



## Reproductive biological traits of the goby *Stigmatogobius pleurostigma* (Bleeker, 1849) from the Mekong Delta, Vietnam

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### ABSTRACT

*Stigmatogobius pleurostigma* (Bleeker, 1849) is an estuarine species of goby and widely distributed in Asian regions, but very little is known about the reproductive biological traits of this species. The present study was conducted along the coastline of the Mekong Delta, Vietnam from November 2015 to October 2016 to elucidate basic reproductive parameters of the species. A total of 155 fish (96 females and 59 males) were collected every month using fixed bag nets from the mangrove forests in the study area. Data analysis showed that the sex ratio is significantly different from 1:1. This gobiid species is an iteroparous fish spawning from March to November in the study region as deduced from the appearance of mature and ripe ovaries during this period. The length at first maturity was estimated as 4.14 cm in total length and absolute fecundity was found to be quite high (3,100-5,650 eggs) with a strong positive relationship with fish length and weight. The results obtained in this study contribute to the knowledge on the reproductive biology as well as stock status of the species.

Keywords: Absolute fecundity, Gonadosomatic index, Length at first maturity, Spawning season, *Stigmatogobius pleurostigma*

### Introduction

Knowledge of reproductive biology of fish species has an important role in fishery management (Miller, 1984; Komolafe and Arawomo, 2007). Wootton (1990) reported that semelparity and iteroparity (synchronous and asynchronous patterns) were the two main reproductive strategies of fish. The iteroparous strategy is common for numerous gobies (Miller, 1984). Fish fecundity and size at first maturity are used for recruitment and abundance estimation (Kovacic, 2001) and fish stock management (Fontoura *et al.*, 2009; Teichert *et al.*, 2014). However, little is known about the reproductive biology of gobiid species inhabiting the Mekong Delta in Vietnam where they have high species diversity and are being overexploited (Trinh and Tran, 2012). Therefore, there is a need to generate in depth knowledge on the reproductive biological traits of gobiids to understand their recruitment strategies in estuaries.

*Stigmatogobius pleurostigma* (Bleeker 1849) (Family : Gobiidae) is a small gobiid species with an elongated body (Larson, 2005) and is widely distributed from freshwater to brackishwater in South-east Asia including Vietnam (Larson, 2005; Froese and Pauly, 2017). The *Stigmatogobius* genus comprises seven species including *S. borneensis*, *S. elegans*, *S. minima*, *S. pleurostigma*, *S. sadanundio*, *S. sella* and *S. signifer*

(Larson, 2005) but only *S. pleurostigma* is recorded in the Mekong Delta, Vietnam (Tran *et al.*, 2013). This species is a targeted species for fishing and displays negative allometric growth (Dinh, 2017), but there is no information on its reproductive biological traits. This study, therefore, aims at providing information on certain aspects of reproductive biology of this gobiid species such as sex ratio, gonadal development, spawning strategy and season, size at first maturity, fecundity and egg size which could contribute towards resource assessment and management of the species in Mekong Delta.

### Materials and methods

#### *Study site and fish collection*

This study was carried out at Tran De, Soc Trang Province (9°28'47.41"N; 106°12'25.96"E) which is fringed by mangrove forest with a large mudflat and semi-diurnal tide. In the dry season (January-May), it rarely rains whereas heavy rains (approximately 400 mm per month) appear in the wet season (June-December). The mean annual temperature is ~27°C. The natural environmental conditions are typical for the Mekong Delta, Vietnam (Le *et al.*, 2006).

Fish specimens were collected every month from the mangrove forest regions using fixed bag nets with 1.5 cm mesh size in the cod-end from November 2015 to October

2016. During high tide, three nets were set, each at a distance of 250 m from the other for 2-3 h and during ebb tide they were retrieved to collect fish. After collection, fish were sexed by examination of genital papilla morphology as described by Dinh (2017) and identified based on their external description and classification (Tran *et al.*, 2013). Fish were then anaesthetised using benzocaine and preserved in 5% formalin before transporting to the laboratory.

#### Fish analysis

In the laboratory, fish specimens were weighed to the nearest 0.01 g and measured in length to the nearest 0.1 cm before removing ovaries and testes. Gonads were then classified visually into six maturation stages based on the criteria description for *Gobius niger* by Vesey and Langford (1985). Fish length at first maturity ( $L_m$ ) was estimated using the logistic curve as  $P=1/(1+\exp[-r(TL-L_m)])$ , where, P is the proportion of mature individuals in a length class;  $L_m$  is the length with 50% of fish reaching sexual maturity, and r is a model parameter (Zar, 1999). All eggs of mature (stage IV) and ripe (stage V) ovaries were placed and counted in a petri dish to determine the absolute fecundity based on the method of Dinh *et al.* (2016). The Motic Image Pro Plus v.2 software was used to determine the diameter of 30 randomly selected oocytes at each oocyte stage to estimate mean oocyte diameter per ovarian stage (Dinh *et al.*, 2016). This software was also used to measure the width of ovaries and testes from the microscopic images. The ovaries and testis were examined histologically as per Carleton *et al.* (1980). For this the gonads were fixed in Bouin's solution for 24 h, then the gonads were washed and stored in 70% alcohol until further processing. The fixed tissues were then dehydrated in ascending series of alcohol, embedded in paraffin wax, trimmed and 6  $\mu$ m thick sections were cut using a microtome. The thin sections were finally mounted on a glass slide for staining with Mayer's haematoxylin and eosin. The developmental stages of the oocytes and spermatocytes were then described following Yamamoto (1956) and Yamazaki (1965).

#### Data analysis

The differences in males and females between the dry (January-May) and the wet (June-December) seasons were examined by  $\chi^2$  test. The variation of oocyte diameters between ovarian development stages was tested by one-way ANOVA. Logarithmic regression was used to examine the relationships between fish total length and weight to fecundity (Metin *et al.*, 2011). All statistical analysis was carried out in SPSS software v.21 at a significance level of 5%.

## Results

### Sex ratio, length at first maturity and fecundity

A total of 155 fish (96 females and 59 males, Table 1) were collected for the present study during the wet and dry seasons. The female to male ratios of *S. pleurostigma* were significantly different from the ratio of 1:1 within the dry (21 females and 12 males) and the wet seasons (75 females and 45 males,  $\chi^2$ ,  $p < 0.05$  in all cases). In this study *S. pleurostigma* was found to attain 4.14 cm in total length at first sexual maturity (Fig. 1). The absolute fecundity of *S. pleurostigma* ranged from 3,100 eggs (4.6 cm in length and 1.18 g in weight) to 5,650 eggs (5.6 cm in length and 2.34 g in weight). This goby displayed a positive relationship between absolute fecundity with total length of fish (Fig. 2a) and body weight (Fig. 2b).

Table 1. Number of *Stigmatogobius pleurostigma* caught from the study site over time

Sampling time	No. of female	No. of male
Nov.15	4	7
Dec.15	2	5
Jan.16	2	2
Feb.16	6	2
Mar.16	6	1
Apr.16	3	1
May16	4	6
Jun.16	4	3
Jul.16	23	19
Aug.16	25	1
Sep.16	8	3
Oct.16	9	9
Total	96	59

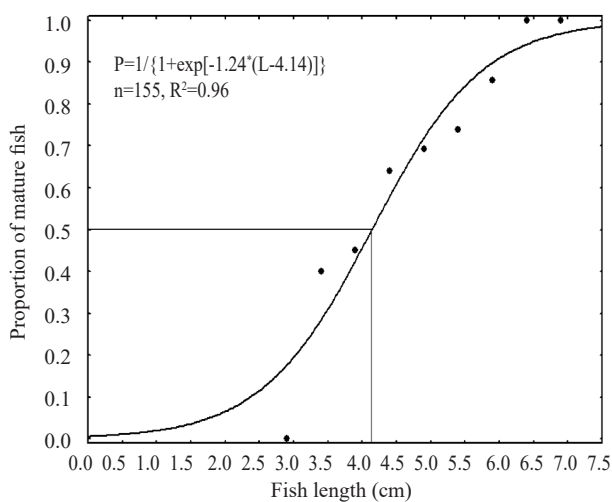


Fig. 1. Length at first maturity of *Stigmatogobius pleurostigma* caught from the study site

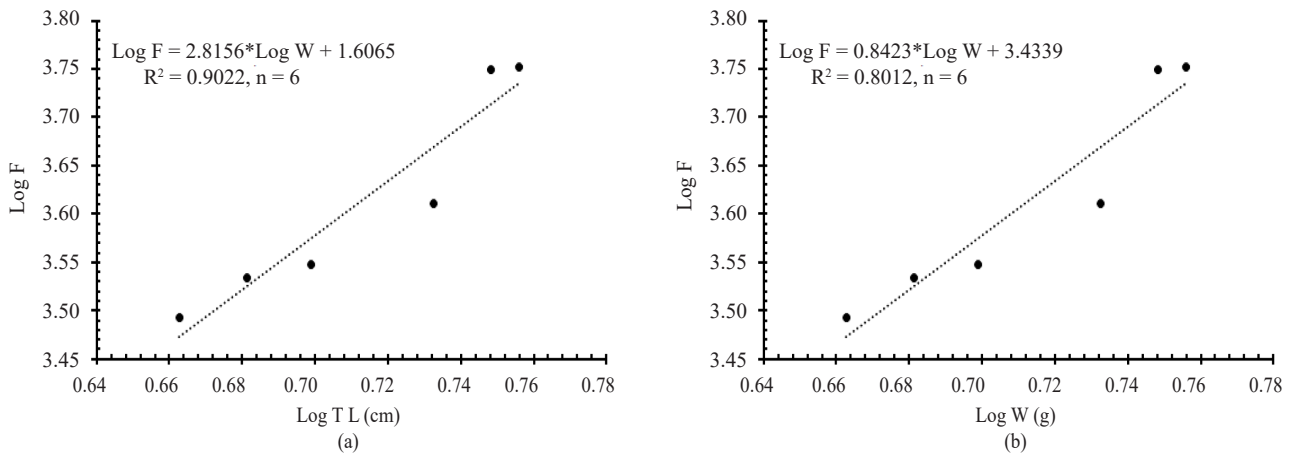


Fig. 2. Relationships between fecundity (LogF) and (a) fish total length (LogTL) and (b) body weight (LogW) of *Stigmatogobius pleurostigma* caught from the study site

*Development of ovary and testis*

The ovaries at stage I were  $519 \pm 29.19 \mu\text{m}$  in width, linked by connective tissue and hard to differentiate from stage I testes. Germ cells, oogonia and primary oocytes were found in ovaries at this stage (Fig. 3a). The ovarian width increased to  $989.25 \pm 98.98 \mu\text{m}$  in stage II when the gonad was yellowish. At this stage, two parts of each ovary were separated and easy to differentiate from testes as small oocytes were found in ovaries under a reflected light microscope. Beside primary oocytes, germ cells and oogonia, stage II ovaries also contained the primary

and secondary vitellogenic oocytes (Fig. 3b). At stage III, ovarian width was  $1,213.49 \pm 63.46 \mu\text{m}$  and two parts of each ovary were separated and became transparent with visible orange-yellow eggs. Secondary and post-vitellogenic oocytes were mainly found in this stage (Fig. 3c). Stage IV ovaries were  $1,826.97 \pm 523 \mu\text{m}$  in width and contained small whitish eggs covered by blood vessels. Post-vitellogenic oocytes with the nucleoli in the center of the nucleus were mostly found in stage IV ovaries (Fig. 3d). Ovaries reached the maximum width of  $3,654.86 \pm 296.44 \mu\text{m}$ , became opaque and were covered by a smooth and firm membrane in stage V.

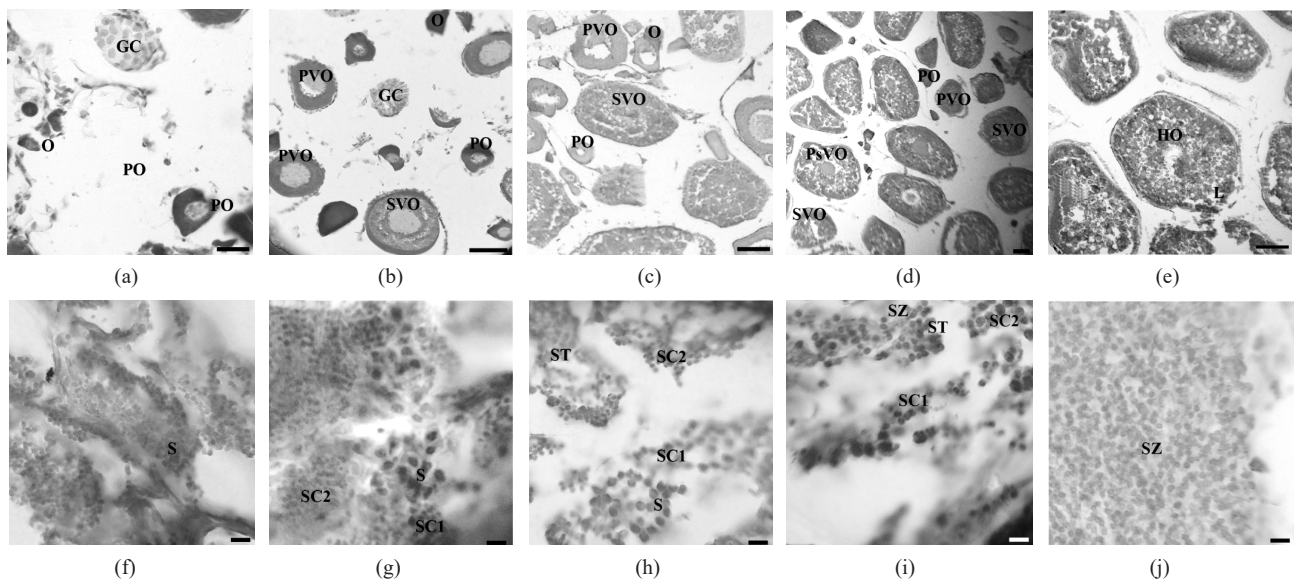


Fig. 3. Histological sections of ovary and testis of *Stigmatogobius pleurostigma* caught from the study site (a, b, c, d and e represent ovary stages I, II, III, IV and V; f, g, h, i and j represent testis stages I, II, III, IV and V respectively). GC: germ cells, O : oogonia, PO: primary oocyte, PVO: primary vitellogenic oocytes, SVO : secondary vitellogenic oocytes, PsVO: post-vitellogenic oocytes, HO: hydrated oocytes, L: lipid, S: spermatogonia, SC1: primary spermatocytes, SC2 : secondary spermatocytes, ST: spermatid, SZ: spermatozoa. Scale bar: Ovarian sections - 100  $\mu\text{m}$ ; Testis sections -10  $\mu\text{m}$

At this stage, ovaries contained mostly hydrated oocytes and some secondary and post-vitellogenic oocytes (Fig. 3e). Resting ovaries (stage VI) were not encountered during the present study.

Testes were  $320.83 \pm 31.26 \mu\text{m}$  in width, elongated and paired in stage I and not easy to differentiate from stage I ovaries. Testes at this stage consisted mainly of spermatogonia (Fig. 3f). Testes width increased to  $323.24 \pm 20.51 \mu\text{m}$  in stage II and the testes were pale pinkish in colour. The number of primary and secondary spermatocytes increased and a few spermatogonia appeared in testes (Fig. 3g). At stage III, testes increased to  $372.36 \pm 44.11 \mu\text{m}$  in width and became translucent with smooth surface. At this stage, primary and secondary spermatocytes and a few spermatids and spermatozoa were found in the sperm ducts (Fig. 3h). Stage IV testes were  $786.01 \pm 51.54 \mu\text{m}$  in width and became milky in colour covered by blood vessels. In this stage, testes comprised mainly spermatids and spermatozoa in the testicular cavity and sperm ducts (Fig. 3i). Testes reached the maximum size of  $1774.62 \pm 542.30 \mu\text{m}$  in stage V and consisted of mainly mature spermatozoa (Fig. 3j). Like ovary, resting testes (stage VI) were also not found in this study.

Mature (stage IV) and ripe (stage V) ovaries and testes were found from March 2015 to November 2016 (Fig. 4). The oocyte diameter increased significantly during vitellogenesis from primary oocytes, primary, secondary and post-vitellogenic oocytes to hydrated oocytes (Table 2).

Table 2. Oocyte diameter at different maturity stages of ovary in *Stigmatogobius pleurostigma*

Maturity stage	Oocyte diameter, $\mu\text{m}$ (mean $\pm$ SD)
Stage I	83.56 $\pm$ 10.95 <sup>a</sup>
Stage II	139.00 $\pm$ 18.61 <sup>b</sup>
Stage III	267.18 $\pm$ 33.80 <sup>c</sup>
Stage IV	360.36 $\pm$ 27.24 <sup>d</sup>
Stage V	401.51 $\pm$ 28.14 <sup>e</sup>

Values bearing different superscripts differ significantly ( $p < 0.05$ )

**Discussion**

According to Kovacic (2007), because of male egg-tending activity in *Gobius vittatus*, more females than males were caught during the spawning season. Likely, after releasing eggs into the roof of the selected caves by female *S. sadanundio* males tend eggs until hatching (Larson, 2005). The number of female *S. pleugostigma* in this study was significantly higher than males, whereas sex ratios of some co-occurring gobiid species such as *Pseudapocryptes elongatus* (Tran, 2008), *Boleophthalmus boddarti* (Dinh et al., 2015), *Periophthalmodon schlosseri* (Dinh, 2016a), *Trypauchen vagina* (Dinh, 2016b) and *Parapocryptes serperaster* (Dinh et al., 2016) are close to 1:1. This indicates that *S. pleurostigma* may share a similar reproductive behaviour with *G. vittatus* and *S. sadanundio* but different from *P. elongatus* (Tran, 2008), *B. boddarti* (Dinh et al., 2015), *P. schlosseri* (Dinh, 2016a), *T. vagina* (Dinh, 2016b) and *P. serperaster* (Dinh et al., 2016).

Longhurst and Pauly (1987) reported that many gobies are serial spawners depositing eggs more than once or one

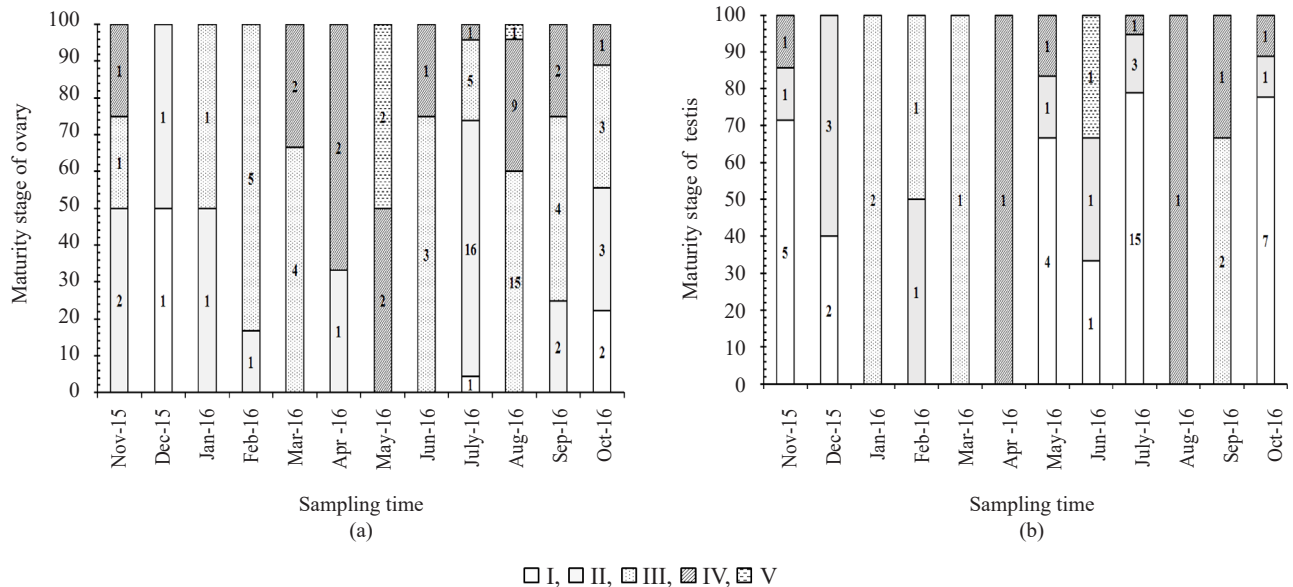


Fig. 4. Gonadal stage compositions of *Stigmatogobius pleurostigma* caught from the study site (n = 96 for ovary and n = 59 for testis)

time during spawning period. The present study showed that *S. pleurostigma* was a multiple spawner as the stage IV and V ovaries contained four oocyte types (oogonia, primary oocytes, vitellogenic and hydrated oocytes). Likely, the iteroparous types were also found in some gobies living in the same habitat, e.g., *P. elongatus* (Tran, 2008), *B. boddarti* (Dinh *et al.*, 2015) and *P. serperaster* (Dinh *et al.*, 2016). Female and male *S. pleurostigma* may release eggs and sperms from March to November due to the appearance of mature and ripe ovaries and testes during this period. In contrast, *P. serperaster* collected in the same region along with *S. pleurostigma* is a multiple spawner and deposits egg in the mid-wet season (Dinh *et al.*, 2016). Similarly, some other co-occurring gobies such as *P. elongatus* (Tran, 2008) and *B. bodarti* (Dinh *et al.*, 2015) also lay eggs during the main wet season (July to October).

Like other gobies, the absolute fecundity of *S. pleugostigma* had a positive correlation with fish total length indicating that the fecundity of this species was strongly correlated on fish size, which was also found in most gobies such as *P. elongatus* (Tran, 2008), *B. boddarti* (Dinh *et al.*, 2015) and *P. serperaster* (Dinh *et al.*, 2016). The fecundity of *S. pleurostigma* is higher than *Neogobius melanostomus* (84 to 606 eggs) in North America (Macinnis and Corkum, 2000), *Crystallogobius linearis* (200-700 eggs) in the Adriatic Sea (Caputo *et al.*, 2003) and *S. sadanundio* (upto 1,000 eggs) (Froese and Pauly, 2017), but lower than *Amblygobius phalaena* (37,000-38,000 eggs) in Japan (Takegaki, 2000), *P. elongatus* (2,600-29,400 eggs) in Vietnam (Tran, 2008), *B. boddarti* (2,100-12,300 eggs in India and 9,800-33,800 eggs in Vietnam) (Chandran *et al.*, 2014; Dinh *et al.*, 2015) and *P. serperaster* (6,000-11,700 eggs) in Vietnam (Dinh *et al.*, 2016). It appears that the fecundity in gobiid species varies with different geographic regions. Indeed, the fecundity of *B. boddarti* is regulated by environmental factors as Chandran *et al.* (2014) reported its fecundity as 2,100-12,300 eggs in India while Dinh *et al.* (2015) reported 9,800-33,800 eggs in Vietnam

*S. pleugostigma* was observed to be a serial spawner releasing eggs throughout the year. This goby matured at 4.14 cm in total length and was found to have quite high fecundity. The understanding of size at first maturity and spawning season are helpful for local people and authorities to set the minimum legal size and time for fishing. The results of the present study fills the scientific gap in knowledge on the reproductive biology of this goby as well as to provide a better understanding of the population dynamics of the species which would help in monitoring and sustainable management of this resource in the future.

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