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Research Article

Histological characterization of sexual reproduction in the coral *Pocillopora damicornis* (Coelenterata: Scleractinia) from the Red Sea

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Abstract

The scleractinian coral is common along the Red Sea coast, and its reproductive mode and period of reproduction were assessed using histological preparations. the sexuality, and reproductive mod timing of reproductive of *pocillopora damicornis* from adjacent to the fringing reefs of the Ubhur Creek in the Red Sea, were assessed using a serial histological section. Sexual reproduction in *pocillopora damicornis* a shallow water hermatypic coral was studied from December 2011 to November 2012. *pocillopora damicornis* is a simultaneous hermaphrodite with ovary and testis in the project into the body cavity on the same mesentery. Sperm and eggs were usually released simultaneously from the same polyp. The onset of the reproductive period of *pocillopora damicornis* was found to be limited (April to May). In the number of eggs and testes observed in this period, the gonads were found in the polyps. The *pocillopora damicornis* egg size ranged from 49.80 µm (in March) to 125.0 µm (in May). Four stages were chosen, to reflect very immature ovaries, the early stages of oocyte development, ova near maturity, and mature ova, and also four distinct stages of sperm development were identified. The state of gonads development (eg. testis and eggs) was measured by a calibrated eyepiece micrometer of a compound light microscope. Zooxanthellae were presented in the mature oocytes in *pocillopora damicornis*. This study aimed to examine the reproduction mode and timing of *pocillopora damicornis*.

Introduction

Coral reefs are ecosystems as productive as tropical rainforests and biodiversity [1-4]. Reef-forming coral reefs have an important role in underwater life as well as for the many human communities that depend on the ecosystem services they provide. [5,6].

Scleractinian corals show a range of sexuality, there are four basic patterns of sexual reproduction, Knowing among scleractinians such as hermaphroditic broadcast spawners (dominant group), hermaphroditic brooders, gonochoric broadcast spawners, and gonochoric brooders [7,8].

Scleractinian corals can reproduce asexually by fragmentation [9-11], buds, polyp bail-out [9], or sexually by gametogenesis. Free-spawning corals release their gametes into the water column, where fertilization and larval development occur. Brooders release only sperm, with fertilization and maturation of larvae taking place within the polyps of females [8,12,13]. Sexual reproduction in corals has been studied in many locations but research has been concentrated on a few geographical regions. By 1986, detailed information on reproduction in a wide range of coral was available primarily from the Great Barrier Reef (156 species), Northern Western Australia (28 species), the Caribbean (20 species), Red Sea (13 species), Okinawa (11species), Hawaii (10 species) and Palau (10 species) [7]. Coral identified as *Pocillopora damicornis* produces asexual planulae and broadcast spawners gametes in Western Australia [14], while is only a broadcast spawner for the same species in the Eastern Pacific [15]. Similarly *Pocillopora verrucosa* brooders at Enewetak Atoll [9,16] and broadcast spawners in the Red Sea [17-19]. *Pocillopora damicornis* colonies are mostly delicately branched with slender cylindrical branches and large wart-like projections that are often difficult to distinguish

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from true branches, as the two intergrade. It occurs in all shallow water habitats from exposed reef fronts [20]. It has a wide range of distribution from East Africa; to the Red Sea to Mexico and to Ecuador (Batangas, Luzon, Philippines) [21]. Fecundity and the ratio of male to female colonies may be regulated by a variety of internal and external processes [22-24] The reproductive mode may be determined by the coral's morphological traits such as colony size, polyp size oocyte diameter, and gonad location [24-26].

The studied species contribute approximately 60 to 80 of the total living coral cover in the Ubhur Creek in the Red Sea and are therefore very important in this ecosystem. furthermore, the present study adds much-needed data about scleractinian corals' reproduction in the Red Sea. The results are discussed in light of different reproductive pathways, with special emphasis on the question of phylogenetic constraints on gonad parameters and the relationship between reproductive strategy (brooding versus broadcasting) and gonad morphology. This study aimed to examine the reproduction mode and timing of *pocillopora damicornis*.

Material and methods

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 (E39 05 46.11 N 21 42 34.26) at 5m depth (Figure 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 northernmost extremity.

All fieldwork has been carried out underwater by using SCUBA diving techniques

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 5m. Readings monthly, from December 2011 to November 2012. The mean monthly temperature was calculated as the average between the mean maximum and minimum temperature for each month.

Salinity: The salinity of seawater was measured monthly on samples of surface seawater, from December 2011 to November 2012, using a hand-held salinity refract meter (Atago Co. ltd) which could be read to the nearest 1.0 ‰.

Reproduction

Samples for the reproductive study of *Pocillopora damicornis* were taken every morning and every two weeks for the duration of one year starting from December 2011 to November 2012.

Coral specimens were cut off coral colony by chisel and hammer and fixed in a container with 7% seawater formalin for three days. Thereafter, they were transferred to a 10% solution of nitric acid to decalcify the skeleton. The decalcified polyps were washed with distilled water and preserved in a solution of 70 % Ethanol for further histological study. The preserved

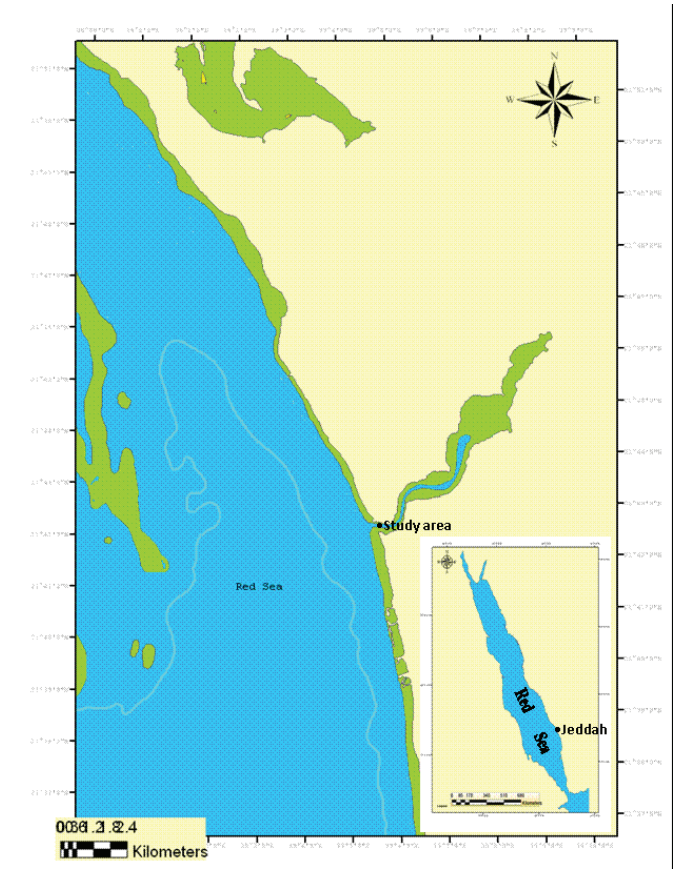

Figure 1: Red Sea map and location of Ubhur Creek, which lies in north of Jeddah city. The study site is marked as (•) at the entrance of Ubhur Creek (Floos, 2012) [27].

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 embedded 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 (Flowchart 1). Thereafter, the stained sections were cleared with xylene and mounted in Canada balsam.

In an attempt to quantify the state of gonad development, the maximum and minimum dimension of a transverse section through a single testis and ovary of six or seven specimens was measured with a calibrated eye-piece micrometer on a compound light microscope. An approximation of gonad size was then obtained from the mean of the maximum and minimum dimensions.

Statistical analysis

I am used one way- ANOVA.

Results

Temperature

The mean monthly temperature together with the mean maximum and minimum temperatures for each month is shown in (Figure 2). The lowest minimum temperature was 24.0 ± 0.25 °C in March 2012 and the highest maximum was 33.5 ± 0.51 °C in August 2012, where the lowest monthly average

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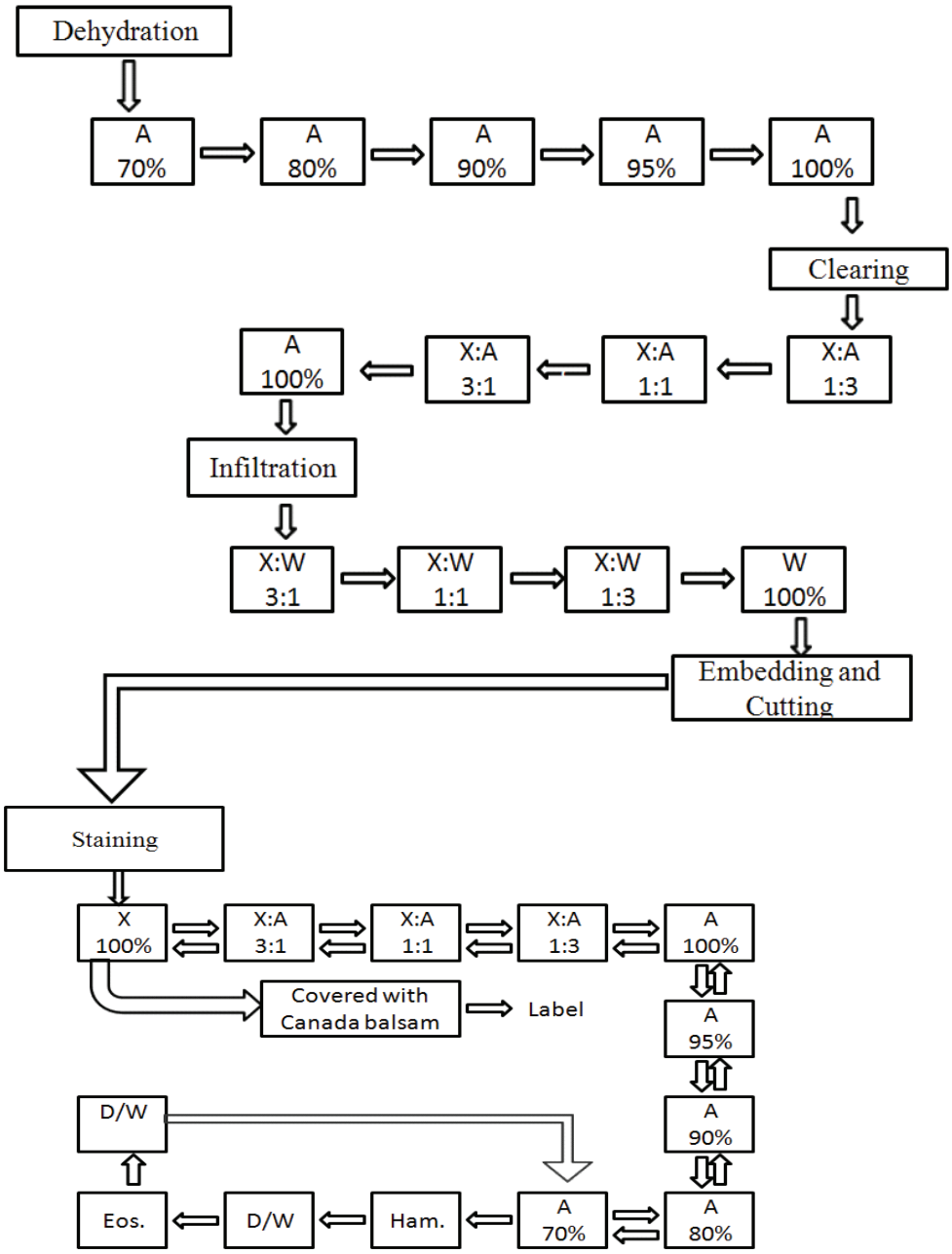

Flowchart : Showing the step by step histology preparation of the sample.
Where X =Xylene, A= Alcohol, Ham.= Haematoxilin , Eos.= Eosin , D/W = Distilled Water W= Wax [27].

was 24.5 °C in March 2012 and the highest was 33.0 °C in August 2012. The mean difference between the maximum and minimum readings was 1.7 °C. The annual range of monthly mean temperature was between 24.6 ± 1.07 and 33.0 ± 0.75 °C, a difference of 8.6 °C.

Salinity

Salinity values ranged from a minimum of 39.0 ± 2.3‰ to a maximum of 41 ± 1.15‰); (Figure 3), the lowest values occurring in the months of April, May, August, and November of the year 2012, and the highest values found through the rest of the year.

Development of gonads and reproduction period

The histological sample revealed that the species *P. damicornis* is a simultaneous hermaphrodite where female and male gonads develop at the same time (Table 1) and (Figure 3). *P. damicornis* has a very short reproductive season (Table 1). In *P. damicornis* the size of the small egg was 49.80 µm ± 7.50 (20) in diameter found in April, then reached its maximum

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of 125.00µm ± 23.00 (20) in May. Development of the testes began in April for species and this increased progressively in size until May (Table 1 and Figure 3). In *p. damcorinis* from the Red Sea is a simultaneous hermaphrodite, with ova and sperm commonly seen in the same polyp. The sperm was spherical in shape in more advanced testes (Figure 5D). Small eggs have their own nucleus in the center, but as they become mature, the nucleus moves towards the surrounding cell wall of the egg (Figure 4 A, D). Zooxanthellae are present in the mature eggs of the species (Figure 4D). The species were broadcast spawners, as planulae were not observed in the coelenteron of the polyp.

Oogenesis

Four stages were chosen, to reflect: very immature ovaries, the early stages of oocyte development, ova near maturity, and mature ova.

Stage I (Figure 5A). Multiple oocyte ovary; oocytes < 30 µm (smallest oocyte were 10 µm in diameter and had a germinal vesicle with a prominent nucleolus. Only a few yolk granules were present, located at one side of the oocyte

Stage II (Figure 5B). Single oocyte ovary; <50 µm although usually >30 µm diameter and Yolk granules were arrayed in a circle around the germinal vesicle

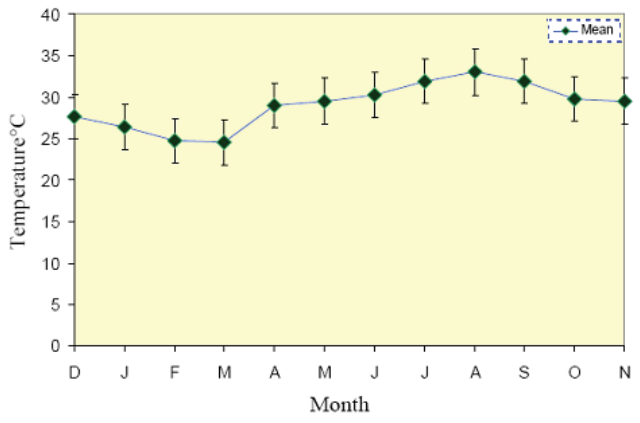

Figure 2: Monthly variation of seawater temperature (Co) at study site at Ubhur Creek depth, from December 2011 to November 2012.

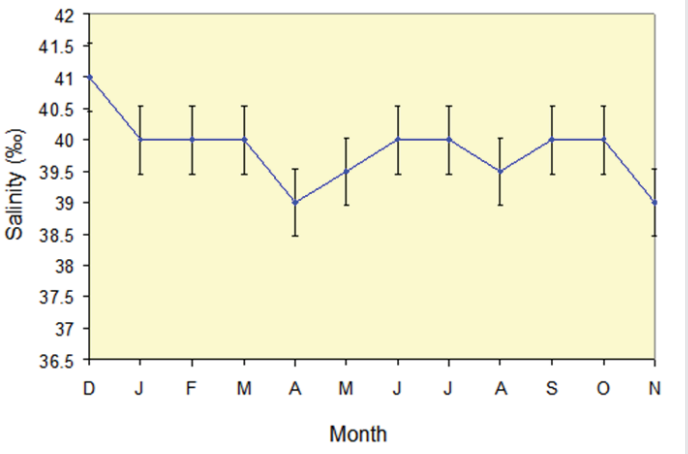

Figure 3: Monthly variation of surface seawater salinity (‰) at study site in Sharm Ubhur, from December 2011 to November 2012.

Table 1: Mean size of eggs and testes (µm) ± SD (n) each month for *P.damicornis* at the study site from March 2012 to June 2012.

Month	Egg size	Testes size
March		
29/03/2012	-	-
April		
15/04/2012	49.80 ± 7.50 (20)	29.7 ± 8.6 (20)
26/04/2012	65.30 ± 8.50	27.8 ± 1.5 (20)
May		
09/05/2012	65.20 ± 4.13 (20)	89.28 ± 19.70 (20)
30/5/2012	125.00 ± 23.00 (20)	177.80 ± 35.90 (20)
June		
9/6/2012		156.80 ± 27.18 (10)

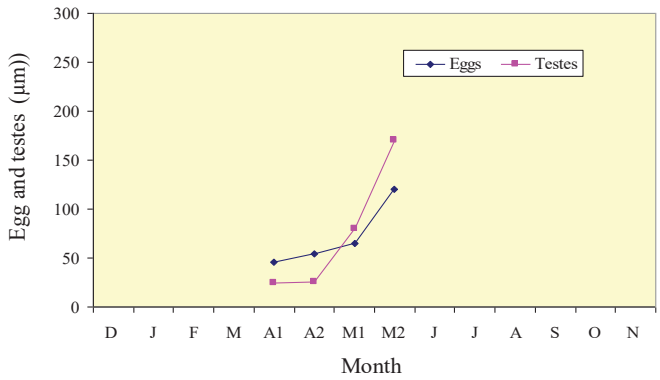

Figure 4: Mean size of oocytes and testes in *P. damicornis* from December 2011 to November 2012 where, A1= 15/4/2012, A2=26/4/2012, M1=9/5/2012 and M2=30/5/2012.

Stage III (Figure 5C). Oocytes from 50 to 70 µm diameter; nucleolus usually well developed and intensely staining, occasionally dispersed throughout the nucleus; granular cytoplasm invested with numerous small vacuoles. A vitelline membrane or cortical layer was observed at the surface of the oocyte

Stage IV (Figure 5D). Oocytes were about 100 µm in diameter usually surrounded by a darker membrane and contracted slightly from the ovary wall; cytoplasmic vacuoles enlarged; nucleus and nucleolus less obvious than in the previous stage.

Spermatogenesis

Four distinct stages of sperm development were identified.

Stage I (Figure 6A). Spermaria usually < 30 µm diameter primary spermatocytes surrounded by a thickened spermatogonial wall

Stage II (Figure 6B). Testes of varying size (up to 200 µm) secondary spermatocytes with a characteristic hollow circle appearance.

Stage III (Figure 6C). large (usually >100 µm diameter) Testes; densely staining spermatids; Testis not vacuolated.

Stage IV (Figure 6D). Mature sperm with tails: the middle

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of the testes is often vacuolated or the whole testes loosely packed with sperm. Stage 1V sperm is sometimes observed free in the coelenterons, although this may be due to histological technique in the field or aquaria.

P. damicornis the arrangement of ovaries and testes on mesenteries was found on different pairs of mesenteries and they were carried on a stalk which is tall and thin in the case of the male and short and thick in the female illustrated in Figures 5,6 respectively."

Small eggs have their own nucleus in the center, but as they become mature, the nucleus moves toward the surrounding cell wall of the egg illustrated in (Figure 5 A, D). Zooxanthellae are present in the mature eggs of the species illustrated in (Figure 5D). The sperm was spherical in shape in more advanced tests illustrated in (Figure 6D).

The species was broadcast spawners, as planulae were not observed in the coelenteron of the polyp. Oocytes and testes developed in mesenteries and were enveloped in mesoglea and gastrodermis. Mature oocytes and testes protruded into the gastrovascular cavity and were connected to a mesentery by a stalk.

Discussion

Studies on the reproduction of *Pocillopora damicornis* indicated that species were hermaphrodite broadcaster spawners with external larval developments. Embryos of species were not observed in the histological sections (Table 1) and (Figure 5). The reproductive strategy of *P. verrucosa*

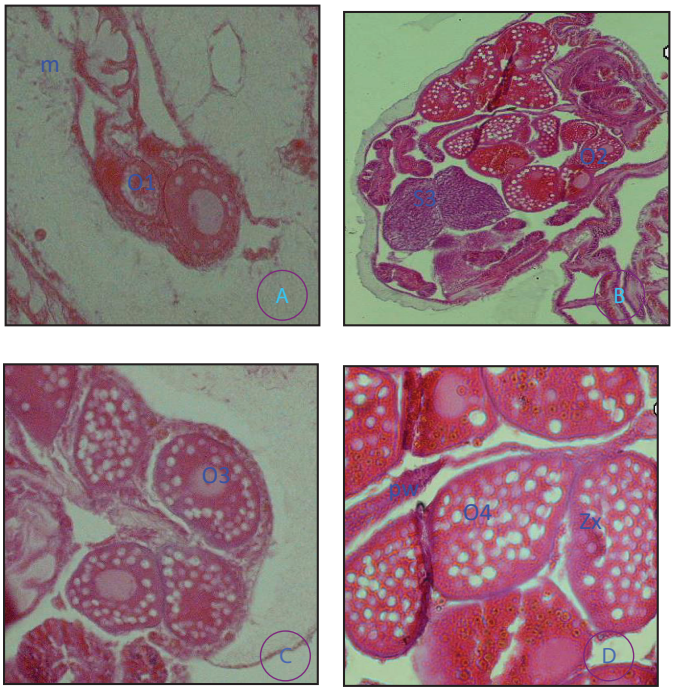

Figure 5: *Pocillopora damicornis* oocyte development. A : Stage 1 and Stage II oocyte embedded in the same mesentery; B Stage II oocytes . C Stage III oocytes undergoing vitellogenesis . D Mature Stage IV oocytes infested with zooxanthellae (m mesentery; O1 Stage I oocyte : O2 Stage II oocyte ; O3 Stage III oocyte: O4 muture Stage IV oocyte; pw: Polyp Wall; zx: Zooxanthellae.

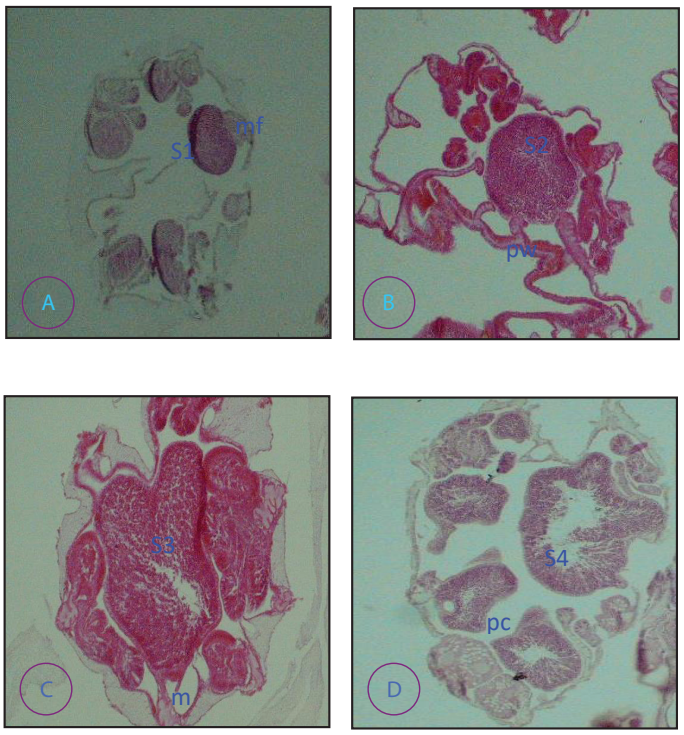

Figure 6: *Pocillopora damicornis* spermary development. A: Stage 1 spermary with spermatogonia (1000x); B Stage II spermary with spermatocytes (1000x); C Stage III spermaries with spermatids arranged on the periphery of the spermaries (250x). D Mature Stage IV spermaries and oocytes in the polyp (250x) (m mesentery; mf mesentery filament; pw: Polyp Wall; S1 Stage I spermary: S2 Stage II spermary; S3 Stage III spermary; S4 mature Stag IV spermary.

from the Red Sea was found to be a hermaphrodite broadcaster spawner [9], while Stimson [16] reported brooding in *P. verrucosa* from Enewetak. On the other hand, most studies on *P. damicornis* from different geographic locations proved that *P. damicornis* is a hermaphrodite brooder [14,28-30] Similar results were in agreement with the present study by Glynn, *et al*, [15] who found a hermaphrodite broadcaster spawner in the eastern Pacific However, in Western Australia *P. damicornis* includes both a brooder and broadcaster spawner [14].

Differences in the reproductive patterns *of P. damicornis* from different geographic areas were related to differences in many parameters such as colony morphology, polyp size, environmental conditions, and habitats [16,31]. None of these factors have been shown to give any explanation for these differences [32]. In the Red Sea *P., verrucosa* displays no different reproductive pattern among the different sites studied (e.g. North of Agaba Gulf, Yanbu, and Jeddah in the present study), whilst *P. damicornis* has a similar reproductive mode as in *P. verrucosa* in the Red Sea, but there are no comparative studies on *P. damicornis* from the Red Sea so that *P. verrucosa* is not the only known broadcasting *Pocilloporidae* as mentioned by Fadlallah [32].

p. damcorinis from the Red Sea is a simultaneous hermaphrodite, with ova and sperm commonly seen in the same polyp. Ovaries were situated on stalks projecting into the coelenterons, as reported for *p. damcorinis* from the Great Barrier Reef [33]. However, testes appeared as an outgrowth

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of mesenteries, although this was not always apparent in the transverse section of polyps as not all of a large testis was connected to the mesentery. Sex pairs of gonads were present within polyps and sperm substantially outnumbered ova in terms of the volume occupied within a polyp, and the number of the polyp with gonads of one sex. Oocytes were classified into 4 stages based on morphology and size (see Figure 5) Stoddart and Black [34].

The onset of the reproductive period of *P. damicornis* was found to be of very short duration i.e. from April to May 2012 (Figure 4), with a negligible number of eggs and testes observed at the end of March and early June (3-5 eggs). These species were also simultaneous hermaphrodites where oogenesis and spermatogenesis occurred at the same time during April. Fadlallah [32] and Shlesinger and Loya [17] noticed a 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. This short duration of gametogenesis occurred with the increase of seawater temperature (29 °C) in April after the minimum average of seawater temperature from 24.5 °C to 24.75 °C during Feb. and Mar.2012 with a narrower range of seawater temperature from 28.5 °C min. to 30 °C, by the end of the reproductive period in May, where the mean temperature reached 30.25 °C (Figure 2) [35].

There is annual variation in salinity during the period of this study from 39‰ to 41‰ (Figure 3), while AL- Sofyani [36] showed a similar range of 39‰ to 40.5‰ in Sharm Ubhur over a broad period of 2 years. The increase from 39‰ to 40‰ in the summer months is probably due to surface evaporation and to the action of the northwesterly winds driving the more saline water from the northern Red Sea to more southerly latitudes [36-38].

Gonad development of *P. damicornis* is stimulated by increasing water temperature. These species prefer a slightly warmer season for their gonads development and this will explain why this species during the bleaching events in 1998 exhibited a sign of temperature stress *P. damicornis* and was bleached, whereas *P. verrucosa* was more resistant to extreme temperature. Fadlallah [32] reported that gametogenesis of *P. verrucosa* may be triggered by rising sea water temperature in March and April. Many suggested factors influence the reproduction among various marine invertebrates, especially corals: these are temperature [39-41], lunar tidal cycle, and day-night cycle [25].

In the present study, *P. damicornis* showed a similar reproductive mode as in *Pocillopora verrucosa*. While The *Stylophora pistillata* and *Seriatopora hystrixhave* have been reported as brooding coral species of the Red Sea [18,19,27,36,42].

In the Red Sea, the South-North gradients of seawater temperature seem to be by far from the most powerful factors influencing the reproductive cycle of scleractinian corals [36,43]. The egg sizes of *P. damicornis* ranged from 49.50 µm in April. to 125µm in May (Table 1). Sier and Olive [44] found zooxanthellae infestation at a later stage in this case, while Harrison and Wallace, 1990 mentioned that, an infestation can occur weeks before the spawning, as observed in the genus *Porites* and *Montipora* sp. or as little as 24 h before spawning as in *Montipora digitata.*

Conclusion

pocillopora damicornis are hermaphrodite broadcasters with external larval development. Differences in the reproductive patterns of *pocillopora damicornis* from different geographic areas were related to differences in many parameters such as colony morphology, polypsize, environmental conditions, and habitats. The onset of the reproductive period of *oscillopsia damicornis* was found to be limited (April to May). In 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 damicornis*

Recommendation

- This study revealed that is not spa ***pocillopora damicornis*** winning throughout the year but limited to particular months. Their settlement rate is 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. A 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 importance of coral reefs will provide a positive result.

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