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Biological study, life cycle and fecundity of *Coralliophila violacea* (Coralliophilidae: Gastropoda: Mollusca) inhabiting the Red Sea coral reefs, Egypt

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ABSTRACT

One of the most common molluscan species in the Egyptian water is *Coralliophila violacea* (Coralliophilidae, Gastropoda, Mollusca). There are very few studies had been carried out on this species and its importance and its effect on coral reefs in the Red Sea. Reproductive studies of *C. violacea* indicated that females produce a number of egg sacs, each sac contains a large number of eggs ranged from 222 to 2893 eggs /sac with an overall average of 1046.33 ± 468.9 eggs /sac. The color of the examined egg masses varied from transparent creamy white to light orange and finally to light brown color. The close examination of the colored sacs indicated that the developmental stage of the eggs is the factor behind the differences in color. Male gonad development comprised the spermatogonia, primary (PSC) and secondary spermatocytes (SSC), spermatids and sperm cells (SP) were also examined and measured. The diameter of the spermatogonia of *C. violacea* varied between 4 and 6 µm with an average of 4.9+0.37 µm.

1. INTRODUCTION

Hexacorallia are the major architects of tropical reefs, acting as ecosystem engineers and providing the structural framework for a highly diverse assemblage of marine organisms especially in the Red Sea of Egypt (Mahdy *et al.*, 2018; 2021; Ghallab *et al.*, 2020). Many gastropods of the genus *Coralliophila* are found feeding on the margins of the living tissues of coral colonies, causing small and localized tissue damage (Schuhmacher, 1992). The impact of predation on the growth of stony corals has gained increased attention in the last decade, although the degree to which coral co-specific density can modify the effects of corallivores remains poorly studied Shantz *et al.* (2010). Oren *et al.* (1998) they reported that gastropods of the genus Coralliophila are found feeding on coral margins.

Many marine gastropods package fertilized embryos into individual egg cases or capsules that are deposited as masses or groups cemented onto hard substrate (D'saro, 1991). Female size has been positively related to fecundity (embryos/ female/ year) both in gastropod species that produce a single egg mass or clutch per year e.g., *Ceratostoma foliatum* (Spight *et al*., 1974); *Thais lamellosa* (Spight and Emlen, 1976) as well as gastropods that produce multiple egg masses per breeding season or year such as *Eupleura caudata* (MacKenzie, 1961); *Buccinum isaotakii* (Ilano *et al*., 2004). Chen *et al*. (2004) studied the fecundity of *C. violacea* on different coral hosts. They reached conclusion that the fecundity of the females on the branching hosts was significantly lower than that on the massive hosts. The size at sex change (male to female) of the snails was smaller on the branching hosts than on massive hosts. Patch composition differences can partly explain the smaller size at sex change for snails on branching hosts; however, there was also evidence that host morphology had a significant effect on the timing of sex change. Miller *et al*. (2005) studied the fecundity of *C. abbreviata* on three coral hosts (*Acropora palmate, Diploria* sp., and *Montastraea* sp.). They reported that the percent of brooding females that contained fully developed veligers was similar for each host; 36% (n = 20) for *A. palmate*, 33% (n = 5) for *Diploria* sp., and 36% (n = 5) for *Montastraea* sp. They also found that veligers ranged in shell length from 0.19 to 0.33 mm and were on average ±SD of 0.28 ± 0.01 mm and that the veligers size did not vary significantly among hosts (One-way ANOVA; df = 2, F = 2.109, P > 0.05). The same authors also stated that the number of egg capsules brooded, the average area of egg capsules and total egg capsule area per female increased with female shell length. Thus, on average, the larger females of *A. palmate* brooded larger egg capsules, thereby having a greater total egg capsule area than females on the other two hosts. Although the reproductive season is unknown for *C. violacea*, females carrying egg capsules in their mantle cavity can be observed year round (Lin and Liu, 1995). Hatched veliger larvae remain actively swimming and strongly photopositive for at least one week in the laboratory, indicating the potential for long-distance dispersal in water column.

Nasution (2003) investigated the intra-capsular development stages of embryos from newly laid eggs and consumption rate of nurse eggs during development. The result of his study show that *Buccinum undatum* embryos develop within the egg capsule through the provision of nurse eggs as an extra embryonic source of nutrition. He also suggested that this nurse-egg feeding mechanism was demonstrated by the reduction of the capsule egg content ingested by developing embryos during development. As development continued, between the fourth and the sixth weeks, the body of the embryo began to coil. Intra-capsular development was completed when the juveniles grew to about 1.5 shell whorls, between the seventh and ninth weeks. After ten weeks, young juveniles started to emerge through a hole underneath the capsule.

Johnston and Miller (2007) studied variation in life history traits of the corallivorous gastropod *C. abbreviata* on three different coral hosts. They reported that brood size increased as a power function with female shell length. Females on *A. palmata* were significantly larger than females on the other two hosts, therefore, produced more offspring per female. Wells and Lalli (1977) they also indicated that females brood a series of unattached egg capsules in the mantle cavity from which, after being released, free-swimming veligers emerge. Although Wells and Lalli (1977) described reproductive characteristics of female *C. abbreviata* inhabiting the coral *Montastraea annularis*, but no comparative study has been done to examine potential host-specific differences in reproductive output. Furthermore, there is no information on early life-history, age-structure, and mortality of *C. abbreviata*. Population structure and dynamics may be substantially affected by predation. For instance, there is often a negative correlation between predation pressure and growth rate in natural populations due to behavioral interference (Nakaoka, 2000).

Members of genus *Coralliophila* are thought to be a protandrous hermaphrodite (Hayes 1989). Sequential hermaphroditism has been documented for a wide range of taxa, especially fish and invertebrates (Charnov, 1982; Allsop and West, 2004) and protandry is common in the family Coralliophilidae (Chen *et al*., 1998). Johnston and Miller (2007) reported that females *C. abbreviata* were larger and older than males on all host taxa and female fecundity increased substantially with size. They also added that, sex ratios were biased toward males with the populations consisting of smaller individuals having a higher proportion of males compared to populations made up of larger individuals. These results conform to the predictions of sex allocation theory as it pertains to sequential hermaphroditism (Charnov, 1982), lending support for the existence of protandry in *C. abbreviata*.

The timing of sex change for sequential hermaphrodites may be influenced by environmental and social factors such as growth, mortality, and density and composition of conspecifics (Charnov, 1982). For instance, the presence of females can inhibit sex change of neighboring males in protandrous species such as *C. violacea* (Chen *et al*., 1998) and the shelf limpet, *Crepidula norrisiarum* (Warner *et al*., 1996). Thus, the higher mortality of adult (female) snails on *Diploria* sp. and *Montastraea* sp. may account for the earlier sex change of individuals in these populations since there are fewer older/larger females in the population. Interestingly, *C. violacea* and *C. meyendorffii* are geographically disparate members of the Coralliophilidae family that, like *C. abbreviata*, have populations characterized by host-specific morphology and life-history.

Molecular evidence suggests that *C. violacea* and *C. meyendorffii*, host-specific variation is indeed a result of phenotypic plasticity and not genetic differentiation (Oleverio and Mariottini, 2001; Chen *et al*., 2004). The evolution of internal fertilization in the animal kingdom is a reproductive strategy that includes the organization of a

complex reproductive system adapted for copulation. The structural complexity associated with the male reproductive system, besides its association with the habit of internal fertilization, includes the organization of a compartmentalized gonad which is able to provide a specialized environment within which highly species specific gametes are formed (Brown, 1990). In many marine gastropods, diverse patterns of organization of the reproductive system are evident among its representatives; these patterns have been considered taxonomically relevant for defining the phylogeny of families Littorinidae (Reid, 1989). In general, the male reproductive systems of the gonochoric representatives include: (1) a branched gonad among the tubules of the digestive gland, (2) a seminal vesicle in which the euspermatozoa are stored, (3) a prostate gland, and (4) a conical penis located under the right tentacle of the animal (Castillo and Brown, 2008). Most of the gametogenic studies in gastropods are mostly limited to commercial species such as *Buccinum undatum* Linnaeus, 1758 from east Canada (Himmelman and Hamel, 1993), from Argentina (Gimenez & Penchaszadeh, 2002), or to imposex indicators such as *Reishia* (*Thais*) *clavigera* (Küster, 1858) from the west coast of Korea (Lee, 1999) and *Nassarius reticulatus* Linnaeus, 1758 from the northwest of Portugal (Barroso & Moreira, 1998).

Johnston and Miller (2007) studied the biology and growth of *C. abbreviata* inhabiting number of different corals species. Their aim was to investigate whether snail life-history and fitness are differentially affected by the coral host, an analysis of the age structure and female reproductive output of snail populations on three coral host taxa (*Acropora palmata*, *Diploria* spp., and *Montastraea* spp.). Their results indicated that the operculum striae have been shown to represent annual growth marks in the studied species as it was proven for other gastropods (Chen and Soong, 2002; Ilano *et al*., 2004). The present study was design to investigate some biological aspects of *C. violacea* on the reefs at the Red Sea og Egypt and determine its potential of forming a threat to coral reefs in the area.

2. MATERIALS AND METHODS

2.1. Study Sites

The samples were collected from the shore of the small Giftun Island, Red Sea, Egypt. The reef where *C. violacea* was collected is located at the south eastern tip of the island (Fig. 1). The reef at this area is of the fringing type extended from the island southward, with a considerable area of reef lagoon followed by a wide area of reef table and ended in the seaward side with a healthy reef crest. The reef characterized with different sclaractena species especially *Porites*. Most of the *Porites* colonies are located in the upper area of the reef wall at depth of 3-6 meters. The three species of *Porites* are located in this site and they infested by a large number of *C. violacea*.

Figure 1. Collection site of the reef area at small Gifton Island, Red Sea, Egypt

2.2. Collection and preservation of C. violacea samples:-

C. violacea samples were collected monthly during October 2008 to November 2009 using SCUPA diving equipment. Collection was made from coral colonies ranged in depth between 4 and 6 m. At each sampling site, about 100 individual/ month randomly sampled and kept in plastic bag underwater. All samples were then preserved in 10% neutral buffered formalin prior to transferring to the laboratory.

2.3. Laboratory studies:

All collected samples were transferred to the laboratory and prior to perform any studies. Samples were preserved using 10% neutral buffered formalin in the same day of collection and prepared for keeping the numbers that have been grouped into morphometric parameters and biological study.

2.3.1. Obtaining of C. violacea egg sacs:

From the literature (Chen and Soong, 2002), it is well known that *C. violacea* produce egg sacs which kept inside the mantel of the females. For obtaining the egg sacs of *C. violacea* large individuals were collected from the reef in separate plastic containers 50 ml each. At the laboratory the containers were examined and all obtained egg sacs were removed, photographed and preserved in 5% formaldehyde. The size of the egg sacs (capsules) was estimated using the elliptical surface area, i.e. ¼ X π AB, where A and B are the long and short diameters of the ellipses, respectively.

2.3.2. Determination of the egg development stages:

For determination of the stages in which eggs reached maturity, the egg sacs were dissected under binocular stereo microscope (Olympus stereo microscopes 16X fitted

with a calibrated ocular micrometer) and the stage of the eggs were determined according to Chen and Soong (2002) as following classification:

Stage I: Gastrula stage: during which the animal inside the egg is in a form of cluster of cells without any recognition of the different parts and not motile. **Stage II: Recognition stage:** during this stage veliger larvae starts to appear with its recognizable shape, however its movement still somewhat sluggish inside the egg. **Stage III: Active Veliger stage:** during this stage the larvae are very active inside the eggs and its movement is recognizable even from the sac wall. **Stage IV: The juvenile stage:** in this stage the larvae of *C. violacea* has developed a thin transparent shell and ready for hatching. The egg cover is rather deteriorating or not present; movement of the individuals is slower than previous stage. The egg capsules color and shape as well as the eggs were documented by photography.

2.3.3. Fecundity:

For the determination of the fecundity, two different techniques were used the first included counting the number of eggs of the different developmental stages in each egg sac (Chen and Soong, 2002). In addition, the total surface area of all capsules of a female was used as its index of fecundity in this study, because the number of veligers in a capsule is positively correlated to the surface area of the capsule.

2.3.4. Histological examination:

The histological examination was conducted on the animals collected from the field after taking the morphometric measurements (Fig. 2) according to Potkamp *et al.* (2017). Sex was determined depending on the extraction of egg sacs as indication of females for the large size animals (1.4 cm). For small size animal, a random sample of 10 individuals was used. Shells were removed mechanically and soft part was extracted and fixed in a separate lab tubes. The following procedures were used for histological preparation:

I. **Fixation**: The soft part or tissues was fixed in 10% formaldehyde solution in a separate glass jar. Another samples were fixed in Bouin's fluid (75% picric acid, 20% formaldehyde, 5% glacial acetic acid), after which they washed in 70% ethyl alcohol and were preserved in 70% alcohol.

II. Dehydration: Soft part tissues were dehydrated in an alcohol series dilution (70%-80%-90%-95%-100%) for 30 minute each, and then cleared in xylene-cedar wood oil mixture for 24 hours.

III. Embedding: Embedding was performed in paraffin wax in oven at 55ºC.

IV. Sectioning: Sectioning was made through different regions of the animal specially gonads at 5-7 micron thickness. Sections were mounted in glass slides.

V. **Staining**: Sections were stained with Harris Hematoxylin and with Eosin as a counter stain. After staining, sections were mounted using Canada balsam and stored to dry oven at temperature degree 50˚ about 24 hour.

VI. Examination: Sections were examined and photographed using Nikon microscope equipped with Digital camera. Samples were examined microscopically mainly to assess gonad status and condition.

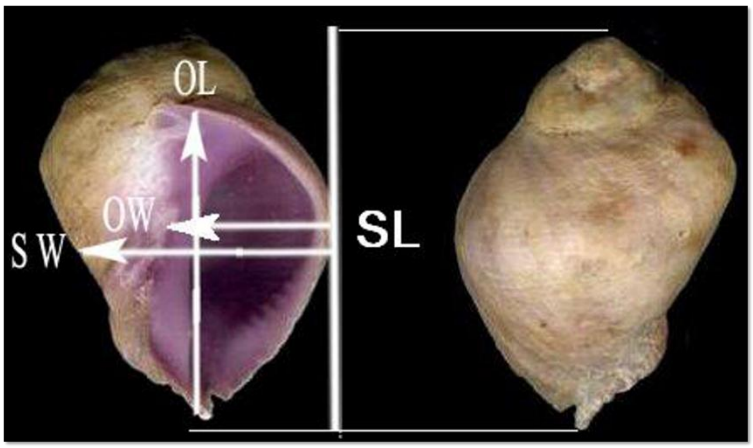

Fig.2. The morphometric measurements of *C. violacea.* SL (shell length), SW (shell width), OL (operculum length) and OW (operculum width).

3. RESULTS

3. Results:

3.1. Histological examinations of the C. Violacea reproductive system:

Examination of the gonad sections taken from males and females of *C. vciolacea* showed that several gametogenesis stages are simultaneous taking place within the same gonad. This pattern was observed throughout the year, showing no annual cycle with particular periods of gonad maturation or resting.

3.1.1. Females:

In females of both of germ cells, Oogonia (OG) and primary oocytes (PO) were observed simultaneously throughout the year. Despite the fact that yolk granules (YG) were abundant in female gonads at all the examined specimens, the mean diameter of these granules ranged between 7.6 and 16.2 µm with an average of 11.4 ± 1.8 µm. The oocyte diameter before vitellogenesis (without the yolk granules) ranged from 32 to 62 µm with an average of 50.25 ± 11.3 µm. During development and maturation of the eggs, the yolk granules that surrounded the oocytes (O) peripherally ranged between 2.17–5.85 µm and averaged 4.57 ± 0.95 µm. These peripheral oocytes are also significantly smaller than the rest of the granules which ranged between 6.1 to 14.8 µm with an average of 9.3 ± 2.3 µm. After complete vitellogenesis, the eggs of *C. violacea* ranged between 152.47 and 454.69 µm in diameter with an average of 383.44 ± 71.8 µm (Fig. 3A-D).

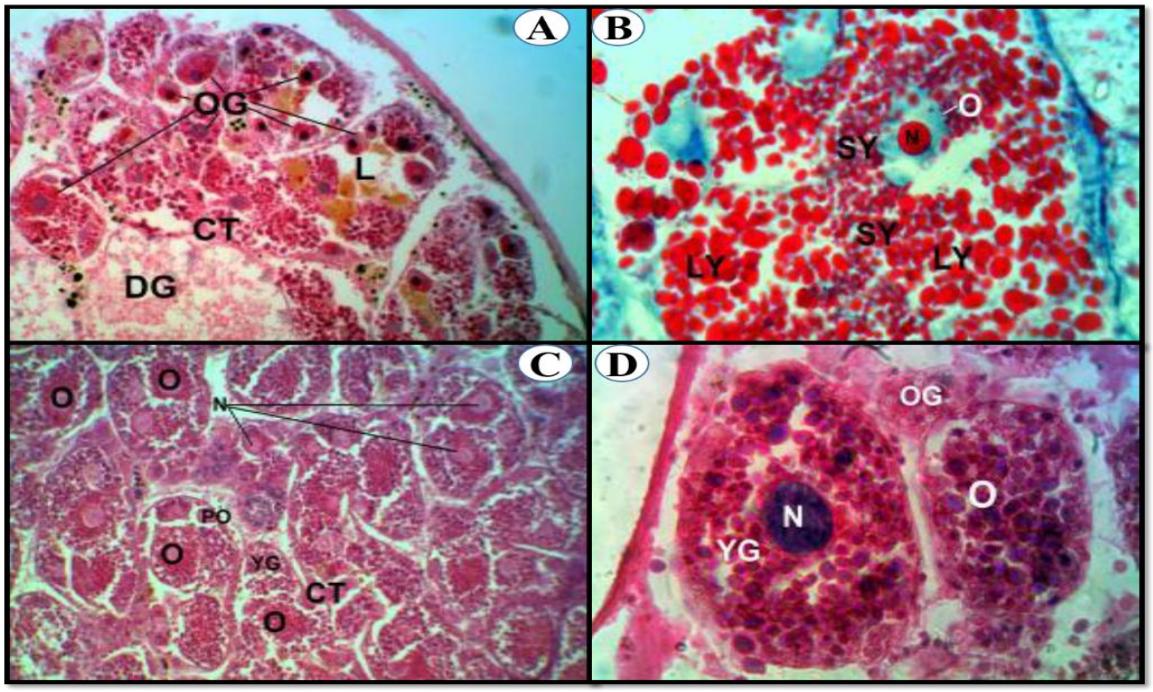

Fig. 3: Histological section of the female gonads of *C. violacea* indicating different stages of gametogenesis: (A) Ovary section at start of the development. Hx/Eos.100X; (B) the start of vitellogenesis and aggregation of yolk granules, Gomri trichrome 400X; (C) fully mature ovary with large eggs Hx/Eos.100X and (D) Mature egg of *C. violacea* Hx/Eos.400X.

3.1.2. Males:

In case of the male of *C. violacea* species, the spermatogonia, primary and secondary spermatocytes, spermatids and sperm cells were also observed with different degrees of intensity within one gonad and throughout the year. Sections through the male's gonads indicated that they have a fundamentally acinar organization among the acini of the digestive gland (Fig. 4A). Three compartments can be distinguished within the gonad into (Figs. 4A): I) gametogenic or acinar; II) Perigametogenic or periacinar and III) intergametogenic or interacinar. The gametogenic compartment (GC) is located among the tubules of the digestive gland (DG); the acinus is its' basic morphological unit, having a globous appearance, and is found intimately interacting with the periacinar (PA) and interacinar compartments (Figs. 4A-C). In this, the germinal cells interact with the somatic Sertoli-like cells (SC) in a centripetal organization towards the lumen of the acinus (Fig. 4 A-C). Seminal vesicles, are organs in a form of coiled ducts (Fig. 4 B), which contains the spermatozoa on its' lumen. It is lined with a monostratified epithelium, which can vary from slightly squamous to cylindrical. In the males of this species, the spermatogonium, primary (PSC) and secondary spermatocytes (SSC), spermatids and sperms (SP) were also measured and the diameter of the spermatogonia of *C. violacea* varied between 4 and 6 µm with an average of 4.9±0.37 µm (Fig. 4A-D).

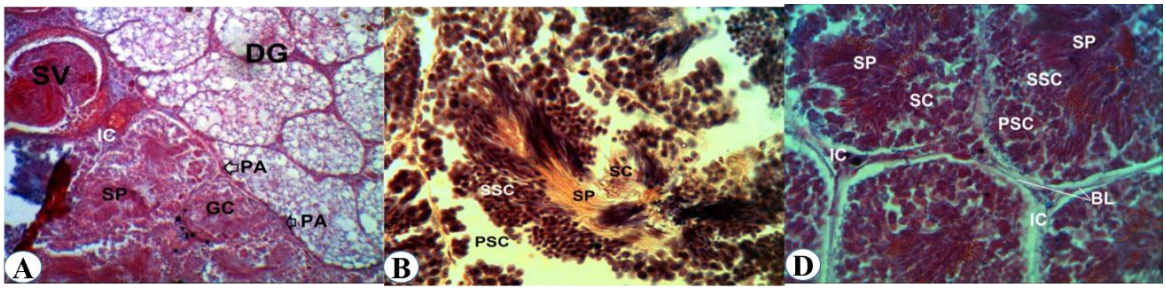

Fig. 4: Histological section of the male gonads of *C. violacea* indicating different stages of gametogenesis and different structure units as indicated in text.

3.2. Larval Development:

The egg sacs of *C. violacea* are oval to elliptical in shape (Fig. 5A). The longer axis of those sacs reaches 5.32 mm, and the wider axis is about 4.52 mm. Once the female releases the egg sac into the mantel cavity it remains attached to the inner surface of the mantel by a viscous material. The newly released sac is filled with fertilized eggs in the same stage of maturity. The examination of the sacs extracted from *C. violacea* females showed that those sacs included a large number of eggs ranged from 222 to 2000 eggs /sac with an overall average of 1046.33 ± 468.9 eggs /sac (Fig 5A).

The coloration of the egg masses varied from transparent creamy white to light orange and finally to light brown colour (Fig. 5B). The close examination of the colored sacs indicated that the developmental stage of the eggs is the factor behind the differences in color, according to the following: I: the transparent creamy white sacs included newly laid sacs and/or sacs containing the first or second stage. II: the light orange sacs included animals is early larval stage (veligers). III: the light brown color sacs most probably well-developed villagers or pre-hatched snails. The examination also revealed that all the sacs collected from the same female were in the same developmental stage. However, the time needed the sacs contents of eggs to develop to the final stage may differ by about 24 hours (Fig. 5C).

3.2.1. Stages of development:

The monitoring of the different stages of development revealed that all individuals within the same egg sac undergo changes at the same time. Also at the hatching, only 1 to 5 % of the eggs may be damaged or less developed. At the initial period of development there were about 1000 cleaving eggs in each capsule. By its end, their number was reduced as a result of resorption and only 900–950 veligers hatched into plankton from each egg capsule (Fig. 5C).

Stage I: represents both of blastulation and gastrulation stages where the fertilized egg starts to develop into a lump of cells aggregated together without any distinction of the parts (Fig. 5D). By the end of this stage the folding starts to form the different body parts of the animal, however, still no differentiation of the parts (Fig. 5E). **Stage II**: During the first part of this stage the animal starts to shape up (Fig. 5F) and the body could be distinguished into the shape of the first Veliger (Fig. 5 G, H). The eye spots (ES) are clear and the movement is noticeable despite its slowness. By the end of this stage the young villagers starts to move within the egg casing and in some cases released into the sac. **Stage III**: This stage is characterized by fast moving veligers within the sac. They

started by carrying transparent porous shell which reveals the body structure from underneath (Fig. 5I, G). The increase of movement activity of the large veliger enable us to see the stretched velum (V), occupied with long cilia and dark pigmentation (P) in the form of large patches distributed chainwise on its edges. The velum lobes were identical in their size and form. Already at this stage the rudiment of the foot and operculum were formed. Dark eye spots (ES), statocysts, and the retractor muscle were observable. The digestive gland was distinguished as a large dark patch in the shell (s). **Stage IV**: This stage is considers the final stage of development where the veligers may be released to the water to complete its development in the planktonic form. The villagers undergo development including the darkening of the shell as result of increase in its thickness and the disappearance of the cilia. In addition, the foot of the animal starts to be distinguishes as a movement organ. The digestive gland of the animal and the rest of the body organs appear in a form of dark mass when the shell is retracted (Fig. 5K, L).

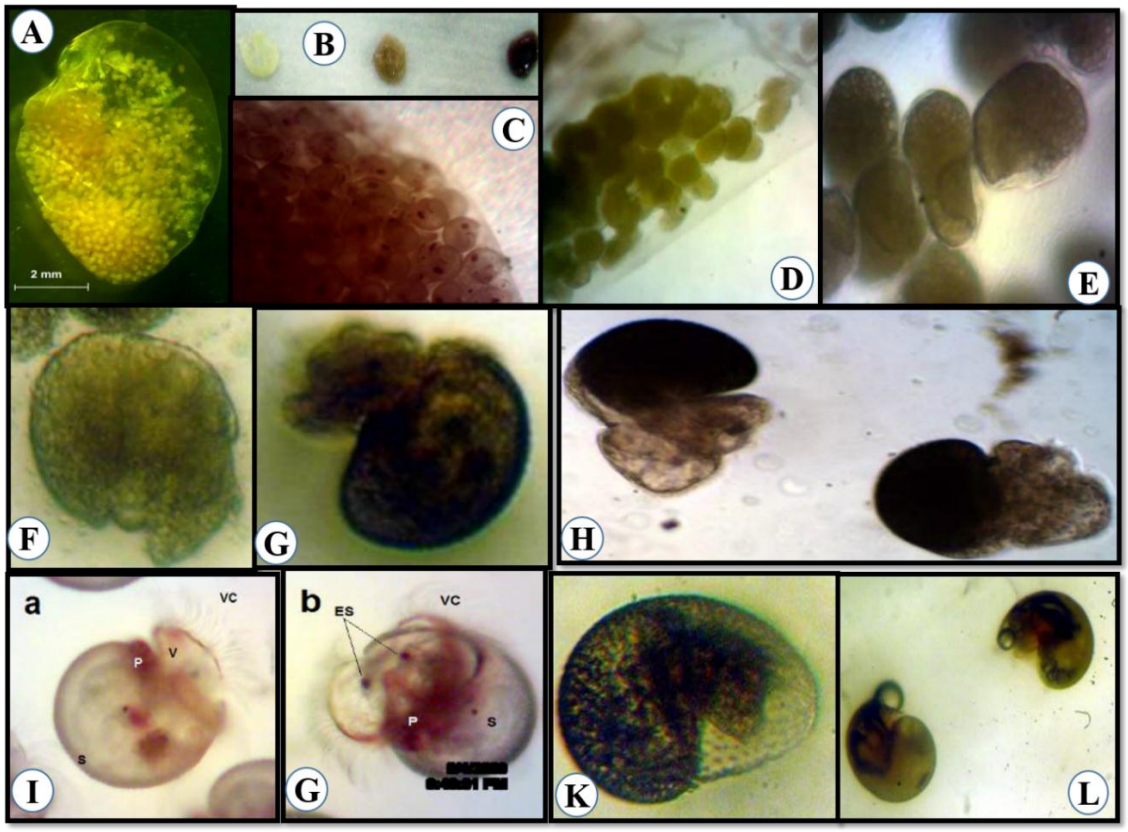

Fig. 5: The different developmental stage of *C. violacea.* A) egg sac, B) the color of egg sacs reflecting the different stages of maturity for the eggs as mentioned in text, C) hundreds of individuals at the same stage within the egg capsule, D, E) the first stage of development, F, G, and H) the Second stage of development, I, G) the third stage of development, and K, L) the fourth stage of development.

3.3. Fecundity

The total capsule area of a female was correlated to female size expressed as shell aperture length (Fig. 6). The data were taken from 150 samples and presented in figure

(6) indicated that the relation could be expressed by the equation: Area of egg capsules = 12.831 X aperture length -88.916, with R^2= 0.935 and P>**0.01.** From the previous data it was clear that the fecundity of females of *C. violacea* depends mainly on the development of the female size which was clear from the regression analysis. However, the size of the female may also play a role in its efficiency in producing eggs. All the examined females contained from one to four egg capsules (sacs) which carry a number of eggs vary in relation to the number of sacs (Fig. 6).

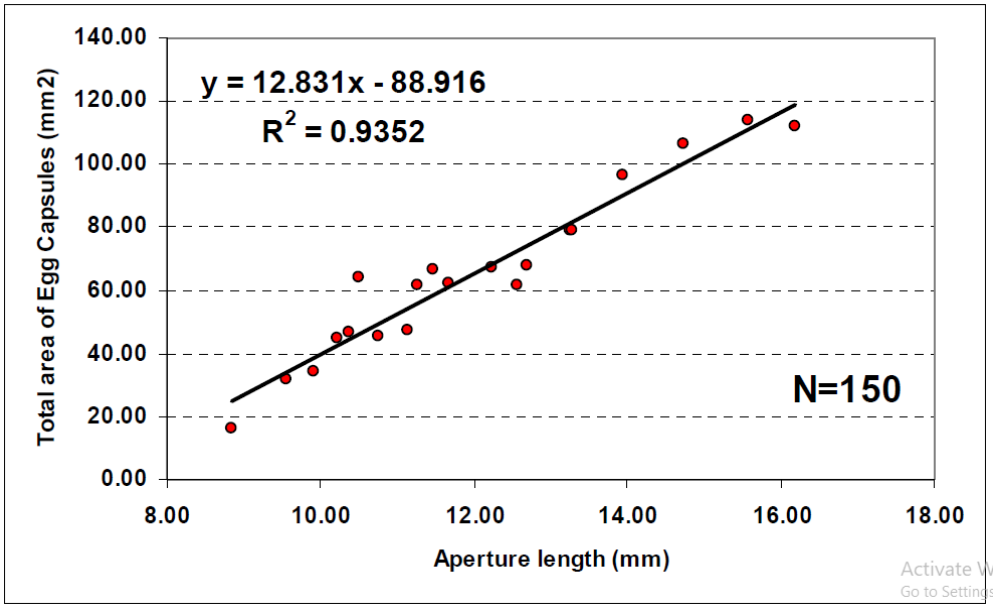

Fig. 6: Relationship between aperture length and area of egg capsules (sacs) of *C. violacea*.

Data in Fig. (7), indicated an increase in the average number of egg per sac till it reaches the maximum number when the animal is carrying three sacs beings 1185.94 ± 455 egg/sac. Meanwhile, animals with 4 sacs of eggs demonstrated a lower average beings 1052.6 ± 393.9 egg/sac. This may express sometimes to the relation between the number of eggs produced by female and its shell height. The data representing this relationship is given in Fig. (7).

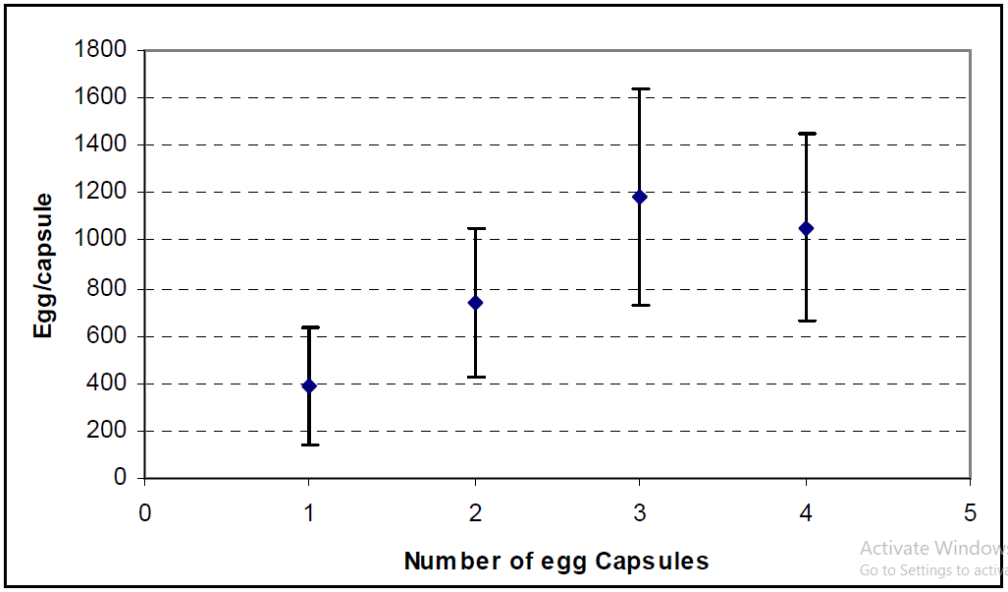

Fig.7: The average ± SD number of eggs per capsule recorded from samples of females *C. violacea.*

The data in Fig. (8) showed that the relation between the female size and its ability to produce eggs is of the positive regression type and could be expressed by the equation: No. of eggs/capsule= 164.5 X shell height-1744.5, with R^2= 0.820 and P>**0.05.**

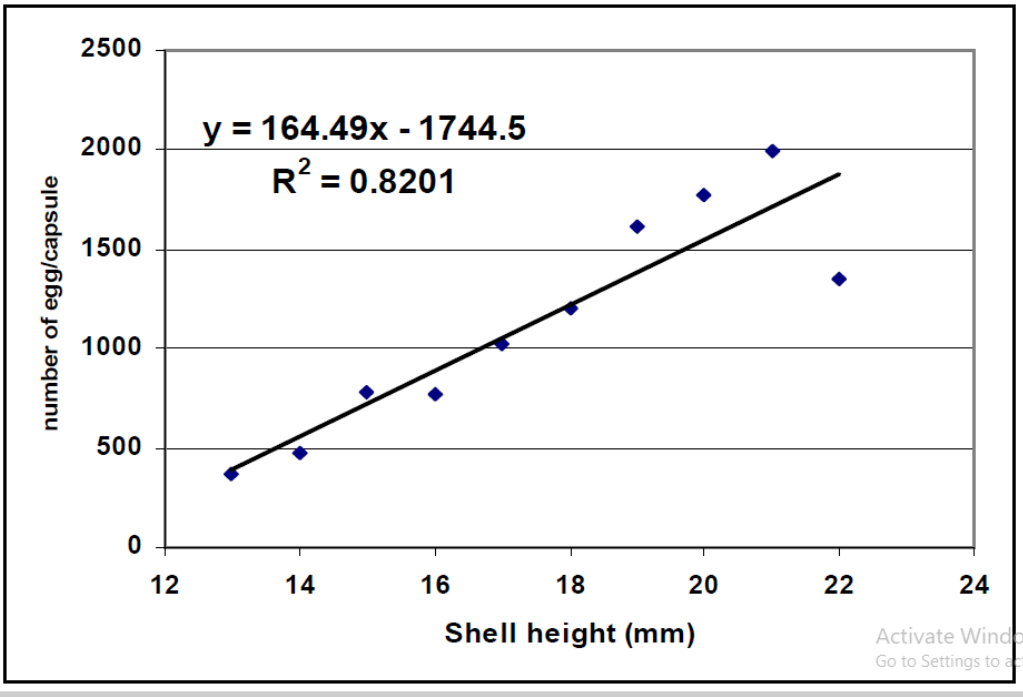

Fig. 8: Relationship between female shell heights and number of eggs produced/sac of *C. violacea*.

4. DISCUSSION

The histological examination of male and female gonad tissues of *C. violacea* revealed that they have a normal structure that can be observed in most Muricidae gastropods. This was clear in the structure of the egg with large amount of yolk granulation characteristic for those having a long incubation period. Also, the continuously active gonads which represent species have high production of offspring was clear from the number of eggs within egg sac (more than 2000 eggs).

The present study demonstrated that each female of *C. violacea* produce number of egg sacs or capsules which increase with an increasing in shell size (aperture length). It was clear from the regression relationship between shell size and number of egg sac, beside the size difference between females could also determine the number of the stages of maturity on which the eggs are in. These results agree with the finding of Wells and Lalli (1977), and Chen *et al*. (2004). The present study also indicated that, although egg capsules from single female contained larvae in a similar stage of development, however, the stage of larvae development varied among families. This was also proved that the number of egg capsule prodded the average area of egg capsules, and a total egg capsule area per female increased with female shell length.

In the present study, the obtained results also provided description to the different development stages recorded inside the egg capsule which agrees with the finding of Nasution (2003). It seems that the time needed for the development of the eggs to reach the swimming larval stage or till the free swimming veligers emerges depends to great extent on the surrounding environmental conditions. Laboratory experiments showed that the development occurs one week and after veligers being released, it took about three days to be settled. This was also confirming the finding of Wells and Lalli (1977), Lin and Liu (1995).

5. CONCLUSION

In conclusion, Corallivores cause minor to severe damage to coral reefs, but there is a growing body of evidence that even minor tissue or skeletal structure removal has growth and/or fitness consequences for a scleractinians coral colony. In light of rising reef stressors and declining coral populations, we believe the role of corallivores in reef trophodynamics is more complicated than previously recognised.

DEDICATION

Special Dedicated to the soul and memory of Prof. Dr. Mohamed Abu Zaid, Prof. of Marine Biology Branch, Zoology Department, Faculty of Science, Al-Azhar University, Cairo.

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