



Ovarian Development, Spawning Characteristics, Size at First Maturity and Fecundity of *Glossogobius sparsipapillus* Akihito & Meguro, 1976 (Gobiiformes: Gobiidae) along Estuarine and Coastal Regions in the Mekong Delta, Vietnam

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Abstract: The ovarian development, spawning pattern and season, size at first maturity and fecundity of *Glossogobius sparsipapillus*, which is an essential commercial fish in the Mekong Delta, have been studied. Totally, 317 fish were collected from the estuarine and the coastal regions including Vinh Hau, Hoa Binh to Dien Hai, Dong Hai (Bac Lieu Province) and Tan Thuan, Dam Doi (Ca Mau Province). Fishes were sampled using gill nets during the dry and wet seasons in April 2019 – January 2020. The results show that the ovaries have different types of oocytes and that this goby was a multiple spawner. It spawned from July to September at three of the studied sites, since most of the mature ovaries appeared at that time. The size at first maturity (L_m) was from 6.50 cm in Vinh Hau and Tan Thuan to 6.78 cm in Dien Hai. The average batch fecundity was $24,966 \pm 2,542$ eggs, ranging from $17,918 \pm 2,258$ SE in Vinh Hau to $28,700 \pm 5,322$ SE in Dien Hai, followed closely by Tan Thuan ($26,352 \pm 3,458$ SE). There was an increase in batch fecundity with fish size. The fish total length and weight had strong positive relationships with batch fecundity. The egg size was 48.20 ± 4.89 SE μm length and 26.39 ± 3.09 SE μm width.

Key words: Bac Lieu, Ca Mau, multiple spawner, oocyte, spawning season.

Introduction

The reproductive strategies of fish play a vital role in fishery management (MILLER 1984, KOMOLAFE & ARAWOMO 2007) and are mainly classified into semelparity (synchronous pattern) and iteroparity (asynchronous pattern) (WOOTTON 1990). Most gobiid species have iteroparous strategies (MILLER 1984, DINH et al. 2016), whereas semelparous strategies rarely occur in gobies, e.g. *Leucopsarion petersi*

in Niigata Prefecture (TAMURA & HONMA 1969, LA MESA 2011), *Aphia minuta* along the Ortona coast (CAPUTO et al. 2001) and *Crystallogobius linearis* along the Adriatic Ocean coast (CAPUTO et al. 2003). The size at first maturity, the batch fecundity and egg sizes are strongly related to the fish stock management (FONTOURA et al. 2009, TEICHERT et al. 2014).

The fish community in the estuarine and coastal regions in the Mekong Delta are diverse and tend to be overexploited (TRINH & TRAN 2012, DIEP et al.

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2014). Hence for practical strategies to recover fish pool is urgently required and the need of data. The knowledge on the reproductive biology of species of this community, however, is limited to five out of 54 gobiiform species, including *Parapocryptes serperaster* (see DINH et al. 2016), *Trypauchen vagina* (see DINH 2018), *Stigmatogobius pleurostigma* (see DINH & TRAN 2018), *Periophthalmodon schlosseri* (TRAN et al. 2019) and *Periophthalmodon septemradiatus* (see DINH et al. 2020).

Glossogobius T. N. Gill, 1859 (Gobiiformes: Gobiidae) is one of the largest genera, with 29 species documented by HOESE et al. (2015). Only three of these species, *G. giuris*, *G. aureus* and *G. sparsipapillus*, have been recorded in the Mekong Delta, Vietnam (TRAN et al. 2013). *Glossogobius sparsipapillus* was described by AKIHITO & MEGURO (1976). The species has a widespread distribution: from brackish to freshwater in the Indo-Pacific regions (TALWAR & JHINGRAN 1991, RAINBOTH 1996, RIEDE 2004, FROESE & PAULY 2019). This species is a commercial fish in the Mekong Delta, especially in the estuarine and coastal regions in the Bac Lieu and Ca Mau Provinces. The population has shown a decline due to of commercial fishing for food supply; however, little is known about its external morphology, environmental requirements and food preferences (RAINBOTH 1996, TRAN et al. 2013). There is a need to study its biology, including ovarian development, spawning pattern and size at first maturity. These results may contribute to the understanding of fish reproduction and adaptation; they can be used for studying artificial reproduction and population management. This study aims to collect data on the ovarian development, spawning pattern and size at first maturity of *G. sparsipapillus* in the estuarine and coastal regions of the Mekong Delta, South Vietnam.

Materials and Methods

Study site and fish collection

Our study was carried out from April 2019 to January 2020 in estuarine and coastal regions from the Bac Lieu to the Ca Mau Provinces, southern Vietnam. These provinces are fringed by mangrove forests with long coastlines and large mudflats and have a semi-diurnal tide with an amplitude of ~1.2 m. There are two main seasons in the study regions: dry and wet, with monthly precipitation of 400 mm in the wet season (June–December). The average annual air temperature in these provinces is ~27°C (LE et al. 2006). Fish specimens were collected monthly from three sampling sites: Vinh Hau (Hoa Binh, Bac Lieu (VH), 9°12'24.8"N 105°42'54.9"E),

Dien Hai (Dong Hai, Bac Lieu (DH), 9°06'03.2"N 105°29'49.1"E) and Tan Thuan (Dam Doi, Ca Mau (TT), 8°58'17.5"N 105°22'51.8"E; Fig. 1). We used bottom gill nets with a mesh of 1.5 cm in the cod end. After 2-3 h setting during the high tide, gill nets were retrieved after 48 h (DINH et al. 2015). After collection, fish were stored in 5% formalin and transported to the laboratory. The biotic and abiotic factors at the studied sites were also recorded. The pH, temperature and salinity were measured using a pHep (HI98127) and a refractometer (950.0100 PPT-ATC), respectively.

Fish and data analysis

In the laboratory, the fish were separated into male and female using genital papilla (triangle in males and oval in females), followed by determination of total length (TL, 0.1 cm) and weight (W, 0.01 g). The six maturation stages of the goby *Gobius niger*, as demonstrated by VESEY & LANGFORD (1985), were used to classify the ovarian developmental stages of *Glossogobius sparsipapillus*. Ovarian stages were histologically examined using the staining method of CARLETON et al. (1980). The gamete terminology followed YAMAMOTO (1956) and YAMAZAKI (1965) and was used when determining the six developmental stages of oocyte of *G. sparsipapillus*. The fish spawning pattern and season were classified using the method of DINH & LE (2017).

The size at first maturity of fish (L_m) was calculated using the equation $P = 1/(1+\exp[-r \times (TL-L_m)])$, where P was the proportion of mature individuals in a length class; TL was fish total length and r – a model parameter (ZAR 1999).

The batch fecundity was estimated as $F=(n \times G)/g$, where, n was the number of eggs in a sub-sample; g – the weight of sub-sample and G – the ovarian weight (BAGENAL 1967). Three sub-samples were collected from each ovary: in the anterior, middle and posterior areas of both left and right ovaries. Eggs in sub-sample of ovaries were counted under the Motic Stereo Microscope, while the oocyte diameter in different developmental stages was measured using the Motic Image Pro Plus v.2 (DINH et al. 2016).

The spatial variation in batch fecundity and oocyte diameter of *G. sparsipapillus* were quantified using a one-way ANOVA. The change of temperature, pH and salinity between the dry and wet seasons at each studied site were confirmed by a t-test. The variation of these three factors among the three studied sites were quantified with a one-way ANOVA. The relationship between fish size (total length and body weight) and batch fecundity were quantified using the logarithmic regression (METIN et al.

2011). All tests were performed with the SPSS software v.21 and were set at a significant value of 5%.

Results

Environmental characteristics

The vegetation in VH and DH was dominated by *Avicennia alba* and *Rhizophora apiculata*, whereas the one in TT consisted of *Avicennia alba*, *Rhizophora apiculata*, *Lumnitzera racemosa*, *Excoecaria agallocha*, *Rhizophora mucronata*, *Aegiceras floridum* and *Nypa fruticans*. The mean temperature did not change among sites (one-way ANOVA, $P>0.05$), ranging from 28.6 ± 0.8 SD in VH to 29.7 ± 1.0 SD in DH and 30.0 ± 0.6 SD in TT. pH and salinity were not significantly different among the three studied sites ($P>0.05$ for all cases), they were 7.6 ± 0.1 SD and 23.8 ± 1.3 SD in VH, 7.6 ± 0.2 SD and 23.8 ± 2.6 SD in DH and 7.5 ± 0.2 SD and 23.8 ± 2.9 SD in TT, respectively. The salinity was significantly different between the dry and wet seasons at each study site (t-test, $P<0.01$ for all cases), but temperature and pH values were not ($P>0.05$ for all cases).

Gonadal development, spawning pattern and season

The ovary in stage I was small and white and was hard to differentiate testis, but under the stereo microscope, the ovary was pale whitish (Fig. 2a). The cross-section of the ovary in this stage consisted of germ cells (GS), oogonia (O) and primary oocytes (PO) (Fig. 3a). The ovary was yellowish with prominent blood vessels at stage II in which some small oocytes could be found in the ovaries under a reflected stereo microscope (Fig. 2b). The histology of ovary in stage II contained GC, O, PO and primary vitellogenic oocytes (PVO) with some yolk granules in the cytoplasm (Fig. 3b). At stage III, the ovary became transparent with small but visible yellowish eggs (Fig. 2c). In this stage, the ovary consisted of mainly PVO and secondary vitellogenic oocytes (SVO) with nucleus and yolk accumulation besides O and PO (Fig. 3c). In stage IV, the yellow eggs were found in the ovary with prominent blood vessels (Fig. 2d), while the ovary consisted of mostly post vitellogenic oocytes (PsVO) with the nucleoli in the centre of

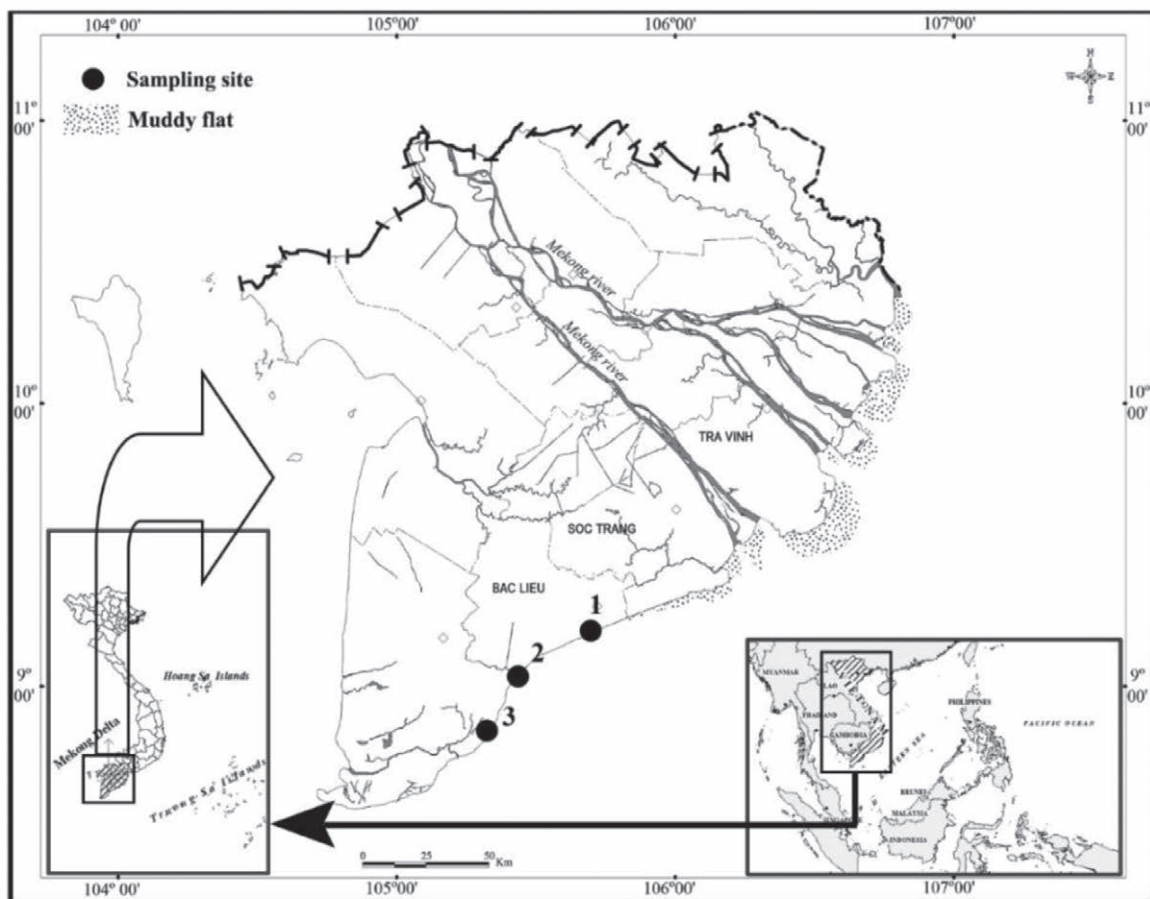


Fig. 1. Sampling map along the coastline in the Mekong Delta (●: sampling area; 1: Vinh Hau, Hoa Binh, Bac Lieu; 2: Dien Hai, Dong Hai, Bac Lieu; 3: Tan Thuan, Dam Doi, Ca Mau)

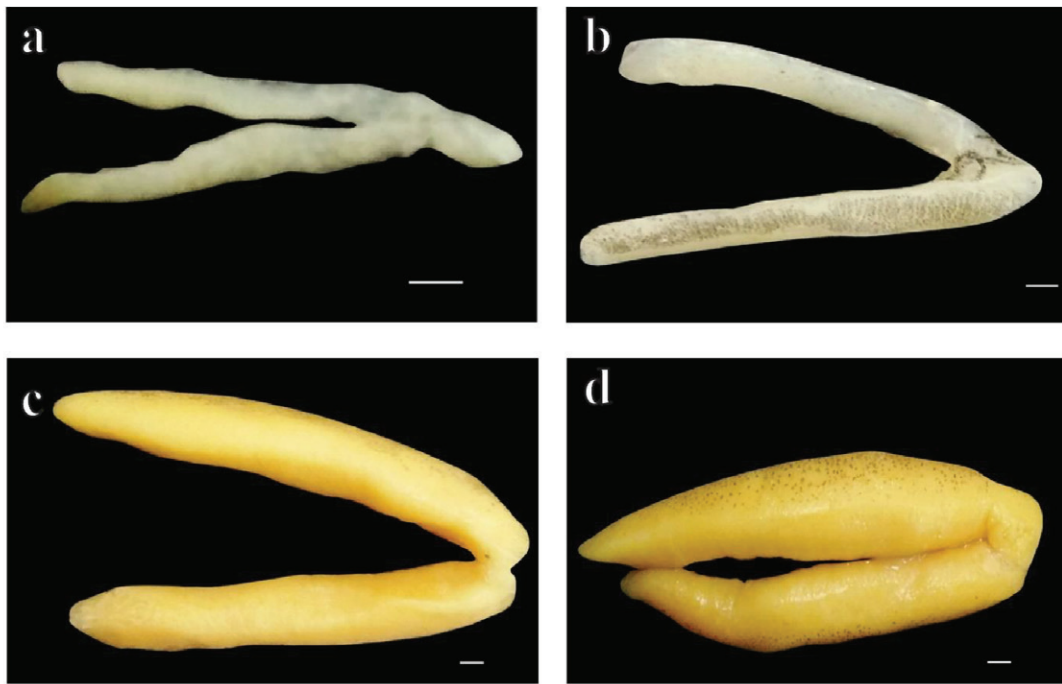


Fig. 2. Ovarian morphology of *Glossogobius sparsipapillus* (a, b, c, and d represent ovarian stages I, II, III and IV; scale bar: 1 mm)

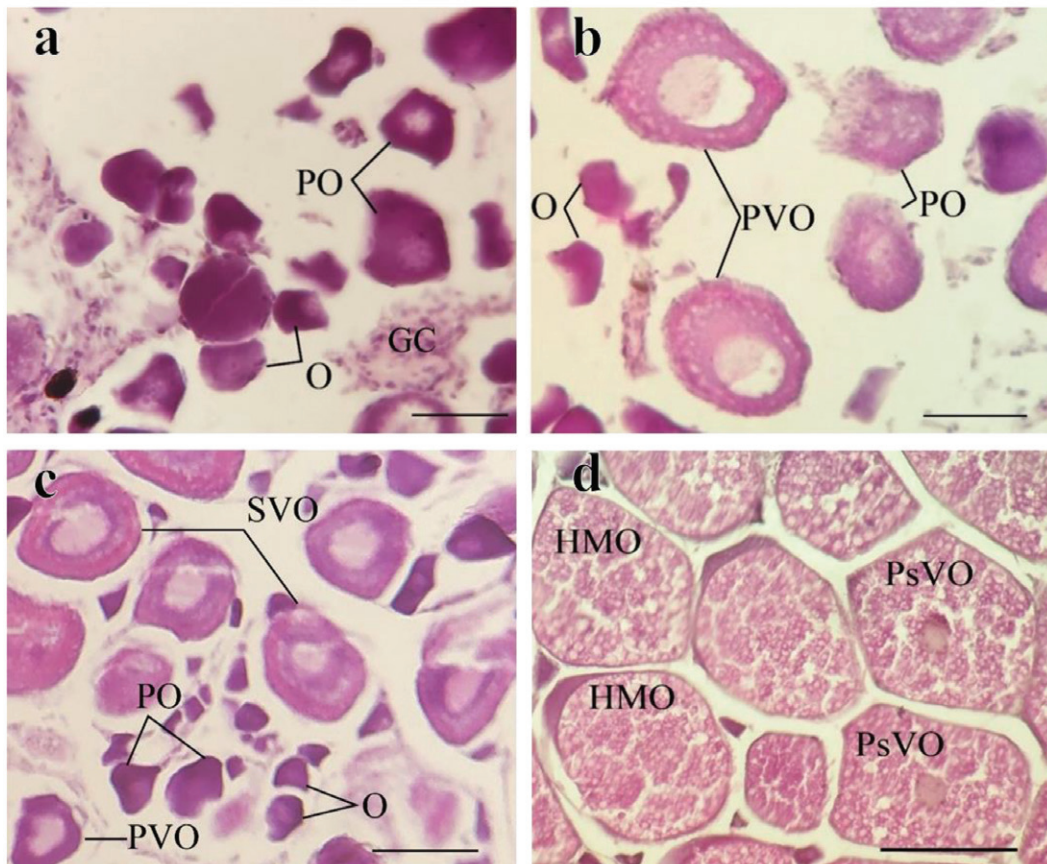


Fig. 3. Ovarian histology of *Glossogobius sparsipapillus* (a, b, c, and d represent ovarian stages I, II, III and IV; GC: germ cells, O: oogonia, PO: primary oocyte, PVO: primary vitellogenic oocytes, SVO: secondary vitellogenic oocytes, PsVO: post vitellogenic oocytes, HMO: hydrated oocytes; scale bar: 50 μ m for histology)

Table 1. The number of fish in each ovarian stage collected at the three studied sites

Months	Vinh Hau, Hoa Binh, Bac Lieu					Dien Hai, Dong Hai, Bac Lieu					Tan Thuan, Dam Doi, Ca Mau				
	Total	SI	SII	SIII	SIV	Total	SI	SII	SIII	SIV	Total	SI	SII	SIII	SIV
04/2019	5		1	2	1	18		2	12	4	16			16	
05/2019	1				1	24	1	1	6	16	19			3	16
06/2019	3	1	1		1	4		1		3	21				21
07/2019	10		3	3	4	4			3	1	15			2	13
08/2019	18	1	3	5	9	20		1	10	9	17			9	8
09/2019	7	1		1	5	9		0	2	7	18			3	15
10/2019	2			1	1	6		2	2	2	13			1	12
11/2019	9				9	16	7	9			6			1	5
12/2019	8	1	1	4	2	2	2				6		1	1	4
01/2020	5			1	4	11	11				5	1	1	1	2

SI: stage I; SII: stage II; SIII: stage III; SIV: stage IV.

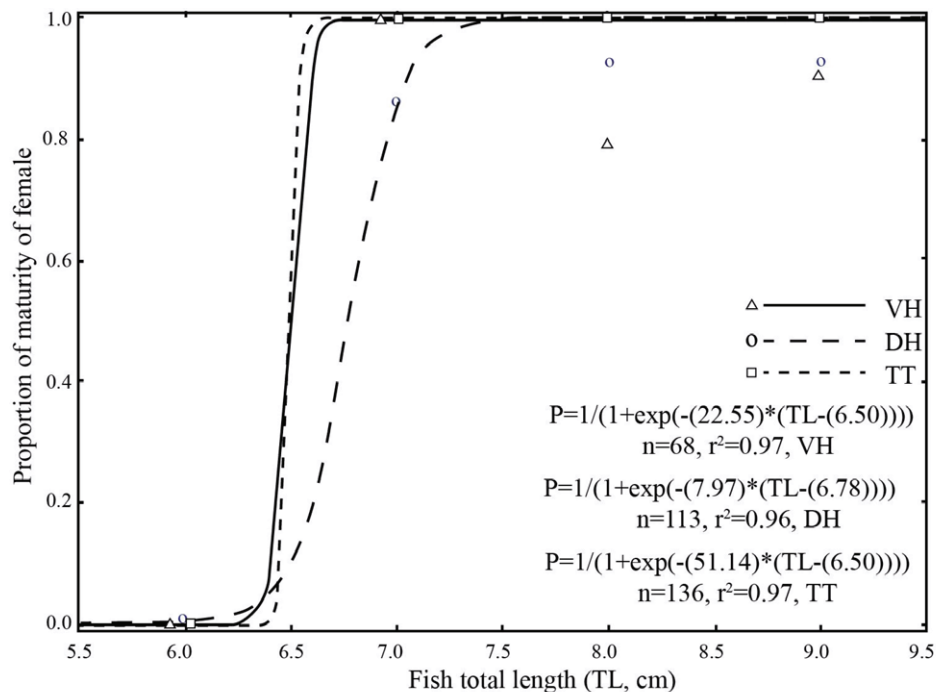


Fig. 4. Length at first maturity of *Glossogobius sparsipapillus* (VH, DH, and TT stand for Vinh Hau, Hoa Binh, Bac Lieu; Dien Hai, Dong Hai, Bac Lieu and Tan Thuan, Dam Doi, Ca Mau, respectively)

the nucleus and hydrated oocytes (HMO) besides some PO, PVO and SVO (Fig. 3d). The nucleus comprising many nucleoli could be easily found in PsVO, but was absent in HMO. Fish with ovaries in stage V and VI were not found in the present study because either their time was relatively short, so they were not detected, or because during the sampling period, the fish moved into the breeding grounds.

In this study, mature ovaries were observed during a period of three months (from July to September) at three sampling sites from VH to DH and

TT (Table 1). Oogonia and PO were mostly found in all stages of ovaries. The mean diameter of oocytes increased from PO, PVO, SVO and from PsVO to HMO.

Length at first maturity, fecundity and egg diameter

Glossogobius sparsipapillus displayed a variation in length at first maturity, increasing from 6.50 cm TL (in VH and TT) to 6.78 cm TL in DH (Fig. 4). The batch fecundity ranged from 8,568 eggs/female (2.93 g W and 7.1 cm TL) to 37,474 eggs/female (9.16 g W and 10.5 cm TL) in VH. In DH, it varied

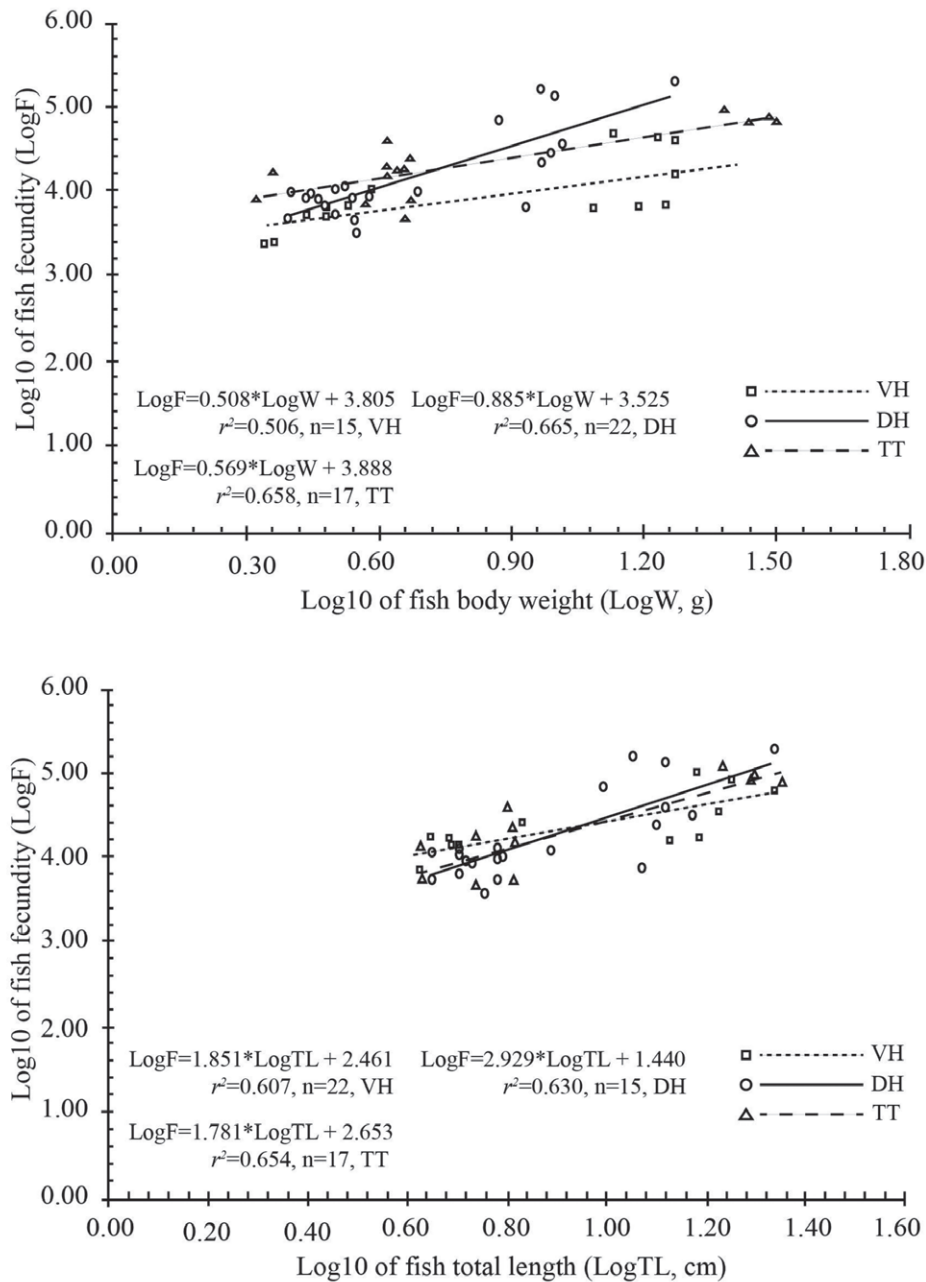


Fig. 5. Relationships between fecundity and weight and total length of *Glossogobius sparsipapillus* (VH, DH, and TT stand for Vinh Hau, Hoa Binh, Bac Lieu; Dien Hai, Dong Hai, Bac Lieu and Tan Thuan, Dam Doi, Ca Mau, respectively)

between 9,305 eggs/female (6.16 g W and 8.8 cm TL) to 95,191 eggs/female (33.76 g W and 14.8 cm TL). In TT, it was from 10,199 eggs/female (5.58 g W and 8.6 cm TL) to 26,352 eggs/female (5.72 g W and 8.6 cm TL) in TT. Batch fecundity of this fish did not vary among the studied sites (one-way ANOVA, $F=1.58$, $p>0.05$), ranging from $17,918 \pm 2,258$ SE ($n=15$) in VH to $28,700 \pm 5,322$ SE ($n=22$) in DH, which was followed closely by TT ($26,352 \pm 3,458$ SE, $n=17$). The batch fecundity of this species was positively related to TL and W as indicated by the

high value of determination parameter (r^2), obtained from the linear regression between batch fecundity and fish TL and W (Fig. 5). The average egg size (μm) was 48.20 ± 4.89 SD in length and width is 26.39 ± 3.09 SD. The egg length diameter did not change among sites (ANOVA, $F=2.97$, $p>0.05$), which was found in the width of egg diameter (ANOVA, $F=2.98$, $p>0.05$). The length and width of eggs (μm) at studied sites were 48.84 ± 5.75 and 26.52 ± 3.59 (in DH), 49.41 ± 3.95 and 25.66 ± 2.74 (in VH) and 47.35 ± 4.63 and 27.00 ± 2.77 (in TT).

Discussion

Similarly to *Periophthalmodon schlosseri* (TRAN et al. 2019) and *P. septemradiatus* (DINH et al. 2020), *G. sparsipapillus*, which is a multiple spawner, the appearance of various types of oocytes such as PVO, SVO, PsVO, and HMO were found in the mature ovaries. Their reproductive strategy was also found to be linked with other gobies living in the Mekong Delta, including *Pseudapocryptes elongatus* (TRAN 2008), *Boleophthalmus boddarti* (DINH et al. 2015), *Parapocryptes serperaster* (DINH et al. 2016), *Trypauchen vagina* (DINH 2018) and *Stigmatogobius pleurostigma* (DINH & TRAN 2018). LONGHURST & PAULY (1987) demonstrated that several of the gobies belonged to serial spawners type, which releases eggs more than one time during the spawning period. Also, *G. sparsipapillus* was an iteroparous fish, a spawning type found in some gobies living in the brackish and freshwater such as *Pseudapocryptes elongatus* (TRAN 2008), *Boleophthalmus boddarti* (DINH et al. 2015), *Parapocryptes serperaster* (DINH et al. 2016), *Trypauchen vagina* (DINH 2018), *Stigmatogobius pleurostigma* (DINH & TRAN 2018), *Periophthalmodon schlosseri* (TRAN et al. 2019), and *Periophthalmodon septemradiatus* (DINH et al. 2020). The changes in environmental conditions may not affect the reproducing activity of *G. sparsipapillus* as it displayed the same spawning pattern at three sampling sites.

Glossogobius sparsipapillus spawned during the wet season from July to September as the mature ovaries were mainly found during this period. It indicated that the spawning season was regulated by the high nutrients transported by rainfall to habitats of the fish in the wet season. Similarly, *Periophthalmus barbarus* in Nigeria spawned mainly in May due to high nutrient transported to its habitat (ETIM et al. 2002, UDO 2002) and *Periophthalmodon schlosseri* in Malaysia reproduced during the southwest monsoon season (April – October) due to abundant food resources in their habitat at the time (MAZLAN & ROHAYA 2008). Furthermore, *Pseudapocryptes elongatus* (TRAN 2008), *Boleophthalmus boddarti* (DINH et al. 2015), *Parapocryptes serperaster* (DINH et al. 2016), *Trypauchen vagina* (DINH 2018) and *Stigmatogobius pleurostigma* (DINH & TRAN 2018), in the Mekong, spawned mainly due to availability of nutrients to the estuarine region. For other gobies living in different habitats such as *Neogobius melanostomus* (MACINNIS & CORKUM 2000), *Cryсталlogobius linearis* (CAPUTO et al. 2003) and *Amblygobius phalaena* (TAKEGAKI 2000), they spawned during a period from May to October. Whereas, *Va-*

lenciennea strigata (REAVIS 1997), *Butis butis* (DINH & LE 2017), *Periophthalmodon schlosseri* (TRAN et al. 2019) and *Periophthalmodon septemradiatus* (DINH et al. 2020) could spawn all year round.

Like *Periophthalmodon septemradiatus* (DINH et al. 2020), the size at first maturity (L_m) of *Glossogobius sparsipapillus* could be related to the changes of biotic and abiotic conditions since L_m of this fish varied with studied sites. This was also found in *Pseudapocryptes elongatus* (TRAN 2008), *Boleophthalmus boddarti* (DINH et al. 2015), *Parapocryptes serperaster* (DINH et al. 2016), *Butis butis* (DINH & LE 2017), *Trypauchen vagina* (DINH 2018), and *Periophthalmodon schlosseri* (TRAN et al. 2019). However, the size at first maturity of these gobiid species was greater than that of *Glossogobius sparsipapillus*. As for the sizes at first sexual maturation, they were also greater than that of *Periophthalmodon septemradiatus*. It is likely that the length at first maturity of those gobies is species-specific.

Unlike *Periophthalmodon septemradiatus* (DINH et al. 2020), the average of batch fecundity (F) of *Glossogobius sparsipapillus* in this study did not change with studied site. It meant that the F value of this species could not be influenced by changes in biotic and abiotic conditions. Meanwhile, F of *Boleophthalmus boddarti* is affected by environmental factors, being 2,100–12,300 eggs in India (CHANDRAN et al. 2014) due to environmental pollution but 9,800–33,800 eggs in Vietnam (DINH et al. 2015).

The results of this study not only increase the knowledge of reproductive biology of the species, but also contribute to the basic understanding of this gobiid population for sustainable management in the regions.

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