

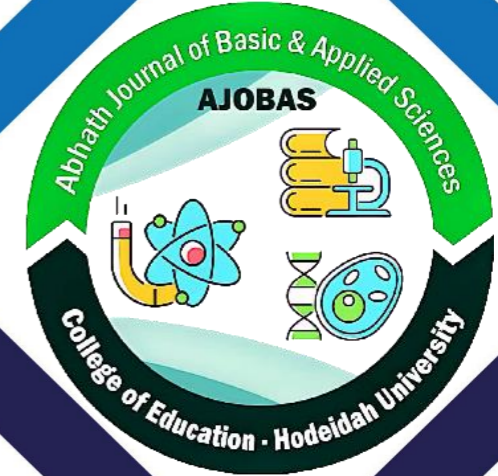


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Introduction of the Issue

We are pleased and delighted to present the researchers with this issue of the 'Abhath' Journal of Basic and Applied Sciences, which is the first issue of the first volume, the issuance of which emanates as an affirmation of moving forward towards issuing specialized quality journals.

The Faculty of Education at Hodeidah University aims, by issuing this journal, to publish specialized researches in basic and applied sciences, from inside and outside Yemen, in the English language.

On this occasion, the journal invites male and female researchers to submit their researches for publication in the next issues of the journal.

In conclusion, the editorial board of the journal extends its thanks and gratitude to Prof. Mohammed Al-Ahdal – Rector of the university – the general supervisor of the journal, for his support and encouragement for the establishment of this journal. Furthermore, thanks are extended to Prof. Mohammed Bulghaith – University Vice-Rector for Higher Studies and Scientific Research – vice-supervisor of the journal, for his cooperation in facilitating the procedures for the issuance of this issue. Nevertheless, thanks are for all researchers whose scientific articles were published in this issue, and for the editorial board of the journal, which worked tirelessly to produce this issue in this honorable way.

Journal Chief Editor

Prof. Yusuf Al-Ojaily



Histological; Mode and Timing Reproduction Studies of *Pocillopora verrucosa* in the Red Sea

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Abstract

At the study site, the lowest mean of seawater temperature is 24.5°C December 2010 to November 2011, while the maximum mean of seawater temperature was 33°C during summer.

The mode of reproduction in *Pocillopora verrucosa* vary between geographically isolated regions. This scleractinian coral is common along the Red Sea coast, and its reproductive mode and period of reproduction were assessed using histological preparation. The study was undertaken from December 2010 to November 2011. Sexual reproduction of the scleractinian corals *Pocillopora verrucosa* was studied for a period of one year in Sharm Ubhur. *Pocillopora verrucosa* is hermaphrodite broadcaster spawner with external larval developments, Embryos of species was not observed at the histological sections studies. They has very short annual cycle of gematogenesis. The onset of reproductive period of *Pocillopora verrucosa* was found to be limited (March to May). The number of eggs and testes observed in this period the gonads were found in the polyps. The *Pocillopora verrucosa* egg size was ranged from 26.2 μm (in March) to 152.0 μm (in May). Zooxanthellae was presented in the mature oocytes in *Pocillopora verrucosa*.

Keywords: coral reef , reproduction, fertilization, hermaphrodite, temperature

Introduction

Corals, which are members of the phylum Coelenterata, class Anthozoa, sub-class Hexacorallia and order Scleractinia, commonly referred to as hard corals, are a group of animals. These organisms are the backbone of coral reefs, which support high species diversity, provide goods and services (e.g., food, coastal protection, tourism), and provide substantive support to people worldwide (Scheer and Pillai, 1983 Veron,

1986 and Veron, 2000; Praveena, et al. 2012; Huang, et al. 2016; Hoegh-Guldberg, et al. 2019 and Lee et al. 2022).

Hermatypic corals are restricted to tropical and sub-tropical seas where the temperature is not lower than 18°C, with optimal reef development between 25° and 29°C this is expressed in latitudinal patterns of coral reefs distribution and diversity (Wells, 1956; Miller, 1995 and Bikerland, 1997).

Knowledge of scleractinian coral reproduction has progressively grown in the past 15 years. As Richmond and Hunter stated in a 1990 review, reproductive data were available for 40% of known species from the tropical Pacific, 30% of Caribbean coral species and only 6% of Red Sea species. There are four basic patterns of sexual reproduction, known among scleractinian such as hermaphroditic broadcast spawners (dominant group), hermaphroditic brooders, gonochoric broadcast spawners and gonochoric brooders (Harrison and Wallace, 1990; Knowlton, 2001 and Thomas, et al. 2019) .

Pocillopora corals have relatively high rate of reproduction and can reproduce asexually by fragmentation, budding, polyp bailout (a stress response involving just the polyps), polyp expulsion (occurs in apparently healthy coral and includes both the polyp and part of the skeleton), and asexually brooded planulae (competent larvae). These corals provide an important habitat for many other reef animals (Borneman, 2001 and Spalding, et al, 2001).

Most Pocillopora species are abundant and they are not considered globally threatened species. However, like many other corals, they may be threatened at a local or regional level. Human activities such as pollution, destructive fishing practices, unsustainable tourism (Harrison and Wallace, 1990 and Cairns, et al, 1999). Since the extensive reviews by Harrison and Wallace, 1990, additional studies have been published on reproduction in corals from the tropical Pacific (Glynn, et al. 1991; Ward 1992 and Tanner, 1996), the Caribbean and the Gulf of Mexico (Szmant, 1991; Van Veghel, 1994 and Knowlton, et al. 1997). However, information on the reproductive biology of corals from the Red Sea is very limited and still lagging (Loya, 1976; Rinkevich and Loya 1979a,b and Shlesinger and Loya 1985).

Population of the same species may display different reproductive modes when geographically separated; for example, coral identified as *Pocillopora verrucosa* are brooders at Enewetak Atoll (Stimson, 1978) and broadcast spawners in the Red Sea (Shlesinger and Loya, 1985). Similarly, *P. damicornis* produces asexual planulae and broadcast spawners gametes in Western Australia (Ward, 1992), but is only a broadcast spawner in the eastern Pacific (Glynn, et al. 1991). Over the

years, researchers have hypothesized why species use certain reproductive modes. The influences that habitat (Stimson, 1978 and Kojis and Quinn, 1981a) morphology (Rinkevich and Loya, 1979), and environmental conditions (Tomascik and Sander, 1987) play in determining the mode of reproduction in corals have been considered. Brooding corals exhibit a very different reproductive strategy. Only sperms are released into the water column and reach the eggs in the maternal colony. The fertilization is taken place in the maternal colony itself and then developed larvae are released into the water as swimming larvae (Knowlton, 2001). In this mode, the larvae are capable of settling on nearby substrate shortly after released. The number of larvae is limited due to the probability of sperms from one colony to reach the eggs of another colony for the successful reproduction. Therefore, reef degradation may be occurred not only because of the lowered gamete production, but also due to the reduced fertilization rate of those gametes released (Knowlton, 2001).

The coral larvae travel distance can be affected by the reproductive mode of their parental colonies. This is because of different mean larval competency periods resulting from brooding versus broadcast spawning coral species (Harrison and Wallace, 1990 and Harrison and Booth, 2007).

Brooding coral species can develop larvae internally by either sexually or asexually and these larvae can often settle immediately or shortly after release from the parental colony (Richmond, 1987 and Harii, *et al.* 2002). Settlement and genetic data support routine dispersal distances of brooding coral larvae in the range of 10-100s of meters (Tioho, *et al.* 2001 and Underwood, *et al.* 2007). Conversely, larvae formed by broadcast spawning and external fertilization have a minimum competency period of > 24 hours (Nozawa and Harrison, 2005), which aids in dispersing propagates away from parental colonies. The aim of this study is to examine the reproduction mode and timing of *Pocillopora verrucosa*.

Material and methods

Study site. The study was carried out in the on shore laboratory of the Faculty of Marine Science, King Abdulaziz University, Jeddah, which is 35 km away from the north of the city and located adjacent to the fringing reefs of the Ubhur Creek (Fig. 1). The study site was located in the northern side mouth of the Creek. The entrance of the Sharm is about 36 m deep and the depth decreases gradually to 3 m at its northern most extremity.

Temperature

Water temperature was measured by using two maximum and minimum thermometers. These were attached to a piece of iron bar and hidden among coral colonies at a depth of 3m. Readings monthly, from December 2010 to November 2011. The mean monthly temperature was calculated as the average between the mean maximum and minimum temperature for each month.

Reproduction

Samples for reproductive study of *P. verrucosa* was taken every two weeks for the duration at one year starting from December 2010 to November 2011. Coral specimens were cut off coral colony by chisel and hammer, fixed in a container with 7% seawater formalin for three days. Thereafter, they were transferred to 10% solution of nitric acid to decalcify the skeleton. The decalcified polyps were washed with distilled water and preserved in solution of 70 % Ethanol for further histological study. The preserved polyps were passed through a series of Ethanol of increasing strength from 70% Ethanol and ending with absolute alcohol, then cleared with xylene. The cleared specimens were infiltrated and imbedded in pure paraffin of melting point 58-60°C.

Serial sections with a thickness of 7 μm were cut through the polyps, and then stained with Haematoxylin and Eosin. Thereafter, the stained sections were cleared with xylene and mounted in Canada balsam (**Flowchart 1**). The state of gonads development (e.g. testis and eggs) was measured by a calibrated eyepiece micrometer of compound light microscope.

Statistical Analysis

I am used (one way- ANOVA , $p < 0.05$) Significant differences .

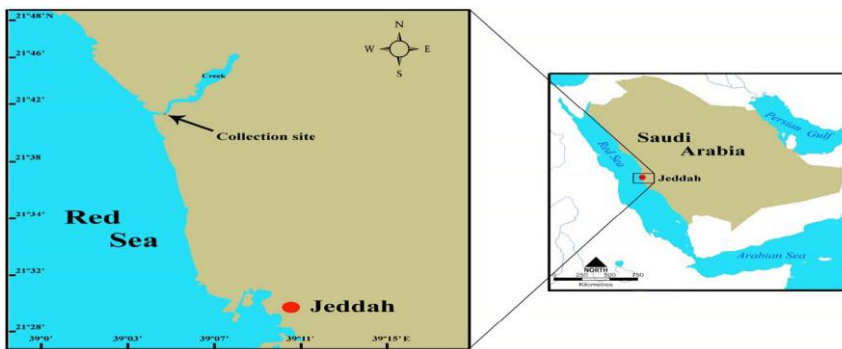
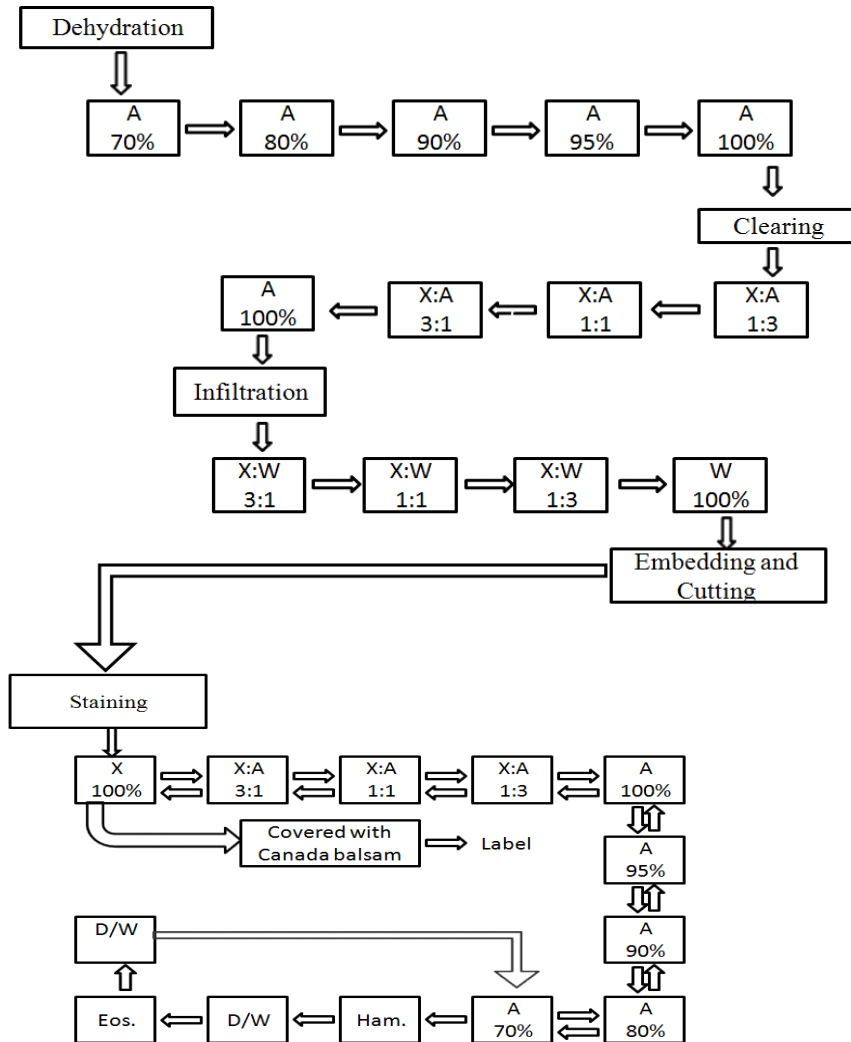


Figure 1 : Map of the Red Sea showing the location of the study site at the entrance of Ubhur Creek (Al-Sofyani and Floos 2013).



X=Xylene , A=Alcohol , Ham.= Haematoxinil , Eos.=Eosin , D/W=Distilled Water
W= Wax

Flowchart 1 : Showing the step by step histology preparation of the sample.

Where X=Xylene, A=Alcohol, Ham.= Haematoxinil , Eos.=Eosin ,
D/W=Distilled Water W= Wax (Floos, 2012)

Results

Temperature

The mean monthly temperature together with the mean maximum and minimum temperatures for each month are shown in ((Fig. 2). The lowest minimum temperature was $24.0 \pm 0.25^{\circ}\text{C}$ in March 2011 and the highest

maximum was $33.5 \pm 0.51^\circ\text{C}$ in August 2011, where the lowest monthly average was 24.5°C in March 2011 and the highest was 33.0°C in August 2011. The mean difference between the maximum and minimum readings was 1.7°C . The annual range of monthly mean temperature was between 24.5 ± 1.06 and $33.0 \pm 0.71^\circ\text{C}$, a difference of 8.5°C .

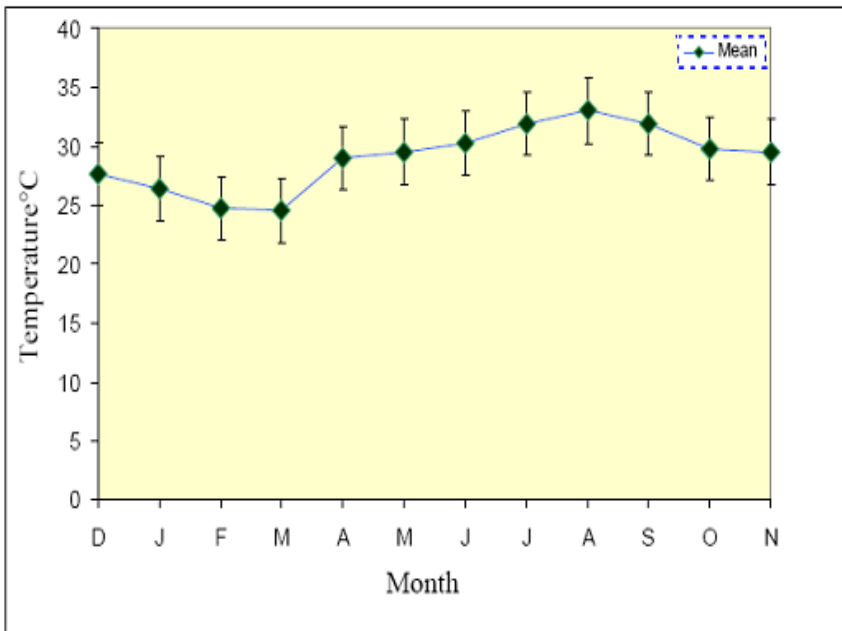


Figure 2 : Monthly variation of seawater temperature ($^{\circ}\text{C}$) at study site at Ubhur Creek depth, from December 2010 to November 2011.

Development of gonads and reproduction period

The histological sample revealed that species *P. verrucosa* is hermaphrodite protogynous gametogenesis where female gonads develop before male gonad (Table 1.). They have very short reproductive season (Table 1.) and (Fig.3). In *P. verrucosa*, small eggs of $26.2 \pm 5.4 \mu\text{m}$ were first observed in March and these increased to a maximum of $152.0 \pm 39.9 (25) \mu\text{m}$ in May. Development of the testes began in April and these increased progressively in size until May and June. In the species the arrangement of ovaries and testes on mesenteries is found on different pairs of mesenteries and they are carried on a stalk (Fig. 4) which is tall and thin in case of the male and short and thick in the female

(Fig. 7) The sperm are spherical in shape in more advanced testes (Fig.8). Small eggs have their nucleus in center, but as they become mature, the nucleus moves towards the surrounding cell wall of the egg (Fig. 5). Zooxanthellae are present in the mature eggs of the *P. verrucosa* (Fig.7). *P. verrucosa* is broadcast spawners, because planulae were not observed in the celenteron of the polyp.

Table 1 :Mean size of eggs and testes (µm) ± SD (n) each month for *P. verrucosa* at the study site from March 2011 to June 2011.

<u>Month</u>	<u>Egg size</u>	<u>Testes size</u>
March		
29/3/2011	26.2 ± 5.4 (5)	-
April		
20/4/2011	43.1 ± 7.8 (15)	18.77 ± 2.9 (8)
30/4/2011	52.6 ± 16.4 (18)	114.3 ± 49. (12)
May		
17/5/2011	69.6 ± 14.7 (18)	152.5 ± 43 (19)
27/5/2011	152.0 ± 39.9 (25)	262.5 ± 29.11 (28)
June		
3/6/2011	69.71 ± 5.79 (7)	

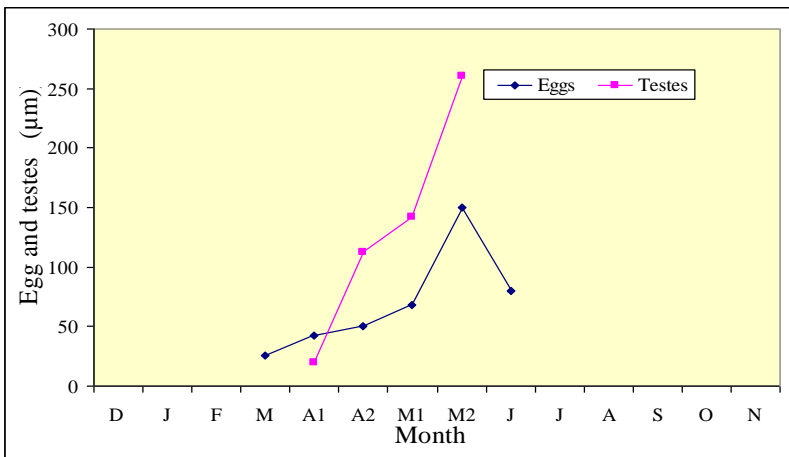


Figure 3 : mean size of oocytes and testes in *P. verrucosa* from December 2010 to November 2011 where, A1= 20/4/2011, A2=30/4/2011, M1=17/5/2011 and M2 27/5/2011.

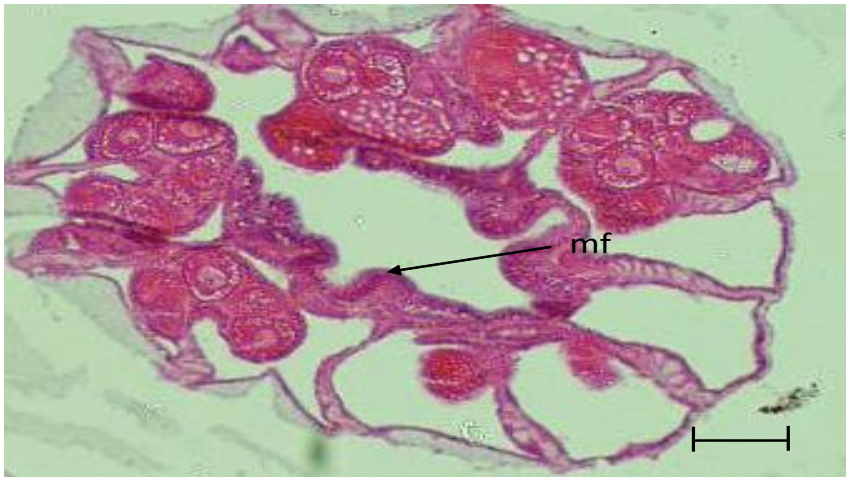


Figure 4 : Histological section through the polyps of *P. verrucosa* showing female gonads , showing mf: mesenteries filament Scale bare = 21.45 μ m.

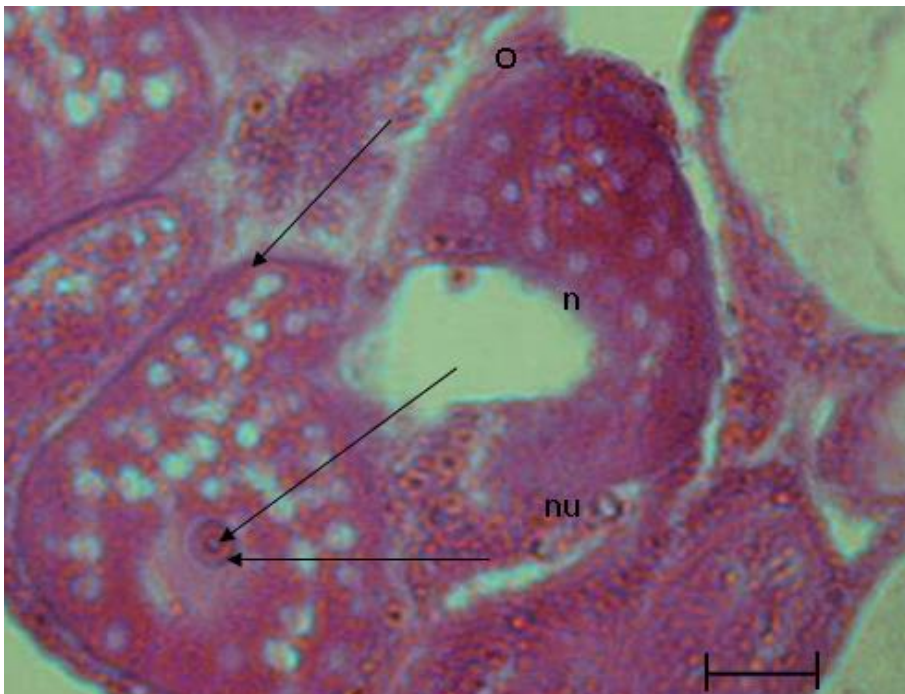


Figure 5 : Histological section through the polyps of *P. verrucosa* showing female gonads. O: oocyte. nu., nucleus; n; nucleolus. Scale bare = 26.45 μ m.



Figure 6 : Histological section through the polyps of *P. verrucosa* showing female gonads on stalks. St: stalk. scale bare = 58.24 μm .

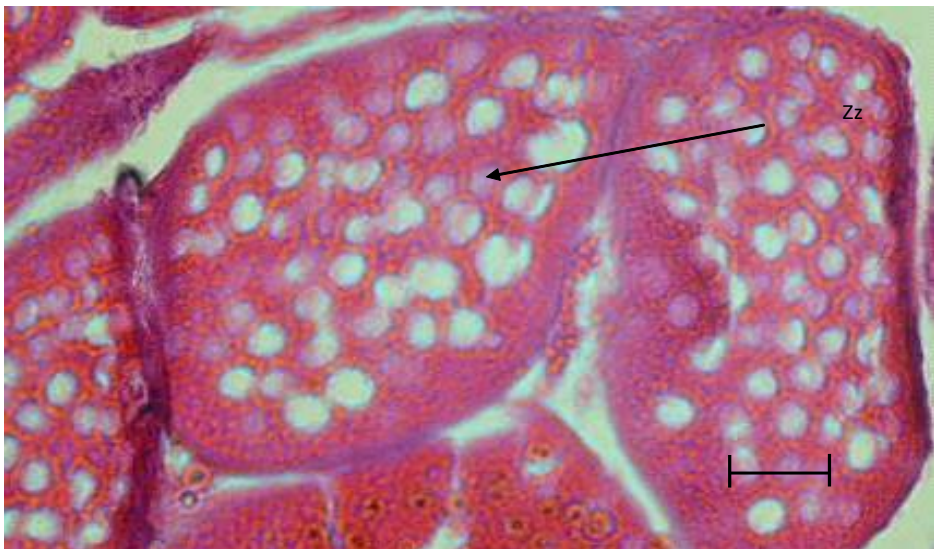


Figure 7 : Histological cross-section through polyp of *P. verrucosa* showing zooxanthellae (zx) in the mature oocytes .Scale bare = 23 μm .

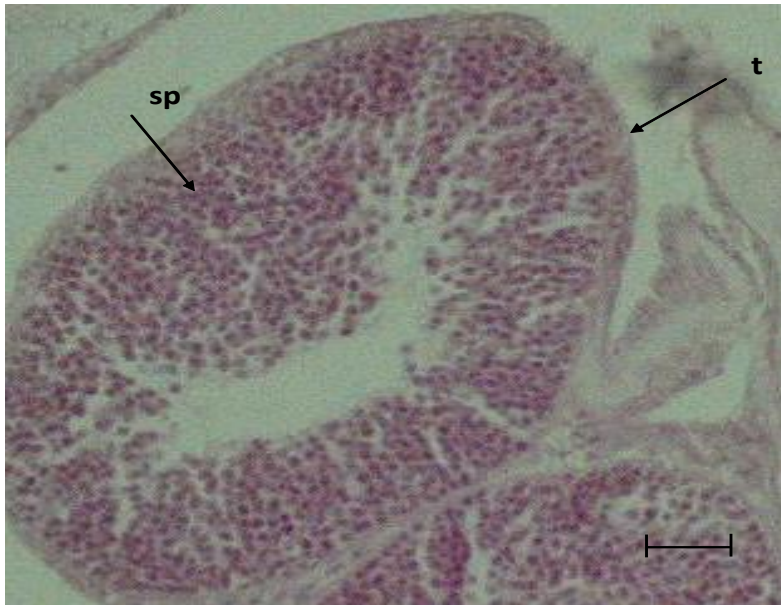


Figure 8 : Histological cross-section through male gonad of *P. verrucosa* showing testes (t). Sp: sperm cells. Scale bare = 26 μ m.

Discussion

The pattern of annual seawater temperature changes at Sharm Ubhur are lowest mean of seawater temperatures at depth 3m (24.5-24.75°C) to occur in Feb. Mar.2011, while the maximum mean of seawater temperature are 30.25 to 33°C seawater temperature. (Floos, 2007) observed the lowest seawater temperatures from February to March 2005 (24.5 -24.75°C) in the Obhur Creek at 3 m depth and the highest seawater temperature from July to August 2005 (30.25 to 33°C). Similarly, (Al-Sofyani, 1991) recorded lowest temperatures during February and March (1990) and the highest in July to October 1990 (30°C to 31°C) respectively. In addition, (Fadlallah, 1985) recorded similar temperatures at Obhur Creek and adjacent Yanbu Coast. Temperature change might be expected to influence the metabolic rate of both animal and plant components of the symbiosis, and the rate of photosynthesis.

Studies on the reproduction of *Pocillopora verrucosa* indicated that is hermaphrodite broadcaster spawner with external larval developments, as embryos of *Pocillopora. verrucosa* was not observed in the histological sections (Table 1) and (Fig. 3). The reproductive strategy of *P.*

verrucosa from the Red Sea was found to be a hermaphrodite broadcaster spawner (Fadlallah, 1985; Shlesinger and Loya, 1985; Kruger and Schleyer, 1998; Al-Sofyani, 1991; Floos, 2007; Floos, 2012 and Floos, et al., 2012), while (Stimson, 1978) reported brooding in *P. verrucosa* from Enewetak. Differences on the reproductive patterns of *P. verrucosa* from different geographic areas were related to differences in many parameters such as colony morphology, polyp size, environmental conditions and habitats (Stimson, 1978; Kojis and Quinn, 1981a ; Rinkevich and Loya, 1979a,b and Tomascik and Sander, 1987), but none of these factors have been shown to give any explanation to these differences (Fadlallah, 1985). In the Red Sea *P. verrucosa* displays no different reproductive pattern among the different study sites) *P. verrucosa* is not the only known broadcasting *Pocilloporidae* as it has been mentioned by (Fadlallah, 1985).

The hermaphrodite brooder reported since in the Red Sea are *Seriatopora caliendrum* (Fadlallah, 1983a and Shlesinger and Loya, 1985), and *Seriatopora hystrix* (Maier et al., 2005 and Maier et al., 2009; Floos, 2012 and Floos, et al., 2012). In worldwide, the *Seriatopora hystrix* was also reported as brooder in the Great Barrier Reef (Ayre and Dufty 1994; Ayre and Hughes, 2000 and Noreen, 2010) and in the northern part of Okinawa Island, Japan (Nozawa and Harrison, 2005).

In the present study, *P. verrucosa* showed similar reproductive mode as in *Pocillopora damicornis*. While The *Stylophora pistillata* and *Seriatopora hystrix* have been reported as a brooding coral species of the Red Sea (Al-Sofyani, 1991; Floos, 2007; Floos, 2012 and Floos, et al., 2012).

The onset of reproductive period of *P. verrucosa* was found to be of very short duration i.e. from April to May 2011 (Figs.3), with negligible number of eggs and testes were observed in the end of March and early June (3-5eggs). *P. verrucosa* was also simultaneous hermaphrodite where oogenesis and spermatogenesis occurring at the same time during April. (Fadlallah, 1985; Shlesinger and Loya, 1985 and Floos, 2007) reported very short reproductive period for *P. verrucosa* at Yanbu north of Jeddah from April to May and at Eilat ,the northernmost tip of the Gulf of Aqaba in the Red Sea from July to August. In the Kwazulu-Notal, South Africa, the reproductive period of *P. verrucosa* lasts for four months from October to January (Kruger and Schleyer, 1998). This short duration of gametogenesis occurred with the increase of

in Sea water temperature (29°C) in April after minimum mean of Sea water temperature from 24.5°C to 24.75°C during Feb. and Mar.2011 with a narrower range of Sea water temperature from 28.5°C min. to 30°C, by the end of reproductive period in May, the mean temperature reached 30.25°C (Fig.2). It appears that gonad development of *P. verrucosa* is stimulated by increasing water temperature. *P. verrucosa* prefer slightly warmer season for their gonads development and this will explain why *P. verrucosa* during the bleaching events in 1998 showed a sign of temperature stress, where *P. verrucosa* was less resistant to extreme temperature and was bleached.

(Fadlallah, 1985) reported that gametogenesis of *P. verrucosa* may be triggered by rising sea water temperature in March and April. Many studies have mentioned many factors influence reproduction among various marine invertebrates specially corals: these are temperature (Jokiel and Guinther, 1978; Fadlallah, 1983a; Richmond and Jokiel, 1984; Hairison et al., 1984 and Al-Sofyani, 1991), lunar tidal cycle (Rinkevich and Loya, 1979b) and day-night cycle (Atoda, 1951b).

In the Red Sea, the South-North gradients of sea water temperature seem to be by far the most powerful factor influencing the reproductive cycle of scleractinian corals (Al-Sofyani, 1987 and Al-Sofyani, 1991).

The egg-sizes of *Pocillopora verrucosa* that was observed; ranged from 26.2 µm in Mar. to 152.0 µm in May (Table 1). (Fadlallah's, 1985) reported for *Pocillopora verrucosa* similar findings to our studies, the maximum diameter of the eggs ranged from 22.1 µm in Mar. to 148.3 µm in May. Zooxanthellae are present in the mature oocytes in *P. verrucosa*. Similar results were in agreement with present study by (Sier and Olive, 1994) who found zooxanthellae infestation have occurred at a later stage in this case. (Harrison and Wallace, 1990) reported that, infestation can occur weeks before spawning, as found in the genus *Porites* and *Montipora* spp or as little as 24 h before spawning as in *Montipora digitata*.

Conclusion

pocillopora verrucosa is hermaphrodite broadcasters with external larval developing. Differences on the reproductive patterns of *P. verrucosa* from different geographic areas were related to differences in many parameters such as colony morphology, polyp size, environmental conditions and habitats. The onset of reproductive period of *pocillopora verrucosa* was found to be limited (March to May). The number of eggs

and testes observed in this period the gonads were found in the polyps. Zooxanthellae are present in the mature oocytes in *pocillopora verrucosa*.

Recommendations

- This study revealed that *P. verrucosa* is not spawning throughout the year but limited to particular months. Their settlement rate also highly challenging. Hence, this is our responsibility to protect the corals by providing suitable substrates for settling.
- Develop a white paper to justify the rationale for an internationally recognized systematic terminology for coral histopathology in consultation with experts in scientific nomenclature.
- Record the normal range of histological characteristics for the priority species of healthy corals in the Red Sea. This would include developing standardized methods to collect corals on a spatial and temporal basis and conduct histology using light microscopy on selected specimens.
- Reef fishing and boat anchoring on reefs should be banned. Separate boat jetty should be arranged (if necessary) in reef areas to avoid reef damage.
- Recreational diving and other related activities should not be allowed in the core reef area.
- Awareness campaign for both public and students will be essential to know the important of coral reefs will provide positive result.

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