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REPRODUCTIVE BIOLOGY OF THE BOMBAY-DUCK *HARPADON MICROCHIR* IN THE COASTAL WATERS OFF SOUTHWESTERN TAIWAN

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Key words: reproductive biology, *Harpadon microchir*, spawning season, fecundity.

ABSTRACT

The bombay-duck, *Harpadon microchir*, is one of the important bycatch species of the trawl fishery in the waters off southwestern Taiwan. However, its biological information, especially reproductive biology, is still lacking. In this study, the reproductive biology of the bombay-duck was described based on 1,697 specimens (1,066 females and 631 males) caught by bottom trawl fishery in the waters off southwestern Taiwan from October 2007 to December 2008. The spawning season of this species was estimated to be in May and from August to December with a peak in November based on the macroscopic appearance, gonadosomatic index, histological examination, and group maturity rate. Oocyte development and ovarian development were categorized into nine stages and four stages, respectively based on histological or macroscopic examination. Mean fecundity and mean batch fecundity were estimated to be 446,373 ± 174,858 and 61,680 ± 60,279, respectively. The relationship between fecundity (F) and body weight (BW) was estimated as: $F = -46.580 + 9.69 \times 10^3 BW$($r^2 = 0.587$; $n = 50$). The sex ratio (female/total), 0.63, was significantly different from 0.5. The sizes at 50% maturity were estimated to be 39.9 and 19.8 cm FL for females and males, respectively.

I. INTRODUCTION

The bombay-duck, *Harpadon microchir* Günther, 1878, a demersal species, is widely distributed in the northwestern Pacific Ocean, including Taiwan and Japan waters [18]. This species can be found in the waters surrounding Taiwan except the north [19]. This demersal species usually inhabits near the continental shelf or in the mud-sand sediment. It mainly preys on small fish and crustaceans [18]. In addition, this species is an important prey for the threadfin bream *Nemipterus spp.* [15] and its trophic level is 4.3 in the waters off southwestern Taiwan [Chen *et al*. unpubl.]. This species can be caught as byctch all year round mainly by trawl fishery in the southwestern Taiwan. It used to have very little economic value (< US$0.5 kg^{-1}), but its unit price increased to about US$4 kg^{-1} during our survey period from 2007 to 2008. Thus, the bombay-duck made considerable contribution in economics to the bottom trawl fishery in recent years [Chen *et al*. unpubl.].

Biological information of the Genus *Harpadon* has been well documented. Several aspects of *H. nehereus* have been described e.g., age and growth [9], food and feeding habits [15, 16], and population dynamics [3]. Liao's (unpubl.) description on age and growth of *H. microchir* in the waters off southwestern Taiwan by using otolith is the only study for this species. However, the reproductive biology information of *H. microchir* is still lacking.

This species is an important element in the marine ecosystem in the southwestern Taiwan waters [Chen *et al*. unpubl.]. However, the life history information which is essential for fisheries management and conservation of this species is lacking. Thus, the objective of this study is to provide the first information on reproductive biology including oocyte and ovarian development, spawning season, sex ratio, gonadosomatic index (GSI), hepatosomatic index (HSI), condition factor (CF), size at maturity, fecundity (F) and batch fecundity (BF) of bombay-duck in the southwestern Taiwan waters. It is anticipated that the results derived from this study can be used to estimate population growth rate with demographic analysis and can be used as input parameters for stock assessment and management of this stock.

II. MATERIALS AND METHODS

The specimens, caught by a commercial trawl vessel (38

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Table 1. Sample size and size range of the specimens of *Harpadon microchir.*

Month/year	Female			Male			Total
	N	Range of FL (cm)	Range of BW (g)	N	Range of FL (cm)	Range of BW (g)	
Oct. 2007	28	19.3-28.5	27.22-84.04	35	15.4-29.2	7.81-93.36	63
Nov. 2007	28	19.8-50.5	29.71-635.00	24	19.9-30.0	29.37-124.27	52
Dec. 2007	39	16.3-49.5	18.77-600.00	19	10.2-28.2	2.81-106.96	58
Jan. 2008	26	15.2-29.5	11.64-102.05	32	14.4-29.1	9.32-115.13	58
Feb. 2008	37	15.5-29.3	11.29-94.01	18	17.1-26.0	17.74-71.94	55
Mar. 2008	28	11.4-27.2	7.40-84.14	28	11.4-28.5	4.45-107.53	56
Apr. 2008	28	16.2-29.9	12.17-102.54	27	13.1-26.2	7.10-65.35	55
May 2008	38	13.9-47.3	9.32-170.40	24	11.9-30.4	5.16-111.62	47
Jun. 2008	29	11.8-34.7	4.87-188.88	22	10.2-29.2	3.58-110.83	51
Jul. 2008	32	11.3-28.0	2.62-75.07	23	12.9-26.6	3.23-69.70	55
Aug. 2008	171	15.7-50.9	9.17-580.00	137	6.4-32.7	16.87-157.61	308
Sep. 2008	132	18.0-39.7	17.91-275.00	52	6.3-36.6	13.60-205.00	184
Oct. 2008	107	15.8-44.3	10.37-420.00	91	21.2-33.7	30.72-152.92	191
Nov. 2008	187	7.2-57.7	0.70-912.00	28	21.0-32.5	37.07-147.77	154
Dec. 2008	156	17.5-48.2	16.90-146.59	71	19.7-35.3	29.83-215.00	225
Total	1,067	7.2-57.7	0.7-912.00	631	6.3-36.6	2.81-215.00	1,697

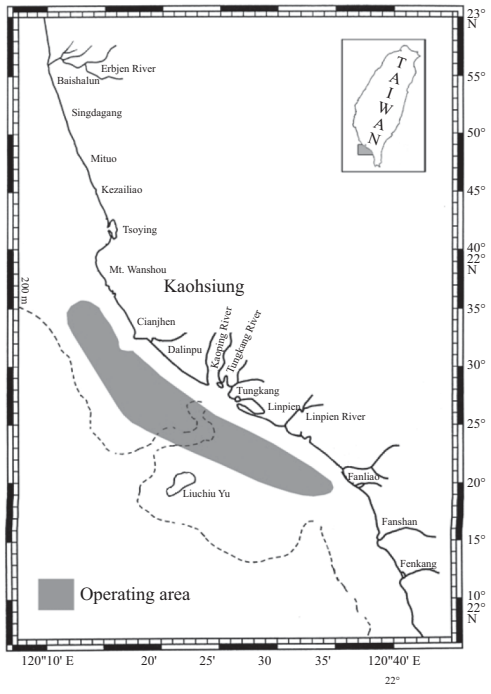

Fig. 1. Sampling area of *Harpadon microchir* in the waters off southwestern Taiwan.

tons, 350 HP) with mesh size of 2 cm in the depth of 50-250 m in the waters off southwestern Taiwan (Fig. 1), were collected randomly in size from the catch on a monthly basis from October 2007 to December 2008 (Table 1). Measurements of specimens were taken on the standard length (SL), fork length (FL), total length (TL) to the nearest 0.1 cm, body weight (BW), and gonad weight to the nearest 0.01 g. The gonads were macroscopically examined to determine the mature condition prior to preservation in 10% formalin until further processing. For some small juvenile individuals, which sex cannot be identified by naked eyes, a microscopic observation (Nikon YS100) coupled with histological examination were used to facilitate the sex identification. Histological procedures in this study follow the method of Lee *et al*. [12]. After cleaning, dehydration, infiltration, and paraffin embedding, the tissue was sectioned to 3-10 μm in thickness and stained with hematoxylin and eosin. A total of 212 gonads (157 females, 55 males) were processed for further examination on gonadal development.

Three ovaries taken from three different individuals (49.5, 49.5, 42.7 cm FL) with different gonadosomatic index (GSI) were selected to examine the homogeneity of oocyte diameter and number of oocytes. Each ovary was divided into six portions (anterior, middle, and posterior portions of each lobe), and an amount of 0.01 g of ovary was taken from each portion. These ovary samples were placed on glass slides with grid scale and all oocytes were measured and counted with a projector (25X) (Nikon V-12B). A two-way analysis of variance (ANOVA) indicated that the mean number of oocytes is not significantly different among different portions of an individual ($n = 18$, $F = 1.17$, $p > 0.05$), but significant difference was found among different individuals ($n = 18$, $F = 102.9$, $p < 0.05$). In addition, the frequency distribution of oocyte diameter showed similar trend among different portions of an individual but obviously different patterns were found among different individuals. These results indicated that oocyte diameter and number of ooctyes were homogeneous among portions for an individual. For consistency, the top portion of the right lobe of each ovary was taken for analysis in this study.

Table 2. Monthly sex ratio of the specimens of *Harpadon microchir* in this study.

Month	Number of		Total	Sex ratio	χ^2
	Female	Male			
Oct. 2007	28	35	63	0.44	0.78
Nov. 2007	28	24	52	0.54	0.31
Dec. 2007	39	19	58	0.67	6.90*
Jan. 2008	26	32	58	0.45	0.62
Feb. 2008	37	18	55	0.67	6.56*
Mar. 2008	28	28	56	0.5	0
Apr. 2008	28	27	55	0.51	0.02
May. 2008	38	24	62	0.61	3.16
Jun. 2008	29	22	51	0.57	0.96
Jul. 2008	32	23	55	0.58	1.47
Aug. 2008	171	137	308	0.56	3.75
Sep. 2008	132	52	184	0.71	34.78*
Oct. 2008	107	91	198	0.54	1.29
Nov. 2008	187	28	215	0.87	117.59*
Dec. 2008	156	71	227	0.68	31.83*
Total	1,066	631	1,697	0.63	111.51*

Gonadosomatic index, hepatosomatic index and condition factor (CF) were calculated as follows: GSI = (gonad weight/ gutted body weight) $\times 10^2$, HIS = (liver weight/ gutted body weight) $\times 10^2$, and CF = (body weight/ fork length^3) $\times 10^5$. The sex ratio was calculated as sex ratio = number of females/ number of both sexes combined. The fecundity (F) was estimated from the following equation: F = (number of oocytes greater than 0.1 mm in diameter for 0.02 g of the ovary) $\times$ (weight of ovary)/(0.02 g ovary). In each ovary, oocyte greater than 0.1 mm in diameter corresponds to the yolk vesicle stage. Batch fecundity was estimated based on the number of hydrated oocytes in the ovary, and was estimated by BF = (number of oocytes greater than 0.44 mm in diameter for 0.02 g of the ovary) $\times$ (weight of ovary)/(0.02 g ovary). In total, 50 and 35 ovaries were used for the estimation of fecundity and batch fecundity, respectively.

The size at maturity (L_{50}), defined as the FL at 50% maturity, was estimated in the spawning season. The following logistic equation was fitted to the proportion of mature per 1 cm FL intervals by a nonlinear regression:

$$\mathrm{Pr} = \frac{1}{1+e^{a+bFL}} \quad (1)$$

where Pr is the proportion of mature fish in each length interval, *a* and *b* are parameters to be estimated.

III. RESULTS

A total of 1,697 specimens including 1,066 females and 631 males were collected in this study. Females ranged from 7.2 to 57.7 cm FL with body weights of 0.7-912.0 g, while males ranged from 6.3 to 36.6 cm FL with body weights of 2.81-215 g (Table 1). Females were mainly in 21-40 cm FL while males mainly ranging from 21-30 cm FL with few larger than 33 cm FL.

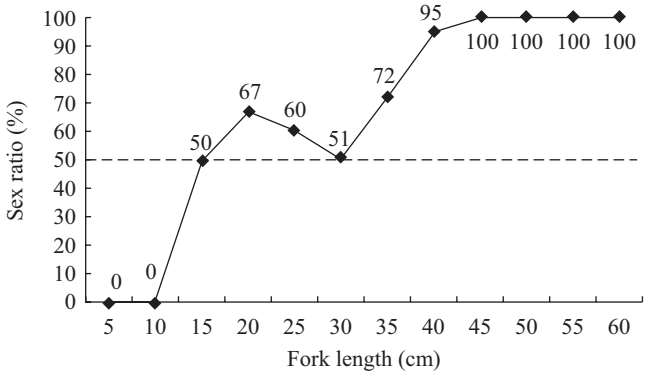

Fig. 2. Sex ratio-at-length of *Harpadon microchir*.

The sex ratio of all specimens, 0.63, was significantly different from 0.5 (n = 1697, $p < 0.05$). However, monthly sex ratio was not significantly different from 0.5 in most months except in December 2007, and in February, September, November, and December 2008 (Table 2). The Chi-square test indicated that females outnumbered males from 35.0 cm FL onward (Fig. 2).

The gonads of female and male bombay-duck were similar in shape and size, but they can be easily identified from their external appearances except some small individuals. Immature ovaries were slender and small and their color was pale yellow, but mature ones were very swollen containing many eggs and their color was deeply yellow-orange. Immature testes were thin with pale yellow color, but mature ones were white and milt occurred in the spermatic duct.

Nine development stages of oocytes for *H. microchir* were determined based on histological observation following Lee [12], Rahman and Tachihara [17], and Yamaguchi [29] as follows:

(1) Chromatin-nucleolus stage: Oocytes are very small, and remain strongly basophilic, so are stained deeply purple by haematoxylin. Oocyte diameter ranges from 0.02 to 0.04 mm (Fig. 3(a)).
(2) Peri-nucleolus stage: The shape of oocyte is rounded. A number of nucleoli of different sizes are situated in the periphery of the nucleus. Oocyte diameter ranges from 0.03 to 0.08 mm (Fig. 3(b)).
(3) Yolk vesicle stage: Oocytes become larger and less basophilic, but cytoplasm is still stained with hematoxylin. At the beginning of this stage, white yolk vesicles start to appear in the cytoplasm near the nucleus. Oocyte diameter ranges from 0.07 to 0.12 mm (Fig. 3(c)).
(4) Primary yolk stage: Yolk globules and oil-droplets start to increase in the cytoplasm, and yolk globules move to

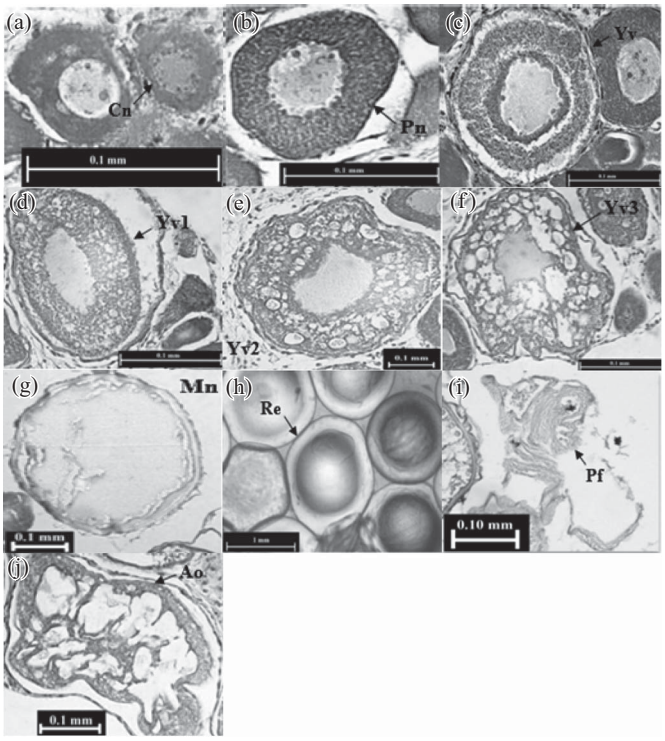

Fig. 3. Histological appearance of ovaries of *Harpadon microchir*: (a) Chromatin-nucleolus stage (Cn), (b) Peri-nucleolus stage (Pn), (c) Yolk vesicle stage (Yv), (d) Primary yolk stage (Ys1), (e) Secondary yolk stage (Ys2), (f) Tertiary yolk stage (Ys3), (g) Migratory nucleus stage (Mn), (h) Ripe egg stage (Re), (i) Postovulatory follicle stage (Pf), (j) Atretic oocytes (Ao).

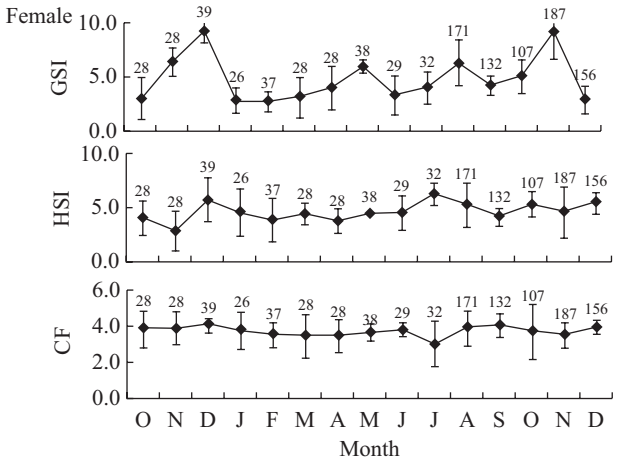

Fig. 4. Monthly variations of gonadosomatic index, hepatosomatic index and condition factor of female *Harpadon microchir*. Numbers indicate sample size.

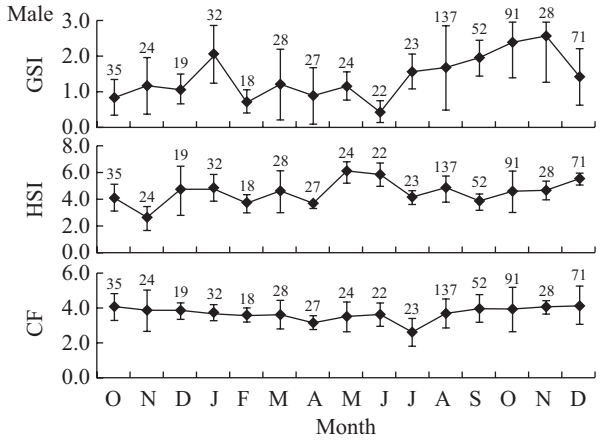

Fig. 5. Monthly variations of gonadosomatic index, hepatosomatic index and condition factor of male *Harpadon microchir*. Numbers indicate sample size.

nucleus. Oocyte diameter ranges from 0.1 to 0.17 mm (Fig. 3(d)).

(5) Secondary yolk stage: Larger oil-droplets start to move to nucleus and yolk globules and oil-droplets continue to increase in number. Oocyte diameter ranges from 0.09 to 0.19 mm (Fig. 3(e)).

(6) Tertiary yolk stage: Yolk globules keep developing and begin to merge into yolk granules. Yolk globules and oil-droplets continue to increase in size and number. The yolk globules begin to coalesce into a yolk mass. Oocyte diameter ranges from 0.16 to 0.32 mm (Fig. 3(f)).

(7) Migratory nucleus stage: Yolk globules and oil-droplets do not change in number, but oil-droplets keep increase in size. The yolk appears as a homogeneous mass containing the oocytes. Oocyte diameter ranges from 0.31 to 0.47 mm (Fig. 3(g)).

(8) Ripe egg stage: Shape was complete circle and transparent. A single yolk mass exists and yolk globules have fused into larger ones (Fig. 3(h)). Postovulatory oocytes are founded in this stage (Fig 3(i)). Oocyte diameter exceeds 0.44 mm.

(9) Atretic oocytes: Oocytes which do not ovulate are absorbed by ovary itself then atrophy (Fig. 3(j)).

Based on the GSI variation, macroscopic examination, and histological analysis, ovarian development can be divided into the following three stages:

(1) Immature stage: Ovaries are small (< 0.2 g), thin and translucent. Ooocytes are very small and in high density. Most oocytes are in the chromatin-nucleolus stage to the peri-nucleolus stage.

(2) Maturing stage: Ovaries become larger (0.2 to 16.8 g) and are deeply white, their macroscopic structure is found to be blood capillary. Most oocytes are in the yolk vesicle stage to the secondary yolk stage.

(3) Mature stage: Ovaries become apparently larger (> 16.8 g), and are yellow-white. Oocytes near the surface of ovary can be seen clearly with naked eyes. Most oocytes are in the tertiary yolk stage to the ripe egg stage.

Monthly changes of GSI were shown in Figs. 4 and 5 for

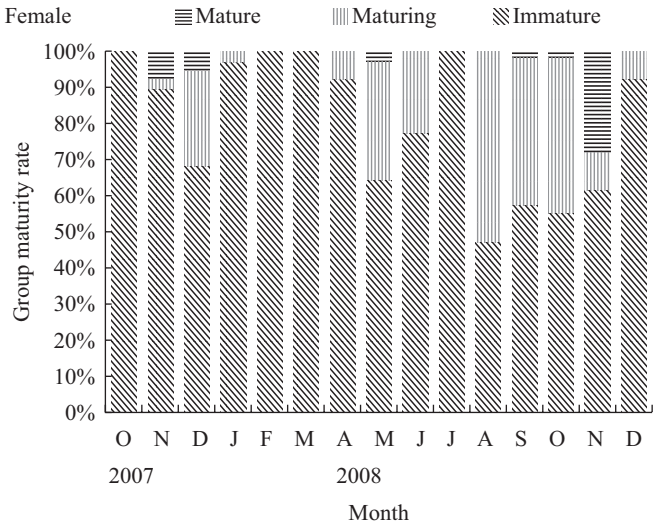

Fig. 6. Monthly variation of group maturity rate of female *Harpadon michrochir*.

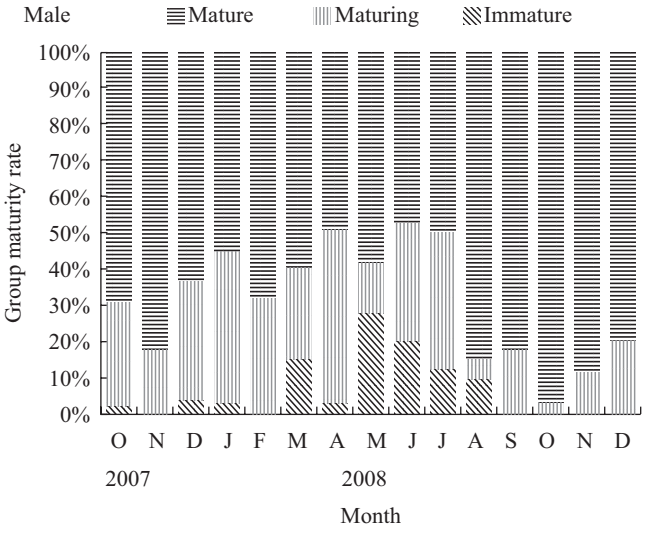

Fig. 7. Monthly variation of group maturity rate of male *Harpadon michrochir*.

females and males, respectively. The mean GSI of females peaked in December 2007 (9.24) then dropped to the lowest values (2.8) in February, 2008. The mean GSI of males dropped to the lowest value (0.46) in June 2008 and peaked in November 2008 (2.6). The mean HSI of females and males ranged from 2.98-6.39 and 2.65-6.14, respectively (Figs. 4 and 5). It peaked in May and August 2008, and had the lowest value in November 2007 for both sexes. Similar trends of monthly changes of GSI and HSI for females indicated that liver development is closely related to ovary maturation. The monthly changes of CF indicated that CF remained stable with the lowest value in July 2007 for both sexes (Figs. 4 and 5).

Monthly percentage of occurrences of ovarian development stages for females and males during October 2007 to December 2008 were shown in Figs. 6 and 7, respectively. Immature females were found in October 2007, February 2008, March 2008 and July 2008, while mature ones were found in May 2008, and from August to December 2008, with a peak in November (26%). Mature males were found all year round in this study with a peak in October 2008 (97%).

The macroscopic appearance of the ovaries indicated that most immature ovaries occurred from February to July, and mature ovaries appeared from August to December. The GSI of females in May, August, November and December was higher than those in other months (Fig. 4). Group maturity rate of females also showed similar pattern that mature ovaries were only found in May and from August to December (Fig. 6). Histological examination showed that the mature oocytes of bombay-duck presented from August to January. Based on above methods, the spawning season of *H. microchir* in the coastal waters off southwestern Taiwan was estimated to be in May and from August to December.

The logistic equations describing the relation between proportion of maturity (Pr) and fork length were estimated as follows:

$$\Pr = 1/(1+e^{5.7971-0.1916FL})(p<0.05, n=1,066)$$

for females (Fig. 8) (2)

$$\Pr = 1/(1+e^{4.4544-0.084FL})(p<0.05, n=631)$$

for males (Fig. 9) (3)

Based on these logistic equations, the sizes at 50% maturity were estimated to be 39.9 cm and 19.8 cm FL for females and males, respectively (Fig. 8, Fig. 9).

The fecundity of *H. microchir*, estimated based on 50 ovaries ranging from 40-57.7 cm FL, was estimated to range from 271,514 to 621,230 eggs with a mean (±SD) as 446,372 ± 174,858. The fecundity increased with body weight and gonad weight, and their relationships were estimated as follows:

$$F = -46,580 + 9.69\times10^3 BW (r^2 = 0.584; n = 50)$$

(Fig. 10) (4)

$$F = 24,767 + 4.27\times10^3 GW (r^2 = 0.573; n = 50) \quad (5)$$

The batch fecundity was also estimated based on 35 ovaries in the spawning season, which body length ranged from 40.4-57.7 cm FL. The batch fecundity was estimated to range from 1,401 to 121,959 eggs with a mean (±SD) as 61,680 ± 60,279. There is a significant relationship between batch fecundity and gonad weight: $BF = -37,164 + 16894GW$(r^2 = 0.672; n = 34) but the relationship is weak for the batch fecundity and body weight: $BF = -34,301 + 174.1BW$(r^2 = 0.158; n = 35).

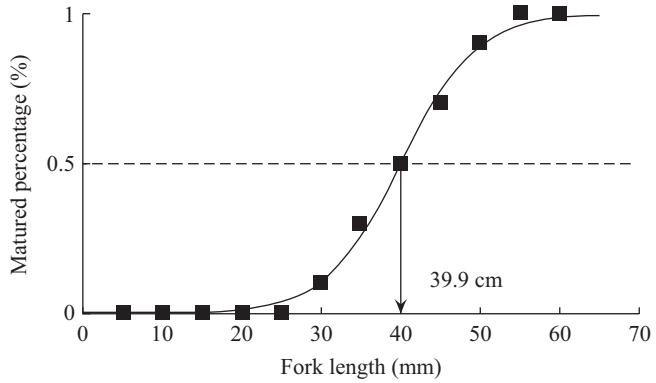

Fig. 8. The relationship between proportion of maturity and fork length of female *Harpadon microchir*.

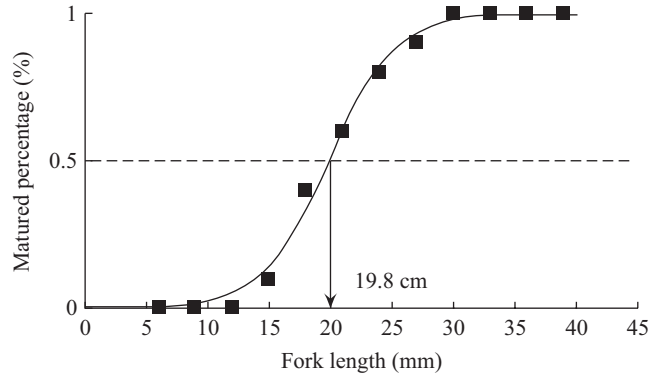

Fig. 9. The relationship between proportion of maturity and fork length of male *Harpadon microchir*.

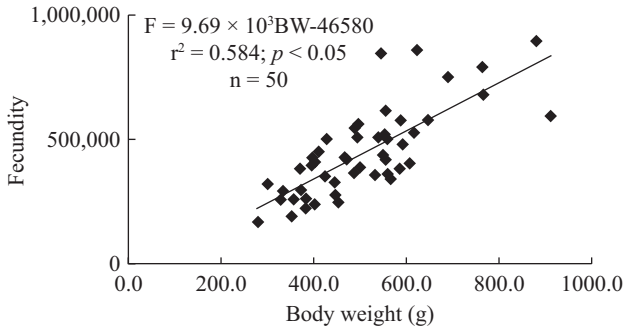

Fig. 10. The relationship between fecundity and body weight of *Harpadon microchir*.

IV. DISCUSSION

Variation of sex ratio may be influenced by a number of factors including mortality, growth rate, life span, sex reversal, seasons, fishing grounds, fishing methods [25], and spawners' behavior change in spawning season [11]. In addition, differences in growth, mortality, or longevity between sexes, sexual dimorphism, and migration may also be the possible influencing factors [27]. In this study, females outnumber males during the spawning season. Other studies have found similar results for those species with high fecundity such as the white croaker (*Argy-rosomus argentatus*) [24], mullet (*Mugil cephalus*) [22], and white-tongued crevalle (*Uraspis helvolus*) [1], the sex ratio in the spawning season is low. On the other hand, the sex ratio in the spawning season is high for those species with low batch fecundity such as the Indian drift fish (*Ariomma indica*) [13] and notchedfin threadfin bream (*Nemipterus peronei*) [27]. However, it is different in the bombay-duck in this study. Other possible factors such as sexual segregation, selective migration, or differential fishing cannot be ruled out and need to be investigated in the future.

An increase in sex ratio with body size has been documented for other species, and is possible due to the higher mortality of males and longer longevity of females [4]. In addition, differences in growth between sexes, sexual dimorphism, and migration may also be influential factors [4]. In the present study, no sexual dimorphism was found. Therefore, the increase of the sex ratio with size might be related to the difference in growth and longevity between sexes.

Although the large individuals collected in this study are all females yet the proportion of mature ones is low and no specimen greater than 60 cm FL was collected. The operation depth of fishing vessel is 50-250 m but the fish can stay in deeper waters (250-800 m) [20]. In addition, we had collected two specimens of mature females (>70 cm FL) which were caught in depth of 500 m in the waters off northeastern Taiwan. Therefore, the operation depth did not cover the habitat of mature females could be a possible reason for this result.

Four techniques commonly used to estimate spawning season are (1) macroscopic appearance of ovaries [2, 7], (2) histological examination of ovaries [14, 25, 28, 30], (3) oocyte diameter measurements [2], and (4) gonodsomatic index [5, 6]. Among them, gonadosomatic index (GSI) was the easiest method, but it cannot indicate gonad mature situation correctly in the later period [26]. In order to estimate spawning season accurately, we used the above three methods (1, 2, 4) to judge reproductive cycling in the present study. Good correspondence of the three methods has indicated that our estimate of the spawning season is reasonable.

Fish can store energy in the liver or viscera to meet the requirement of spawning. In this study, a similar trend between the GSI and HSI of females (Figs. 4 and 5) suggests that the required energy for oocytogenesis by female bombay-duck may be derived from their livers. On the other hand, the CFs are stable all year round for both sexes indicating that the CF is not closely related to the spawning behavior of the bombay-duck.

The coexistence of oocytes in the post-ovulatory and migratory stage indicated this species is an asynchronous type. Similar results have been reported for many species such as *Apogon lineatus* [10], *Leiognathus equulus* [12], *Sillago aeolus* [17], *Atherina presbyter* [14], *Pennahia argentata* [29], *Gerres equulus* [7] and *Ilisha elongata* [31].

Wu [27] suggested that spawning may be a rapid process, and hydration in many teleost fishes may occur as early as 12

hours before spawning. Hence, it was very difficult to collect female specimens with running hydrated oocytes. In the present study, one ovary sample with hydrated ripe oocytes was found indicating that the waters off southwestern Taiwan could be the spawning ground for bombay-duck. However, more specimens with hydrated oocytes need to be collected to test this hypothesis.

The logistic curve [8] has been successfully applied to estimate the size at 50% maturity for many species [5]. In this study, the estimated the size at 50% maturity of females is much larger than that of males. Tormosova [23] and Lee [12] suggested that food, stock density, and water temperature may influence the size at 50% maturity. That males and females have different ages and sizes at maturity is common for many fish species [21]. Stearns [21] pointed out that females mature later and larger than males because they continue to gain fecundity after males have grown to the region of diminishing return.

The major spawning season of *H. microchir* in the southwestern Taiwan waters is from August to December and peaked in November. To ensure sustainable utilization of this species, we recommend protecting the adults during the major spawning season. A seasonal closure of trawl fishery from August to December can provide better breeding opportunities for adults and is believed to be a good fishery management measure for this species.

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