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Reproductive biology of the spotted guitarfish *Rhinobatos punctifer* from the Gulf of Suez, Egypt.

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ABSTRACT

Guitarfishes are an important component of the artisanal elasmobranch fisheries in the Gulf of Suez and are commonly caught as trawl fishery bycatch throughout the Gulf. However, no studies have been undertaken to investigate the life history of the species. To address this lack of critical biological information, the reproductive biology of the spotted guitarfish *Rhinobatos punctifer* was investigated in the Gulf of Suez. 320 specimens were collected from the trawling fishing gear. Development of claspers, testes, ovaries and uterus width indicated that males and females reach sexual maturity at 87.5 cm total length. R. punctifer is an aplacental viviparous species, with each female having two ovaries and two uteri, both functional. Ripe oocytes in the ovaries, ova, embryos and fully developed fetuses in the uteri are symmetrically distributed. The gestation period could last for a maximum of ten months. Ovarian egg size and male gonadosomatic index both peaked in February and March, indicating that mating occurs in winter. Uterine eggs were present from October through February. Females with full-term embryos were observed from November to February. Ovarian fecundity ranged from 79 to 1898 (mean 19) eggs per fish, whereas uterine fecundity ranged from 2 to 10 (mean 6) embryos per fish. The average size at birth was 19.4 cm TL. The estimated overall sex ratio was 1.2:1 male to female.

INTRODUCTION

The spotted guitarfish *Rhinobatos punctifer* is the most abundant ray species in the Gulf of Suez, accounting for more than 95% of the total ray's biomass. However, little is known about the life history of this species despite its common occurrence in the bycatch of the trawl and artisanal fisheries of the Gulf of Suez. The Spotted Guitarfish is endemic to the Arabian Seas region, occurring from the northern Red Sea, including Sudan, Egypt and possibly Saudi Arabia, to the Sea of Oman and the Arabian Gulf (**Bonfil and Abdallah 2004; Last *et al.*, 2016**). The species has been frequently misidentified in the literature, sometimes confused with the Bengal Guitarfish (*R. annandalei*) (**Moore *et al.*, 2012**). This species inhabits the inshore continental shelf to depths of 70 m, reproduction is viviparous and the species reaches at least 90 cm total length (**Weigmann 2016**). The International Union for

Conservation of Nature (IUCN) Red List of Threatened Species recently assessed the spotted guitarfish *Rhinobatos punctifer* as near threatened species (**Ebert *et al*., 2017**).

As a group, elasmobranchs can be classified as equilibrium (K-selected) strategists and typically share the biological characteristics of low fecundity, late maturity, high juvenile survivorship, slow body growth, and long life span (**Hoenig and Gruber 1990; Winemiller and Rose 1992; Camhi *et al*., 1998; King and McFarlane 2003**). Equilibrium strategists tend to have slow rates of population growth and generally cannot sustain high levels of fishing pressure (**Holden 1973; Adams 1980; Hoenig and Gruber 1990**). Elasmobranchs typically have life history characteristics that make them susceptible to overfishing.

Fisheries in the Red Sea are primarily artisanal, although industrial fleets (trawlers and purse seines) also operates in the Gulf of Suez and Foul Bay. Sharks and rays are usually caught by gillnets, hooks and lines and longlines. They also retained as incidental catch in trawlers and sometimes in purse-seiners. Most of the fisheries resources in the Red Sea and Gulf of Suez are suffering from overexploitation (**El Ganainy, 2017**). Many studies highlight that increased fishing intensity and technological advancement of fishing gear has resulted in a decline in many chondrichthyan species captured by various gears (**Bonfil 2003, Henderson *et al.* 2004, Spaet and Berumen 2015, Mohamed and Veena 2016**).

Biological studies on *R. punctifer* is very rare, only **Henderson et al. (2004)** reported the maturation size of the species from Omani waters. Most of the literatures focused on the taxonomy and identification of the species (**Compagno &and Randall, 1987; Golani and Bogorodsky, 2010; Bineesh *et al*., 2017**)

The current study is the first to investigate the reproductive biology of the spotted guitarfish *Rhinobatos punctifer* as an essential component of effective stock assessment and management in the Gulf of Suez. The main objectives are to focus on spawning, maturity, and fecundity of the species.

MATERIALS AND METHODS

Samples of The guitarfish *Rhinobatos punctifer* were collected monthly from the commercial trawlers landed at Attaka fishing harbor in the Gulf of Suez (Fig 1). A total of 320 specimens were collected, where total length (TL) was measured as the distance from the tip of the snout to the end of the tail, and disc width (DW) was measured as the distance from wingtip to wingtip. Total weight (TW) was recorded. For males, Inner clasper length (ICL) was measured from the clasper tip to the point of insertion, outer clasper length (OCL) was measured from the clasper tip to the notch created by the pelvic fin, and testis were weighed (Fig 2). Claspers were classified into flexible, semi rigid or rigid for maturity assessment. For females, the weight of ovary, uterus and the largest egg were recorded. In case of presence of embryos their weight, length and number were also recorded.

Fig. 1. The sampling locations in the Gulf of Suez

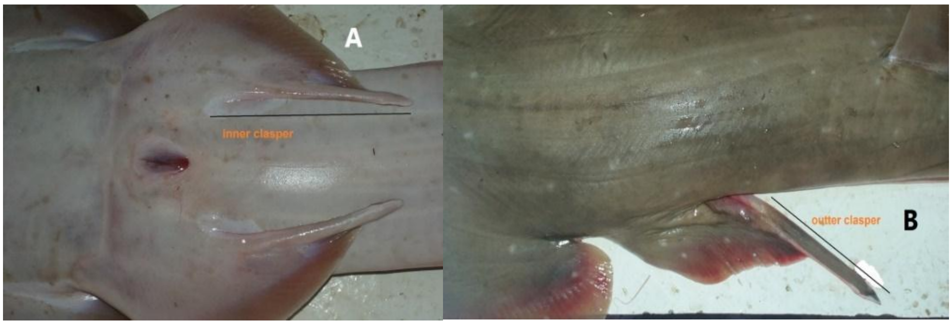

Fig. 2. Measurement for inner clasper (A) and outer clasper (B) length

The sex ratio was calculated monthly for male and female samples, it was determined as the percentage of males to females (M: F), this percentage was also calculated according to the total fish length.

Gonado somatic index (GSI) is the method of expressing the variation of the condition of gonads in the different periods of the year and it is the application of what is generally referred to gonad index (**Batts, 1972**), Such parameters considered as the percentage of weight of the gonad whether testes or ovaries in the total fish weight.

G.S.I. = gw / GW *100

Where, gw is the gonad weight and GW is the gutted weight of the fish.

Maturity stages were determined, based on descriptions by **Zeiner and Wolf (1993)** with some modifications and summarized in Table (I) and Figure (3) for male and Table (2) and Figure (4) for females.

Table 1. Description of the maturity stages for males

Maturity Stage	Description
Immature (I)	Undifferentiated testis. Claspers were flexible and shorter than the pelvic fin.
Maturing (II)	Testis started to enlarge and differentiate. Claspers were semi rigid and as tall as the pelvic fin.
Mature (III)	Testis was fully developed and reproductively functional. Claspers were fully rigid and taller than the pelvic fin.

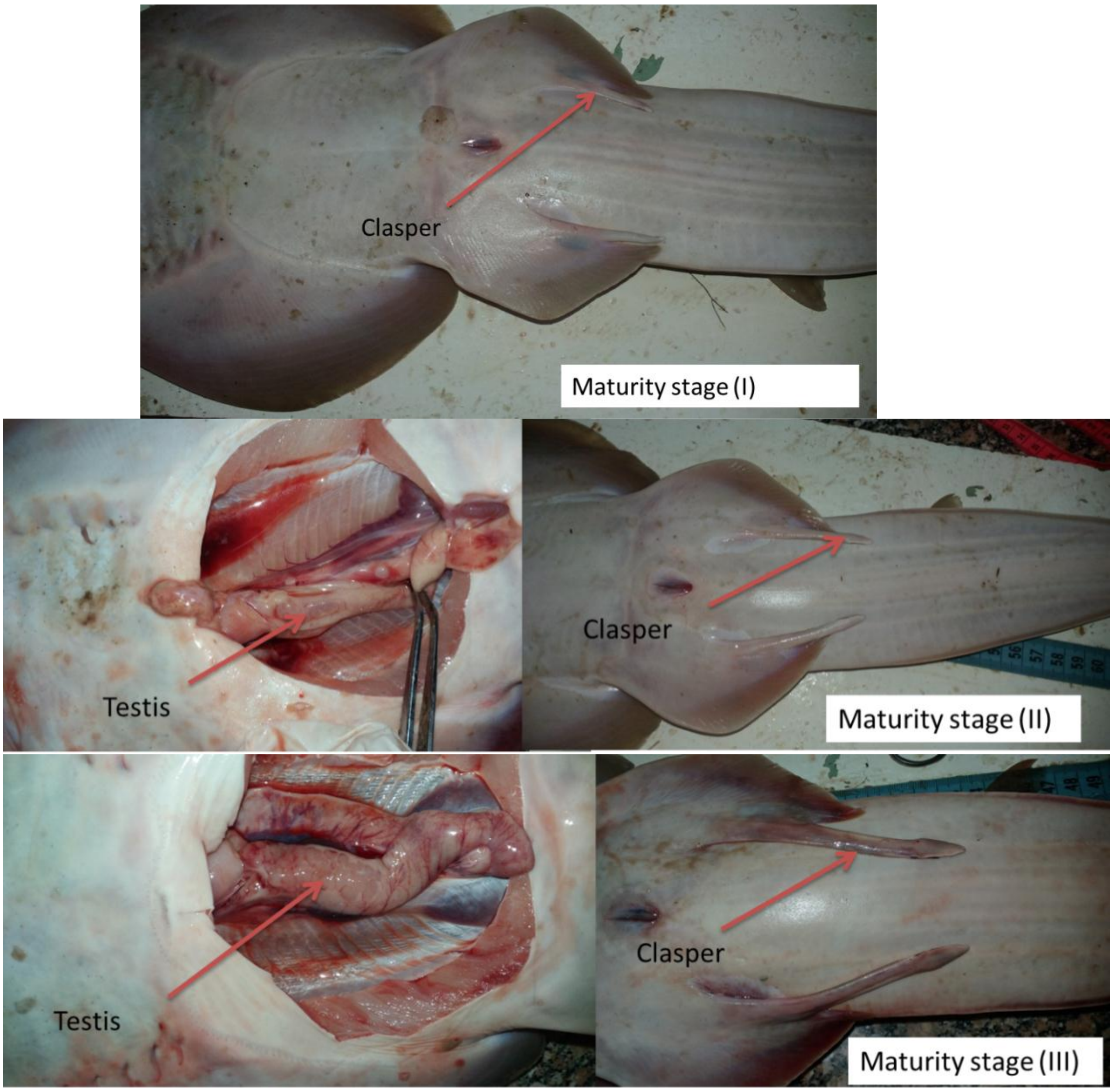

Fig. 3. Different maturity stages of males *Rhinobatos punctifer*

Table 2. Description of the maturity stages for females

Maturity stage	Description
Immature (I)	Undifferentiated ovary.
Maturing (II)	Ovary started to differentiate but still had no egg. Uterus wasn′t present.
Mature (III)	Ovary was fully developed with egg. Uterus was present; its size was ranging from small to large. Embryos were present.
Spent (IV)	Ovary was fully developed with egg. Uterus was present, but they were empty after delivering. Embryos weren′t present.

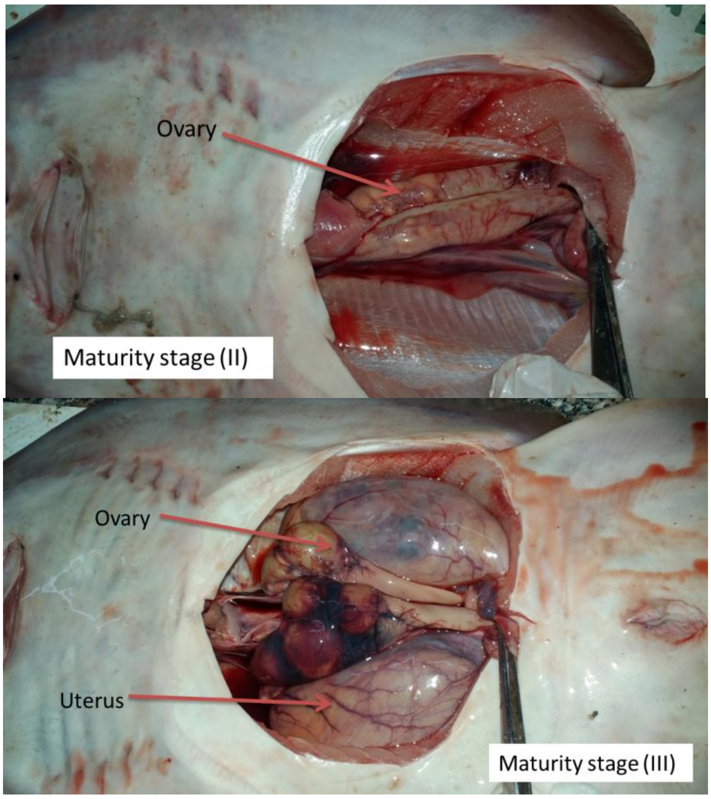

Fig. 4. Maturity stages of females

For estimating the length at first maturity a logistic curve was fitted to the relationship between the proportion of mature males or females as a function of TL: $P_{TL}=1/(1-e^{(a+b*TL)})$, where P_{TL} is the fraction of mature individuals in length class TL, and *a* and *b* are model parameters. In this model, median size at maturity, TL_{50}, is given by *-a/b*, which represents the body size at which 50% of the population was mature (**Restrepo and Watson 1991**).

The fecundity of females was calculated by counting the number of ova found in the ovary and the number of embryos found in the uterus per month.

The clasper length was used to construct and detect many relations, to convert field measurements, the relationship between total length (TL) and inner clasper length (ICL) and the relationship between inner clasper length (ICL) and outer clasper length (OCL) were conducted.

RESULTS

Sex ratio

The estimated overall sex ratio was 1.1: 1 male to female. For the total 237 specimens 179 individuals of them were males and 158 individuals were females (Figure 5). The sex ratio in relation to mid length showed that females dominated in small length interval 45 cm and large length intervals from 100 to 110 cm while, the males predominated in length intervals from 60 to 85 cm (Figure 6). The chi-square value was 0.253 ($P<.05$).

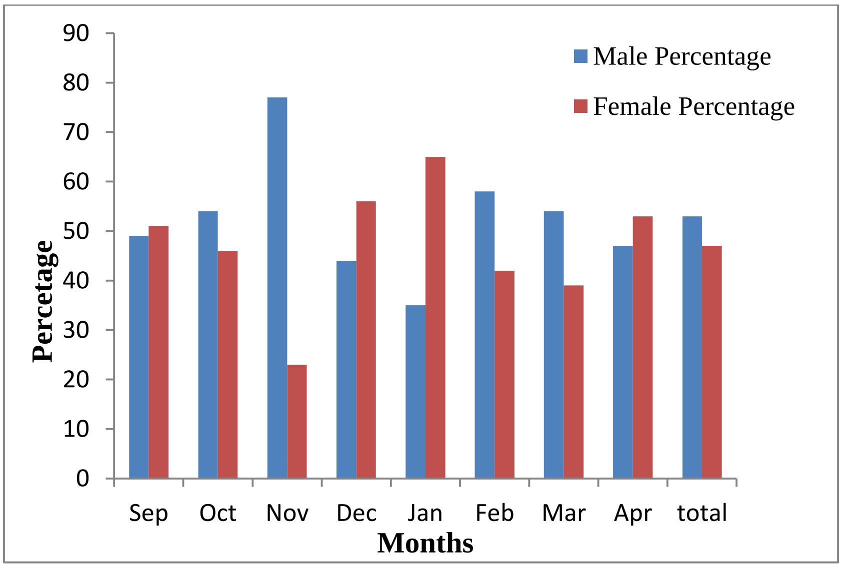

Fig. 5. Monthly sex ratio for male and female *R. punctifer* collected from the Gulf of Suez

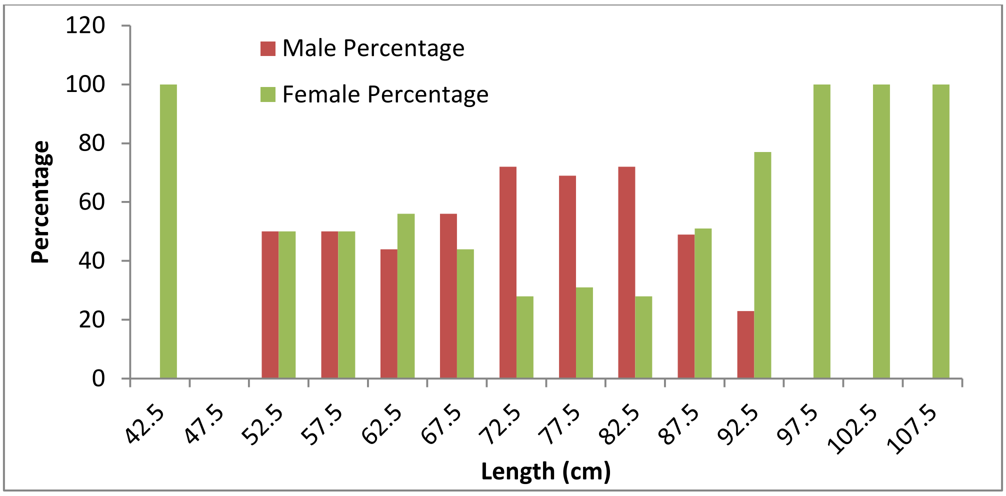

Fig. 6. Sex ratio for male and female *R. punctifer* in relation to mid length.

Clasper Length Relations

In the relation between Total Length (TL) and Inner Clasper Length (ICL) of males *R. punctifer* (n= 179) (Figure 7), the increase in clasper length with respect to total length, begin around 70 cm TL, indicated the onset of maturation.
The regression of outer clasper length on inner clasper length (Figure 8) resulted in the following equation, with an r^2 of 0.885: OCL = 0.6906 (ICL) + 0.2973.

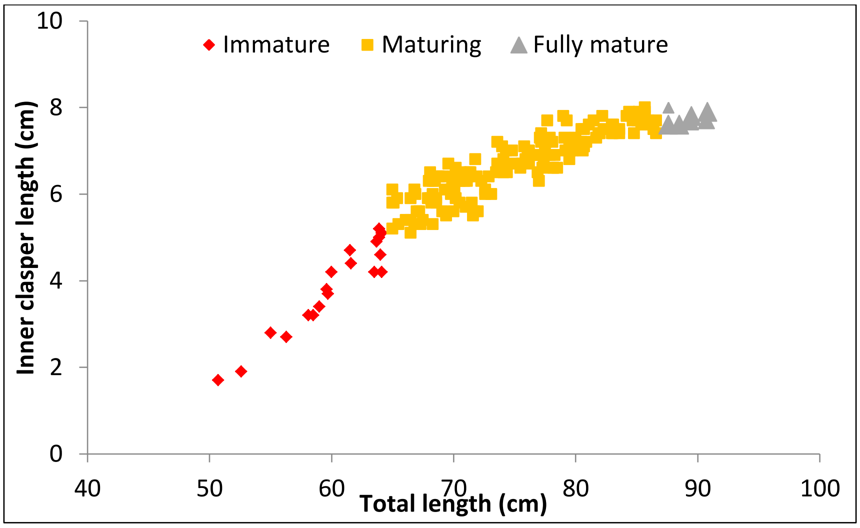

Fig. 7. The relation between total length and inner clasper length

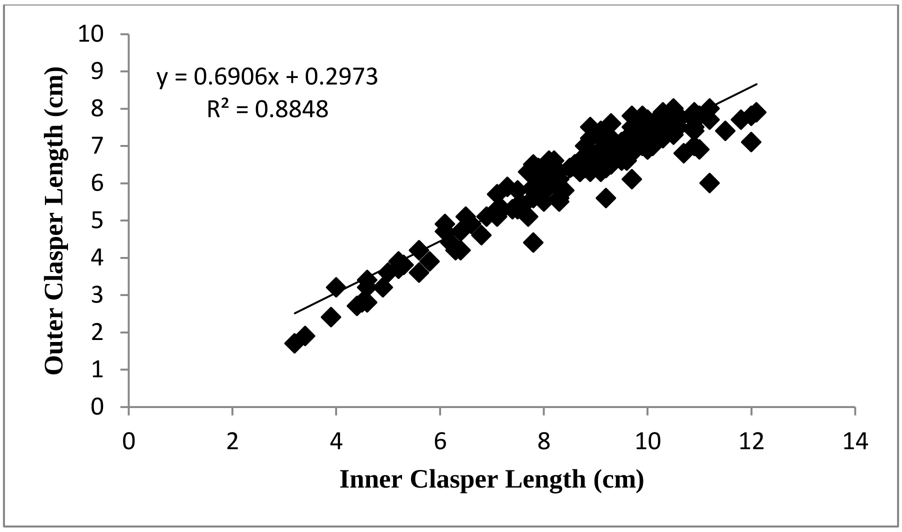

Fig. 8. The regression of outer clasper length on inner clasper length

Maturity stages

Maturity stages were recorded monthly for male and female samples. For males, the three maturity stages were found around the sample collection period with high occurrence of maturity stage III from December to April (Fig. 9). For female individuals the maturity stage IV was encountered from December to April (absence in March due to lack of data) while, maturity stage III was represented during the whole fishing season and dominated the catch in January with complete absence of maturity stage I in this month (Fig. 10).

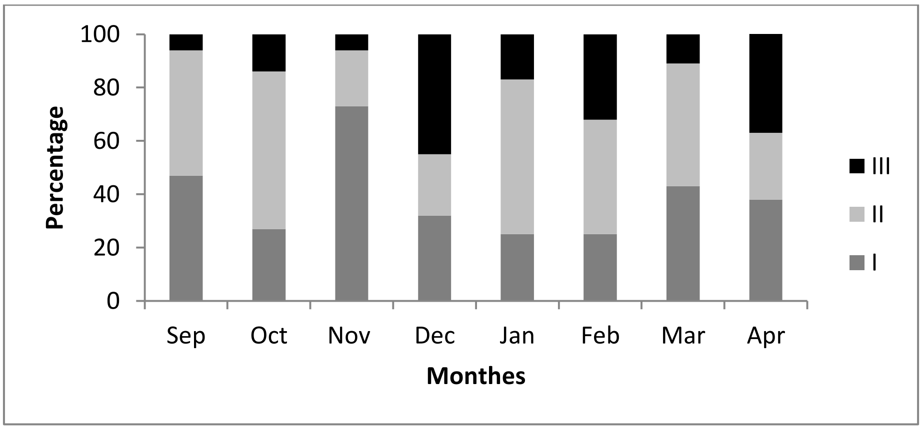

Fig. 9. Monthly distribution of maturity stages for male *R. punctifer* from the Gulf of Suez.

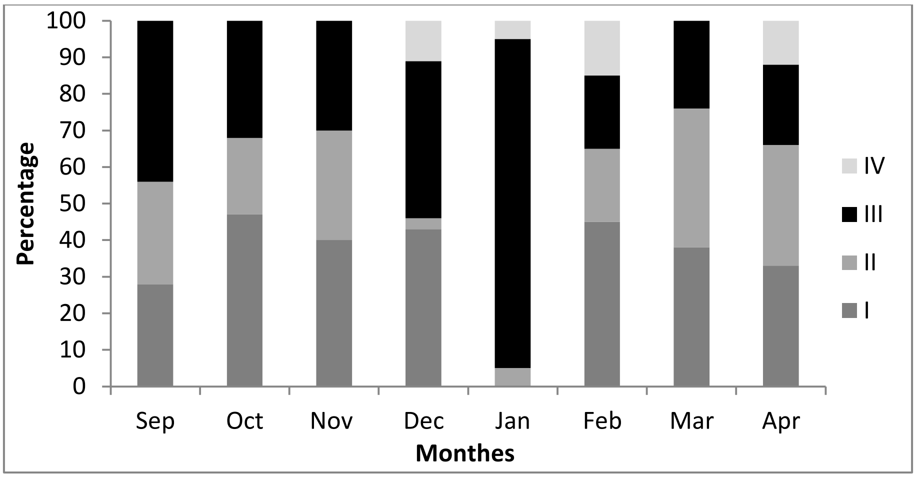

Fig. 10. Monthly distribution of maturity stages for female *R. punctifer* from the Gulf of Suez.

Gonado Somatic Index (GSI)

The analysis of gonado – somatic index (GSI) showed a gradual increase og GSI values from November to February, and the highest values of GSI for males and females occurred in January and February, indicating that the reproduction occurs from November to March with a distinct beak in January-February (Fig. 11).

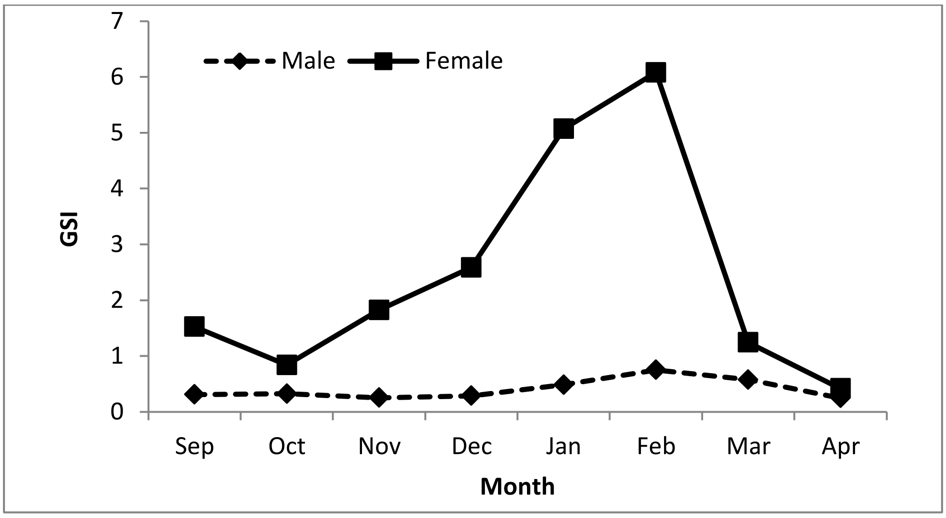

Fig. 11. Monthly values of GSI for male and female *R. punctifer* collected from the Gulf of Suez

The length at first sexual maturity

The percentage of maturity in male and female samples increased with increase in length. The maturity started in male samples at length 72.5 cm while in female samples started at 82.5 cm. the length at which 50% of males were mature was 86 cm. while that of females was 87.5 cm. (Fig. 12).

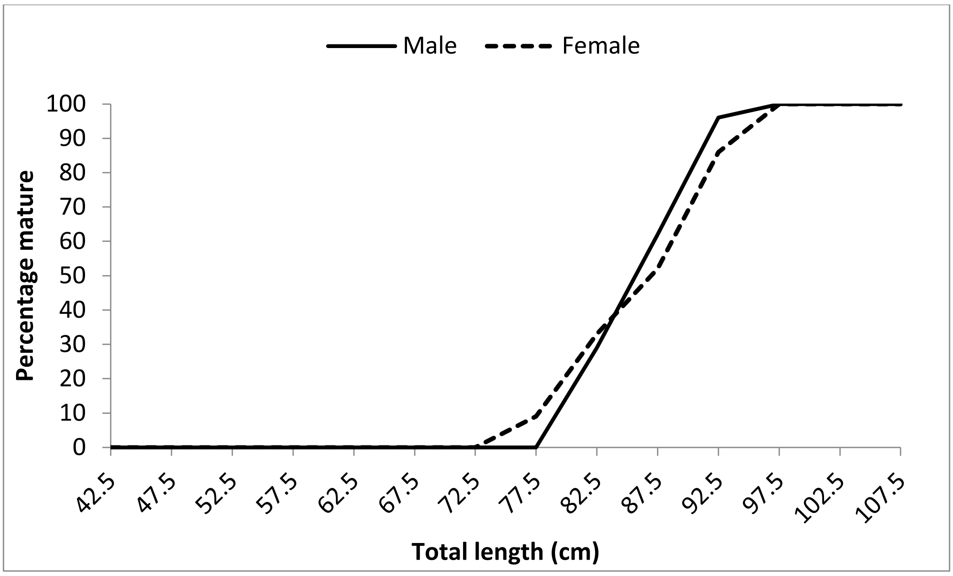

Fig. 12. Length at first sexual maturity for males and females of *R. punctifer*

Fecundity

The fecundity was calculated by counting the number of eggs found in the ovaries and the number of embryos found in the uterus monthly. The results showed that, during sample collection period there were 3000 egg in ovaries with highest occurrence in January by 1898 egg and lowest occurrence was in April by 78 eggs while there were 85 embryos in uterus with highest occurrence in January by 30 embryos. Female samples littered of 8 - 10 pups. For the relation between number of eggs and total length the results showed that the highest number of eggs was 145 at mid length 87.5 cm (Table 3 and Fig. 13).

Table 3. The number of eggs and embryos per month

Month	No. of eggs	No. of embryos
Sep	241	0
Oct	140	0
Nov	79	9
Dec	255	28
Jan	1898	30
Feb	98	18
Mar	211	0
Apr	78	0

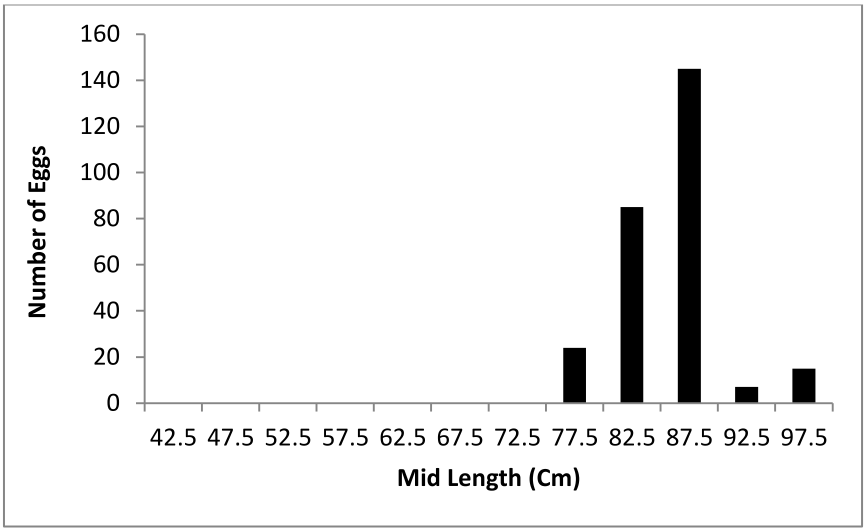

Fig. 13. The number of eggs per mid length

DISCUSSION

As there are neither known studies on the reproductive biology of the spotted guitarfish *R. punctifer* in the Red Sea nor worldwide all the results of this study will be compared to the closest relatives from the same family (Rhinobatidae).

The sex of *R. punctifer* was always determined from external characters where males possessed a pair of claspers which were visible from an early stage of development on the inside edge of pelvic fins. The females did not have claspers. The sex ratio between males and females for overall samples was 1.1: 1 with no significant difference between both sexes as chi square was 0.253. This result agreed with **Çek *et al.*, (2009)** for the common guitarfish *Rhinobatos rhinobatos* from İskenderun Bay (Turkey, Eastern Mediterranean).

Analysis of GSI demonstrated that *R. punctifer* is a placental viviparous species exhibit partially defined annual cycle with one peak in February during the year. This is supported by the fact that all maturity stages of sexual development are not represented throughout of the year by the same percentage. Additionally, a reproductive resting phase (e.g. 'spent' gonads) was observed in large females and wasn′t observed in large males. This strategy of reproduction also occurred in *Rhinobatos cemiculus* (**Capapé and Zaouali, 1994; Seck *et al.*, 2004**), *R. rhinobatos* (**Abdel-Aziz, *et al.*, 1993; Ismen *et al.*, 2007**), *R. productus* (**Timmons and Bray, 1997**), *R. hynnicephalus* (**Wenbin and Shuyuan, 1993**), In the present study, females *R. punctifer* acquired higher means of GSI than males because the size of female genital system was larger than that of testes at the same maturity stage,

Length at 50% maturity (L_{m50}) of *R. punctifer* from the Gulf of Suez occurred at approximately 52% of the observed maximum TL for males and at 61.5% for females. Length at 50% maturity was 87.5 cm for female and 86 cm for male samples. The first mature male was observed at length 77.5 cm while, the first mature female length was 82.5 cm. **Henderson et al., (2004)** indicated that females *R. punctifer* taken during autumn off Muscat (Omani waters) were all mature at less than 77 cm TL while males matured at less than 71 cm TL. **Cortés (2000)** noted that elasmobranchs generally mature at 75% of their maximum size, while **Frisk *et al*., (2001)** observed that dogfishes, skates and rays mature at 73% of their maximum size.

According to the present results, the male *R. punctifer* in the Gulf of Suez reach maturity at size less than that of female. These results agreed with the results of most of the authors who studied the reproductive biology of rays. **Timmons and Bray (1997)** for the shovelnose guitarfish *Rhinobatos productus* near Long Beach, California, analyzed the reproductive tracts and showed that female guitarfish matured at 99 cm. and the clasper length and width indicated that males matured at 90-100 cm. For the same species from the eastern Gulf of California, México, (**Márquez – Farías, 2007**) found that L_{50} for males is 53 cm while for females >57 cm. **Seck, *et al*., (2004)** for the blackchin guitarfish, *rhinobatos cemiculus*, from the Coast of Senegal (Eastern Tropical Atlantic), indicated that L_{50} for males is 1.55 m while for females 1.63 m. delayed onset of maturity in females, known as 'bimaturism', could be related to the need of females to carry the pups, and as such, to invest on growth in favour to reproduction **(Cortés 2000**). However, exceptions exist within the Rajidae family. For instance, **Ebert (2008) and Ruocco *et al*., (2006)** noted that in *Bathyraja* species, males and females attained maturity at the same size. A review of the literature on batoids suggests that it is also common for males or females to reach maturity at differing sizes **(Braccini and Chiaramonte 2002a, 2002b; Mabragana and Cousseau 2004; Frisk and Miller 2006, 2009; Ruocco, *et al*., 2006; Colonello, *et al*., 2007).** Selection for a larger female size in viviparous species may result in increased internal volume and a greater number of offspring.

R. punctifer is an aplacental viviparous species, with each female having two ovaries and two uteri, both functional. Ripe oocytes in the ovaries, ova, embryos and fully developed fetuses in the uteri are symmetrically distributed. The gestation period could last for a maximum of ten months. Uterine eggs were present from October through February. Females with full-term embryos were observed from November to February. Ovarian fecundity ranged from 79 to 1898 (mean 19) eggs per fish, whereas uterine fecundity ranged from 2 to 10 (mean 6) embryos per fish. Average size at birth was 19.4 cm TL. These results of *R. punctifer* agreed with the general description of the family Rhinobatidae as yolksac viviparous **(Musick and Ellis 2005)** with females produce 4 to 18 pups in a litter **(Villavicencio-Garayzar 1995; Dowton-Hoffman 1996)**. In *R. cemiculus* and *R. productus,* females possess two ovaries and two uteri, both functional ; the ovarian fecundity ranged from 20 – 39; the uterine fecundity ranged from 8 – 10; and average size at birth 24 – 17.5 cm respectively **(Seck, *et al*., 2004 ; and Márquez – Farías, 2007)**. Fecundity is still likely to be low, as is typical for most elasmobranchs.

CONCLUSION

Reproductive studies allow stock assessment biologists to create models for evaluating stocks and to predict the impact of fishing pressure. However, very little life history information has been published for any ray species in the indo Pacific. It is imperative that basic life history information be collected prior to the initiation of a fishery to form a baseline for management and prevent a possible stock collapse should a targeted fishery arise. The guitarfish *Rhinobatos punctifer* as a typical elasmobranch species is characterized by late maturity and low fecundity, so it needs a specific management plan to conserve this important resource and further life history parameters is essential for this purpose.

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