Testicular development, reproducing references and length at first maturity of Acentrogobius viridipunctatus (Actinopteri: Gobiiformes) in the southwest Viet Nam

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1. Introduction

Acentrogobius, a fish genus of the Gobiidae [14], plays a fundamental role in fish food supply due to its high nutritional content in the southwest Viet Nam known as the Vietnamese Mekong Delta (VMD) [27]. Acentrogobius consists of 22 species globally living from the Indian Ocean to the western Pacific Ocean [14]. In VMD, there are three species, including Acentrogobius globiceps (Hora, 1923), Acentrogobius caninus (Valenciennes, 1837) and Acentrogobius viridipunctatus (Valenciennes, 1837), are recorded [4, 33, 35, 36]. Among them, A. viridipunctatus is the most common species caught for food supply [27, 34]. This fish displays a dark curved line under the eyes and many bright blue spots on the head and body described by Tran et al. [33]. This species reaches a maximum total length of 16.5 cm in Israel [3] and decreases in recent years [32]; therefore, there is a need to find a suitable strategy for conserving and exploiting fish resource there.

Reproduction of fish and other organisms is crucial to ensure the survival and development of the species [23]. Information on the maturation and degeneration of the gonads helps us better understand and determine the changes and development of fish populations in the wild [29]. Nikolsky [28] indicates that the testis in most fishes is divided into six stages based on their external morphological characteristics. The fish length first maturity ($L_m$) is also an important indicator in managing fish stocks [13, 31]. The $L_m$ is species-specific and varies accordingly to location and reproductive behaviour [6]. Reproducing reference is closely related to producing and capturing aquatic species [19, 24]. On the other hand, A. viridipunctatus and other gobies are being over-exploited in VMD [37]. Therefore, studying reproductive biology, i.e., testicular development, type and time of sperm releasing, and size at first maturity in A. viridipunctatus is essential. The findings will help set up a suitable strategy for conserving and exploiting the fish resources.

2. Material and methods

Fish samples were collected from four sites, including Long Huu-Tra Vinh (LHTV), Trung Binh-Soc Trang (TBST), Dien Hai-Bac Lieu (DHBL) and Tan Thuan-Ca Mau (TTCM) (Figure 1). In these sites, the
precipitation varies significantly with the season, ranging from ~0 in the dry season (January–May) to ~400 mm monthly precipitation in the wet season (June–December). The tide of these sites was semi-diurnal tide with a range of ~1.2 m, the annual temperature was ~27 °C temperature, and the pH was ~8 pH, as documented by Le et al. [20].

A total of 960 individuals (528 males and 432 females) were sampled at four sites from January to December 2020. Five hundred twenty-eight males were used in the present study (Table 1), whereas the number of females was used in another study. Samples were collected once a month using trawl nets and were anaesthetized with MS222 before fixing in 5% formalin and shifting to the laboratory for analysis. Herein, fish samples were classified based on their external morphology, documented by Tran et al. [33] and sex determination based on genital spines (male: triangle and female: oval) [5]. Then the total length (TL) and weight (W) of fish

| Month    | Long Hoo-Tra Vinh | Trung Binh-Soc Trang | Dien Hai-Bac Lieu | Tan Thuan-Ca Mau |
|----------|-------------------|----------------------|-------------------|------------------|
| No.      | W range (g)       | TL range (cm)        | No.               | W range (g)       | TL range (cm)        | No.               | W range (g)       | TL range (cm)        |
| January  | 19                | 6.18-20.68           | 8.1-12.4          | 15               | 4.44-17.73         | 6.5-10.5           | 16               | 5.97-33.26         | 7.1-13.3           |
| February | 7                 | 5.87-8.84            | 7.5-8.8           | 24               | 6.25-19.18         | 7.9-11.7           | 7               | 9.69-27.80         | 8.9-11.9           |
| March    | 12                | 3.33-14.65           | 6.3-10.3          | 18               | 6.25-18.44         | 7.9-11.1           | 8               | 3.82-26.19         | 6.4-12.6           |
| April    | 12                | 6.84-54.07           | 8.7-11.2          | 14               | 3.88-37.88         | 6.7-15.0           | 8               | 6.56-40.24         | 7.5-15.4           |
| May      | 11                | 6.87-24.16           | 8.6-12.9          | 5                | 3.16-25.80         | 6.5-13.5           | 8               | 6.76-24.50         | 7.4-12.6           |
| June     | 8                 | 6.74-19.90           | 7.8-12.0          | 16               | 4.85-33.13         | 7.7-14.5           | 13              | 4.57-24.59         | 7.3-12.0           |
| July     | 11                | 5.2-17.38            | 7.7-11.3          | 11               | 9.04-29.15         | 8.8-12.8           | 7               | 4.11-44.56         | 7.1-15.9           |
| August   | 8                 | 4.86-22.24           | 7.3-13.0          | 19               | 7.79-24.33         | 8.9-14.4           | 12              | 6.93-20.86         | 8.8-12.3           |
| September| 14                | 3.02-16.89           | 6.7-10.8          | 10               | 3.50-31.21         | 6.3-14.2           | 10              | 7.79-22.56         | 9.9-13.8           |
| October  | 17                | 2.38-41.24           | 7.0-15.6          | 5                | 6.91-10.40         | 9.4-10.4           | 7               | 7.49-14.79         | 8.6-11.0           |
| November | 10                | 6.16-17.37           | 8.1-11.3          | 6                | 7.15-9.23          | 8.6-10.1           | 18              | 7.35-22.53         | 9.3-13.4           |
| December | 14                | 2.74-18.37           | 6.2-11.2          | 11               | 3.92-17.23         | 6.6-12.1           | 12              | 7.35-21.69         | 9.3-13.0           |
| Total    | 143               | 2.38-54.07           | 6.2-17.2          | 154              | 3.16-37.88         | 6.3-15.0           | 126              | 3.82-44.56         | 6.4-15.9           | 105              | 2.71-47.34         | 6.5-16.2            |

Figure 1. Allocation map of sampling sites (1: Long Huu-Tra Vinh; 2: Trung Binh-Soc Trang; 3: Dien Hai-Bac Lieu; 4: Tan Thuan-Ca Mau; Dinh, 2018).

Table 1. The weight (W) and total length (TL) of male Acentrogobius viridipunctatus collected at the four sites in 2020.
were measured to the nearest 0.1 cm and 0.01 g, respectively. After dissecting, testis was visually classified into five-stage development using criteria description for *Parapocryptes serperaster*, documented by Dinh et al. [5]. After this, the testis was weighed (GW) to the nearest 0.01 mg using an analytical balance and measured diameter to the nearest 0.01 mm using Motic Image software v2.0 integrated with microscopy.

**Figure 2.** The morphology and histological in testes of *A. viridipunctatus* (a, b, c, d, and e: stage I-V of testis; f, g, h, i, and j: histology of the testis in stages I-V; S: spermatogonia, SC1: primary spermatocytes, SC2: secondary spermatocytes, ST: spermatid, SZ: spermatozoa).
Twenty-five testis (five per stage) was examined histologically using the staining procedure (i.e., fixing, dehydrating, paraffin wax embedding impregnating, 6-μm thick cutting and staining with Hematoxylin and Eosin-Y) of Ho et al. [15] for gamete developmental determination.

The length at first mature \( (L_{m}) \) was the length in which 50% of fish reached sexual maturity and determined from the formula of King [18]: \[ P = \frac{1}{1 + e^{-r(TL-L_{m})}}, \]

where \( P \) was the percentage of mature fish in a given length, \( TL \) was fish total length, and \( r \) was a model parameter. The fish reached sexual maturity when its testis was in stage III onward. The relationship between \( P \) and \( TL \) was qualified using the non-linear regression of the SPSS v21 to obtain the \( L_{m} \).

The reproducing pattern was estimated from the appearance of the gamete in the testis histological slides [28]. The sperm releasing time was determined based on the apparent frequency of testis and gonadosomatic index (GSI) [6, 30]. The GSI was determined from the formula: \[ \text{GSI} = \frac{GW}{W} \times 100 \]

One-way ANOVA determined the change over the months of the GSI at a 5% significance level. SPSS v.21 software was used for statistical processing.

3. Results

3.1. Testicular development

The testes of \( A. \ viridipunctatus \) consisted of two chambers located close to the spine in the fish’s abdominal cavity fixed by various
Stage VI was also the period when duration might have been relatively short, so it was not seen. Besides, with a peak in the April – July period (Figure 3a), sperm catching occurred mainly from April to July (one-way ANOVA, \( p < 0.001 \)). Two different types of gamete, i.e., S (large cells) and SC1 (small cells), were found in the testis (Figure 2g).

Stage III Testis in this stage was milky white, rough and wavy surface, increasing to \( \sim 2.05 \) mm in diameter (Figure 2e). ST also appeared in this stage. The vas deferens were increasingly evident and easier to find (Figure 2h).

Stage IV Testis occupied \( \frac{1}{3} \) of the abdominal cavity volume, with a diameter of \( \sim 3.98 \) mm, and was milky white (Figure 2d). ST was differentiated from spermatozoa (SZ) in the testis. The size of SZ the very small and was stained bright with Hematoxylin (Figure 2i).

Stage V Testis reached the maximum diameter of \( \sim 4.82 \) mm, and the surface was distended (Figure 2e). Spermatozoa could come out when pressing on the fish belly from the outside. In the testis, there were clusters of SC1 and SC2 (Figure 2f). It can be seen that, after the ejaculatory phase, the testis had a recovery and returned stage similar to stage II.

Stage VI was not detected during the study period. This stage’s duration might have been relatively short, so it was not seen. Besides, stage VI was also the period when fish rest after a spawning cycle, so catching fish at this stage was unlikely.

3.2. Reproducing reference and length at first maturity

The testis stage composition and gonadosomatic index (GSI) could determine the spawning season in \( A. \) viridipunctatus. The testis stage composition provided information about the period during which fish were most fertile. Unlike the testis stage composition, the GSI provided information about the period during which these individuals in each length group with testis reaching stage III onward).

The results of the analysis of GSI at LHTV showed that GSI displayed a low value in the dry season and a higher value in the wet season, especially in December (One-way ANOVA, \( F = 2.33, p < 0.001 \)). This showed that although these fish spawn here all year round, the primary reproducing season was in the wet season, from June to December. This change occurred similarly at TBST; however, the main sperm releasing period at TBST began later than LHTV. The GSI in TBST was low from January to August but gradually increased from September to December (\( F = 3.66, p < 0.001 \)). At DHBL, the reproducing season of fish occurred mainly from April to July due to the high GSI in these months (\( F = 2.64, p < 0.001 \)). At TTCM, the GSI remained low in the dry season and increased in the wet season from July to October (\( F = 2.87, p < 0.001 \)), which was the main sperm releasing of this fish.

The analysis results of \( L_m \) in \( A. \) viridipunctatus in four study areas showed that \( L_m \) at LHTV, TBST, and TTCM had similar values (Figure 5a–d). Specifically, this value was \( 9.0 \pm 0.3 \) cm at LHTV (Figure 5a), \( 9.3 \pm 0.3 \) cm at TBST (Figure 5b), and \( 8.9 \pm 0.2 \) cm at TTCM (Figure 5d). Meanwhile, at DHBL, \( L_m \) exhibited the lowest value of \( 6.5 \pm 0.5 \) cm (Figure 5c).

4. Discussion

The histological analysis of \( A. \) viridipunctatus showed that this fish could release sperms many times during the spawning season because...
different stages of spermatocytes were detected in the testis in stages IV and V [6]. After the SZ was released, SC2 and ST continued developing and forming SZ for the next cluster of sperm-releasing cycles. The testicular developmental and sperm releasing patterns of *A. viridipunctatus* were also found in some gobies living in and out of VMD, i.e., *Eleotris melanosoma* [38], *Oxyeleotris urophthalmas* [39], *Glossogobius giuris* [12, 16], *Butis butis* [6], *Stigmatogobius pleurostigma* [8], *G. sparsipapillus* [25], and *G. aureus* [11].

The sperm-releasing season of *A. viridipunctatus* in the four study sites varied between months, but the sperm-releasing season of this species occurred mainly in the wet season. It could be that more favourable environmental conditions in the wet season (low ambient temperature, abundant water) helped these fish spawn mainly in the wet season. The main sperm releasing period in the wet season was also found in *G. giuris* (August to November) [12, 16], *E. melanosoma* (April to June) [38], *O. urophthalmas* (May to June) [39], *Trypauchen vagina* (June to August) [7], *S. pleurostigma* (March to November) [8], *G. sparsipapillus* (September to November) [26], *B. koloiomadon* (August to December) [10], and *G. aureus* (August to October) [11]. The *A. viridipunctatus* showed spatial changes in sperm-releasing due to variation of GSI among these sites, which was found in its congener, *A. pflammi*, and some fishes like *Chasmichthys dolichognathus* and *Tridentiger sp.* in Korea—a temporary region where they spawned in the spring-summer season from May to July [1, 2, 17].

The Lm shared a similar pattern at LHTV, TBST, and TCTC and was higher than DHBL. The environment in this area might have influenced the Lm of this species. According to Dinh et al. [10], the salinity displayed the highest at DHBL (23.5 ‰), followed by TCTC (23.1 ‰), and was higher than at TBST (14.0 ‰) and LHTV (12.3 ‰). This showed that when salinity exceeded the tolerance threshold of fish, Lm tended to decrease. Variation of Lm with salinity was also found in several other fish species in VMD. In *G. aureus*, Lm tends to decrease from freshwater (12.45 ± 1.48 cm) to saltwater (10.45 ± 0.34 cm) [11]. In addition, there was a similar change in Lm in *Periophthalmodon septemradiatus*—a fish of the family Oxudercidae in VMD. Specifically, *P. septemradiatus* exhibited an increase of Lm from saltwater (8.2 cm) to freshwater (9.2 cm) [9]. To sustain the source of *A. viridipunctatus* in the study region, the local government should ask fishermen to catch fish with a total length higher than Lm.

5. Conclusion

*Acentrogobius viridipunctatus* could release spemns year-round, with a prominent peak in the wet season. This species had the length at first mature in males depending on environmental changes. Capturing the Lm changes of this fish in the study areas was the basis for local authorities to determine the appropriate size of the catch. Since then, there have been measures to conserve and develop this fish species. This information is also beneficial for further studies on the artificial reproduction of this fish.

Declarations

**Author contribution statement**

Ngon Trong Truong: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

Ton Huu Duc Nguyen: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Quang Minh Dinh: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

**Funding statement**

Quang, M. Dinh was supported by Can Tho University.

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**Data availability statement**

Data will be made available on request.

**Declaration of interest’s statement**

The authors declare no conflict of interest.

**Additional information**

No additional information is available for this paper.

**Acknowledgements**

The authors would like to thank the local fishers.
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