dropped to its lowest value in Jan.2009. It gradually began to increase in Mar., reached a peak in May 2009, and

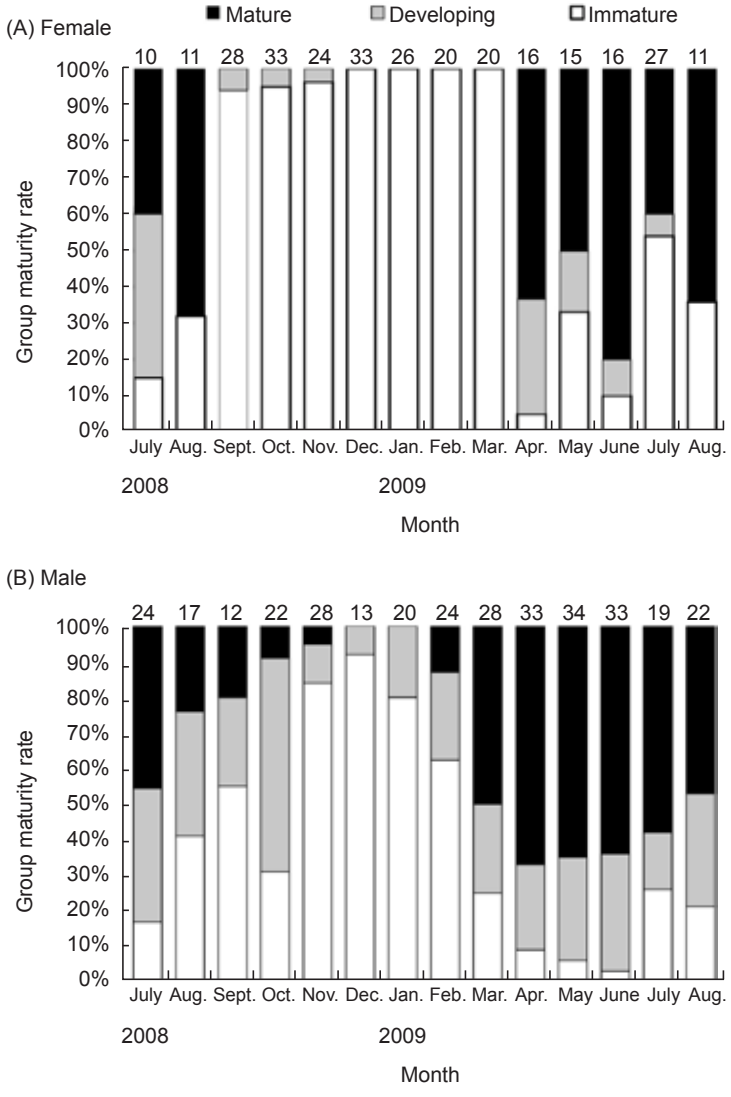

Fig. 9. Monthly variations in the group maturity rate for *Tylosurus acus melanotus*. (A) Females and (B) males.

gradually declined from July (Fig. 8B).

Monthly changes in the HSI of females are shown in figure 8A. Although the HSI exhibited variations, it did not show a distinct trend. A similar pattern was also found for the HSI of males (Fig. 8B). Mean HSI values of females were 15.79-28.06. The HSI was highest in Apr. 2009, and lowest in Feb. 2009.

Monthly changes in the CF for females and males are shown in figure 8A and 8B, respectively. Values were lowest in Aug. 2008 in both sexes. However, only a slight fluctuation was noted.

Group maturity rate

Monthly percentages of the occurrences of gonad development stages in July 2008 to Aug. 2009 for females and males are shown in figure 9A and 9B, respectively. The mature stage was only found in July and Aug. 2008 and Apr.-Aug. 2009 for females with a peak in June 2009 (78%). Females with immature or resting ovaries were found throughout the year. Only the immature stage for females occurred in the period from Dec. 2008 to Mar. 2009, while Dec. 2008 and Jan. 2009 were the only months without the mature stage in males. The mature stage was found in males from July 2008 to Aug. 2009 except in Dec. 2008 and Jan. 2009, and the peak was in May 2009 (65%).

Spawning season

The macroscopic appearance of the ovaries indicated that most immature ovaries occurred from Sept. to Mar., and mature ovaries appeared from Apr. to Aug. The group maturity rate of females also showed a similar pattern of mature ovaries only being found from Apr. to Aug. (Fig. 9A). The histological examination showed that mature oocytes of needlefish were present from Apr. to Aug. Oocyte diameter measurements indicated that 2 modes of oocytes existed in Apr.-Oct. The GSI in Apr.-Aug. was higher than values of other months. Based on the results of the above methods, the spawning season of the needlefish in the waters around Hsiao-Liu-Chiu,

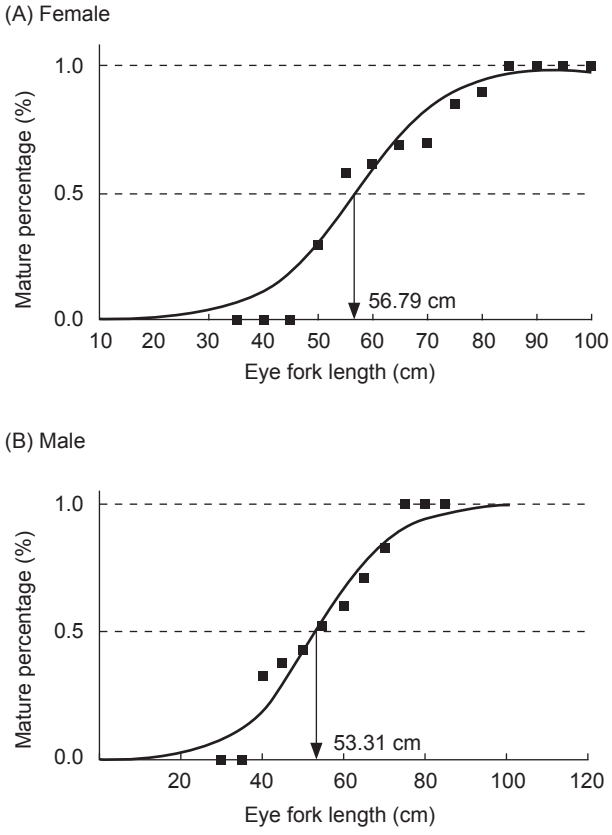

Fig. 10. Relationship between the mature percentage and eye fork length (EFL) for *Tylosurus acus melanotus*. (A) Females and (B) males.

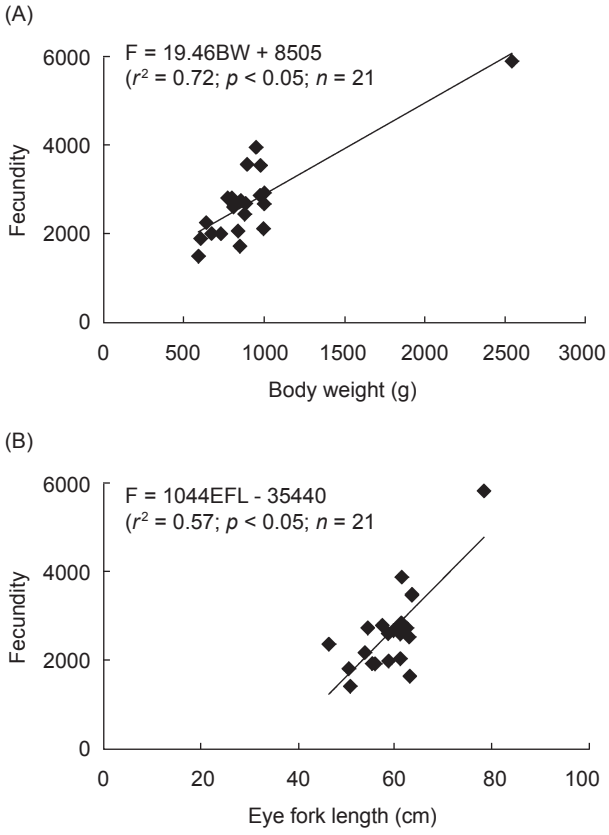

Fig. 11. Relationships of fecundity with body weight (BW) (A) and eye fork length (EFL) (B) for female *Tylosurus acus melanotus*.

Taiwan appeared to be from Apr. to Aug. with a peak in June.

The size at 50% maturity

The minimum sizes at maturity were 50.6 and 46.4 cm for females and males, respectively. The logistic equations fitted to *P* were calculated as $P = 1/(1 + e^{6.79 - 0.12EFL})$ ($p < 0.05$, $n = 329$) for females (Fig. 10A) and $P = 1/(1 + e^{5.54 - 0.11EFL})$ ($p < 0.05$, $n = 329$) for males (Fig. 10B). Based on these equations, EFLs at maturity equivalent to the L_{50} were estimated to be 56.8 and 53.3 cm for females and males, respectively.

Fecundity and batch fecundity

The fecundity of needlefish in this study was estimated based on 21 ovaries with distinct modes of size-frequency distributions of oocytes, which ranged 50.6-132.3 g. The fecundity was estimated to range 14,836-41,117 eggs with a mean ± S.D. of 25,629 ± 6902. Fecundity increased with the BW and EFL (Fig. 11), and their relationships were estimated as:

Fecundity = 8505 + 19.46BW ($p < 0.05$, $n = 21$) and

Fecundity = -35,440 + 1044EFL ($p < 0.05$, $n = 21$).

Batch fecundity was also estimated using the same 21 ovaries. The batch fecundity was estimated to range 1062-24,576 eggs with a mean ± S.D. of 5748 ± 4670. The relative fecundity, expressed as the number of oocytes per gram of gutted body weight, was estimated to be 20-43 (mean, 29) oocytes/g, and there was no significant relationship between the relative fecundity and BW.

DISCUSSION

The largest specimen collected in this study was 102.8 cm EFL, which is close to the maximum size of 115 cm TL recorded in Fishbase (2005), but no specimen smaller than 35 cm EFL was found in this study. It is likely that the lack of larval specimens was due to the study area not covering the breeding ground.

Variations in the sex ratio may be influenced by a number of factors including differential mortality, the growth rate, longevity, sex reversal, seasons, fishing grounds, and fishing methods (Wenner 1972, Huang and Su 1986, Miu et al. 1990, Wu et al. 2001, Micale et al. 2002, Chiou et al. 2004, Koutrakis et al. 2004). In addition, differences in growth among sexes, sexual dimorphism, and migration may also be influencing factors (Wu et al. 2008). In this study, no sexual dimorphism was found. Therefore, the increase in the sex ratio with an increase in size might be related to differential growth, mortality, and longevity between sexes.

The GSI is the easiest method to judge the spawning season, but it cannot correctly indicate gonad maturity in the later period (West 1990). In order to accurately determine the spawning season, we used 4 methods to judge reproductive cycling in the present study. Good correspondence of the 4 methods suggested that our estimate of the spawning season (Apr. to Aug.) is reasonable.

Liu et al. (2001) suggested that the development of ovaries and the process of oocyte development may be stimulated by a change in the water temperature. The spawning season of needlefish began in Apr. which coincides with the time when water temperatures begin to rise in the waters around Taiwan. A similar finding was mentioned by Liu et al. (2001) for *Priacanthus macracanthus*.

Fish can store energy in the liver or viscera to meet the requirements of spawning. In this study, an opposite trend between the GSI and CF (Fig. 6) of females suggested that the required energy for spawning by female needlefish might be derived from their fat reserves. However, an opposite trend between the GSI and HSI (Fig. 7) of males suggested that the required energy for spermatogenesis by male needlefish may be derived from their livers.

In general, the ovaries of multiple spawners simultaneously have both postovulatory and vitellogenic oocytes, after the postovulatory follicles gradually disappear as the vitellogenic oocytes develop (Iqbal et al. 2007). Yoneda et al. (1998b) indicated that the Japanese anglerfish *Lophiomus setigerus* in the East China Sea had ovaries with a tertiary yolk, and migratory-nucleus and mature stage oocytes with postovulatory oocytes during the spawning season, which provided evidence of multiple spawning. The needlefish showed similar results. Some of the advanced oocytes were found together with postovulatory oocytes in ovaries during the spawning season (Apr.-Aug.), which indicates that the needlefish in waters of Hsiao-Liu-Chiu off Taiwan is a multiple spawner

during the spawning season. Murua and Saborido-Rey (2003) indicated that ovarian development belonging to the group-synchronous type should exhibit at least 2 populations of oocytes at any 1 time. Such ovaries contain a fairly synchronous population of larger oocytes as a clutch and a more-heterogeneous population of smaller oocytes from which the clutch was recruited. They also pointed out that the determinate fecundity of batch spawner species should be the number of yolked oocytes remaining in the ovary, which decreases with each spawning time because the standing stock of yolked oocytes is not replaced during the spawning season. From the macroscopic appearance and development of the oocyte size distribution (Fig. 6), 2 distinct modes of oocyte diameter distribution of mature females during spawning season suggest that the needlefish is a group-synchronous type of spawner. A high proportion of reproductively active females only being found in the spawning season and the appearance of a gap between pre-vitellogenic and vitellogenic oocytes demonstrated in figure 7 indicate that the needlefish is a determinate spawner. Therefore, we concluded that the needlefish belongs to the group-synchronous and determinate batch-spawner type.

Wu et al. (2008) suggested that spawning may be a rapid process, and hydration in many teleosts may occur as early as 12 h before spawning. Hence, it is very difficult to capture female fish with running hydrated oocytes. In the present study, no ovarian samples which had hydrated or running ripe oocytes were found. Therefore, the Hsiao-Liu-Chiu waters might not be the spawning ground for needlefish.

Fecundity is an important field of study in fish reproduction and population dynamics (Yoneda and Yoda 2006). Large variations in batch fecundity in teleosts were documented by many authors. For example, the ratio of batch fecundity and fecundity of *Psenopsis anomala* was 31%-46% (Wang and Chen 1995), of *Leiognathus equulus* was 20%-33% (Lee et al. 2005), and of *Nemipterus peronii* was 5%-48% (Wu et al. 2008). The fecundity of needlefish was estimated to range 14,836-41, 117 eggs, batch fecundity ranged 1062-24,576 eggs, and the ratio ranged 7%-60%. The high variability of the batch proportion may change with different spawning stages. Differences between fecundity and batch fecundity can be influenced by many factors, such as stocks and individuals (Johnson et al. 1997), the water temperature of the habitat, the food supply, and food quality (Maack and George 1999, Liu et al. 2001). However, the actual factors may be complex, and further investigation is needed for needlefish in the future.

The spawning season for needlefish in waters of Hsiao-Liu-Chiu off southwestern Taiwan extended from Apr. to Aug. with a peak in June. We recommend protecting adults with seasonal closure from Apr. to Aug. during the major spawning season, thereby providing better breeding oppor-tunities in order to ensure sustainable utilization of this species.

Acknowledgments: We would be like to thank Dr. Liu, Dr. Joung, Dr. Chiou, and Dr. Fan for their useful suggestions. Also the authors wish to thank the fisherman, Mr. Chen, and his wife for their kind help in collecting the monthly samples.

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