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PROOF RESEARCH PAPER

Reproductive Biology of *Pomadasys stridens* (Forsskal, 1775) in the Northern Coasts of the Persian Gulf, Iran

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Abstract

This study aims to investigate the reproductive cycle of striped piggy (*Pomadasys stridens*) along the northern coasts of the Persian Gulf (Bushehr Province, Iran) from October 2014 to September 2015. A total of 276 specimens were collected on a monthly basis and their length, weight, sex, gonad weight, and maturity status were recorded. The mean monthly values of GSI indicated that the main spawning period occurs during December to June. Based on ovarian histology and macroscopic analysis of gonads five stage of maturity development were identified. The result of present study demonstrated that, similar to several other tropical species with a distinct annual reproductive cycle, *P. stridens* characterized by prolong spawning season and females are the predominant sex in the population. The percentage frequency of different developmental oocyte stages within oocyte cluster verifies that the *P. stridens* is a batch spawner with asynchronous oocyte development.

Keywords: Gonadosomatic index, asynchronous, reproductive cycle, *Pomadasys stridens*, Persian Gulf.

Introduction

The Persian Gulf is considered to be one of the richest areas in fishery resources where large quantities of fish and shrimps are concentrated in different locations, particularly in the territorial waters of the south of Iran (Hashemi, Taghavimotlagh, & Eskandary, 2011). Annually, several species of grunt are caught in the Persian Gulf and Oman Sea which they are one of the common types of fish which target by the artisanal and industrial trawlers (Valinassab, Keyvan, Sedghi Marouf, & Kamali, 2007). Among the species of grunt, striped piggy (*Pomadasys stridens*) which belongs to Haemulidae family has a poor commercial value due to its small size. The species is spread in shallow tropical seas around the coastlines of the Indian Ocean: Red Sea (including the eastern Mediterranean Sea), South Africa, and western India. *P. stridens*, which is found in coastal waters down to a depth ranging from 65 to 68 m (Weitkamp and Sullivan, 2003), feeds mainly on crustaceans and fishes (Fischer *et al*., 1990; Vahabnezhad, Kaymaram, Taghavi motlagh, Valinasab, & Fatemi, 2016). Usually, they caught by bottom trawls, hook and line (Carpenter, Krupp, Jones, & Zajonz, 1997). The largest length of stripped piggy among the commercially discarded fishes is 20.0 cm (TL) (Fischer *et al*., 1990).

Bycatch of small fishes constitute approximately 16% (11109 tons) of the total catches in the Persian Gulf (Eskandari *et al*., 2014). In spite of low economic value of these small species in comparison with big species, they play an important role in food web. Grunts, snapper, threadfin, and white pomfret are among small-sized species which are economically valuable (Valinassab, Daryanabard, Dehghani, & Pierce, 2006). Catch limits for non-target fish and other marine animals with depleted populations must be based on scientific information. Once these limits are reached, fisheries should be shut down for the remainder of the season (Keledjian *et al*., 2014).

Despite its great ecological importance in the Persian Gulf ecosystems as a prey in of other species, there is little information about the biology of striped piggy. According to a review of resources by Hashemi *et al*. (2011), different aspects of biological parameters of *P. stridens* have been studied by different researchers such as Pauly, Froese and Albert (1998) in Philippines waters, Fischer *et al.* (1990) in Mozambique waters, and Ben-Tuvia and McKay (1986) in north-eastern Atlantic Ocean. However, few studies have been performed on the biology of *P. stridens* in the Persian Gulf. The feeding biology

investigated by Vahabnezhad *et al*. (2016), population parameters and length-weight relationship by Hashemi *et al*. (2011), and reproductive biology by Karimi, Mahbobi Soofiani, Paykanheirati and Katiraei (2013). The present study is aimed to investigate reproductive cycle of *P. stridens* in the Persian Gulf and defining its spawning season and size at the first maturity.

Materials and Methods

In this study, a total of 276 specimens of *P. stridens* were collected monthly from October, 2014 to September, 2015. Data were collected from the coastal waters of Bushehr province (south coastal waters of Iran in the Persian Gulf) (Figure 1).

The data collected from commercial trawling nets and gill nets (mesh size, 12 cm) as bycatch, which utilized during shrimp fishery season that usually, take place for 45 days each year (August to September) and other seasons, respectively. Female specimens were examined for gonad development stages, spawning season, and length at sexual maturity. The total length (T_L,1 cm), body weight (B_W, 0.1g), and gonad weight (G_W, 0.1g) of each fish were measured, while sex was determined by examination of the gonads, either with the naked eye or with the aid of a binocular microscope. Moreover, the gonads of the female fishes were macroscopically classified into various development stages based on appearance of ovary. Ovaries were placed in Bouin's solution for 48 hrs and transferred to 70% ethanol. Dehydrated ovaries were embedded in paraffin, and the gonad sections (5 µm thick) were stained with Mayer's haemalum and Young's Eosin balances (Winsor, 1994).

Macroscopic character traits (for example, gonad size, shape, and volume) are listed on the left as they were estimated, using histological characters on the right side. The origins of this scheme were studied by Afonso-Dias, Reis and Andrade (2005) (Table 1). The timing and duration of the spawning season were derived from the monthly data on the incidence of mature individuals and proportions of each maturity stage. Gonadosomatic index (GSI= (G_W/B_W) ×100) was calculated to assess maturity (King, 2007). Based on histological sections, oocyte development was classified. The spawning season was established on the basis of the mean monthly variation of GSI and ovarian maturity stages. To estimate the average size at which 50% of *P. stridens* female individuals attain the first sexual (fishes in stage II of gonad development and above were considered mature) maturity according to the equation, P=1/ (1+exp [-r(L-L_m)]), formulated by King, (2007). The overall sex ratio (♂/♀) different from the expected 1:1 ratio was evaluated using the Chi-square test.

Results

Length Distribution

Figure 2 shows the length distribution of *P. stridens* (89 male and 187 female) fishes. According to sample analysis, females attained larger sizes than males. The total length (TL) and weight of females ranged from 8 to 20.50 cm (mean±SE, 17.01±1.65 cm) and 35 to 151.5 g (mean±SE, 86.32± 25.28 g), respectively. The sex ratio was mostly biased towards females, and the overall sex ratio (M: F) was 1:2.5. In addition, Chi-square analysis showed a significant difference in sex ratio from the expected ration of 1:1 (c2=1.9517, P<0.05).

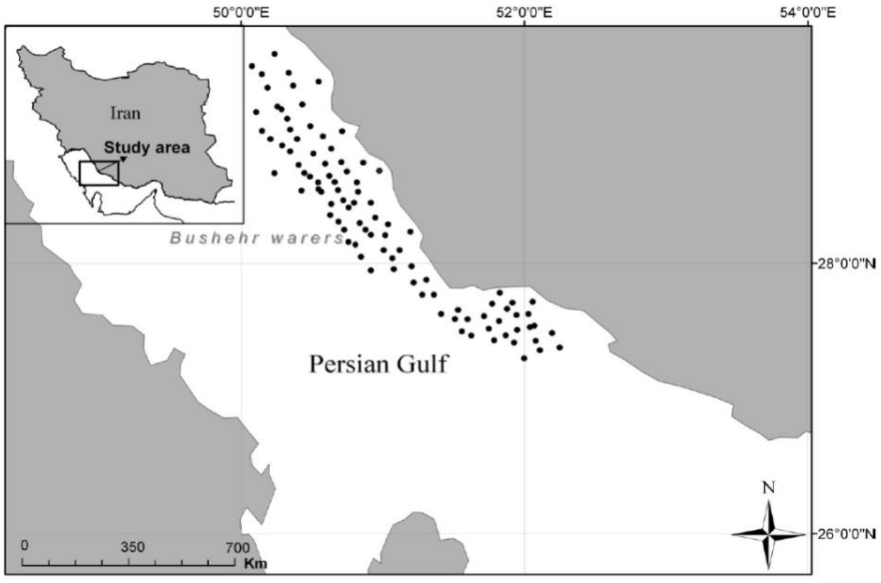

Figure 1. Location of sampling in coastal waters of Bushehr coastal water province, Persian Gulf, Iran.

Table 1. Classification of maturity for female *P. stridens* according, Afonso-Dias et al. 2005

Macroscopic class and criteria	Gonad histology class and criteria
I–Immature No female smaller than 10 cm was mature. The ovary wall was thin and tore easily, smallest in length; the ovary was clear, transparent. Color was evident in larger immature fish, pinkish. Small translucent. Organ located at posterior curve of gut cavity. There were no visible oocytes	I–Immature Oogonias and small oocytes in chromatin nucleolus stage (CNS); no oocytes beyond the perinucleolus stage (PNS) and early perinucleolus (EPN) were found. Observed in spring to autumn
II–Developing Ovaries grew rapidly in this class; increase in length and in width, they become thicker and opaque with blood vessels becoming prominent, small opaque specks are visible as ovary develops to occupy 2/3 of ventral cavity. Ovary extending posteriorly from gut cavity; bright yellow and firm texture. The fully vitellogenic oocytes were large enough to be visible to the unaided eye	II–Developing Some oocytes in CNS but most of the small oocytes were in PNS with one or more nucleoli in the periphery of the nucleus. The larger oocytes were in the cortical alveoli stage (CAS). Observed in spring to autumn
III–Maturing The ovaries increase considerably in volume and usually distend the body. Many opaque and a few hydrated oocytes are visible	III–Maturing Oocytes continue to increase in size, most in CAS. Vitellongenic (Vit) and small numbers of mature (Mat) oocytes with a migrating nucleus are present. Small PNS oocytes are still present. Could be observed in all season, but mostly in spring to autumn
IV–Ripe Ovary may fill body cavity. Large, turgid, and full ovary with hydrated oocytes visible. Hydrated oocytes were darker than the background of yellow to orange yolked eggs. In the running stage the oocytes were extruded copiously under light pressure of the ovary.	IV–Ripe Most advanced oocyte (MAOS) was a hydrated oocyte still within the follicle and lamellae. Many mature and hydrated oocytes were present. Numerous oocytes in CAS and in an advanced stage of vitellogenesis; pre-vitellogenic oocytes were still present. No post-ovulatory follicles (POF) were evident. The gonad wall was stretched. Observed in autumn to winter with the further in winter
V–Spent Ovaries were empty or partially empty and flaccid. If the ovary was cut open to confirm, the sac-like nature of the ovary was apparent. There may be residual hydrated or larger vitellogenic oocytes scattered in a state of reabsorption with a lot of slime. The ovary was usually discolored and red-purple.	V–Spent Numerous post-ovulatory follicles (POF) were present. Many oocytes were in different stages of perinucleolus and cortical alveoli. Many empty spaces in the ovaries. Mature oocytes that were not released in different stages of atresia; observed in spring to summer

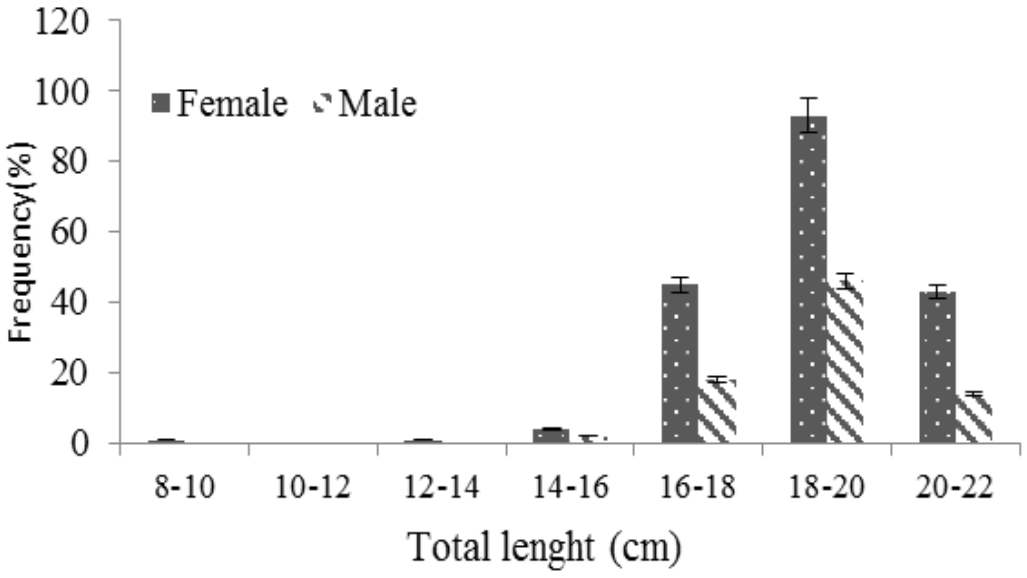

Figure 2. Total length distribution of *P. stridens* sampled in 2014–2015

Size at First Maturity

The mean length at the first sexual maturity (50% of all individuals are sexually mature) was estimated to be 19.84 cm (TL). Figure 3 demonstrates the percentage of the occurrence of mature females. It is evident following figure that all the fishes below 10 cm were immature. The percentage of the mature fishes increased up to the length group of 18-20 cm when all the female specimens were observed to be mature.

Spawning Season

Histological study of the gonads, calculation of gonadosomatic indices and percentage occurrence of mature fishes during the year were taken into consideration to determine the spawning season of *P. stridens*.

Histological Study of Gonads

Monthly histological observation of the gonads indicated that *a P. stridens* start spawning from December and gradually continues up to June. Stage (I) includes the immature or primary growth of a single layer of follicle cells surrounding the oocyte. The oocyte cytoplasm is strongly basophilic during this period, and the nucleus contains many easily distinguishable nucleoli next to the nuclear membrane, which are observed in the spring-to-summer period (Figure 4a). Stage (II) is the developing stage, and the average size of nucleus increases with 7 to 11 nucleoli. The nucleoli arranged in the periphery of the nucleus could be observed in all seasons, but generally in the spring-to-summer period (Figure 4b). Stage (III) is maturing or vitellogenesis which is revealed by the formation of oil droplets, yolk vesicles, and cortical alveoli vesicles surrounding the nucleus, which are observed in the spring-to-autumn period. Stage (IV) is the oocyte maturation stage where the oil droplets are concentrated around the nucleus; following that, they fuse into an oil drop that migrates, together with the nucleus, towards the animal pole. The yolk vesicles progressively fuse to form a continuous mass of fluid yolk, which are observed in the autumn-to-winter period and more frequently in winter (Figure 4c). Stage (V) is spent: the ooplasm, restricted to a narrow rim, lies beside the zona radiata at the oocyte periphery, which is observed in spring (Figure 4d). These results suggest that *P. stridens* are batch spawners exhibiting asynchronous oocyte development and spawning multiple batches of oocytes over the course of the reproductive season.

Gonadosomatic Index

Gonadosomatic indices were calculated for female fishes and demonstrated in Figure 5. It was found that they increased gradually from 0.55 (October) to 3.62 (December). A sudden decrease in GSI in February in comparison to December (3.62 to 0.63) was indicative of the onset of spawning activity. In March, the GSI values decreased to 0.63, which the sign of the cessation of the spawning acts in *P. stridens*. The specimens were mature from the December-February period to the March-Jun period when the fishes started to spawn.

Percentage Occurrence of Mature Fishes

Figure 6 depicts the percentage of the occurrence of fishes with different ovarian stages. The first ripe females were found in December and increased abundantly in January and reached to maximum level in February. However, a gradual increase in the incidence of the occurrence of the individuals in the spent condition indicated the proximity of spawning. During May, the individuals were found in the spent phase, while the rest were in the recoupment phase. From June onwards, no ripe individuals were procured during the present study. The immature fishes were observed throughout the year except October, April, and May.

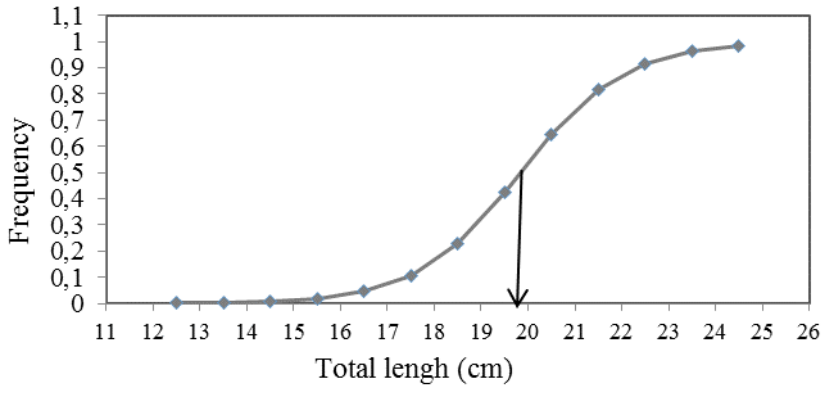

Figure 3. Changes in the proportion of mature *P. stridens* females with size.

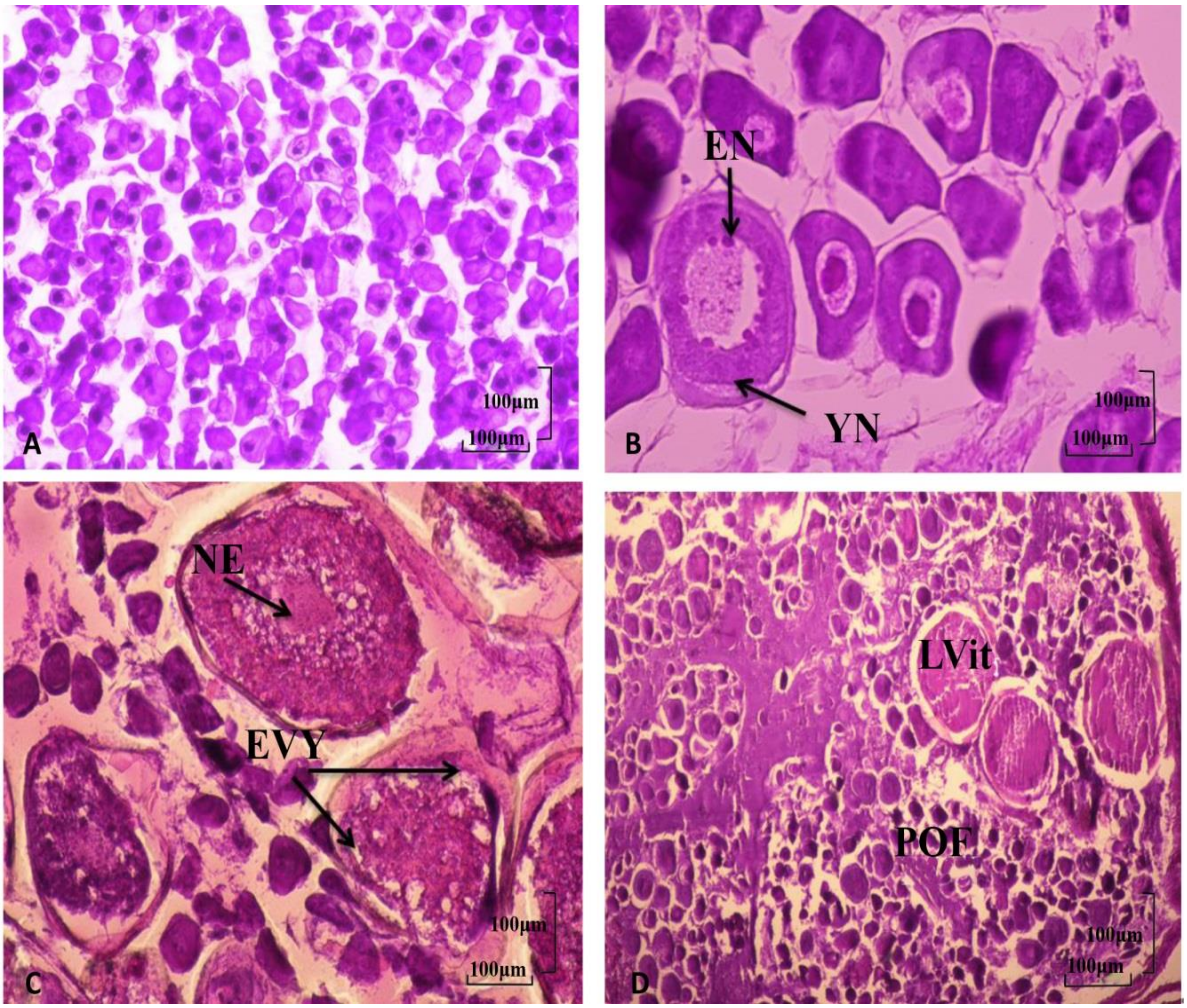

Figure 4. Photomicrographs of cross-sections of *P. stridens* ovaries at different developmental stages. (A) Immature (I) female, (B) developing female, (C) ripe (IV) female, (D) spent female (V).
EVY, extra vasicular yolk; EVit, early vitellogenic stage; LVit, late vitellogenic stage; POF, post-ovulatory follicle; N, nucleus; Nu, nucleolus.

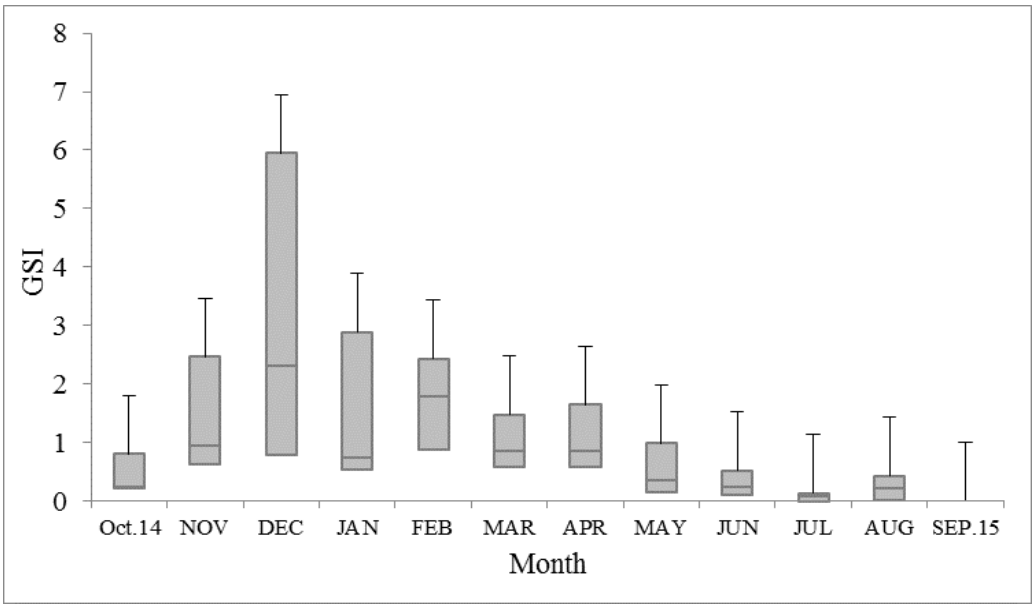

Figure 5. Monthly changes in Gonadosomatic indices (mean±SE) of female *P. stridens*

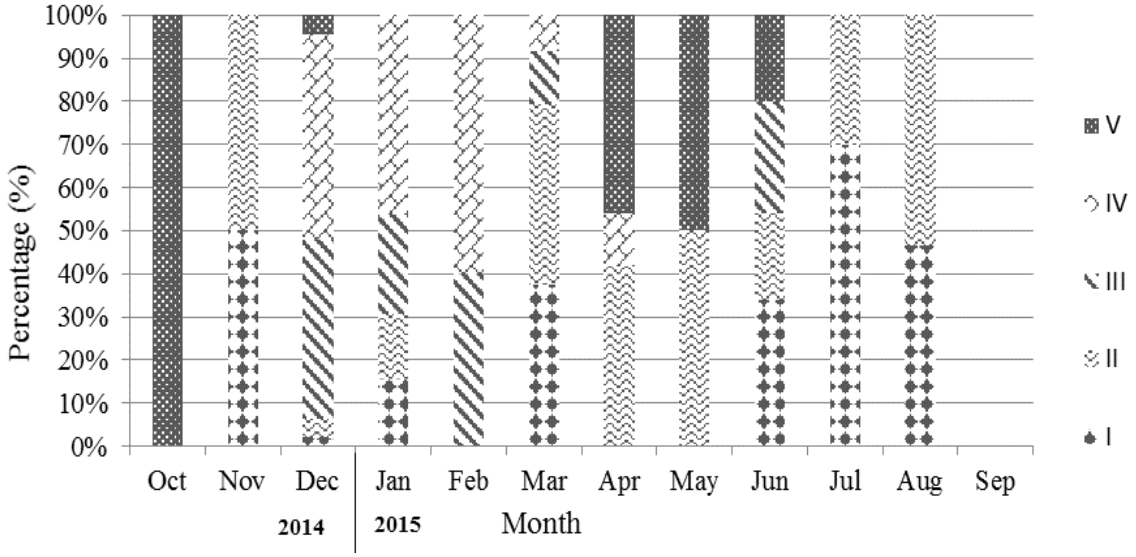

Figure 6. The occurrence of the maturity stages of *P. stridens* by month for females (2014-15).

Discussion

The present study is the first attempt to present detailed information about the reproductive biology of *P. stridens* in the northern coasts of the Persian Gulf (Bushehr province, Iran). The percentage of mature females was described by logistic curve, and the length sexual maturity of *P. stridens* (Lm50%) on the coastal waters of Bushehr province in the Persian Gulf was calculated to be 19.84 cm. According, Karimi *et al.* (2013) female *P. stridens* began to mature at lenght 12.6 to 23 cm and males 11.7 to 22 cm. The ratio of male to female *P. stridens* fishes was significantly different ($P<0.05$) from the expected ratio of 1:1 and population reveals female dominance in sex determination , the results of sex ratio is similar to the findings of Karimi *et al.* (2013). The results of the present study demonstrated the frequently of spawning during December-to-June which suggest these months as the birth-months of all striped piggy in the Persian Gulf. Moreover the result show that, *P. stridens* has a prolonged spawning season extending from December to June. According to Amtyaz, Atiqullah Khan, Zaheer, Usman Ali and Hashmi (2013), the majority of the *P. stridens* fishes spawn during the late summer, i.e. the September-to-February period, in the Karachi Coast, Pakistan. Karimi *et al.* (2013) found that *P. strident*s fishes spawn from December to March in the Persian Gulf waters. High gonadosomatic indices were recorded for *Pomadasys jubelini* in the period of July to September (Adebiye, 2013). According to Fehri-Bedoui and Gharbi (2008), Bastard grunt *Pomadasys incisus* had high gonadosomatic indices which were observed in the period of July to September. Al-Ogaily and Hussain (1990) found high GSI for trout sweet lip grunt, *Plectorhynchus pictus*, from March to May, which is not in conformity with the results of present study. According to Abu-Hakima study (1984) mean GSI of female silver grunt (*Pomadasys argenteus*), was highest in March, with an additional small peak in October. The study suggested spawning months of *Pomadasys argenteus* as February, April, and October. The results of present study suggest longer spawning period for *P. stridens,* (from January to June) showing a spawning period longer than six months. Diversity in the spawning season and periodicity exists due to the varied ecological environments (Agarwal, 2008).

Ben-Tuvia and McKay (1986) found that striped piggy had a distinct pairing during breeding with one clear seasonal peak per year, being in agreement with the result of the present study.

The cytological events associated with oogenesis observed in this research were similar to those of other studies on grunts (Falahati Marvast, Poorbagher, & Lokman., 2011; Falahati marvast, Vazirizadeh, & Fakhri, 2008; Valinasab *et al.*, 2007; Abu-Hakima, 1984). In the present study, it was observed that during different stages of gonadal maturation and development of oocytes, as a result of an increase in weight ovaries increased in volume. However, *P. stridens* has batch spawning during the spawning, and the ovaries have an asynchrony pattern. It appears that the increase in weight and ovarian volume is related to the vitellogenic stages in the first oocyte development (Branco, Viana, Félix, Véras, & Hazin, 2013). The cortical alveoli stage was observed in the ovaries of *P. stridens* at the end of the previtellogenic stage; nevertheless, this structure in other species may appear only in the late previtellogenic stage (Cardenas, Chávez, González, Espinosa, & Jiménez-garcía, 2008) or only in later stages of the oocyte development (Tricas and Hiramoto, 1989). The

vitellogenic stage of *P. stridens*, as in the majority of teleosts, was marked by a significant increase in the oocyte sizes, mainly by the inclusion of lipid droplets in cell cytoplasm, a process that begins in the previous stage (II, III and IV) by the exogenous accumulation of proteins **(**Branco *et al.*, 2013). The final oocyte development stage was identified in the present work and was characterized by mature oocytes. The presence of mature oocytes fully hydrated was registered similar to what is usually observed in marine fish, which was also reported by Robillard, Reiss, and Jones (2008) and Branco *et al.* (2013). In the majority of teleosts, the mature oocyte development stage is featured by a rapid increase in follicular volume, depending on the hydration and accumulation of macromolecules (Murua and Saborido-Rey, 2003). Atretic follicles observed during the oocyte development of *P. stridens* were more frequent in ovaries in the months of October and April to June. These atretic follicles were close to the final maturation or already matured.

In conclusion, this study indicated that striped piggy has a distinct annual reproductive cycle and a prolonged spawning season and that the females are the predominant sex in the population. The frequency percentage of different developmental oocyte stages within oocyte cluster verifies that the *P. stridens* is a batch spawner fishes with asynchronous oocyte development. The identification and characterization of different oocyte development stages and ovarian maturation found in this survey play an extremely significant role in designing future studies related to the reproductive cycle of other species of Grunts.

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