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REPRODUCTIVE BIOLOGY OF THE TROPICAL SEA CUCUMBER *HOLOTHURIA ATRA* (ECHINODERMATA: HOLOTHUROIDEA) IN THE RED SEA COAST OF EGYPT

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ABSTRACT

Although the echinoderms have been the subject of various investigations by many authors, those of the Red Sea species till now; have received little attention. Sea cucumbers (Holothuroidea) are marine animals that present in the group of echinoderms. They are distributed from the shore to the greatest depths. The importance of detailed reproductive biological studies on *Holothuria atra* are necessary due to its important role in the conservation of the marine environment. Samples of the present study was collected from three sites at north Hurghada City, Red Sea during the period from January to December 2003. Four maturity stages were assigned for each sex as immature, maturing, ripe and spent stages. From the monthly and size distribution of these maturity stages of both sexes, it was found that the ripe stage was observed most time of the year for *Holothuria atra* with high abundance in June and November for males and from June to December for females. The observed size at first sexual maturity was found at 16.5 cm and 15.5 cm total length for males and females, whereas the calculated size was 16.5 cm for females and 17.5 cm for males respectively. The maximum observed oocyte diameter was observed during the intensive period of spawning season for *Holothuria atra* being in the range from 175 to 200 (μm). Fecundity of *Holothuria atra* was found to be in the range from 12110 to 1342278 oocytes for individuals of size range from 21.0 to 25.0 cm total length. The relation between absolute fecundity and gonad weight is best fitted by a linear regression.

INTRODUCTION

Echinoderms are one of the most interesting organisms among all invertebrates. They are the most conspicous and characterized marine organisms. It comprises 5 classes, Asteroidea (sea stars), Ophiuroidea (Brittle stars), Crinoidea (sea feather), Echinoidea (sea urchins) and Holothuroidea (sea cucumbers). The sea cucumber are marine animals and are abundant group of motile macro-invertebrates. They can be found from very superficial waters to great depths. However, they are most common in the Indian Ocean and the Southwest Pacific (Indo-Pacific tropical region). At present, nearly 1400 species of sea cucumbers are known from the seas in the world (James, 2001).

Holothuroid fauna of the Red Sea attracted attention of many investigators for many years ago. Identification of species and species lists had been made by some authors, although taxonomy of holothuroids was faced by many difficulties. The various habitats of the Red sea from Cherbonnier (1955) till Hassan (2001) recorded 98 holothuroid species. However, our knowledge of the general biology and ecology of holothurians is still incomplete in the Red Sea area.

The importance of detailed studies on Holothuroids are necessary due to their

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important role in the conservation of the marine environment. They are economically of important value and used as food, also they are important members of the benthic communities, and responsible for causing significant changes in the composition of the sea floor. They have also medical importance include treating weakness, impotence, debility of the aged, constipation due to intestinal dryness, and frequent urination.

Severe over fishing of sea cucumber has occurred in most countries of the world. Even though they were abundant along the Red Sea coast of Egypt in the mid 1990's, sea cucumber populations are now significantly reduced and some species have almost disappeared. Howaida *et al.* (2004) *Holothuria atra* Jaeger is widely distributed in the Indo West Pacific (Clark and Rowe 1971) and is the most common aspidochirotid holothurian on tropical reef flats (Conand and De Ridder 1990).

Few studies have been carried on the reproductive biology of *Holothuria atra* (Hyman 1955; Conand, 1990, 1993; Smiley *et al.* 1991, Chae 1993; Chang and Lee 1986, 1987; Harriott, 1982, 1985; Chao *et al.* 1994; Conand and Deridder 1990).

The goals of the present study are: to describe gonad development in *Holothuria atra* in the Red Sea coast of Egypt and determine its spawning season and Fecundity. These information are important for the sustainable management of the fishery and aquaculture of this species.

MATERIALS AND METHODS

For this study samples of *Holothuria atra* were collected monthly during the period January 2003-December 2003) from three sites at north Hurghada city Red Sea as shown in Map (1). A total number of 60 males and 48 females were collected.

All measurements were done after relaxation of sea cucumber in 2.5% $MgCl_2$ (w/v) in a plastic container. This was found to overcome the error resulting from contraction and relaxation of the animal's body. The same method was applied and proved to be efficient by Sewell (1994) during her study on *leptosymapta clarki*.

The specimens were dissected to obtain gonad weight, gutted weight (body wall of the animal after removing all viscera). The gonads either male or female were fixed to 7% buffered formalin then rinsed in tap water and stored in 70% ethanol according to (Ramofafia *et al.* 2000).

Macro and microscopic features of the gonads was used to assess maturity stages. Gonad color, thickness size, shape, the length and diameter of tubules to determine the different maturity stages. Based on these criteria, four stages of maturity were determined for both sexes of studied species as follows:

Immature stage (I), maturing stage (II), ripe stage (III) and spent stage (IV). Each gonad was examined and assigned to one of these stages. In ripe female of *Holothuria atra* a diameter of 50 oocytes were measured by using eyepiece micrometer.

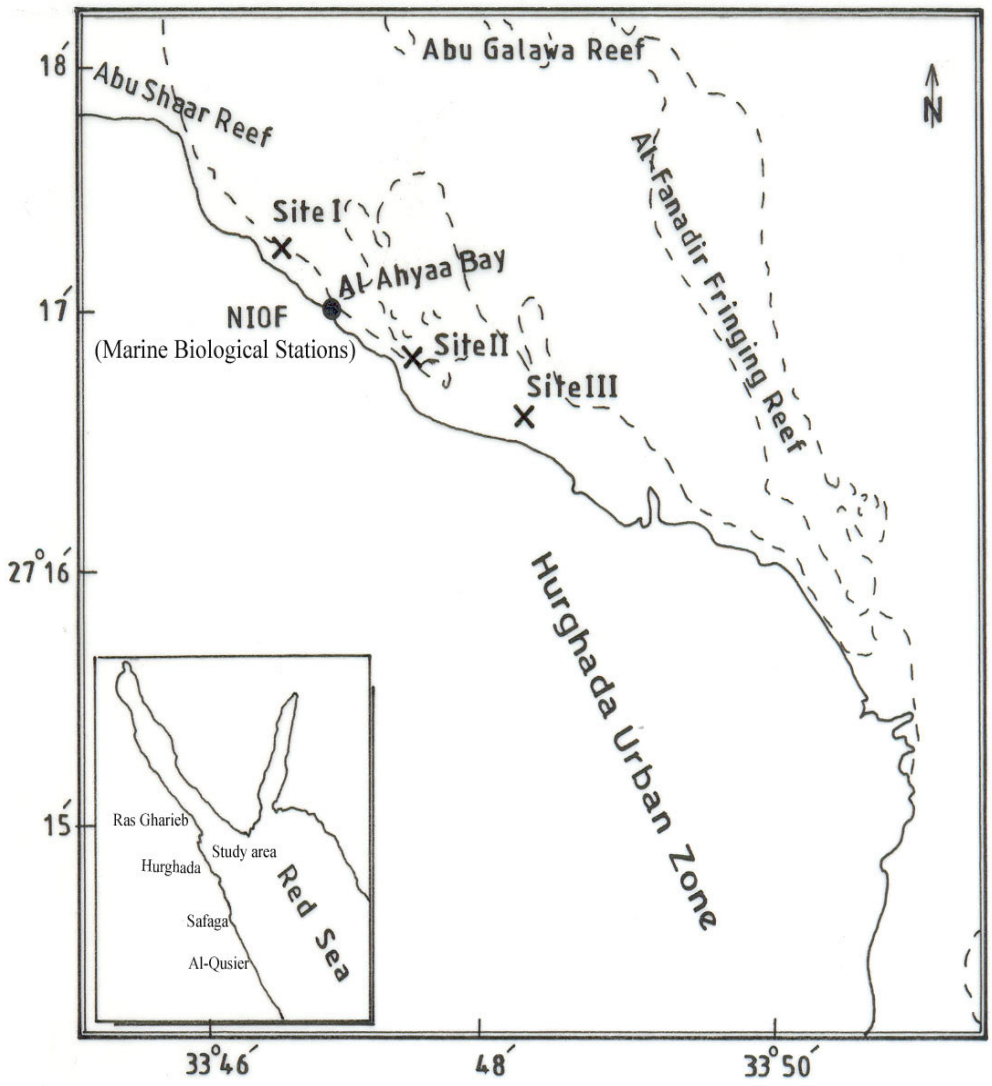
The gonado-somatic index (G.S.I) has been calculated monthly for either male and female of *Holothuria atra* using the following formula:

G.S.I= [weight of gonad (gm)/Gutted weight (gm)] x 100

Fecundity of *Holothuria atra* was studied by using three subsamples of ripe ovary, weighing approximately 0.1 gm. The samples were taken from the median part of preserved tubule for counting the ripe oocytes. Before counting them rinsing was done to remove fragments of ovary wall and oocyte clusters were separated with a pair of tweezers. The numbers of the oocytes in the subsamples are taken as X_1, X_2, X_3.

The average number of oocytes in the three subsamples in ($\bar{X}$). The absolute fecundity (F) was calculated according to the following formula:

F= [total weight of the preserved gonad x average no of oocytes in subsamples]/ weight of the subsample.



Map 1: Map of the study area.

RESULTS

Sexes are separate in *Holothuria atra* and it is not possible to differentiate the males and females externally. Gonads are single, consisting of numerous filamentous tubules united basely into one tuft attached to the left side of the dorsal mesentery and hangs freely in the coelom. The tubules are elongated and branched. In advanced stages of maturity the filaments of the ovary have red color, in which the oocytes are visible as small as white spots. The tests consist of long creamy white beaded filaments.

1- Maturity stages of *Holothuria atra* was studied in both sites according to the gross morphology and microscopic examination of the gonads, as immature (I), maturing (II), ripe (III) and spent (IV) stages.

a. Male maturity stages:

Immature stage (I): The morphology and micropscopic features of immature gonads of male *Holothuria atra* as showing Fig. (1A). the gonad was characterized by shorter, thinner, branched and yellow-white color tubules. The tubule length ranging from 6.5-17 mm with a diameter range from 0.4 to 0.6 mm, G.S.I. ranging from 1.2 to 2.7 according to gutted weight.

Maturing stage (II): As shown in Fig. (1B), maturing gonad of male *Holothuria atra* was characterized by long, thin, branched and creamy-white color tubules. The tubule length ranging from 14.0 to 47.0 mm with a diameter from 0.4 to 1.2 mm, G.S.I. was ranging from 1.0-29.4.

Ripe stage (III); Ripe gonad of male *Holothuria atra* as Fig. (1C) were characterized by maximum volum tubules long white beaded filaments, swelling and sperm may be present in gonoduct, tubule length ranging from 33-60 mm with diameter from 1-1 to 2-5 mm, G.S.I. ranging from 7.8 to 27.2, with numerous spermatozoa swimming from a tubule section.

Spent stage (IV); As in Fig. (1D) spent gonads of male *Holothuria atra* showed an empty but have residual, branched, white color tubules, transparent, tubule length ranging from 5.0 to 36 mm with diameter from 0.2 to 0.9 mm, G.S.I. ranging from 0.3 to 6.2, some spermatozoa are remaining.

b. Female maturity stages

Immature stage (I), As in Fig. (2E) the female immature gonads *Holothuria atra* were characterized by thin tubules, short, branched and yellow-white color. Tubule length ranging from 4.0 to 12.0 mm with diameter from 0.2 to 1.0 mm. G.S.I. ranging from 2.2 to 7.7 according to gutted weight.

Maturing stage (II), According to Fig. (2F), this stage in *Holothuria atra* was characterized by long, thick, branched and yellow color tubules. Tubule length ranging from 21.0 to 51.0 mm. with diameter from 0.7 to 2.0 mm. G.S.I. ranging from 4.1 to 14.3, opaque spherical oocytes were observed.

Ripe stage (III), This stage as shown in Fig. (2G) was characterized long, thick, branched and more red color. Tubule length ranging from 24 to 130 mm with diameter from 1.0 to 2.5 mm. G.S.I. ranging from 9.8 to 13.7, spherical oocytes with diameter ranging from 75 to 175 microns, clearly visible nucleus was observed.

Spent stage (V), As in Fig. (2H) this stage characterized by empty, branched and some tubules more limp and yellow in color, some oocytes are remaining. Tubules length ranging from 20 to 24 mm with diameter from 0.4 to 1.0 mm, G.S.I, ranging from 1.7- to 8.4.

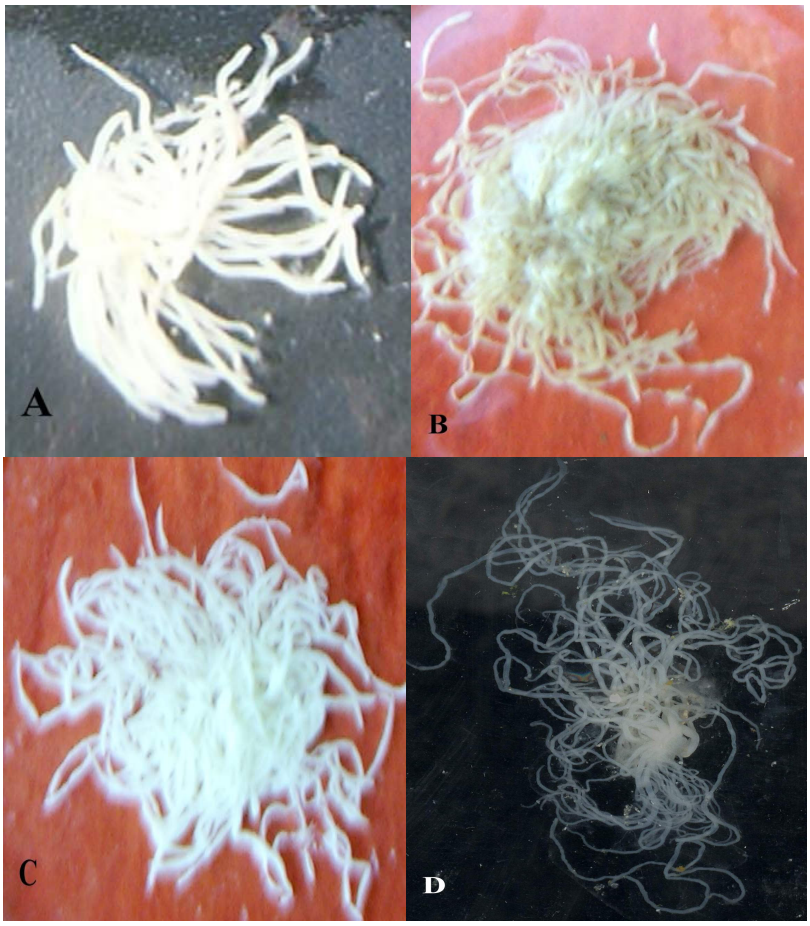


Fig 1: Macroscopic features of Holothuroidea male gonads (*Holothuria atra)*

A: Stage I
B: Stage II
C: Stage III
D: Stage IV

Fig. 2: Macroscopic features of Holothuroidea female gonads (*Holothuria atra)*

E: Stage I
F: Stage II
G: Stage III
H: Stage IV

c. Monthly distribution of different maturity stages

Fig. (3) describe the percentage distribution of different maturity stages of male *Holothuria atra* during the period of study. Ripe stage (III) was observed most time of the year with highly abundance in June and November, while the immature individuals were observed between January and March, followed by maturing individuals between April and May. Spent stage (IV) was observed between October and December.

On the other hand, Fig. (4) showed the monthly distribution of female maturity stages of the studied species. Most of the ripe females were observed between June and December followed by spent stage during October and December.

The immature stage in females was found between January and March and maturing stage between April and May.

d. Size distribution of different maturity stages:

Fig (5) illustrate the size distribution of different maturity stages of male *Holothuria atra* in area of study.

The small size individuals of size range from 9.5 to 17.5 cm were immature while maturing stage was represented with individuals of size range from 18.5 to 21.5 cm. Ripe stage individuals were with a size range from 16.5 to 24.5 cm and spent stage of a size between 23.5 to 27.5 cm.

In case of females *Holothuria atra* as in Fig. (6) most ripe individuals were observed between size 15.5 cm and 28.5 cm and usually followed by spent stage individuals of size from 19.5 to 27.5 cm. Immature stage mostly with small individuals of size range from 13.5 to 17.5 cm while maturing stage with a range from 17.5 to 22.5 cm.

2- Size at first sexual maturity

The size at first sexual maturity can be determined for each sex by estimating the percentage frequency of ripe males and females of *Holothuria atra* in one year samples and plotting the average total length against the percentage of ripe individuals.

The observed size at first sexual maturity of *Holothuria atra* during the period of study was 16.5 cm for males and 15.5 cm for females, while the Fig. (7) show the calculated size at first sexual maturity for both sexes as 16.5 cm for females and 17.5 cm for males.

3- Gonado-somatic index

Monthly distribution of gonad index (G.S.I) for males of *Holothuria atra* as represented in Fig. (8) showed an increase from February to April with a peak in May and June, then decreased after that. While in females as in Fig. (9) the (G.S.I) increased gradually from February to May with a peak in June followed by another peak from August to October and than decreased in November, December. Which indicate that ripe females of *Holothuria atra* were found all the year round with a distinct period of activity during June and August – September.

4- Monthly distribution of oocyte diameter

The size frequency distribution of oocyte diameter of *Holothuria atra* during the spawning season as in Fig. (10) showed that the average oocyte diameter in June was of a range from 112.5 to 187.5 (μm), the maximum oocyte diameters were observed in June and July as 137.5 (μm). From August to December the average oocyte diameter ranges from 112.5 to 187.5 (μm). The maximum observed oocyte diameter during the intensive period of spawning for *Holothuria atra* was 175-200 (μm).

5- Fecundity

In the present study, fecundity of *Holothuria atra* was found to be in the range of 12110 to 1342278 oocytes with individuals of size range from 21.0 to 25.0 cm total length. Three forms of correlations were tried to detect the most appropriate correlation between fecundity and total length, gutted

weight and total gonad weight. The regression constants "a" and "b" and correlation coefficient (r^2) were described in Table (1):

The relation between absolute fecundity and gonad weight is the best fitted by a linear regression, it gives the highest correlation coefficient r^2= 0.874 and expressed by the following equation:

F= -672005.5 + 105907 G.Wt.

Where, G.W. is the weight of gonad of *Holothuria atra.*

Table (1): The different correlation of relationships between Fecundity and total length, gutted weight, and gonad weight of studied species.

The relationship	*Holothuria atra*		
	a	**b**	**r^2**
F & Total length	-1508485	85938.8	0.613
F & gutted wt.	3010406	5152.05	0.301
F & gonad wt.	-67200.5	105907	0.874

DISCUSSION

Reproduction characteristic is one of the most important, aspects in dealing with the fishery biology of sea cucumber. Male and female sexes are usually separate, but there have been a few reports of hermaphrodites in some species (Smiley *et al.* 1991).

While sea cucumber can reproduce sexually, the ability to reproduce asexually by transverse fission has been known to occur in eight aspidochirotide species (Uthicke, 1997) and may are artificially induced in several others (Reichenbach *et al.* 1996).

The term "maturity" has a peculiar, but generally accepted, meaning fisheries biology. It is taken to show the degree of ripeness of the ovaries and tests of a sea cucumber as well as in other organisms.

Most of the aspidochirote holothorians in both temperate areas and in the tropics tends to have an annual reproduction cycle with spawning in warm months (Hyman 1955, Conand 1990; Smiley *et al.* 1991, Chao 1993).

Smiley *et al.* (1991) attributed the adaptation of indirectly developing holothurians to a summer reproductive cycle to the sea-water temperature and food availability for development of the planktotrophic larvae.

In general, towards the spawning season, the gonads in both males and females increase in size whereby the so-called mature gonads are formed in advance to the spawning season. Routine assessment of maturity stages is normally done by assigning individuals to stages by characters which can be differentiated with the naked eye and

bionocular microscope, these maturity stages are applicable for discrimination between the different conditions of the gonads at different periods.

A large number of keys for maturity staging sea cucumber have been recommended as Costelloe (1985), Conand (1993), Chao *et al.* (1994), Baskar (1994), Sewell and Chia (1994), Ramofafia *et al.* (2000), Thierry and Conand (2001), Purwati and Luong-van (2003) and Howaida *et al.* (2004).

In the present study a special key was followed using four stages of maturity as immature (I), maturing (II), ripe (III) and spent (IV) stages. In addition to the unsexed cases are recorded for *Holothuria atra.*

Determination of the spawning season of *Holothuria atra* was done by following the variation in the monthly distribution of maturity stages. The results showed that ripe male stage (III) was observed most time of the year with higher occurrence during June and November. The monthly distribution of the different female stages showed that ripe stage (III) was observed between June and December as the some period of males.

Chao *et al.* (1994) recorded five stages in *Holothuria atra* as recovery, growth, mature, spawning and post-spawning from southern Taiwan.

Determination of spawning season of *Holothuria atra* in the present study was done also, by size distribution of different maturity stages. In case of males most ripe individuals were observed between size 16.5-24.5 cm. While in case of females most ripe individuals were observed between size 15.5 (cm) and 28.5 (cm).

The monthly changes in G.S.I in *Holothuria atra* during the present study as male increased from February to April with peak in May and June and decreased from October to December. While in female it increase from February to May with a peak in June then decrease in November and December. These results confirms the previous conclusions of the monthly distribution by maturity stages.

Conand (1993 b) reported that G.S.I increased from January to February and decreased from march to September then increase in October to December with a peak in November for *Holothuria atra* collected from the major communities of the New Caledonian lagoon.

Chao *et al.* (1994) studied G.S.I. for *Holothuria atra* at two contrasting sites in southern Taiwan as, from March to August 1990, the G.S.I was less than 0.5. No gonads were found from September 1990 through April 1991. one month later, mature gonads were found with gonad index of $2.31 \pm 0.92\%$ in June and July, the index decreased to $1.49 \pm 0.62\%$ and $0.88 \pm 0.44\%$ respectively.

In case of *Holothuria atra*, a number of authors studied the time of breeding of this species in different areas. Chang and Lee (1986, 1987) reported that *Holothuria atra* spawns from June to September in southern Taiwan, corresponding with the summer phytoplankton blooms. Pearse (1968) reported that *Holothuria atra* living near the equator has mature gonads throughout the year and referred that spawning is asynchronous. He predicted that populations distant from the equator would have restricted spawning periods.

Harriott (1982, 1985) reported that, except in September, *Holothuria atra* at Heron Island reef (23^{o} 27`S, $151^{o}$ 55`E) has mature gonads throughout the year but that gonad maturity peaks in summer and in early winter months (a semiannual reproductive cycle). At new Caledonia (22^{o} 0.5`S, $166^{o}$ 24`E) which has as a similar latitude to the Heron Island reef, spawning of *Holothuria atra* is restricted to the warm season (Conand and DeRidder 1990). In southern Taiwan has a latitude similar to Heron Island reef and New Caledonia, *Holothuria atra* reproduces annually in summer, as does the population of New Caledonia.

The following table summarized the spawning period of *Holothuria atra* in different areas.

Sources	Location	Breeding months	Latitude
Pearse (1968)	Red Sea	All	10° 15`N
Chao, *et al.* (1994)	Southern Taiwan	June –September	21° 27`N
Conand and DeRidder (1990)	New Caledonia	Warm months	22° 20`S
Harriott (1982, 1985)	Heron reef	May-June and Nov. Jan.	23° 27`S
Present study	Hurghada	June – Dec.	27° 15`N

The timing of reproduction in *Holothuria atra* as a warm water time is determined by proximate factors e.g. the ability of adult to acquire food reserues allowing them to devote energy to reproduction and by ultimate factors e.g., timing of spawning such that optimal conditions are available for settlement and development of larvae (Giese & Pearse, 1974; Reese, 1966). Temperature, salinity, food abundance and photoperiod have all been cites as factors regulating holothurian reproductive cycle (Harriott, 1985; Holland, 1994).

The estimation of the size at first sexual maturity has its practical application in the determination of the minimum legal size needed to protect an adequate spawning stock and to ensure at least one spawning for the ripe individuals. The minimum biological size at first sexual maturity in the present study for both males and females of *Holothuria atra* was 16.5 (cm) and 15.5 (cm) respectively, while the calculated size at first sexual maturity of both sexes as 16.5 (cm) for females and 17.5 (cm) for males.

Conand (1993 b) estimated the size at first sexual maturity of *Holothuria atra* collected from the major communities of the New Caledonian Lagoon at 165 (mm) LT_{50}.

In the present study, the monthly distribution of oocyte diameter in *Holothuria atra* the modal size of the ripe oocytes from August to December ranges from 112 to 187 (μm) which is considered the maximum observed oocyte average diameter during the active spawning period of *Holothuria atra.* Harriott (1985) recorded the modal size of egg diameter for *Holothuria atra* was 88 (μm) at Heron Reef, Great Barrier Reef.

Conand (1993) reported that the oocyte diameter of *Holothuria atra* was 150 (μm) in the New Caledonian lagoon.

Fecundity is one population parameter with potential influence in recruitment (FAO, 1990). In the present study, fecundity of *Holothuria atra* was found be in the range of 12110 to 1342278 oocytes with a size range from 21.0 to 25.0 (cm) total length. The relation between absolute fecundity and gonad weight is the best fitted by a linear regression, it gives the highest correlation coefficient (r^2= 0.874).

Study of Hassan, 2005 on *H. Scabra* at Abu Rhamada Island in the Red Sea (coast of Egypt) revealed a strong relationship between fecundity and body size, with fecundity increasing with the increase of the body size, as also found by Conand (1989, 1990, 1993) at other localities.

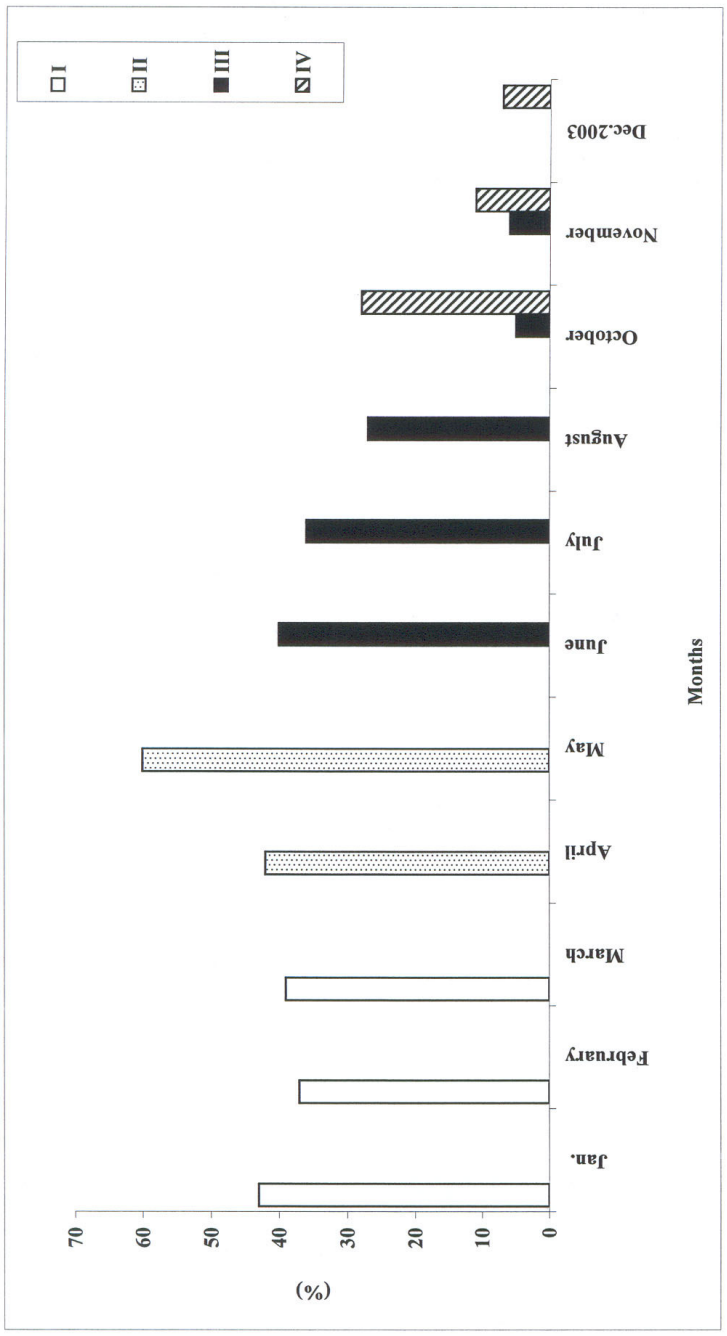


Fig. (3): Monthly % distribution of different maturity stages for male of *Holothuria atra* during the of study.

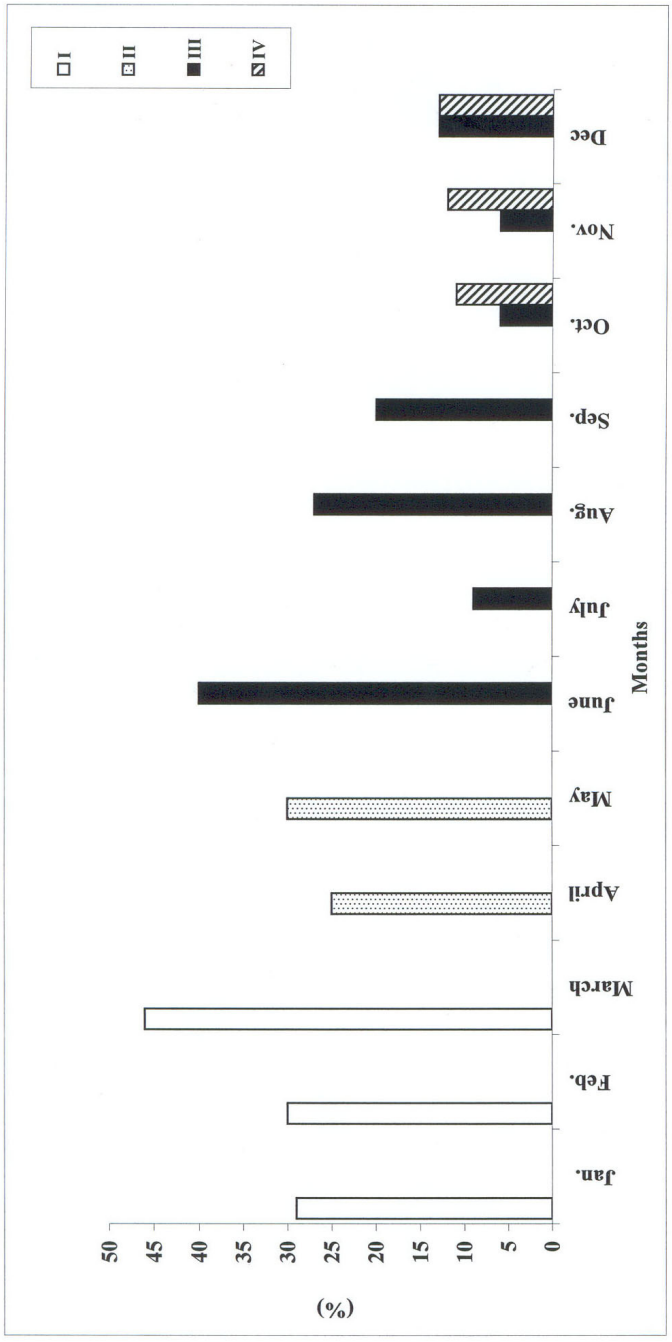


Fig. (4): Monthly % distribution of different maturity stages for female of *Holothuria atra* during the period of study

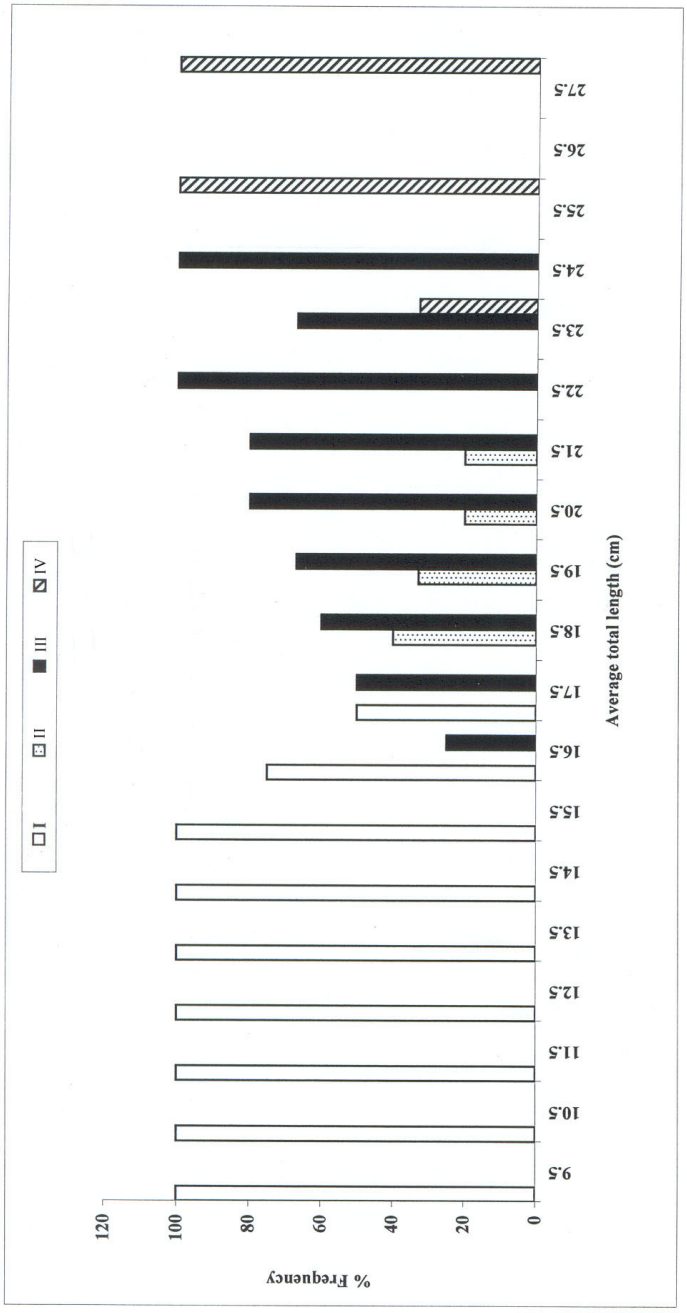


Fig. (5): Size frequency distribution of different maturity stages of male (*Holothuria atra*) during the period of study.

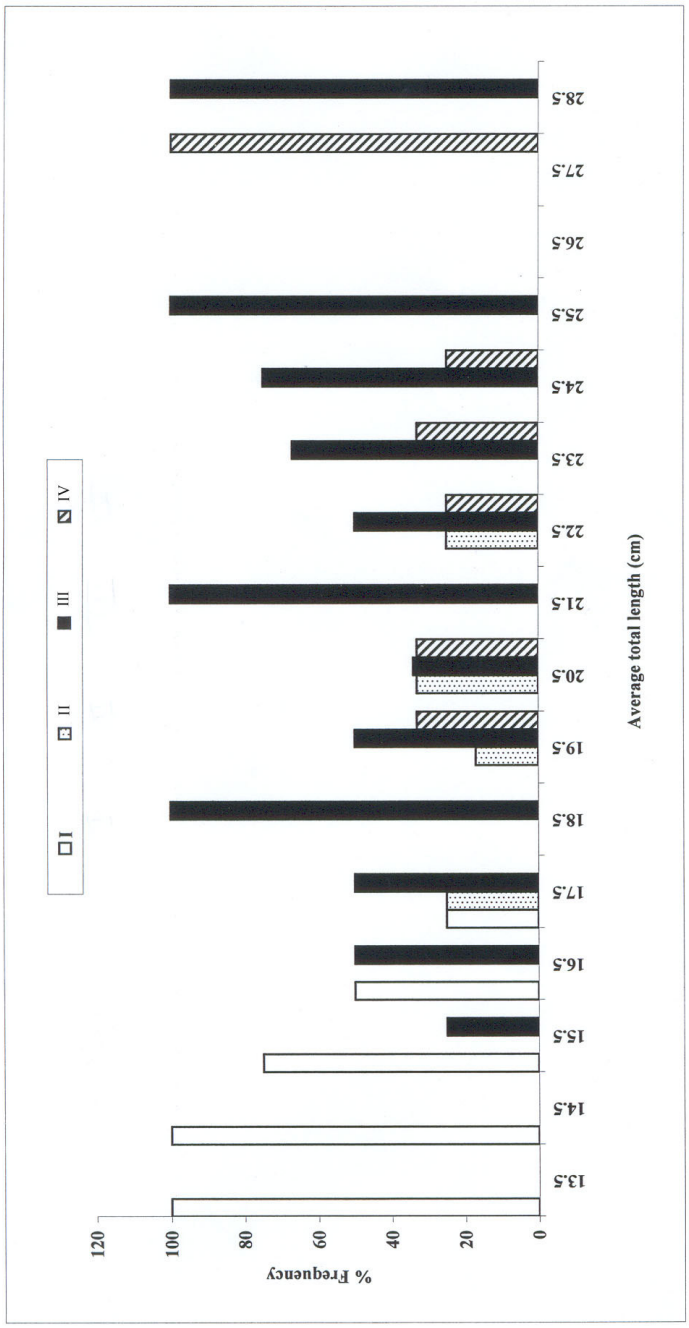


Fig. (6): Size frequency distribution of different maturity stages of female (*Holothuria atra*) during the period of study.

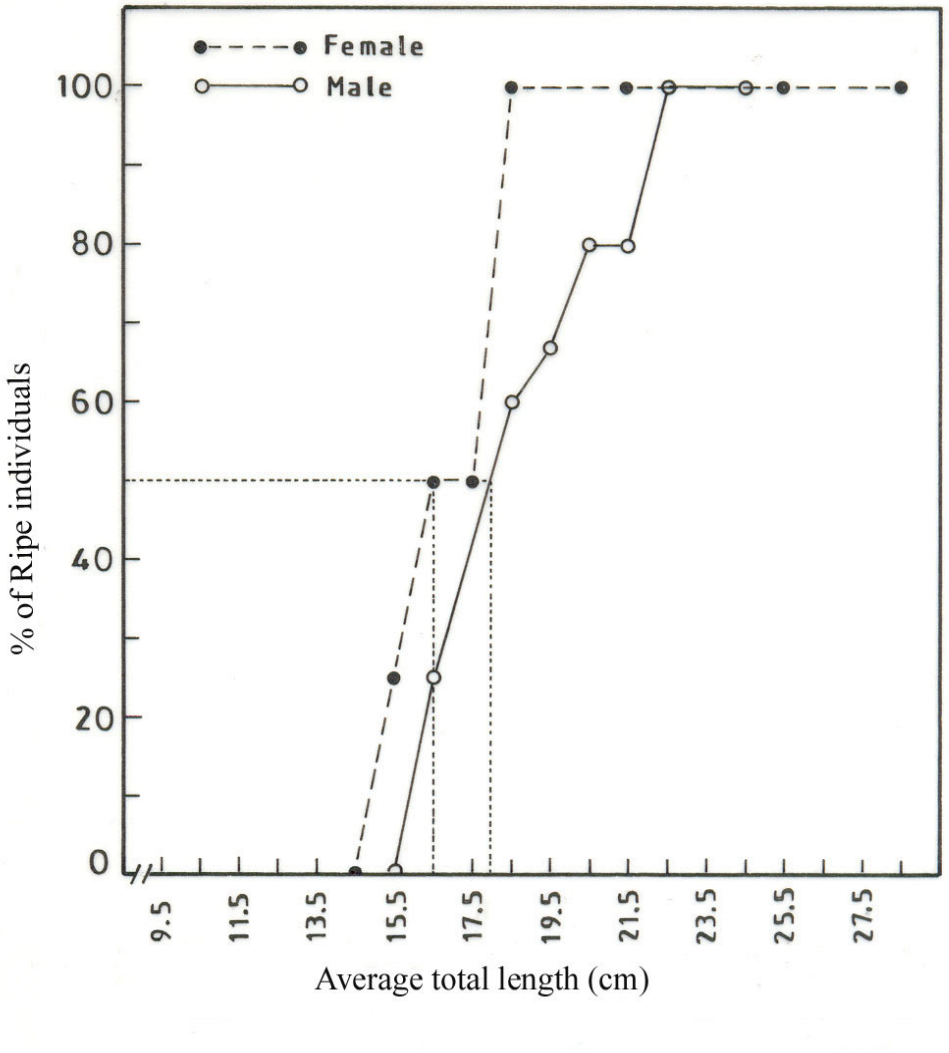


Fig. (7): Size at first sexual maturity of both sexes of *Holothuria atra*

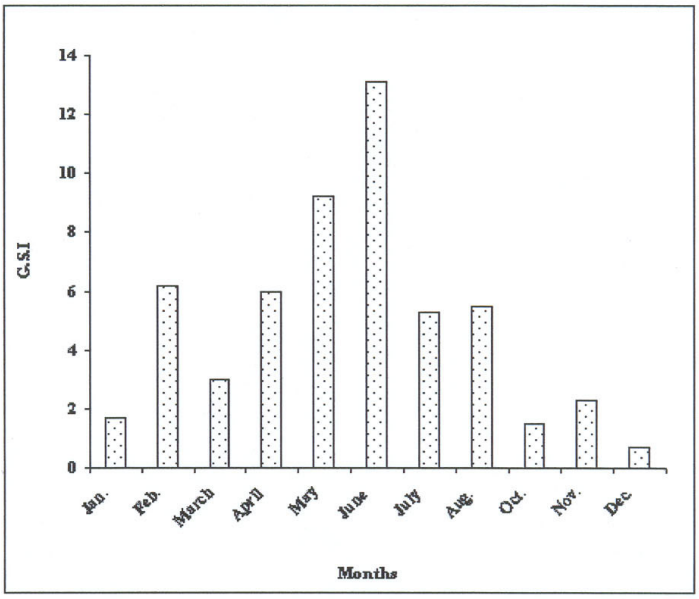


Fig. (8): The average gonado-somatic index for male of *Holothuria atra* based on the gutted weight

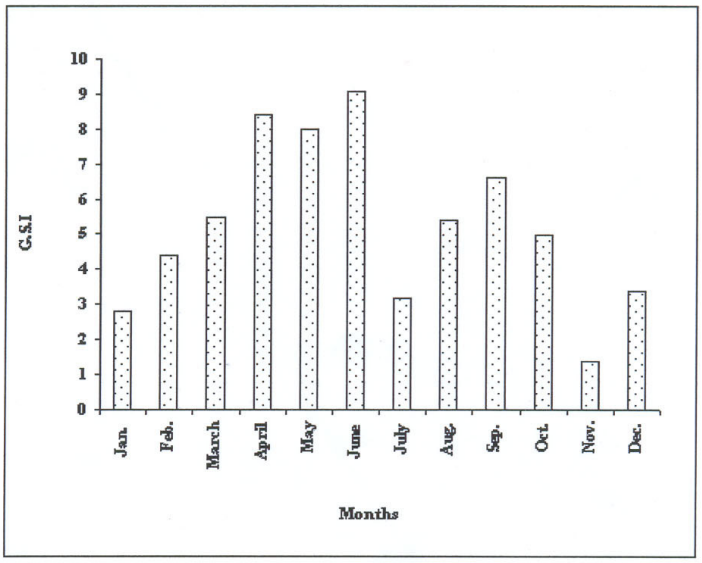


Fig. (9): The average gonado-somatic index for female of *Holothuria atra* based on the gutted weight

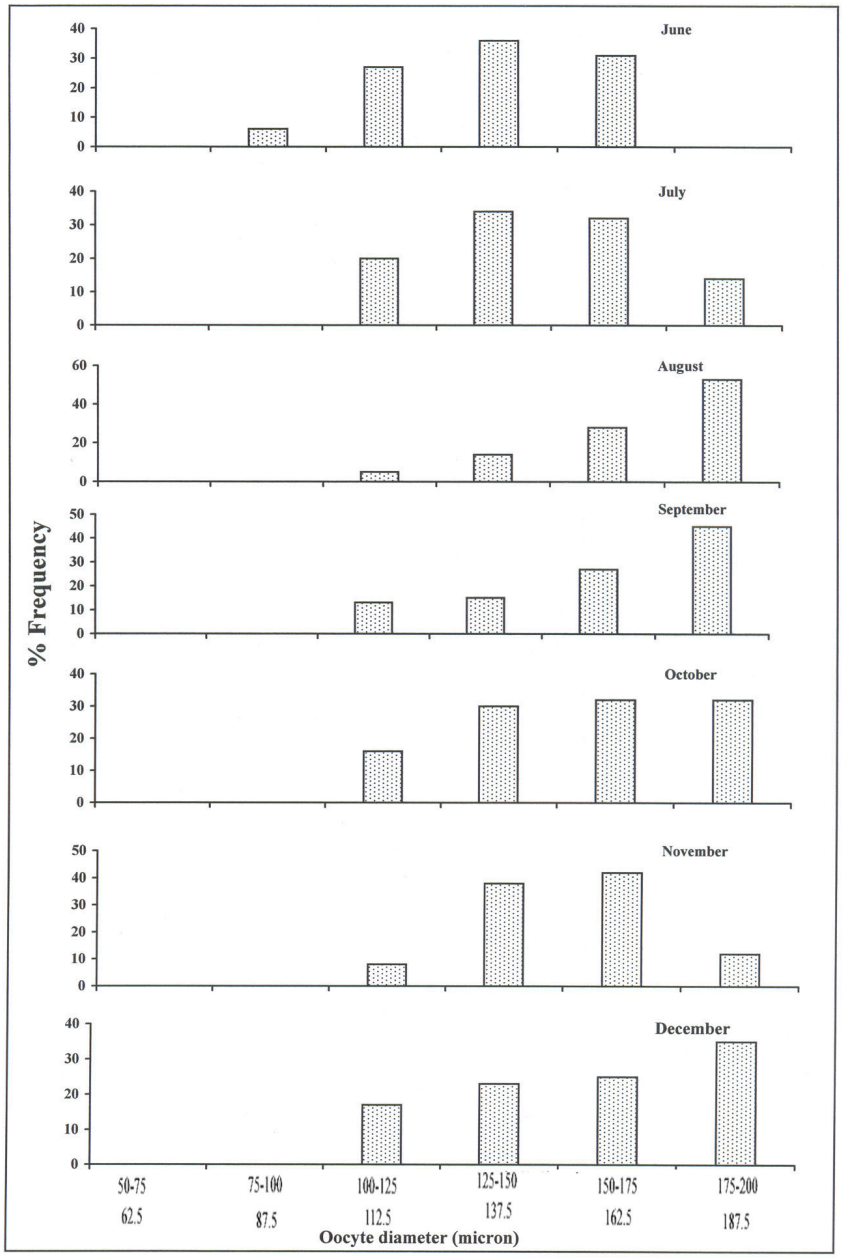


Fig. (10): Size frequency distribution of oocyte diameter of ripe individuals of *Holothuria atra* during the spawning season.

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