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Original Article

Reproductive Cycle of *Spondylus varius* (Sowerby, 1827) in Lianga Bay, Surigao Del Sur, Eastern Mindanao, Philippines

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Abstract: The reproductive cycle of the *Spondylus varius* in Lianga Bay, Surigao del Sur, Philippines, was studied from September 2018 to October 2019. Histological examination showed that this species is gonochoric. The spawning occurred at the size of 80-100 mm shell length. *Spondylus varius* showed a continuous breeding season from December to April. The highest peak was recorded in April 2019. From August to October, the early and late gametogenesis stage predominates. Annual gonad development and spawning period coincided with the increase in sea surface temperature. In terms of management, it is important to leave a proportion of reproductively mature individuals in the population to allow breeding and recruitment. Thus, it is recommended that the collection of individuals smaller than 65 mm should be restricted and a ban on fishing imposed from February to May.

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Introduction

Spondylus varius (Sowerby, 1829) commonly known as spiny oyster or giant variable thorny oyster belongs to the family Spondylidae. It is relatively large compared to other spondylid species and grows to about 38-50 cm. This species can produce water gas-filled chambers in one or both valves in the matured specimens (Lamprell, 2006). *Spondylus varius* inhabits rocky reefs from the intertidal area up to 10-50 meters depth. Economically, it is a source of income for fishermen in the coastal areas of Surigao del Sur.

There are few works on the *Spondylus* species focusing on their taxonomy and habitat (Keen, 1971; Skoglund and Mulliner, 1996; Lamprell, 2006; Huber, 2009; Coan and Valentich-Scott, 2012). Of the 75 species of *Spondylus* species, very scarce information is available regarding their reproductive biology (Villalejo-Fuerte and Garcia-Dominguez, 1998; Rodriguez-Astudillo et al., 2002; Villalejo-Fuerte et al., 2002; Shabtay et al., 2015). The reproductive biology of an organism is the basic remedy to formulate policies to protect and avoid the over-exploitation of marine invertebrates. In addition, it is essential to support the management efforts of any fishery resource (Stoner et al., 2012). Thus, this study was conducted to describe the reproductive features of *S. varius* such as the sex ratio, annual gonad development, and size at the onset of reproduction by sampling during the months of September 2018 to October 2019 from Lianga Bay, Surigao Del Sur, Eastern Mindanao, Philippines.

Materials and Methods

This study was conducted at Barobo, Surigao del Sur, the Southern part of Lianga Bay, Philippines. The study was focused on sampling from the Southern part of the Bay since this area is one of the top collection sites of the *S. varius.*

Sample collections and processing: The specimens were found within the depth range of 5-25 m and were collected by snorkeling and SCUBA diving. The samples were randomly collected within the reef stretch of Lianga Bay. Approximately, 20 individuals monthly (Villalejo-Fuerte et al., 2002; Shabtay et al., 2012) were collected for histological examination

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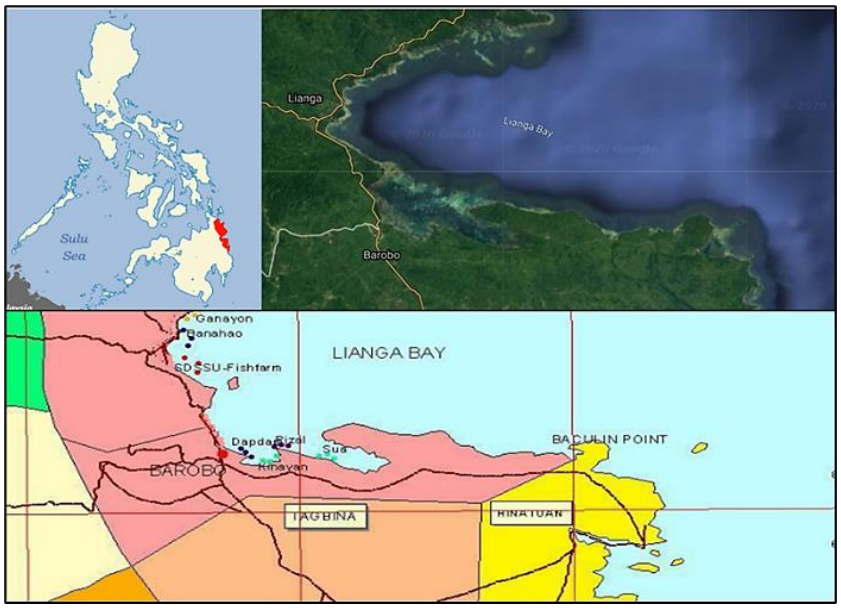

Figure 1. Map of the study area, Southern part of the Lianga Bay.

from September 2018 to October 2019. The live specimens were placed in tanks equipped with oxygen supply diffusions and transported to the Surigao del Sur State University, Lianga Campus Marine Research Station for further study.

Reproductive Features

Sex ratio: The sexual proportion analysis was calculated by dividing the total of collected females by the total number of males, the result was expressed as female to male (F:M) ratio. For the sex ratio equity test, a Chi-square test at a 5% level of significance was performed.

Histological examination of gonads: Before removing the gonads, the samples were measured by a vernier caliper and weighted using a digital scale by including the meat and the gonads. Specimens have distinct gonads that can be removed intact from animals. Then, the gonads were fixed into Bouin solution (Formalin, Glacial Acetic Acid, and Saturated Picric Acid in ratio 5: 45:7 5) for 24 hours at room temperature. Afterward, the gonads were rinsed in tap water and transferred into 70% ETOH for histological preparations. The gonad samples were processed for histological preparation based on Eagderi et al. (2013). The samples were observed under the microscope to study the gonad development and their maturity stages. The gonad development stages were classified based on Dix and Ferguson (1984).

Gonadosomatic Iindex (GSI): GSI was calculated from the ratio of the wet weight of the gonads to the total animal wet mass (Sastry, 1979) using the following formula:

$$GI = (M_{gonad} / M_{total}) X 100$$

Data analysis: The data was tested for normality and homogeneity of variances. The ad hoc test showed that data do satisfy the second assumption. Thus, the variables were analyzed by the ANOVA test at $P<0.01$ probability levels. The size at the onset of sexual maturity was determined using the gonadosomatic indices. A scatterplot of the GSI against the shell lengths (mm) determined the minimum length at onset of active gonadal development which could be a proper indicator of size at sexual maturity.

Results

Sex ratio: A total of 379 individuals were collected from September 2018 to October 2019. Of these, 188 were males and 191 females. The sex ratio in all sampling sites was 1:1 indicating no significant differences between sites. Based on our observations, *S. varius* is gonochoric, and no hermaphrodite specimen was observed. The number of males and females during the sampling period indicated a synchronized reproduction timing.

Spawning period and gonad development: The monthly observations of GSI are shown in Figure 2.

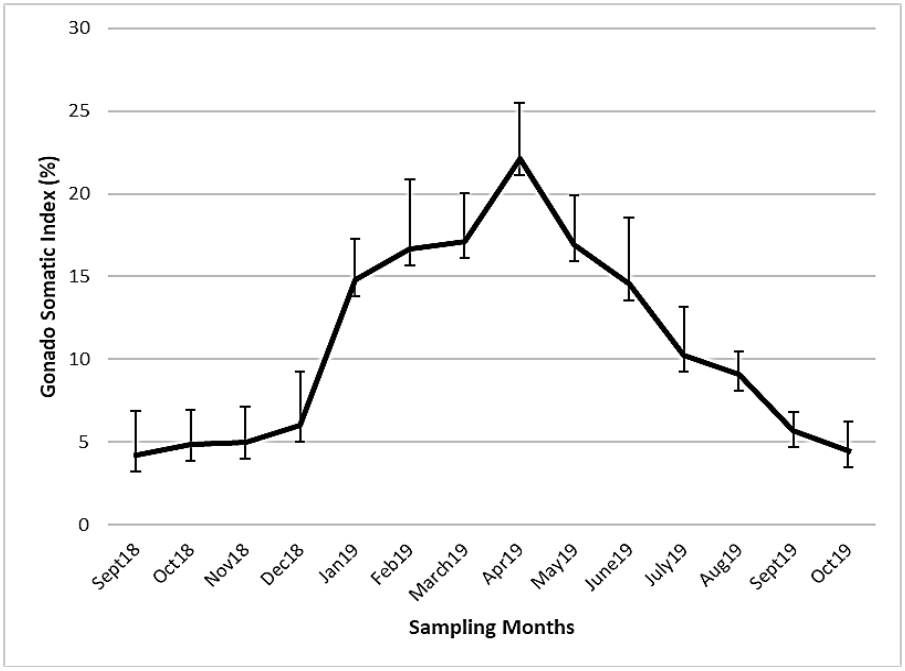

Figure 2. The gonadosomatic index (GSI) of *Spondylus varius* in Lianga Bay, Surigao del Sur from September 2018 to October 2019.

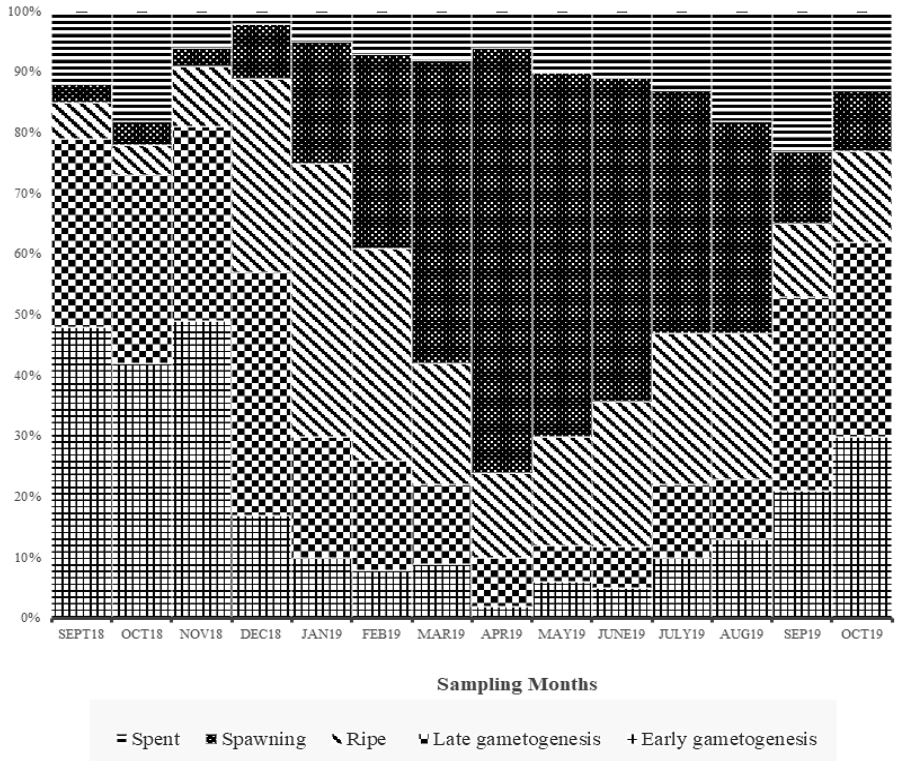

Figure 3. Percentage (%) of gonad stages of *Spondylus varius* in Lianga Bay, Surigao del Sur, September 2018- October 2019.

Continuous peaks were observed from December 2018 to April 2019. The highest peak was recorded in April 2019. The presence of multiple peaks suggests continuous gonad development and spawning. From May 2019 to October 2019, an abrupt decrease in GSI was noted.

Figure 3 shows monthly changes in the percentage of ovary stages of *S. varius*. From September to November 2018, the early and late oocytes had the highest percentage. The ripe stages dominated from December 2018 to February 2019 and the spawning stage was found during these months. The abrupt

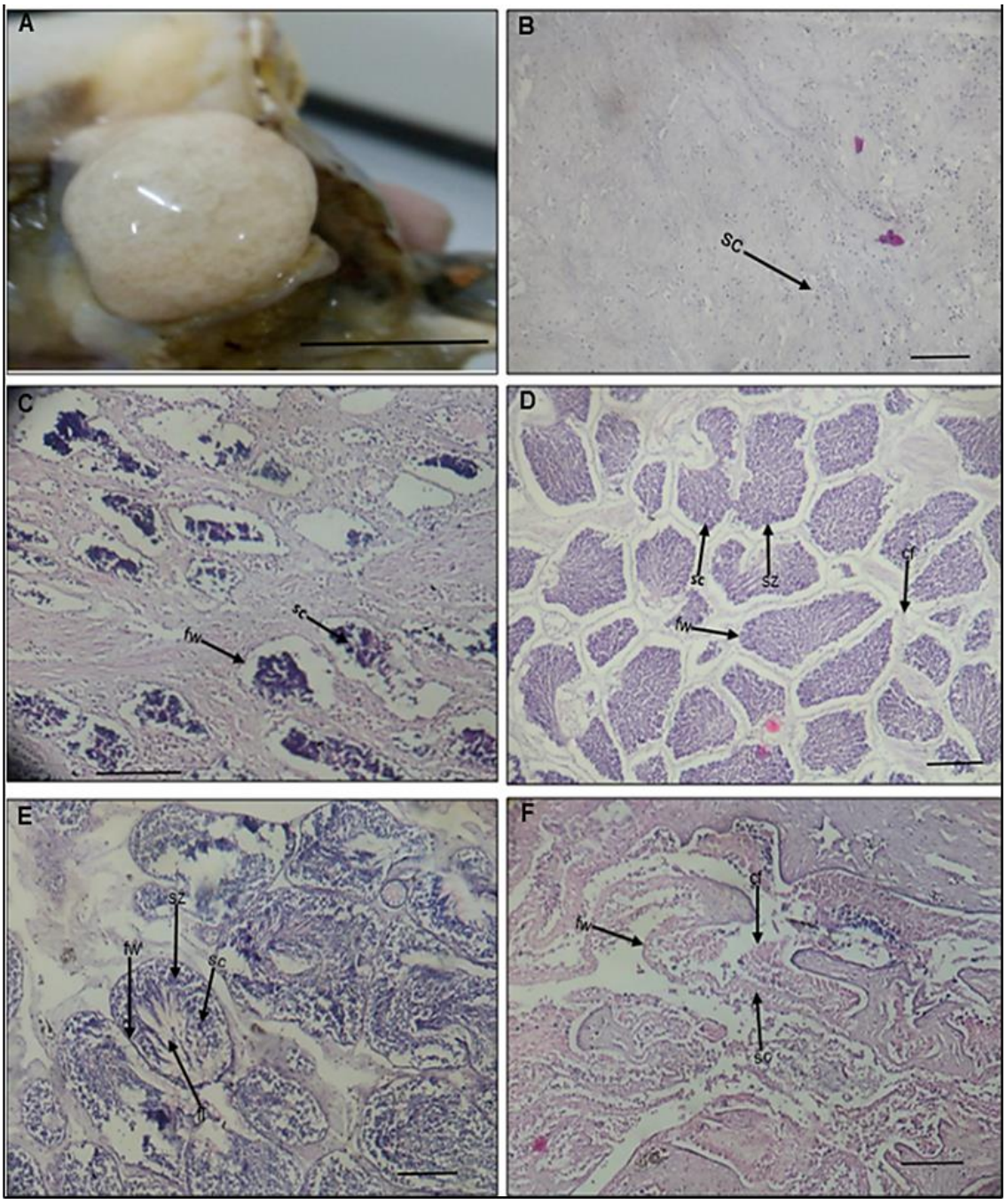

Figure 4. Gonad histology of male *Spondylus varius* (A, live ripe gonad; B, early spermatogenesis; C, late spermatogenesis; D, ripe stage; E, spawning stage; F, spent stage (FL = Follicle Lumen, Sc = Spermatogenesis, and Sz = Spermatozoa) (Scale bars at A = 100 mm B-E = 1000 µm).

increase in the spawning stage was recorded in March 2019 and the highest spawning rate occurred in April 2019. The minimum spawning was observed from May to August 2019. Also, during the spawning stage, ripe gonads were almost dominant. From September to October 2019, the developing gonads were recorded.

Figure 4 shows the histological section of the testis having a cream-white color (Fig. 4A). Early spermatogenesis exhibited a cell layer on the thick follicle wall, undergoing spermatogenesis, and the follicles themselves were empty at that stage (Fig. 4B). The subsequent late spermatogenesis exhibited a thick sperm follicle wall and follicles loaded with spermatocytes undergoing maturation (Fig. 4C). The following ripe stage exhibited a thin sperm follicle wall and follicles packed with mature spermatocytes (Fig. 4D). The spawning stage revealed partly empty

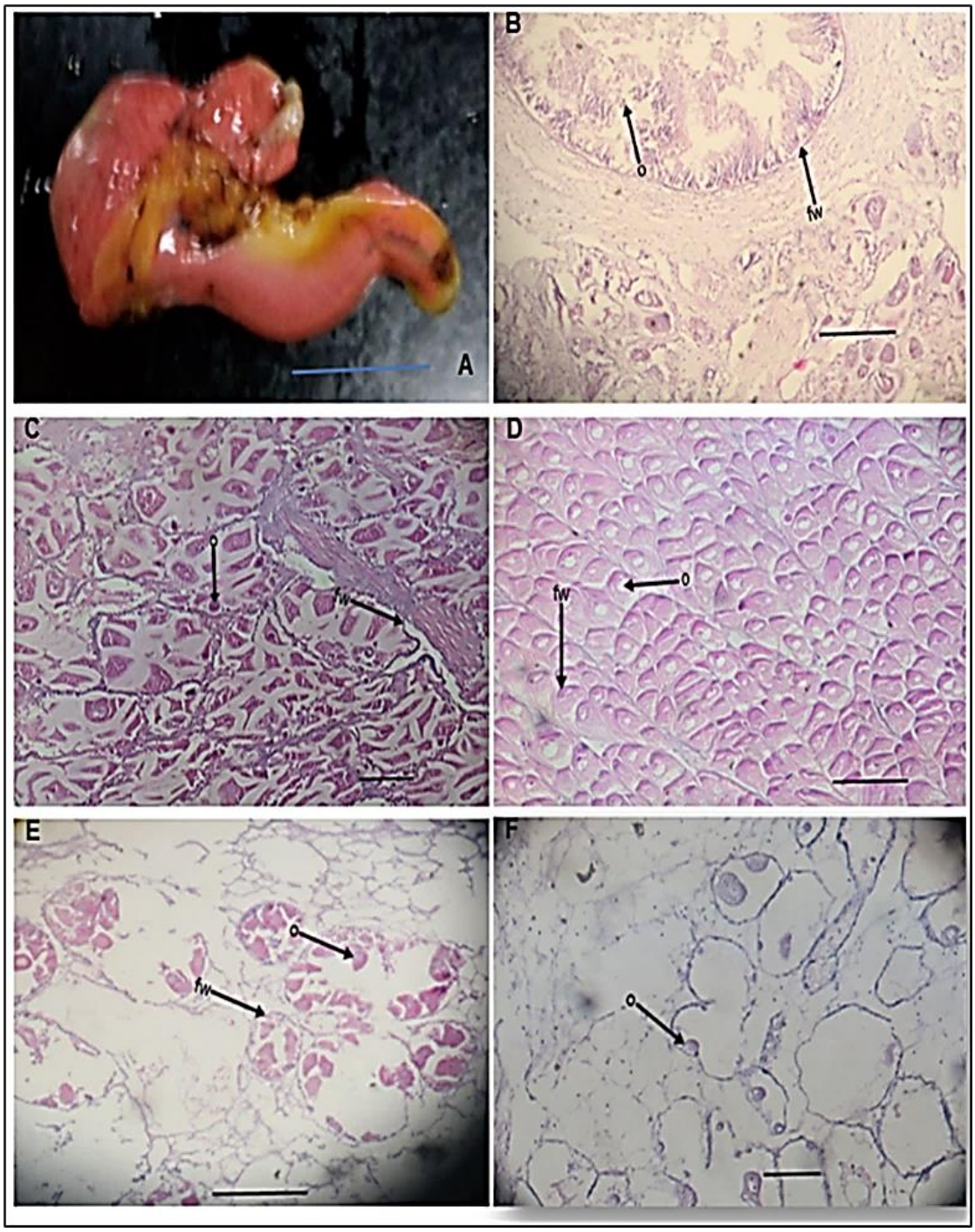

Figure 5. Gonad histology of female *Spondylus varius* (A, live ripe gonad; B, early oocytes; C, late oocytes; D, ripe stage; E, spawning stage; F, spent stage (O = oocytes and FW = Follicle wall) (Scale bar: A =100 mm B-E = 1000 µm)

sperm follicles with no or only a thin wall (Fig. 4E). The spent stage featured a collapsed follicle wall with a few spermatocytes (Fig. 4F).

Figure 5 shows a ripe ovary in red-orange color (Fig. 5A). The early oogenesis exhibited follicles with immature oocytes on their wall surface (Fig. 5B). Subsequently, late oogenesis featured mature oocytes filling most of the follicles, where walls could still be observed (Fig. 5C). The ripe stage displayed swollen follicles packed with mature oocytes and only a small amount of connective tissue between them (Fig. 5). At the spawning stage, some follicles were empty while others had retained some mature oocytes (Fig. 5E). The spent stage featured empty follicles with a

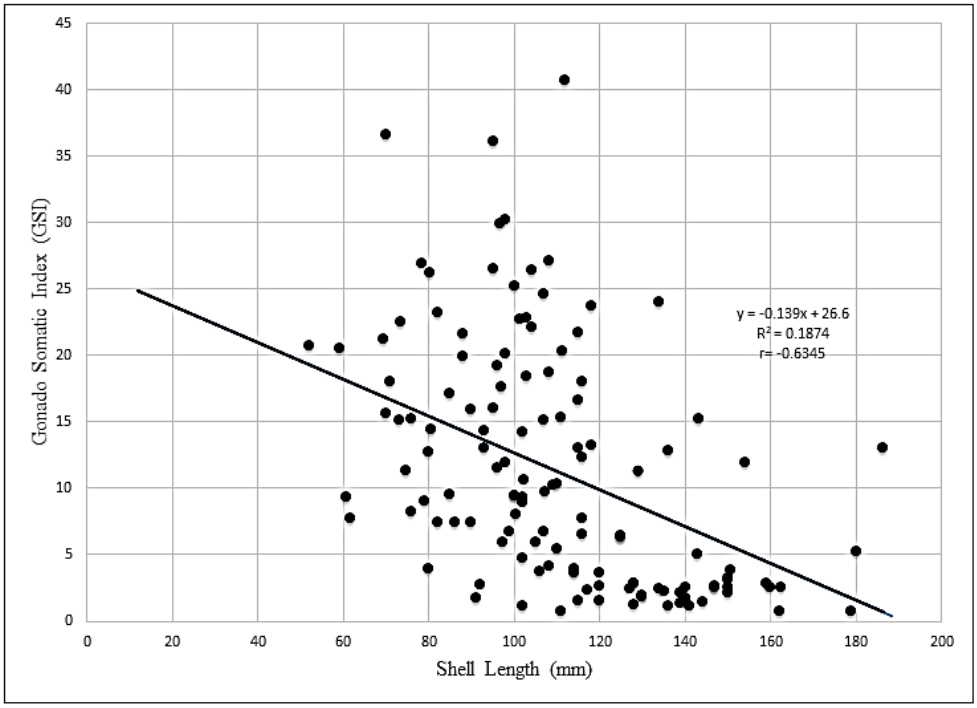

Figure 6. Scatter plot of gonadosomatic index (GSI) against shell lengths (mm) of *Spondylus varius* (n = 379).

collapsed wall and a certain amount of connective tissue (Fig. 5).

At early oogenesis, the average diameter of the oocytes was 65±5 µm and gradually increased to 69±3 and 78±3.5 µm during the late oogenesis and ripe stages, respectively. During the spawning months from February to May, their size was decreased to 73±6 µm. No significant differences were found between the oocyte diameters of the different stages (P>0.05).

Size at the onset of its reproduction (GSI and shell length): Based on the scatterplots of gonadosomatic indices (GSI) against shell lengths, active gonadal development was found around 80-100 mm shell length (Fig. 6). The morphology, color, and size of the gonads are evident to distinguish the onset of the reproduction of *S. varius*. As supported by the histological examination of the gonads, individuals were considered to have mature gonads when abundant motile sperm were observed in males and large, and round oocytes with a well-defined surrounding gelatinous membrane in females. The correlation between the GSI against the shell length (r = - 0.63; P<0.01) was negative.

Discussions

The *S. varius*, a gonochoric species, exhibited a 1:1 sex ratio. A sex ratio of 1:1 among gonochoric organisms is a result of natural selection that acts in favor of organisms (Alexander and Borgia, 1978; Uyenoyama and Bengtsson, 1979). Among bivalves, sex ratio is related to reproductive success as it may affect gamete dispersal and fertilization success (Arnaud-Haond et al., 2003). Similar to other bivalves such as *Chlamys islandica* (Pedersen, 1994) and *Adamussium colbecki* (Heilmayer et al., 2003) exhibit the same ratio as *S. varius.* For the genus *Spondylus* (e.g. *S. calcifer*, Villalejo- Fuerte et al., 2002; *S. spinosus,* Shabtay et al*.,* 2014; *S. princeps,* Cudney-Bueno and Rowell, 2008) also inhibit 1:1 sex ratio. This ratio contributes to high fertilization rates and fecundity, thus contributing to the maintenance of its populations in the new environment (Shabtay et al., 2014).

The relatively long period of spawning activities (December 2018 to April 2019) suggests a continuous reproductive cycle. These results were in agreement with other bivalves such as *Argopecten purpuratus* (Cantillanez et al., 2005), and *Pecten maximus*

*(*Devauchelle and Mingant, 1991). Also, in the family Spondylidae, *S. spinosus* spawned during a relatively long period of several months (Shabtay et al., 2014). A lengthy spawning period of several months was found in *S. princeps* and *S. calcifer* in the Gulf of California (Cudney-Bueno and Rowell, 2008).

Based on the results of the current study, after a long period of spawning period, from August to October, the early and late gametogenesis stages predominate. These results also showed that redevelopment of gonads occurs after spawning, however, a few spent stages were observed during this period. This supports that gonads redevelop after spawning and that gonad development is protracted or continuous. The observed monthly variation of oocyte stages in different months shows that gonad development in *S. varius* is continuous with variation in the intensity of the reproductive activities. These results show that seasonality has a major effect on the variation of gonad development.

A decrease in average oocyte diameter from ripe to spawning stage from 78 to 73 μm from February to May was observed in our results. The results showed that oocyte size was relatively larger than *S. spinosus* (65-68 μm, Shabtay et al., 2014). Although the increase in oocyte size from early gametogenesis to the ripe stage and decrease to the spawning stage was not significant, the degree of variability of the oocyte's sizes in terms of the reproductive stages was clear. In the family Spondylids, the Pectinids, and other bivalves, the oocyte growth is related to food intake and energetic storage in specialized organs (Sastry, 1968; Barber and Blake, 1983; Ruaza and Dy, 2016; Ruaza, 2019) e.g., in *P. magellanicus* and *A. circularis*, the gamete maturation comes from both stored reserves and ingested food (Thompson 1977, 1981; Luna- Gonzalez et al., 2000). The size of the matured gonad in *S. varius* was 80-100 mm in shell length, although some individuals may start to spawn at 65 mm in shell length. These findings were in agreement with *S. calcifer,* a larger species of the genus *Spondylus* which spawned at 113 mm (Villalejo et al., 2002).

Conclusion

The *S. varius* showed a 1:1 sex ratio and all examined samples were gonochoric i.e., no hermaphrodite was observed. The reproductive stages during the sampling periods (September 2018- October) indicated a synchronized timing spawning in males and females. Continuous peaks were observed from December 2018 to April with the highest peak recorded in April showing a relatively long period of several months of spawning activities. After this long period of spawning period, from August to October, the early and late gametogenesis stages predominate. The size of the matured gonad in *S. varius* is around 80-100 mm in shell length, although some individuals may start to spawn at 65 mm in shell length. In terms of management measures, it is suggested to leave a proportion of reproductively mature individuals in the population to allow breeding and recruitment. Thus, in this species collection of individuals smaller than 65 mm must be banned, and collection must also be regulated during maturation and spawning months.

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