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# Assessment of maturity, spawning, and ovarian cycle of sand whiting (*Sillago sihama*) from the north coast of Gulf of Oman

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## Abstract

*Sillago sihama* is the most widespread and abundant member of the smelt-whiting family Sillaginidae and major economic importance throughout the Indo-Pacific. Investigations on the sexual maturity, spawning, and fecundity of *S. sihama* from the Northern coast of Gulf of Oman were carried out from September 2016 to August 2017. The total number of 676 fish with a total length of 10.44-25.4cm and the total body weight ranged between 11.86-124.53 g were used for this study. Females' species predominated in the sampled population and were larger than the males. The study found that the overall sex ratio was 1 male to 1.2 females, diverging significantly from 1:1. Comparison in the histological findings between macroscopic observations and GSI values determined five developmental stages for males and females. These findings revealed that *S. sihama* spawn in April and May and continued until June. The ova diameter frequency distributions in mature component indicated that its spawning is prolong and the release of ova is a single batch. Nearly, all males and females became maturate approximately in over 165 mm and 167 mm, respectively. The results showed that the fecundity had positive correlation with the length and body weight. Furthermore, the absolute fecundity varied from 21345 to 73781 ova and there was a linear relationship between absolute fecundity and fish size for all species tested.

**Keywords:** Sand whiting; Maturation; Spawning; Ovary; Length-weight.

## 1. Introduction

*Sillago sihama*, Silver sillago, locally known as Shoort in Iran, belonging to the family Sillaginidae is an important recreational and commercial marine and brackish species. It lives in coastal areas at a depth of 20 meters and rarely lives up to 60 meters (Mirzaei *et al*., 2017a).

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This species has a wide global distribution, and found in temperate and tropical waters in the Indo-Pacific region from the South Africa in the west to Japan and Indonesia in the east. *S. sihama* is the most popular recreational fish species and good aquaculture potential because of its rapid growth and good flesh quality. For the past decade, it has been subjected to increasing exploitation. Consequently, overfishing is effective in reducing the spawning biomass of a fishery under desired ranges including maximum sustainable or economic yields. Therefore, detailed reproductive knowledge of *S. sihama* will provide valuable information for fishery management and stock assessment, which will lead to a sustainable natural resource in the north coast of the Oman Sea.

Different studies over the past two decades have provided important information on estimation of growth and mortality parameters of this species (Liu *et al*., 2010), age and growth (Panhwar *et al*., 2012), catch and effort data of fishery (Panhwar *et al*., 2012), feeding habits (Taghavi Motlagh *et al*., 2012). More recently, some attempt to gather data on spawning season of *S. sihama* carried out by Hosseinzadeh Sahafi *et al*. (2001) in the northern Persian Gulf. They reported this species got a prolonged breeding season from March to May, with peak spawning activity during the period of April. Shamsan and Ansari (2010) revealed the Oocyte maturation of this specimen from Zuari estuary in India and they found that *S. sihama* have a prolonged breeding season from June to December, with spawning activity peaked during the period of September to November.

In regards to importance of this species and lack of reliable information on its status in the fishery of the Persian Gulf and Oman Sea, the current study is based on the reproductive outcome and spawning patterns of the *S. sihama*. In particular, examine the sex ratios and length at first maturity, maturity stages, and Gonadosomatic Indices (GSI) for fish from the entire north Oman seacoast.

## 2. Material and methods

### *2.1. Study site and sampling*

Monthly samples were collected from the

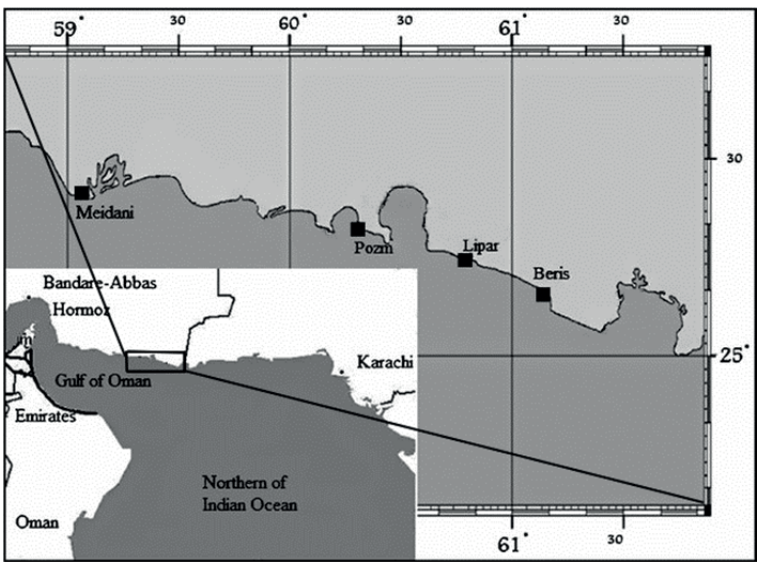


Figure 1. Location of sampling site (■) from the north coast of Oman Gulf

landings of the largest four fishing ports along the north coast of Oman Gulf during one-year study period from September 2016 to August 2017 (Figure 1).

## *2.2. Biological Data Collection*

All specimens were measured to the nearest 0.1 mm of TL and weighed to the nearest 0.01 g. Monthly sex ratio departure from the expected 1:1 ratio was testified. The total length-weight relationship was calculated using regression between these variables for each sex using Kumolu-Johnson and Ndimele (2010) method:

$W = aL^b$

where, W = fish weight (g), L = fish length (cm), a = y-intercept or the initial growth coefficient, and b = slope or the growth coefficient.

## *2.3. Morphological and histological Analysis*

Maturity stages were assessed according to criteria represented by Vahabnezhad *et al*. (2018). Macroscopic classification established by gonadal inspection following the five-step method description is available in Table 1. A histological analysis was carried out according to the method described by Mirzaei *et al*. (2017b). The specimens were fixed in Bouin solution, washed in 70% ethanol, and then were dehydrated in a series of ethanol (from 70% to 95%). The clearing process performed by immersing the gonad specimen in a xylene solution and embedded in paraffin blocks. Sections mounted on glass slides, stained with Hematoxylin to aid observations, and then sealed with a cover slip using DPX glue (Table 2).

## *2.4. Length at first sexual maturity*

The relation between length and maturity in size classes (20mm) are demonstrated on a logistic diagram for estimating the total lengths at 50% maturity. The length at which 50% of fish individuals are at sexual maturity ($L_{m50}$) is known as the length of the first maturity.

## *2.5. Gonadosomatic index*

The Gonadosomatic Index (GSI) of the female fishes of the collected samples are calculated

Table 1. Macroscopic characteristics of ovaries at five developmental stages

| | |
|---|---|
| Immature | Ovary walls are narrow; Ovary and testis occupy nearly a 3/4th length of the body cavity; Tiny and close to the spinal column; Thin Ova are transparent and invisible to the naked eye; Whitish-yellow color; Ribbon like |
| Developing | Ova is visible through the capsule; occupy most of the abdominal cavity; white-colored; and Oocytes usually have yellow color and are easily distinguished by naked eye |
| Ripe | Ovary and testis at this stage are quite thickened; blood vessels with red-pink color; testis are darker |
| Spawning | occupy almost the entire length of the abdominal cavity; Ovary attaining its maximum weight and size with large transparent eggs; large testes; milt flows freely from testes |
| Spent | Testes and Ovaries shrunk with wrinkles; collapsed; reduced in size and volume |

Table 2. Histological characteristics of ovaries at five developmental stages

| | |
|---|---|
| Immature | Ovary having nests of Oogonia and immature Oocytes in large numbers; early and late perinucleolar are dominant |
| Developing | Follicles start to grow and fill with Oogonia and developing Oocytes; most of the small Oocytes were in perinucleolus stage with one or more nucleoli in the periphery of the nucleus |
| Ripe | Ovary dominated by tertiary Oocytes; Oocytes continue to increase in size; fully mature eggs with yolk globules |
| Spawning | Oocytes at late vitellogenic and mature stages are dominant; Oocytes are released from the follicle |
| Spent | Follicle walls look broken and empty, but still distended; there are several post Ovulatory follicles with growth Oocytes, |

separately according to Stoumboudi *et al*. (1993) as follows:

$$GSI = \frac{gonad\ weight\ (g)}{fish\ weight\ (g)} \times 100$$

## *2.6. Fecundity*

Absolute fecundity is estimated by counting the number of mature ova contained within the ovary of individual females. The three ovary subsamples obtained from the anterior, middle, and posterior regions of the ovary. Samples were weighed and the number of mature eggs per subsample was counted under a stereo microscope. Then the total number of eggs in the ovaries is obtained from the following equation (Witthames *et al.*, 2009):

$$F = \frac{\text{Average number of eggs in each subsample} \times \text{ Ovarian dry weight (g)}}{Subsample\ weight\ (g)}$$

Relative fecundity is calculated by the following equation (Jakobsen *et al*., 2016):

$$R = \frac{\text{Absolute fecundity}}{\text{Total body weight (g)}}$$

## *2.7. Ova diameter*

For measurement of ova diameters, small pieces from the anterior, middle, and posterior regions of ovary were taken and then ova were teased out on microslides. Measurement of ova diameter is carried out following the methods of Agarwal (2008) using an ocular micrometer.

## *2.8. Statistical analysis*

Using simple linear regressions described the relationships between fecundity (F) - total length (TL) and fecundity (F) - total weight (TW). One-way ANOVA and group t-tests for independent samples concluded the differences of the gonad weight and GSI value. The statistical analyses were carried out with SPSS software package and a significance level of 0.05.

## 3. Results

A total of 676 individuals, 304 (44.9%) males, and 372 (55.1%) females subjected to the analysis. All observed specimens varied in total length from 10.44-25.4cm (males), 11.17-24.8 cm (females) and between 11.86-124.53g (males) and 13.96-114.05g (females) in total body weight (Figure 2). The overall sex ratio was significantly different from the expected 1:1 ratio (Female/Male=1.2:1) ($p<0.05$). Females were significantly in highest frequency than males except for the months of June and July.

The length–weight relationship was determined for both sexes. The parameters 'a' and 'b' in the power curve equation derived from length-weight relationships of males and females were estimated at 0.008 and 2.98 (males) and 0.007 and 3.03 (females), respectively (Figure 3). The variations in b values from 3 were not statistically significant and indicated an isometric growth for both sexes.

Females: $W= 0085TL^{2.98}$ $R^2 = 0.970$ $P< 0.05$ (Figure 3)

Males: $W = 0.0075TL^{3.03}$ $R^2 = 0.966$ $P< 0.05$ (Figure 4)

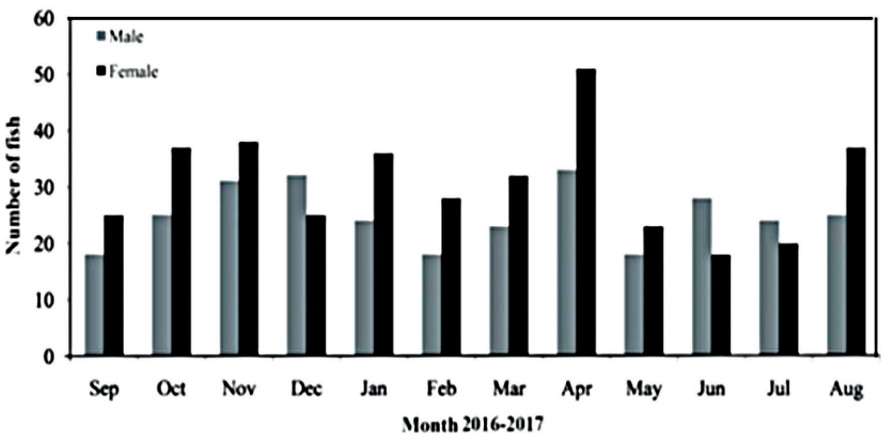


Figure 2. Monthly variation of sex ratio for *S. sihama* (Female: Male) from the north coast of Gulf of Oman

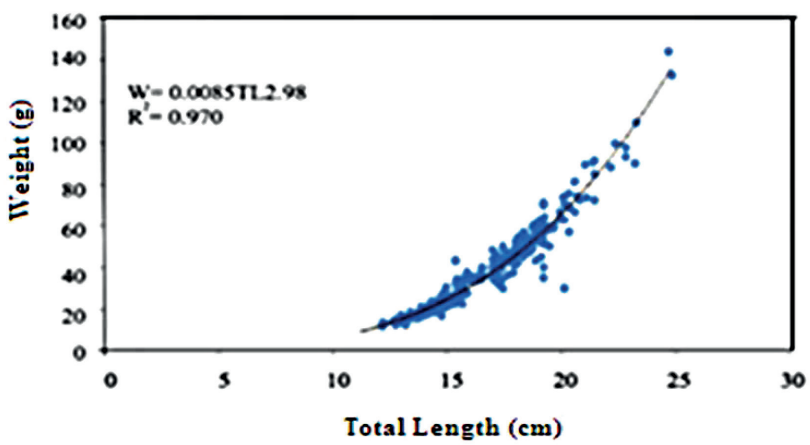


Figure 3. Length-weight relationship of *S. sihama* (Male) sampled from the north coast of Gulf of Oman

The morphological maturity stages of the testis and ovary showed immature and maturing phases were present during September to February (I, II). The ripe ovaries showed a significant increase from December to March (III). While, the highest percentage of the ripe testis were observed in March and gradually decreased until July, spawning condition in males and females began to appear in March and reaching to higher percent in late April and early July (IV). Most of the individuals were in stage V (gonad completely spent) in July and August (V). Spent testes were present during May and reached to the highest percentage in August (Figure 5 and Figure 6).

Histological analysis displayed that the ovary contains an ovarian wall and developing oocytes embedded into the connective tissue. When the Vitellogenic phase increased, the Oocyte size was found throughout the year except Jun and July. During the Oocyte maturation stage, hydration phase (occurs when the yolk granules break) and Ovulation phase (release of a mature egg from the surface of the ovary) were observed from March to August. These results were similar to the monthly macroscopic observation, which obtained in maturity stages for this species (Figure 7).

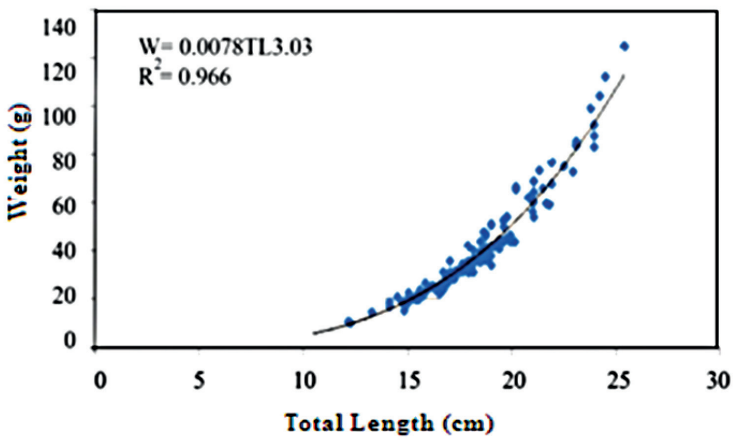


Figure 4. Length-weight relationship of *S. sihama* (Female) sampled from the north coast of Gulf of Oman

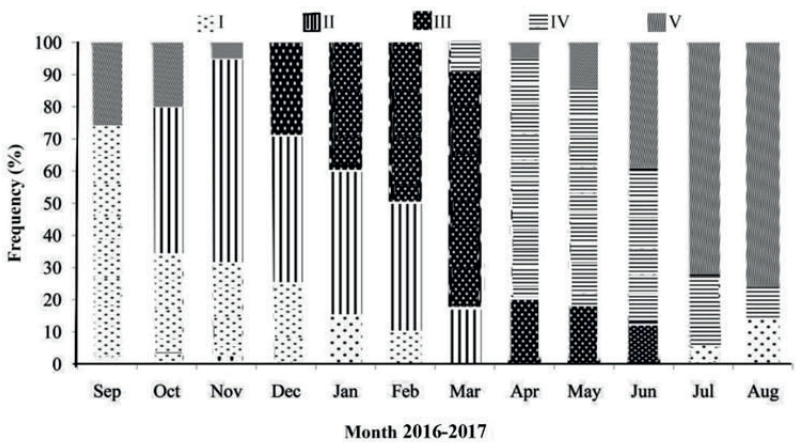


Figure 5. Monthly variations of ovarian maturity stages of *S. sihama* from the north coast of Gulf of Oman during 2016-2017

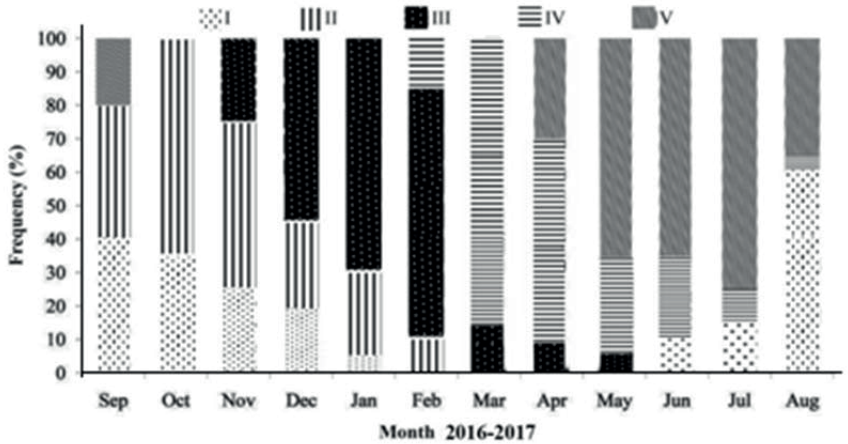


Figure 6. Monthly variations of testis maturity stages of *S. sihama* from the north coast of Gulf of Oman during 2016-2017

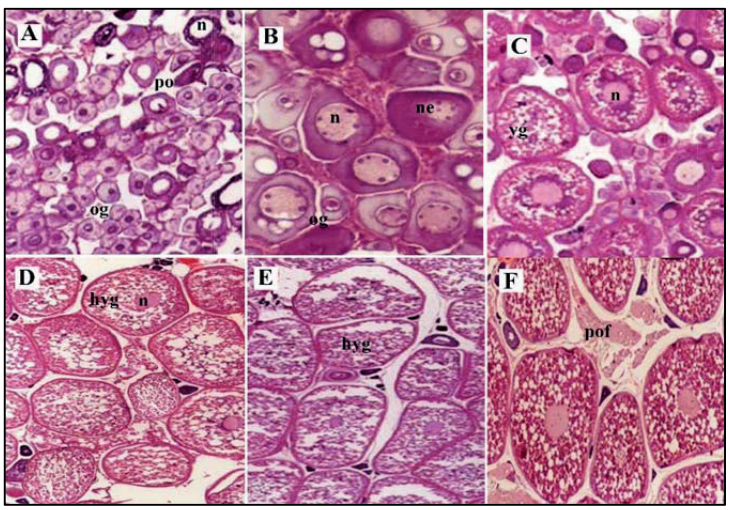


Figure 7. Photomicrographs of cross-sections of S. sihama ovaries at different developmental stages, (A) Immature (B) Maturing stage; (C) End of maturing stage; (D) Ripe stage; (E) Spawning stage; (F) Spent stage;

Description: n: nucleus; ne: Nucleolus; og: oogonia; po: primary growth oocytes; yg: Yolk globule; hyg: hydrolysed yolk granules; pof: post ovulatory follicles.

In stage I, the size of ova ranged from 0.02 to 0.1 mm, majority of them varying in size from 0.016 to 0.076 mm, while in maturing phase the ova diameter increased and the size of ova ranged between 0.07-0.21 mm at the beginning and 0.21-0.31 mm at the end. In stage III, a group of larger ova showed a mode at 0.31-0.43 mm, up to a whopping 0.53 mm in few instances. Fully mature ova in spawning phase was well demarcated from the immature stock of the eggs by modes 0.41-0.55 mm while the largest ova measured about 0.65 mm (Figure 8).

Due to different sizes of ova in different months, the immature and mature ova were present during August-December and February-May. Furthermore, the amount of mature ova dropped and the spawning began based on the ova diameter in the month of June.

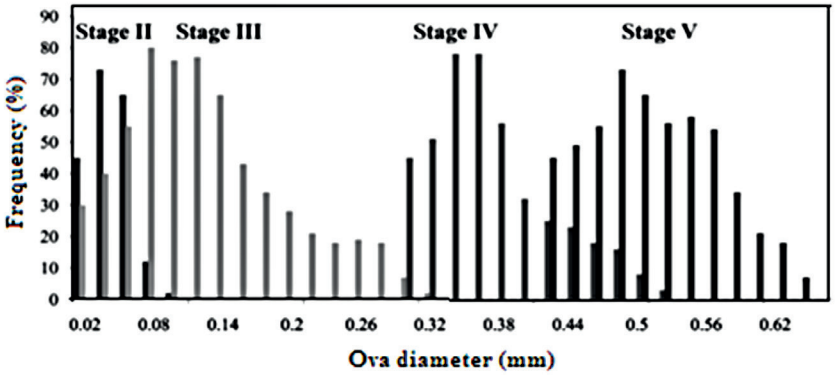


Figure 8. Size-frequency distribution of ova diameter of *S. sihama* from the north coast of Gulf of Oman

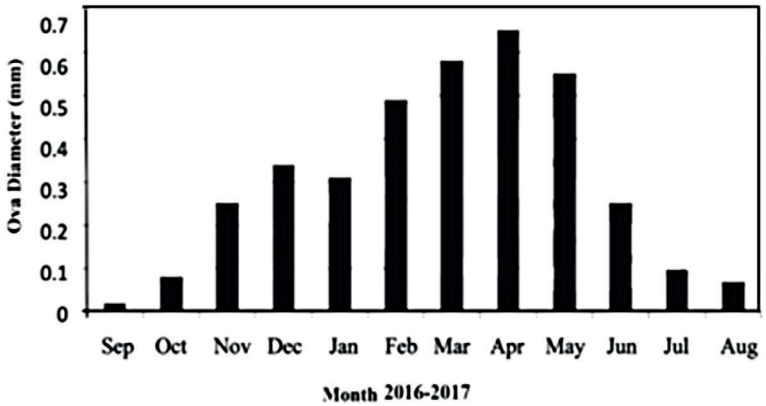


Figure 9. Monthly variation of ova diameter for *S. sihama* from the north coast of Gulf of Oman

Monthly variation in GSI revealed that both sexes followed nearly the same trend. The lowest monthly mean GSI values were recorded in October: 0.7 (Female) and 0.3 (Male). The highest GSI values were 4.1 for females and 1.7 for males in the beginning of April. The higher GSI for females was significant during the study period (Figure 10). The results of this study showed that spawning period started in mid-April and May and continued until early June. There was no significant difference in GSI values among male and female along the months ($p>0.05$).

Size at first maturity (TL) occurred at 132 mm and 138 mm for males and females, respectively. Nearly all males and females were mature approximately over 165 mm and 167 mm, respectively. The results showed that males reached to sexual maturity at a smaller size than females (Figure 11).

Absolute fecundity was determined by counting the number of eggs in the ripe gonads of 62 fish through a gravimetric sub-sampling method. The absolute fecundity of *S. sihama* ranged from 21345 to 73781 with the corresponding total lengths of 13-24 cm and total weight of 14.01-110g.

The minimum relative fecundity (384.29 eggs per gram) was recorded with fish of mean total length 16.8cm and mean body weight 112.14g. While the highest eggs per gram 832.65, was observed in species by mean total length of 21.3cm and mean total weight of 118.44g.

Fecundity showed positive correlation with

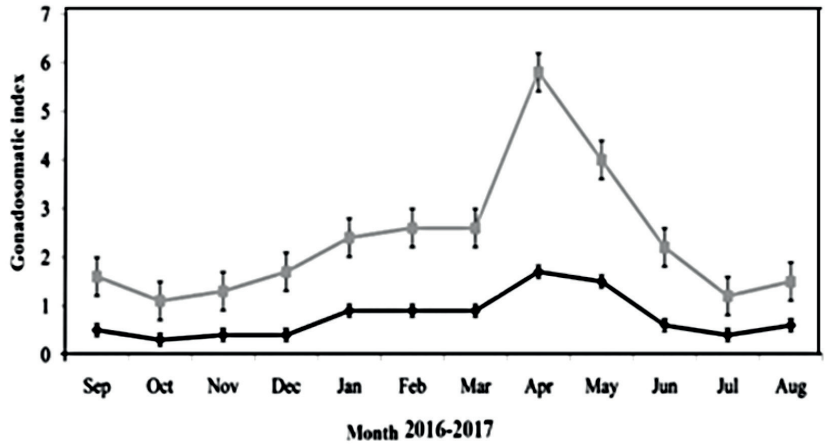


Figure 10. Monthly variations of the GSI values of *S. sihama* from the north coast of Gulf of Oman

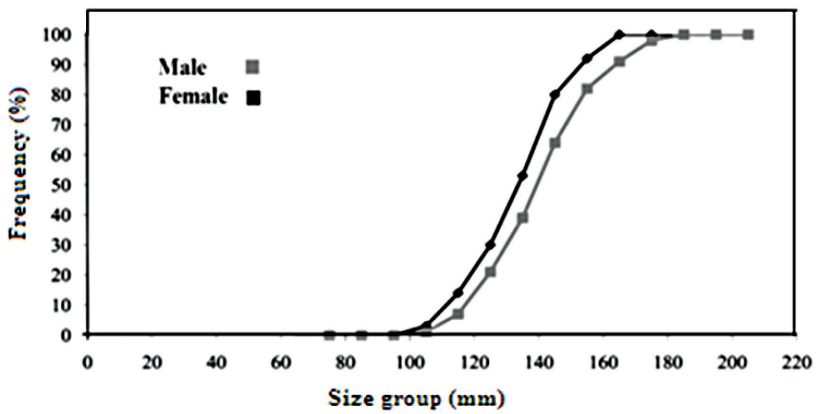


Figure 11. Comparing the length at first maturity (Lm50) of male and female *S. sihama* from the north coast of Gulf of Oman

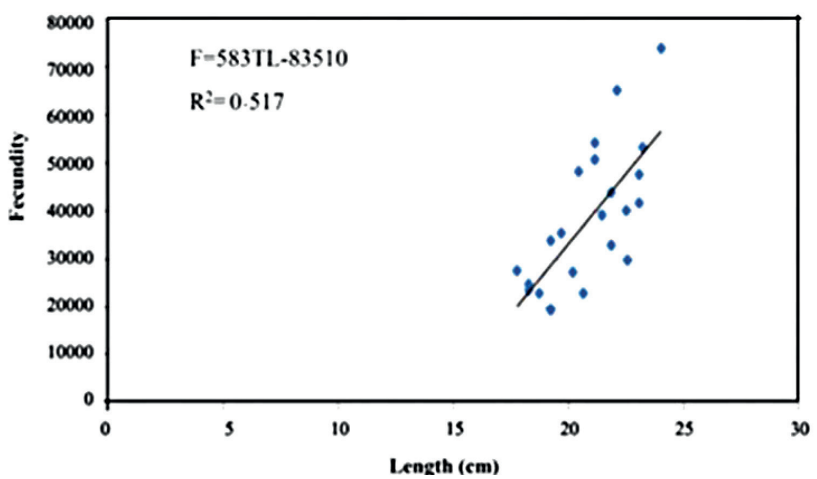


Figure 12. Fecundity - total length relationship in *S. sihama* from the north coast of Gulf of Oman

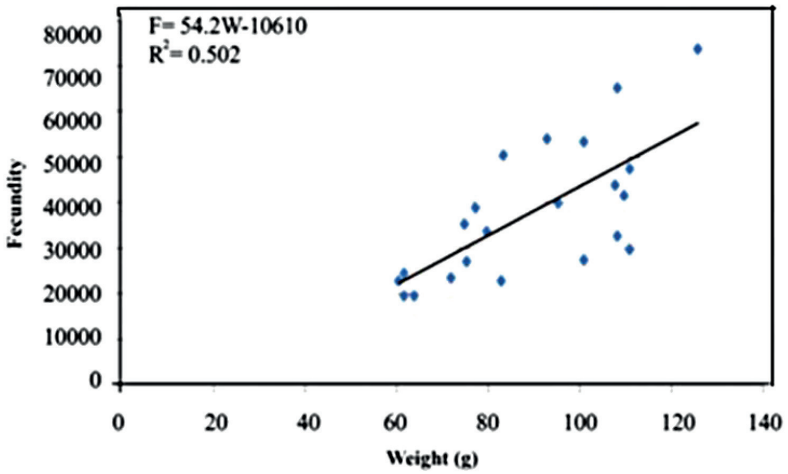


Figure 13. Fecundity - total body weight relationship in *S. sihama* from the north coast of Gulf of Oman

length and body weight. Coefficient of determination ($R^2$) was 0.517 and 0.502 for fecundity-length relationship and fecundity-weight relationship, respectively. Figure 12 illustrates the relationship between fecundity and length which the regression equation is F=5835TL-83510 ($R^2$ = 0.517). Figure 13 illustrates the fecundity-weight relationship. The regression equation was F= 542.6W-10610 ($R^2$ = 0.502).

## 4. Discussion

Length-weight relationship plays a significant role in fisheries sciences as it helps in estimating weight of fish from its length (Chuctaya *et al*., 2018). In general, the regression coefficient b for fish which exhibits isometric growth (b= 3) shall not differ significantly from three, and the weight of fish should vary as the cube of length (Mortuza and Al-Misned, 2013). The b value in length-weight relationship of male and female of *S. sihama* showed that the growth is isometric. The correlation coefficient values were very close to 0.99, which indicated that the relationships between total length and weight of this species was highly significant ($p<0.05$).

In the present research, the sex ratio of male per female was M: F= 1.2:1. This sex ratio changed between different months, but female predominated males. Dominance of females over the males complies with the results of Vinod and Basavaraja (2010).

Results of this study showed that the GSI recorded its highest value during April and May which is the peak breeding season of the fish, then it decreased gradually up to July attaining its lowest value in July Furthermore, females showed higher GSI value than males. The Gonadosomatic Index values increased with the maturation of fish and reached the maximum value during the peak of maturity. The Index values decreased sharply when the fish becomes spent. Furthermore, females generally exhibited comparatively higher GSI values than males. The findings about the reproductive cycle of *S. sihama* in this study are in agreement with those obtained by Hosseinzadeh Sahafi *et al*. (2001) who examined *S. sihama* spawning period in

Persian Gulf and showed spawning period expanded around three months with the highest values of GSI from March to May. Moreover, Radhakrishnan (1957) mentioned that the spawning occurred frequently; it reached to the high values during August to November. These variations might be associated with changes in water temperature, salinity, and food supply, or perhaps to changes in maturity stage.

The findings of the histological experiments of ovary confirmed the existence of Oocytes at most of maturity stages. The majority of Oocytes of a matured group commonly spawned simultaneously and a small number of Oocytes may retain for repeated spawning period. These results are in agreement with Jayasankar and Alagarswami (1994) findings, which showed monthly frequency distribution of Oocytes in the *S. sihama* ovaries and confirmed continuous spawning period.

The estimation of the size at first sexual maturity revealed that both sexes achieved sexual maturation at 130-140 mm length. The size at first maturity in *S. sihama* reported by other investigations is 110-120 mm (Hosseinzadeh Sahafi *et al*., 2001), 155-165 mm (Shamsan and Ansari, 2010), and 170-210 mm (Vinod and Basavaraja, 2010). The geographical variations can explain these differences in the size.

Another important finding was that there was a reasonable difference in ova diameter among female specimens. A possible explanation might be that the ova diameter changes are attributed to the stage of development, temperature, salinity, timing of the spawning period, and the age of spawners (Lee, 1981; Pankhurst and Munday, 2011).

In the current study, the absolute fecundity varied from 21345 to 73781 ova. The relative fecundity varied from 384.29 to 832.65 ova/g body weight. This outcome is contrary to that of James *et al*. (1976) who found that the ovaries of ripe specimens of *S. sihama* included 11304-100593 ova per fish. Furthermore, Vinod and Basavaraja (2010) reported that the absolute fecundity of *S. sihama* varied between 24,439 and 59,372 eggs per female. A possible explanation for this might be that fecundity is not a constant feature but it fluctuates with variations in environmental conditions and specific factors in species (Qadri *et al*., 2015). Shah *et al*. (2018) stated that even within a stock, fecundity can vary annually within individuals. Furthermore, fecundity varies due to spawning period and geographical location (Rijnsdorp and Vingerhoed, 1994).

## Conclusion

This study has shown that *S.sihama* has a long spawning period and a high reproduction rate. The knowledge on length at maturity and spawning season detects when and at which length the fish should be protected which is important for the proper management and conservation of fish stocks. The knowledge on length at first maturity stage and spawning period provides considerable issues for the proper management and conservation of fish stocks such as when and at which length the *S. sihama* should be protected for prohibition of fishing season and regulations of the fishing rules.

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