Reproductive biology of nocturnal reef fish *Pempheris* sp. (Pempheridae) in Okinawa Island, Japan

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Abstract Reproductive characteristics of *Pempheris* sp. were studied in 233 specimens collected from May 2006 to October 2010 on Okinawa Island, Japan. The main spawning season was estimated during April to June, but spawning occurred year round. Standard length at first maturity occurred at ca. 110 mm in both sexes. Batch fecundity of *Pempheris* sp. was ca. 2,000–19,000 per female, and was higher in the main spawning season than in other seasons. No relationship between spawning and lunar periodicity was observed. The spawning interval of *Pempheris* sp. was estimated to be ca. 2 days, with spawning occurring shortly after sunset.

Keywords *Pempheris* sp., Pempheridae, reproduction, spawning season, maturation size, spawning interval, nocturnal, coral reef, Okinawa

Introduction

Fish of the genus *Pempheris* are one of the most abundant species in rocky and coral areas, specifically in the Indo-Pacific Ocean and western Atlantic Ocean comprising 21 recognized species (Nelson, 2006). *Pempheris* species school in caves or crevices during the day and swim out to open water at night to prey primarily on zooplankton (Fishelson et al., 1971; Gladfelter, 1979;

Golani and Diamant, 1991; Fishelson and Sharon, 1997; Platell and Potter, 1999; Platell and Potter, 2001; Annese and Kingsford, 2005; Sazima et al., 2005).

Golani and Diamant (1991) showed that the spawning season of *P. vanicolensis* (Cuvier, 1831) is from April to September in the Mediterranean Sea and throughout the year in the Red Sea. However, they did not conduct histological research, and little is known about the life history of *Pempheris* species in the Pacific Ocean.

Recently, two Japanese new recorded species, *P. oualensis* (Cuvier, 1831) “Yume-hatampo” in Japanese name and *P. vanicolensis* “Kibire-hatampo” in Japanese name, were collected (Koeda et al., 2010 a; Koeda et al., 2010 b) from Okinawa Island and Iriomote Island, respectively. Additionally, another unknown *Pempheris* species (Fig. 1) was recorded in Okinawan water. This species (*Pempheris* sp.) was clearly distinguished from other Pempherid species by its number of pored lateral-line scales (54–62), scale rows above the lateral line (4 1/2), and other taxonomic characters (Koeda et al., unpublished data). Therefore, authors will report this species as undescribed species in the near future. This species named “Ryukyu-hatampo” in Japanese name, and is abundant in the coral reef areas around Okinawa Island and makes mixed schools with *P. schwenkii* Bleeker, 1855. The objectives of the present study were to reveal reproductive traits such as spawning season, first maturation size, batch fecundity, and spawning interval to clarify a part of the

Fig. 1 A school of *Pempheris* sp. in cave at Maeda, Okinawa Island, Japan on 30 July 2010

Fig. 2 Sampling sites in Okinawa Island

lifecycle of *Pempheris* sp. in Okinawan waters.

Materials and Methods

Specimens were caught using a trammel net (mesh size: inside 18 mm, outside 90 mm), spear gun and angling at four sites in Okinawa Island (Maeda, Mizugama, Odo, and Ginowan fishing port: 26°04'–26°52', 127°36'–128°

20': Fig. 2, Table 1) from May 2006 to October 2010 (excepting the period of May to December 2007). A total of 233 specimens (female: $n=171$: 65.1–156.4 mm in standard length: SL; male: $n=62$: 66.2–145.4 mm SL) were collected in this study. Fish were brought back to the University of the Ryukyus in a cooler filled with ice prior to measurement of SL to the nearest 0.1 mm and body weight (BW) to the nearest 0.1 mg. Fish larger than 50.0 mm SL were sexed as possible from the shape of the gonads.

Water temperatures were gathered from data of the Japan Meteorological Agency (<http://www.jma.go.jp/jma/index.html>) every month. Day length and lunar day were gathered from data of the National Astronomical Observatory of Japan (<http://www.nao.ac.jp/>).

The spawning season was estimated using 167 fish (125 females and 42 males) larger than 110.0 mm SL. Gonad weight (GW) for each fish was recorded to the nearest 0.1 mg, and the gonadosomatic index (GSI) was calculated as follows:

$$\text{GSI} = \text{GW} \times 100 / (\text{BW} - \text{GW})$$

For females, ovaries were fixed in Bouin's solution or 10% neutral formalin, dehydrated through an ethanol series, and embedded in paraffin. Ovaries were sectioned to a thickness of 6 μm with a microtome and then stained with Mayer's hematoxylin-eosin. Then, the histologically sectioned specimens were observed under a binocular microscope. Oocytes in the ovary were classified into one of seven stages (perinucleolus, yolk vesicle, early yolk globule, late yolk globule, migratory nucleus, pre-maturation, and maturation stage) following the criteria of Yamamoto (1954) for *Liopsetta obscura*, Hattori et al. for *Gadus macrocephalus*, and Shirafuji et al. (2007) for *Spratelloides gracilis*. The maturity stage of each individual was determined using the most advanced oocytes in its ovary and the presence or absence of postovulatory follicles. The largest diameters of fifty oocytes in each maturational stage were measured to the nearest 0.1 μm under an all purpose projector.

The spawning interval was calculated from the number of spawnable ovaries, i.e., ovaries from late yolk globule to mature stages, divided by the number of hydrated

Table 1 Number of individuals and standard length (mean±SD) according to sampling site and sampling gear.

Sampling site	Female				Male			
	Trammel net	Spear gun	Angling	Total	Trammel net	Spear gun	Angling	Total
Cape Maeda	3 (116.4±21.3)			3 (116.4±21.3)	7 (105.0±16.2)			7 (105.0±16.2)
Mizugama	16 (108.4±29.5)	108 (131.5±13.7)		124 (128.5±18.3)	13 (113.3±27.9)	29 (131.6±8.8)		42 (125.3±18.9)
Ginowan F. P.			15 (115.5±11.2)	15 (115.5±11.2)			6 (115.0±15.2)	6 (115.0±15.2)
Odo Beach	21 (103.6±22.7)	8 (125.9±7.9)		29 (109.7±22.1)	4 (104.2±23.2)	3 (120.3±8.4)		7 (111.1±20.1)
Total	40 (106.5±25.8)	116 (131.1±13.4)	15 (115.5±11.2)	171 (123.9±20.0)	24 (109.4±24.7)	32 (129.6±9.3)	6 (115.0±15.2)	62 (123.1±19.9)

ovaries (Demartini and Fountain, 1981). Sampling period was separated to 4 periods of time, such as morning (sunrise to 12:00), afternoon (12:00 to 15:00), sunset (15:00 to sunset), and night (sunset to sunrise).

Only specimens collected from sunset, which was estimated as the possible spawning time, were used for calculating the spawning interval. Only mature-stage ovaries were used for batch fecundity estimates. Ovarian walls were removed and 100.0 mg of oocytes were weighed to the nearest 0.1 mg. Then, hydrated oocytes larger than 700 µm were counted and the batch fecundity (BF) was calculated using the following formula. The samples were taken five times, the highest and lowest data were excluded, and the remaining samples were averaged.

$$BF = GW / SW \times NMO$$

where SW is the sample weight, and NMO is the number of oocytes larger than 700 µm in the sample.

Results

Oogenesis

Perinucleolus stage (Fig. 3a)

The oocytes in this stage ranged from 16.5 to 122.8 µm (mean±SD: 70.1±25.0 µm; Table 2). The cytoplasm gradually increased in volume and became deeply stained with hematoxylin early in this stage, and it was less reactive to hematoxylin later in this stage. The GSI of the females in this stage ranged from 0.01 to 1.28 (mean±SD: 0.45±0.30; Table 2).

Yolk vesicle stage (Fig. 3b)

The oocytes ranged from 158.9 to 326.5 µm (222.4±45.2 µm; Table 2). Small yolk globules occurred on the periphery of the cytoplasm and increased in volume and number centripetally as the oocyte grew. The GSI of the females in this stage ranged from 0.45 to 1.45 (0.90±0.30; Table 2).

Early yolk globule stage (Fig. 3c)

The oocytes ranged from 256.2 to 441.5 µm in size (344.2±39.8 µm; Table 2). Yolk globules became apparent among the yolk vesicles, forming a layer on the periphery of the cytoplasm. This stage coincided with the primary yolk globule stage in Shirafuji et al. (2007). The GSI of the females in this stage ranged from 1.10 to 1.93 (1.33±0.27; Table 2).

Late yolk globule stage (Fig. 3d)

The oocytes ranged from 402.6 to 656.8 µm in size (518.8±66.6 µm; Table 2). The yolk globules rapidly increased in volume and number throughout the cytoplasm and the yolk vesicles increased against the periphery to form a layer. Ovaries with more developed late yolk globule oocytes were considered to be mature. This stage includes both secondary and tertiary yolk globule stages in Shirafuji et al. (2007). The GSI of the females in this stage ranged from 0.63 to 5.84 (2.67±1.43; Table 2).

Migratory nucleus stage (Fig. 3e)

The oocytes ranged from 419.7 to 889.7 µm in size (678.5±93.3 µm; Table 2). The nucleus began to move from the center to the animal pole. The yolk globules started to fuse. Oil globules appeared throughout the cyto-

Fig. 3 Photographs of different-stage oocytes and postovulatory follicles in *Pempheris* sp. (a) perinucleolus stage; (b) yolk vesicle stage; (c) early yolk globule stage; (d) late yolk globule stage; (e) migratory nucleus stage; (f) pre-maturation stage; (g) maturation stage; (h) postovulatory follicles. Scale bar indicates 100 μ m.

Table 2 Oocyte diameter and gonadosomatic index at different maturational stages. Open circle: mean; Box: standard deviation; Bars, maximum and minimum. Pn, perinucleolus stage; Yv, yolk vesicle stage; Ey, early yolk globule stage; Ly, late yolk globule stage; Mn, migratory nucleus stage; Pm, pre-maturation stage; M, maturation stage.

Maturation stage	Oocyte diameter			Gonadosomatic index		
	Mean $\pm$ SD	Max	Min	Mean $\pm$ SD	Max.	Min
Pn	70.1 $\pm$ 25.0	122.8	16.5	0.45 $\pm$ 0.30	1.28	0.00
Yv	222.4 $\pm$ 45.2	326.5	158.9	0.90 $\pm$ 0.30	1.45	0.45
Ey	344.2 $\pm$ 39.7	441.5	256.2	1.33 $\pm$ 0.28	1.93	1.11
Ly	518.8 $\pm$ 66.6	656.8	402.6	2.68 $\pm$ 1.43	5.84	0.63
Mn	678.5 $\pm$ 93.3	889.7	419.7	3.36 $\pm$ 1.20	4.98	1.94
Pm	707.6 $\pm$ 117.1	857.3	450	2.72 $\pm$ 1.60	4.32	1.11
M	926.8 $\pm$ 114.5	1195.3	681.2	6.87 $\pm$ 3.06	13.24	2.02

plasm. The GSI of the females in this stage ranged from 1.94 to 4.98 (3.36 ± 1.20 ; Table 2).

Pre-maturation stage (Fig. 3f)

The oocytes ranged from 450.0 to 827.3 μ m (707.6 ± 117.1 μ m; Table 2). The yolk globules coalesced further. The oil globules started to fuse and increased in volume and number. The GSI of the females in this stage ranged from 1.11 to 4.31 (2.72 ± 1.60 ; Table 2).

Maturation stage (Fig. 3g)

The oocytes ranged from 681.2 to 1195.3 μ m (926.8 ± 114.5 μ m; Table 2). The oocyte became translucent with the coalescence of the yolk globules and the hydration

of the cytoplasm. The GSI of the females in this stage ranged from 2.02 to 13.23 (6.87 ± 3.06 ; Table 2).

Seasonal changes in ovarian maturation

Monthly changes of GSI, water temperature, and day length are shown in Fig. 4. Mature females ($GSI > 2.0$) were observed throughout the year, except for January. The GSIs from April to June were significantly higher than other months (one-way ANOVA: $p < 0.01$). In April, the GSI rapidly increased with increasing water temperatures and day length. However in July, the GSI rapidly decreased with the increase in water temperature to 26°C and the decrease in day length.

Monthly changes in the maturational stage of ovaries

Fig. 4 A, Monthly changes in water temperature (solid triangle) and day length (open square) at Okinawa Island in 2009, from Japan Meteorological Agency and National Astronomical Observatory of Japan, respectively; B, Monthly changes in gonadosomatic index of females from Okinawa Island, Japan. C, Monthly changes in gonadosomatic index of males. Data from 2006 to 2010 are combined.

are shown in Fig. 5. Mature females appeared through the year, except for September. The frequency of mature females gradually increased from February to June. Hydrated ovaries appeared in February to August, October, and December. Postovulatory follicles were observed in March, April, June to August, and November (Fig. 3h). High GSI values, mature females, and postovulatory follicles were observed during several lunar days (Fig. 6).

Maturation size, spawning time, and spawning interval

The first appearance of mature females was at 111.1 mm SL (GSI=2.64; Fig. 7). In males, the first appearance of high GSI fish occurred at 114.8 mm SL (GSI=1.74).

The frequency of hydrated ovaries and mature females gradually increased with the time of the day and was highest in sunset period (Fig. 8). However, the percentage

Fig. 5 Monthly changes of histologically observed maturity stages. Numbers on bars represent sample numbers, and numbers in parenthesis show ovaries with postovulatory follicles. M, maturation stage; Pm, pre-maturation stage; Mn, migratory nucleus stage; Ly, late yolk globule stage; Ey, early yolk globule stage; Yv, yolk vesicle stage; Pn, perinucleolus stage.

Fig. 6 Relationship between lunar day and gonadosomatic index of mature females (solid circle) and immature females (open circle) from Okinawa Island, Japan. Asterisks indicate the day when postovulatory follicles were observed. Data from 2006 to 2010, and all months are combined.

of mature females rapidly decreased, and hydrated ovaries were not observed after sunset. The spawning interval was calculated as ca. 2 days.

Batch fecundity

The months with batch fecundities with high GSI values (April to June) were calculated as 2,935–18,621

Fig. 7 Relationship between standard length and gonadosomatic index of *Pempheris* sp. collected from Okinawa Island, Japan. Solid and open circles indicate mature and immature females, respectively. Data from 2006 to 2010 are combined.

Fig. 8 Relationship between sampling period and maturity stages of ovaries. Numbers on bars represent sample numbers, and numbers in parenthesis show ovaries with post-ovulatory follicles. Data from all months are combined. Morning: Sunrise–12:00; Afternoon: 12:00–15:00; Sunset: 15:00–sunset; Night: sunset–sunrise. M, maturation stage; Pm, pre-maturation stage; Mn, migratory nucleus stage; Ly, late yolk globule stage; Ey, early yolk globule stage; Yv, yolk vesicle stage; Pn, perinucleolus stage.

Fig. 9 Relationship between batch fecundity and standard length of *Pempheris* sp. collected from April to June (Solid circle), August to March (open circle), and July (open triangle) from Okinawa Island, Japan.

eggs (119.2–156.4 mm SL, $n=12$; Fig. 9). On the other hand, months with low GSI value (August to March) were calculated as 1,749–7,538 eggs (132.4–145.6 mm SL, $n=6$). In July, just after months with peak GSI values, batch fecundity had a middle range between the high (May and June) and low (August to March) GSI monthly values. Relationships between the batch fecundities and standard length were expressed as follows.

$$\text{BF (High GSI months)} = 378 \times \text{SL} - 43,528 \quad (r^2 = 0.75)$$

$$\text{BF (Low GSI months)} = 225 \times \text{SL} - 28,126 \quad (r^2 = 0.81)$$

Discussion

The spawning interval of this species was about two days, and spawning was not related to the lunar cycle. Most coral reef fish spawn according to the lunar cycle (Johannes, 1978; Doherty, 1983; Takemura et al., 2004 a; Takemura et al., 2004 b; Nanami et al. 2010). The spawning pattern of this species has previously been reported in a few coral reef fish, such as butterfly fish (Ralston, 1981). A relatively high ratio of hydrated ovaries appeared in fish collected before sunset, and hydrated ovaries were not observed during the night. These facts suggest that *Pempheris* sp. spawn just after sunset. Several studies suggest nocturnal migration of the fish in this family; some *Pempheris* species emerge in open water from the diurnal shelter, and a high amount of fish gather 15 to 45 minutes after sunset (Fishelson et al., 1971; Gladfelter,

1979; Golani and Diamant, 1991).

Therefore, nighttime observation during the main spawning season is necessary to demonstrate the exact spawning time of this species. These characteristics, such as the short spawning interval, the spawn timing apparently unaffected by the lunar day, and spawning just after sunset, may be common reproductive traits of nocturnal fish. However, it is necessary to clarify the reproductive characteristics of other nocturnal fish.

The gonadosomatic index and the presence of mature females gradually increased from April to June and were low after July. Additionally, the appearance of mature females and postovulatory follicles suggests that the main spawning season of *Pempheris* sp. was April to June, but it seems that spawning occurred throughout the year in Okinawan waters. However, batch fecundity was higher in the main spawning season than in other spawning seasons. In this study, the smallest sizes of mature females and males were ca. 110.0 mm SL in both sexes, and the age of these specimens were 2 years old based on otolith observation (Koeda et al. unpublished data). These reproductive characteristics are significantly different from those of *Pempheris schwenkii*, which makes mixed schools with *Pempheris* sp. (Koeda et al., unpublished data). This fact indicates that it is necessary to clarify the reproductive traits of other Pempherid species to understand the life-cycle strategies of this genus.

Acknowledgments

We are especially grateful to Associate Professor T. Yoshino and Associate Professor H. Imai (Faculty of Science, University of the Ryukyus) for comments and advice, especially in taxonomical part. The authors thank the students of the Laboratory of Fisheries Biology & Coral Reef Studies, Faculty of Sciences, University of the Ryukyus and the members of the University of the Ryukyus Diving Club, for their help with fish collection and for providing information. Associate Professor J. D. Reimer (Rising Star Program, University of the Ryukyus) proofread a final version of this manuscript. This study was supported in part by the Incentive Project in Scholarship and Education from the Sponsorship Group of

University of the Ryukyus.

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