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Gametogenesis Cycle, Spawning Time, and Gametes Microstructure of Two Acroporid Species (*Acropora digitifera* and *Acropora gemmifera)* from the Red Sea, Egypt

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ABSTRACT

Synchronization and gonadal development of broadcasting acroporid species (Acropora digitifera and Acropora gemmifera) were studied in northern Hurghada, the Red Sea. Histological investigations of the gonads were examined every month for approximately two successive spawning seasons. Forty tagged colonies were collected from the two selected coral reef sites in Hurghada. Coral branches were collected from March 2019 to March 2020 for dissection and histological examination. Small living colonies of both studied species were collected and put in plastic aquaria to observe the synchronization of the studied species between the colonies in the field and the aquaria. The current study revealed that *A. digitifera* and *A. gemmifera* colonies were broadcast species with one annual gametogenic cycle (10 months), which started in July 2019 and ended in March 2020 for Oogenesis, while spermatogenesis developed in 7 months. Both species exhibited prominent synchronization between their colonies in both aquaria and the field. The spawning timing of *A. digitifera* and *A. gemmifera* was affected mostly by surface sea temperature, the lunar cycle, and photoperiod (day length). They released their egg-sperm bundles around the April 2019 full moon. Both studied species spawned two days before the April 2019 full moon (17 April 2019). This study represents the first integrated work employing multiple techniques to accurately detail the gonad development and spawning timing of acroporoid corals in the Red Sea. It is also the first to describe the structure of their egg-sperm bundles and the process of spermatogenesis in this region.

INTRODUCTION

Coral reefs are considered marine forests with high productivity and diversity among different ecosystems on this planet. Scleractinian corals are one of the main structural architects of reefs. They afford shelter and food for numerous other marine organisms. The attribution of reef resilience depends on their capacity to sustain or produce a genetically diverse population through sexual reproduction **(Rashad *et al*., 2020)**. The

main reproductive pattern of scleractinian corals in the Red Sea is the release of egg-sperm bundles in the water column followed by external fertilization **(Hanafy *et al*., 2010; Rashad *et al*., 2020)**.

The spawning timing and synchronization within and between reef species can vary extensively among sites and along latitudinal inclines. For example, **Willis *et al.* (2006)** described this phenomenon on a single reef at the Great Barrier Reef, where 30 species spawned their gametes within hours of each other, and more than 150 species released their gametes after one week of October and November full moons. Multi-species synchronous spawning was observed in 2008 and 2009 at two sites in the northern Red Sea, Hurghada. All sampled colonies in early April and late May of 2008 had no oocytes, indicating that all colonies released their gametes a few days after the full moon of April 2008. Hurghada species were observed to mature in late April, and all were empty in early May 2009 **(Hanafy *et al*., 2010)**. Broadcast coral species have an annual gametogenic cycle and participate in multi-species spawning events **(*Guest et al.*, 2012)**. The coral spawning documented in almost all regions was focused on a particular time of the year **(Baird *et al.*, 2009; Rashad *et al*., 2020)**.

Gametogenesis of scleractinian corals is controlled by different environmental factors such as sea surface temperature and day length (photoperiod), while the maturation of gonads to be ready to spawn is under the control of the lunar cycle, and the release of the spawning products is usually under the control of the time of sunset **(Wai Shan, 2010; Shakara, 2015; Rashad *et al*., 2020)**. The northern Egyptian Red Sea region is characterized by high coverage of the genus *Acropora* among coral genera **(Ghallab *et al*., 2020)**.

Most previous reproductive biology studies focused on determining the spawning timing through field observation, while they poorly covered the gametogenic cycle and synchronization within and between species. Therefore, this study was conducted to follow the gametogenic cycle, synchronization, spawning timing, and egg-sperm bundle structure of two abundant acroporid corals, namely, *Acropora digitifera* and *Acropora gemmifera*, located in the northern Red Sea, Hurghada, Egypt.

MATERIALS AND METHODS

Investigation sites

Samples of coral reef branches were collected for histological work from two study sites during predicted spawning months and *in situ* coral spawning observation from March 2019 to March 2020. (27º09'99"N & 33º85'24"E), which is 15 kilometers southeast of Small Giftun Island and east of Hurghada Port, and Small Giftun Island (27º

11'09" N & 33º58'53"E), which is east of Hurghada (Fig. 1). The study sites were chosen because they have high coverage of coral reef species.

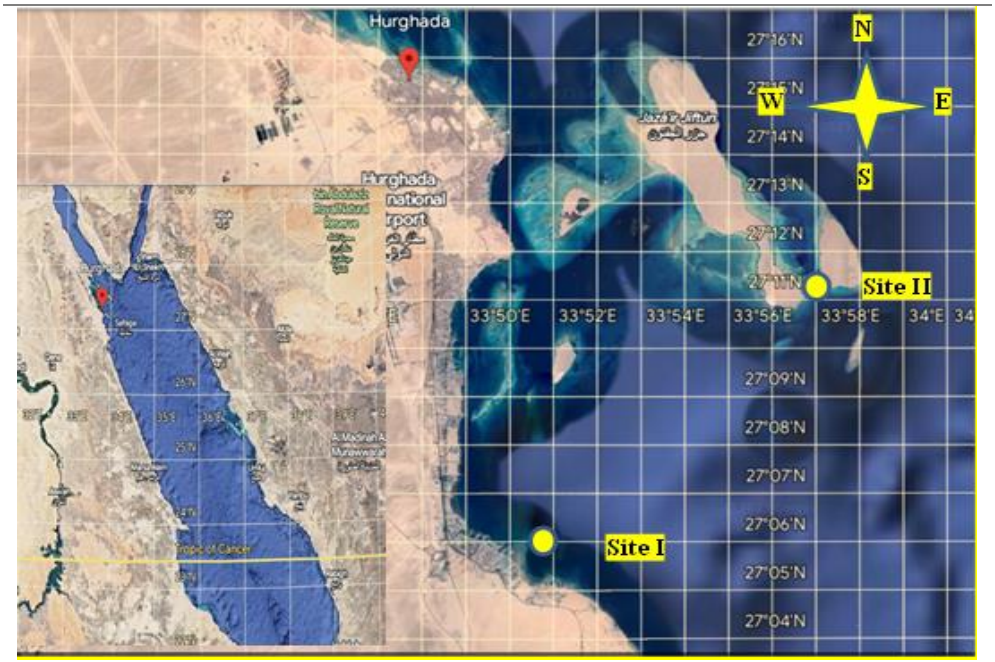


Fig. 1. Location of study sites for coral sampling and coral reef spawning observation (Remevyera Resort Site I and Small Giftun Island Site II), Hurghada, Egypt

Variations in ecological factors at study sites

Several environmental parameters influence coral reef spawning and gametogenesis, including sea surface temperature (SST), day length (photoperiod) (hrs), salinity (ppt), dissolved oxygen (DO). These environmental factors were found to correlate with different stages of the reproductive cycle as follows: sea surface temperature and day length (photoperiod) were associated with the control of gametogenesis **(Nozawa, 2012; Sakai *et al.*, 2020; Gouezo *et al.*, 2020)**, while twilight spectra (lunar cycle) regulated the release of egg–sperm bundles **(Gouezo *et al.*, 2020)**. Additionally, sunset time was identified as a key cue for egg–sperm bundle release **(Gouezo *et al.*, 2020; Hanafy *et al.*, 2010)**.

Surface seawater temperature, dissolved oxygen (DO), and salinity of the water column at the study sites were measured using a HANNA multi-parameter meter (HI 9892). Measurements were taken at a 2-meter depth during the full moon week of each month throughout the study period.

Coral reef species sampling and histological analysis for coral spawning prediction

Ten coral colonies were tagged with plastic plates at each study site. To effectively monitor the gametogenic cycle, only colonies of *Acropora digitifera* and *Acropora gemmifera* exceeding 30cm in diameter were selected, ensuring sufficient tissue for repeated sampling. Species-level identification of the studied *Acropora* species was initially performed based on external morphological features using Veron's identification guide **(Veron, 2000)**. This was followed by confirmation under a dissecting microscope, focusing on differences in axial corallite and axial polyp characteristics as described by **Rashad *et al.* (2020)**. All coral tagging, sampling, and monitoring activities were conducted via snorkeling.

From March 2019 to March 2020, small coral fragments (>5 cm in length) were collected from both *Acropora* species during the full moon week (three days before to three days after the full moon) and preserved for histological analysis to determine their gametogenic cycle. Additionally, at each study site, one colony from each species was enclosed with a plankton net to collect egg–sperm bundles upon release, allowing for microstructural investigation and determination of spawning timing. The study start date was based on a year-long preliminary observation of spawning patterns and informed by previous studies **(Harrison *et al.*, 1984; Mangubhai & Harrison, 2008a; Kongjandtre *et al.*, 2010)**.

Coral fragments collected in the field were returned to the laboratory and fixed in 10% seawater formalin for 24 hours. The skeletal material was removed by soaking the samples in 10% buffered HCl for 4–24 hours, depending on the structure and density of the calcium carbonate skeleton **(Hanapiah *et al.*, 2020)**. Samples were then processed using a Thermo Scientific automated tissue processor. Once dehydrated and cleared, they were embedded in paraffin wax and sectioned at a thickness of 7 μm using a Thermo Scientific microtome. Two sections from each sample were mounted on slides, stained with hematoxylin and eosin, and examined using an Olympus CX31 compound microscope connected to Olympus Soft Imaging Solutions. Observations were made at magnifications ranging from ×10 to ×100 using three different imaging software packages. The presence of oocytes was recorded and counted twice in the mesenterial filaments of each sample.

Coral specimen preparation for structural organization of *A. digitifera* and *A. gemmifera* egg-sperm bundles using a Transmission Electron Microscope (TEM)

Transmission Electron Microscopy (TEM) was used to investigate the structure and organization of egg–sperm bundles in *Acropora digitifera* and *A. gemmifera*. Small pieces of fresh specimens were carefully excised and immediately fixed in 4F1G (4%

formaldehyde, 1% glutaraldehyde) in phosphate buffer solution (pH 7.2) at 4°C for 3 hours. The specimens were then post-fixed in 2% osmium tetroxide (OsO_4) in the same buffer at 4°C for 2 hours. After thorough rinsing with the buffer, samples were dehydrated at 4°C through a graded acetone series. The dehydrated samples were then embedded in resin for polymerization. Ultrathin sections (~90 Å) were cut, mounted on copper grids, and stained with uranyl acetate for 5 minutes followed by lead citrate for 2 minutes. The prepared sections were examined using TEM to observe the ultrastructural organization of the gametes **(Tahmasebi *et al.*, 2015)**.

Statistical analysis

Data were coded and entered using SPSS version 22. The assumptions of parametric testing were evaluated using the Shapiro-Wilk and Kolmogorov-Smirnov tests to assess the normality of continuous variables. Data are presented as means ± standard deviations.

One-way ANOVA was used to analyze differences in physical parameters, oocyte diameters, and sperm diameters. Where significant differences were found, post hoc comparisons were performed using Tukey's pairwise test. A *P*-value of <0.05 was considered statistically significant. Additional data visualization was carried out using RStudio version 2022.02.4. Statistical analysis for some comparisons was also performed using MiniTab version 14.

RESULTS

Histological examination of tissue sections showed that oocytes and spermaries of *Acropora digitifera* and *A. gemmifera* developed on separate mesenteries, consistent with the reproductive pattern described in other *Acropora* species. Oogenesis lasted approximately 9–10 months at both study sites, while spermatogenesis was completed in 6–7 months.

The Process of Oogenesis

From March 2019 to March 2020, histological analysis of tagged colonies of *A. digitifera* and *A. gemmifera* revealed four distinct gametogenic stages (I, II, III, and IV) of oocyte development. Each stage persisted for approximately 2–3 months in the mesenterial tissues (Fig. 2 & Table 1).

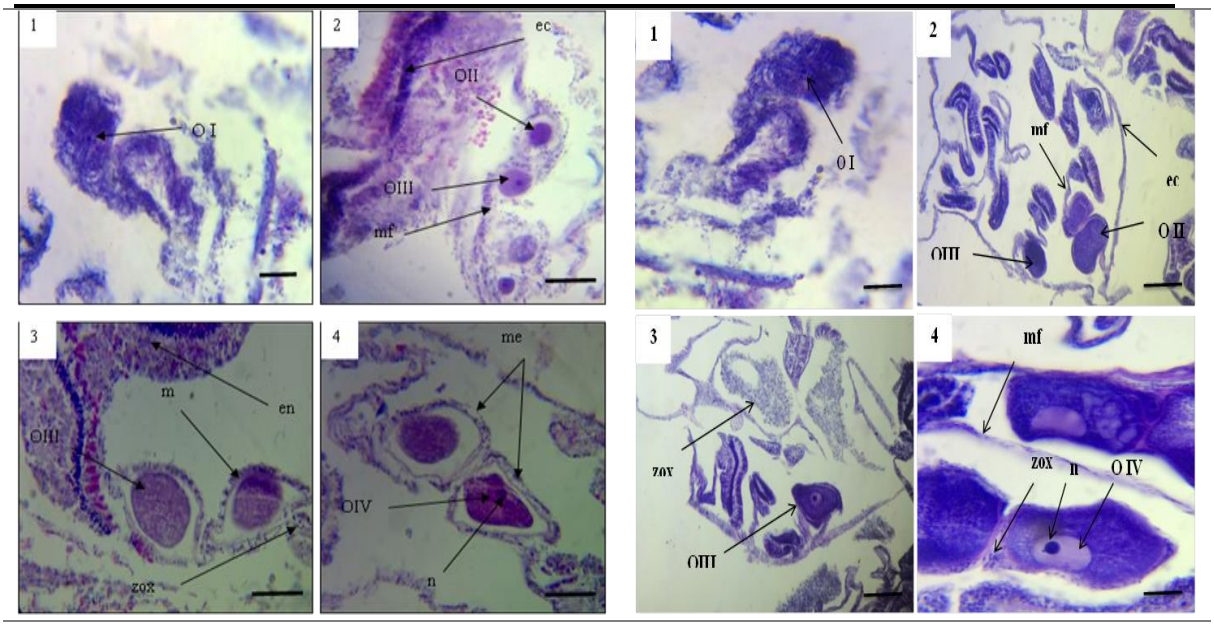
Stage I oocytes were first observed in mid-July 2019, located in the mesoglea or endodermal tissue adjacent to the mesoglea (Fig. 2A.1, B.1). These early-stage oocytes appeared as oval structures with a thin cytoplasmic layer. The nucleus stained reddish-

pink, the nucleolus bright red, and the surrounding cytoplasm gray-blue to purple. The peak of Stage I formation was recorded in September 2019 (Table 1). Maximum diameters (mean ± SD) of Stage I oocytes ranged from 60.89 ± 5.15 to 130 ± 12.67µm for *A. digitifera* and from 70.34 ± 2.85 to 150.79 ± 17.58µm for *A. gemmifera* (Tables 2, 3).

Stage II oocytes, observed from October 2019, were fully enclosed within the mesoglea and surrounded by granular cytoplasm. Their appearance and staining characteristics remained similar to those in Stage I (Fig. 2A.2, B.2). Diameters ranged from 56.59 ± 21.98 to 130 ± 12.67µm for *A. digitifera* and from 150.79 ± 12.67 to 229.72 ± 17.64µm for *A. gemmifera*. The maturation peak for Stage II was noted in late November 2019 to early December 2020 (Table 1).

Stage III oocytes reached their maturation peak in February 2020. These oocytes varied in shape—round, oval, or irregular—and exhibited cytoplasm with a fine-grained grey-blue patch near the nucleus (Fig. 2A.3, B.3). For *A. digitifera*, diameters ranged from 268.64 ± 28.11 to 305.39 ± 14.62µm, while for *A. gemmifera*, they ranged from 266.03 ± 10.91 to 347.82 ± 53.76µm (Tables 2, 3). At this stage, zooxanthellae were present in adjacent endodermal tissue but not yet inside the oocytes.

Stage IV oocytes—large, irregularly shaped, and often appearing squashed together—were first recorded in March 2019 and 2020, with maturation peaks occurring in April during the full moon week (Fig. 2A.4, B.4 & Table 1). Oocyte diameters ranged from 361.82 ± 51.93 to 459.93 ± 31.93µm for *A. digitifera* and from 338.23 ± 34.16 to 479.34 ± 12.37µm for *A. gemmifera*. A key feature of this stage was the presence of zooxanthellae within the oocyte cytoplasm. The nucleus migrated toward the periphery of the cell, becoming saddle-shaped. The cytoplasm appeared to retract from the nucleus, forming an empty halo surrounding it.



A **B**

Fig. 2. Transverse sections in *A. digitifera* (A) and *Acropora gemmifera* (B) soft tissue show oocyte development. 1) stage I oocyte in the mesenterial filament of the endoderm, 2) stage II and stage III oocytes, 3) stage III oocytes, 4) stage III and IV oocytes. Abb: ec = ectoderm, me = mesoglea, mf = mesenterial filament, n = nucleus, o = oocyte, o II = stage II oocyte, o III = stage 3 oocyte, o IV = stage IV oocyte, zox = zooxanthellae. Scale bar: (1, 4) = 200 µm; (2, 3) = 100 µm. Sections stained with HX- eosin- Orange G.

The process of spermatogenesis

Histological examination of both *Acropora digitifera* and *A. gemmifera* revealed that Stage I spermaries appeared as small bundles of 5–10 cells, either adjacent to or engulfed by the mesoglea, and were first observed in October 2019 (Fig. 3A.1, B.1). The formation peak of this stage was recorded in December 2019 (Table 1). The mean diameter (±SD) of Stage I spermaries ranged from 39.13 ± 8.39 to 73.26 ± 7.30µm for *A. digitifera*, and from 41.79 ± 11.07 to 74.67 ± 7.55µm for *A. gemmifera* (Tables 2, 3).

Stage II spermaries measured 73.26 ± 7.30 to 100.35 ± 11.13µm in *A. digitifera*, and 74.67 ± 7.55 to 98.81 ± 11.21µm in *A. gemmifera* (Fig. 3A.2, B.2). These testes were surrounded by mesoglea and appeared to enlarge due to the migration of primary spermatocytes from the endodermal tissue or through cell division. The peak formation of this stage occurred in early January 2020 (Table 1).

Stage III spermaries measured between 100.35 ± 11.13 and 134.76 ± 24.35µm in *A. digitifera*, and from 98.81 ± 11.21 to 132.76 ± 25.98 µm in *A. gemmifera* (Fig. 3A.3, B.3 & Tables 2, 3). At this stage, cell proliferation and migration toward the periphery of the

testis resulted in the formation of a central lumen. The peak formation of Stage III was observed in late February 2020 (Table 1).

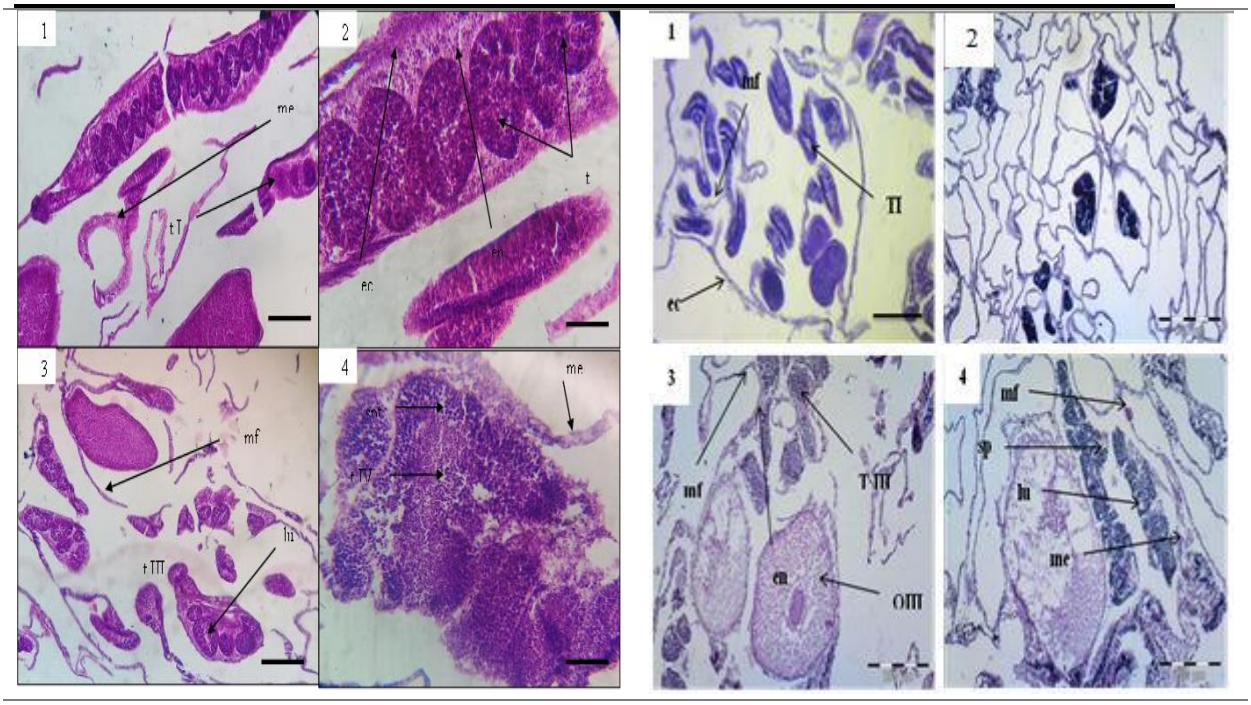
Stage IV spermaries were first detected in March 2019 and reached their maturation peak in April 2019. In this stage, spermatocytes had completed division and appeared smaller than those in Stage III (Fig. 3A.4, B.4). The mean diameter of Stage IV testes ranged from 134.76 ± 24.35 to 183.59 ± 9.45μm in *A. digitifera*, and from 132.76 ± 25.98 to 175.87 ± 11.27μm in *A. gemmifera* (Tables 2, 3). Nuclei were condensed, cells stained dark magenta, and tails light pink. Testes were typically oval, teardrop-shaped, or irregular, with spermatozoa aligned in a bouquet-like arrangement, tails pointing in the same direction.

All coral colonies examined were fully mature and ready to spawn during the April full moon week.

Summary of gametogenic cycle

The present study revealed that gametogenesis in female gonads of both *A. digitifera* and *A. gemmifera* lasted approximately 10 months, beginning in July 2019 and ending in April 2020. In contrast, male gametogenesis spanned around 7 months, from October 2019 to April 2020. Both species exhibited a single annual gametogenic cycle, with a peak in reproductive maturity during the full moon week in April.

The mean diameters of oocytes and spermaries for both *A. digitifera* and *A. gemmifera* throughout their developmental stages are illustrated in Fig. (4A, B), respectively.



A **B**

Fig. 3 (A). *A. digitifera* soft tissue histological section.1) In the endodermal mesentery, the horizontal section contained stage I testes, 2) the horizontal section contained stage II and stage III testes, 3) the male gonad contained stage IV testes, and 4) the mature polyp contained stage IV testes. Abb. ec = ectoderm, en = endoderm, lu = lumen, me = mesoglea, mf = mesenterial filament, spt = spermatids, t = testes, t I = stage I testes, t II = stage II testes, t III = stage III testes, t IV = stage IV testes. Scale bar, (1, 3) = 500µm, (2, 4) = 200 µm. Sections stained with Hx-eosin- Orange

(B). A soft tissue histological section of *A. gemmifera* is shown.1) A cross-section of the endodermal mesentery contained stage I testes, 2) a cross-section of the male gonad contained stage II and stage III testes, 3) a horizontal section of the male gonad contained stage IV testes, and 4) a horizontal section of a mature polyp contained stage IV testes. ec = ectoderm, en = endoderm, lu = lumen, me = mesoglea, mf = mesenterial filament, sp = sperm, t I = stage I testes, t II = stage II testes, t III = stage III testes, t IV = stage IV testes. Scale bar, (1, 2) = 500µm, (3, 4) = 200 µm. Sections stained with Hx-eosin-Orange G.

Table 1. Dates of development stages and spawning of *Acropora digitifera and Acropora gemmifera* gametes observed at the two study sites over two years (2019 and 2020)

Date	*Acropora digitifera*				*Acropora gemmifera*				Egg color
	Site I		Site II		Site I		Site II		
	Oocyte stages (n)	Spermary stages (n)	Oocyte stages (n)	Spermary stages (n)	Oocyte stages (n)	Spermary stages (n)	Oocyte stages (n)	Spermary stages (n)	
Mar. 2019	III (1) IV (10)	III (8) IV (5)	III (1) IV (9)	III (2) IV (10)	IV (10)	III (10) IV (7)	IV (10)	III (10) IV (6)	**Light pink**
Apr. 2019*	IV (10)	IV (10)	IV (10)	IV (10)	IV (10)	IV (10)	IV (10)	IV (10)	**Pink**
May 2019	0	0	0	0	0	0	0	0	**White**
Jun. 2019	0	0	0	0	0	0	0	0	**White**
Jul. 2019	I (5)	0	I (4)	0	I (6)	0	I (5)	0	**Light creamy**
Aug. 2019	I (8)	0	I (9)	0	I (10)	0	I (9)	0	**Light creamy**
Sep. 2019	I (10) II (2)	0	I (10) II (3)	0	I (10) II (4)	0	I (10) II (5)	0	**Light creamy to creamy**
Oct. 2019	II (7)	I (6)	II (7)	I (4)	II (8)	I (5)	II (8)	I (5)	**Creamy**
Nov. 2019	II (10)	I (6)	II (10)	I (7)	II (10)	I (8)	II (10)	I (8)	**Creamy**
Dec. 2019	II (4) III (6)	I (10) II (4)	II (5) III (6)	I (10) II (4)	II (6) III (7)	I (10) II (6)	II (10) III (7)	I (10) II (5)	**Creamy to light pink**
Jan. 2020	III (8)	II (10) III (5)	III (9)	II (10) III (5)	III (9)	II (10) III (6)	III (8)	II (10) III (5)	**Light pink**
Feb. 2020	III (10) IV (3)	III (8)	III (10) IV (4)	III (8)	III (10) IV (5)	III (8)	III (10) IV (6)	III (8)	**Light pink to pink**
Mar. 2020	IV (9)	III (10)	IV (9)	III (10)	IV (10)	III (10)	IV (10)	III (10)	**Pink**

The number of colonies (n) contained in each gametogenic development stage is shown in parenthesis. I, II, III, and IV represent different stages of oocytes and spermaries, whereas 0 represents spawned colonies. Asterisks denote the spawning months.

Table 2. Oocyte diameter and spermaries diameter (µm) with Standard Deviation (±SD) of *Acropora digitifera* during 13 months at the study site

Date	Oocyte diameter (µ)	Sperm diameter (µ)
March 2019	366.7 ± 49.8^{b}	135.5 ± 33.6^{b}
Apr. 2019	459.9 ± 31.9^{a}	183.02 ± 9.77^{a}
May 2019	282.12 ± 11.92^{c}	135.74 ± 11^{b}
Jun. 2019	224.85 ± 21.98^{d}	86.65 ± 23.12^{de}
Jul. 2019	65.53 ± 4.89^{g}	-
Aug. 2019	127.62 ± 15.08^{f}	-
Sep. 2019	130.18 ± 9.85^{f}	-
Oct. 2019	136.43 ± 10.66^{f}	39.67 ± 8.34^{f}
Nov. 2019	156.59 ± 12.67^{ef}	42.53 ± 12.68^{f}
Dec. 2019	191.5 ± 28.11^{de}	72.95 ± 7.31^{e}
Jan. 2020	268.64 ± 12.98^{c}	99.39 ± 11.02^{cd}
Feb. 2020	301.12 ± 16.26^{c}	118.68 ± 13.8^{bc}
March 2020	364.8 ± 45.2b	134.76 ± 24.35b

Means in a column that do not share a letter are significantly different.

Table 3. Mean of oocyte diameter and spermaries diameter (µm) with Standard Deviation (±SD) of *Acropora gemmifera* during 13 months at the study site

Date	Oocyte diameter (µ)	Sperm diameter (µ)
March 2019	392.61 ± 24.8^{b}	128.45 ± 24.33^{bc}
Apr. 2019	479.34 ± 12.37^{a}	175.87 ± 11.27^{a}
May 2019	349.67 ± 25.98^{c}	139.72 ± 14.01^{b}
Jun. 2019	283.72 ± 13.76^{d}	91.61 ± 19.71^{ef}
Jul. 2019	91.61 ± 19.71^{h}	-
Aug. 2019	121.21 ± 11.05^{gh}	-
Sep. 2019	151.92 ± 8.08^{fg}	-
Oct. 2019	180.32 ± 17.58^{f}	42.43 ± 10.56^{g}
Nov. 2019	185.1 ± 23.49^{f}	44.46 ± 11.31g
Dec. 2019	229.72 ± 17.64^{e}	74.67 ± 7.55^{f}
Jan. 2020	269.93 ± 10.82^{d}	98.81 ± 11.21^{de}
Feb. 2020	347.8 ± 53.8^{c}	118.1 ± 11.06^{cd}
March 2020	386.21 ± 25.03^{b}	132.76 ± 25.98^{bc}

Means in a column that do not share a letter are significantly different.

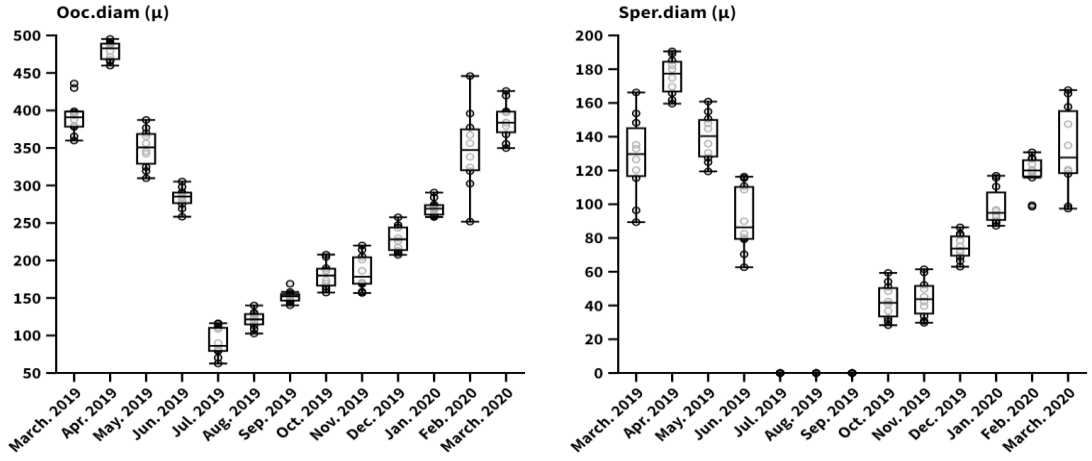


Fig. 4 (B). Mean of oocyte diameter and spermaries diameter (µm) with Standard Deviation (±SD) of *Acropora gemmifera* during 13 months at the study site

Ultrastructure of *Acropora digitifera* and *Acropora gemmifera* egg-sperm bundles

Egg–sperm bundles released by *Acropora digitifera* measured approximately 311.60µm in diameter, while those of *A. gemmifera* were slightly larger, recording 315.98µm. These bundles contained both oocytes and spermatozoa, separated by a thin membrane. Histological sections revealed that large quantities of lipids—staining brown—were present in the bundle structure (Fig. 5A.1, 2, 3, and 4) for both species. These lipids are believed to play a crucial role in maintaining buoyancy, allowing the egg–sperm bundles to float in the water column.

Observations using Transmission Electron Microscopy (TEM) indicated that the surface of the bundles, as well as the space between oocytes, was covered with a mucus layer (Fig. 5B.1, 2, 3, and 4). This mucus had no defined structural organization. The separation between oocytes and spermatozoa within the bundles was variable. In some cases, oocytes appeared directly adjacent to each other without clear separation (Fig. 5B.1), while in others, a significant amount of mucus separated the oocytes from the spermatozoa (Fig. 5B.1, 4).

No fusion of spermatozoa nuclei with oocytes or fusion of plasma membranes was observed. Additionally, no zooxanthellae were found within the egg–sperm bundle structures (Fig. 5B.1, 4).

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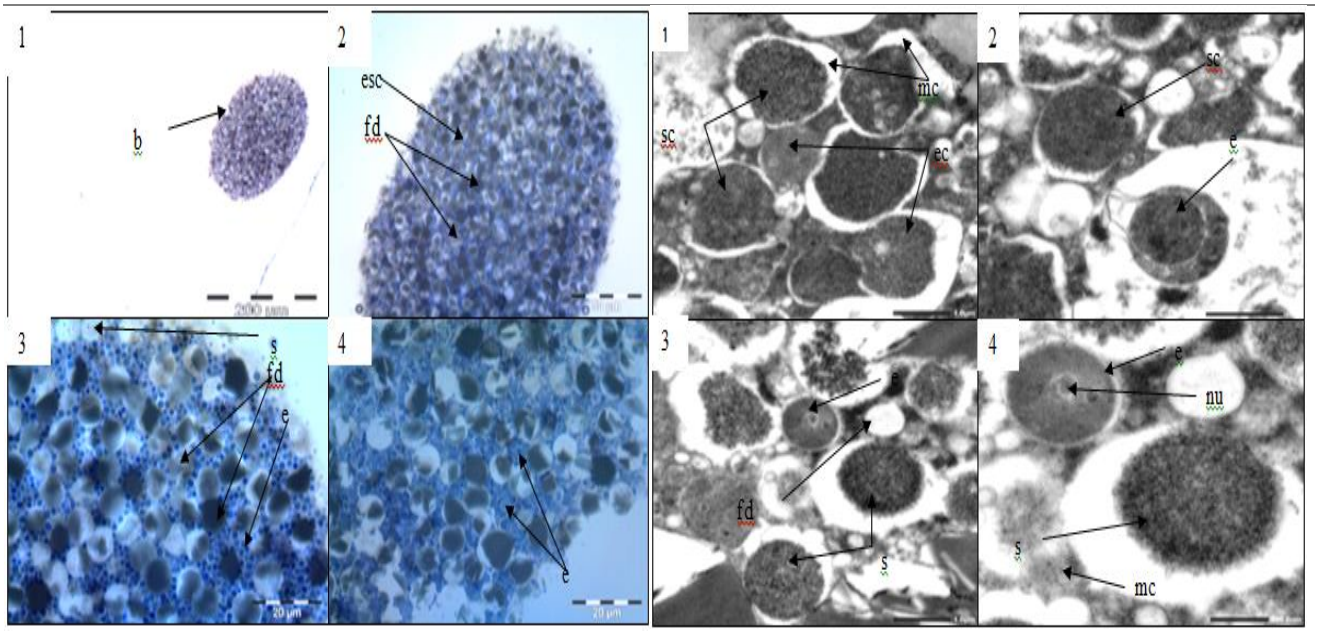

A **B**

Fig. 5(A). Light microscope images showing the structure of *A. digitifera* (Fig. 4.1, 2) and *A. gemmifera* (Fig. 4.3, 4) egg-sperm bundles histologically. Abbr: b, bundle; esc, egg-sperm capsule; fd, fat droplets; s, sperms; e, eggs. Scale bar: 1 = 200µm, 2 = 50µm, 3&4=20µm.

(B). Image from a transmission electron microscope showing the ultra-structure of *A. digitifera* (Fig. 4.1, 2) and *A. gemmifera* (Fig. 4.3, 4) egg-sperm bundles. Abbr: sc, sperm capsule; ec, egg capsule; mu, mucus material; s, sperms; e, egg; fd, fat droplets; nu, nucleus. Scale bar: (1, 2 & 3 =1µm) and (4 = 500nm).

Spawning and dissociation of *A. digitifera* and *A. gemmifera* egg-sperm bundles

The spawning of *A. digitifera and A. gemmifera* colonies occurred two days before the April full moon. Before spawning within 2 hours, polyps relaxed and protruded outward and were seen to produce mucus. Approximately, 10-15 minutes before the spawning event, the egg-sperm bundles become apparent under the oral disc of polyps. The oral disc greatly protruded when the *A. digitifera* colonies spawned, and the polyp tentacles contracted. The egg-sperm bundles were shed through the mouth opening and released into the water column. The released bundles of the coral reef population in the field lasted from 20 to 35min (Fig. 6) for *A. digitifera* and *A. gemmifera*, respectively.

Positively buoyant egg-sperm bundles (pink color) floated to the seawater surface, breaking apart and releasing spermatozoa and oocytes for external fertilization (Fig. 6.1, 4). The breakage of the egg-sperm bundles was initiated by the release of spermatozoa, which was seen as a white cloud released from one or more small openings between oocytes (Fig. 6.2, 5, respectively). The oocytes were separated from one another, and the egg-sperm bundles fully dissociated within 10 -30 minutes (Fig. 6.3, 6) for *A. digitifera*

and *A. gemmifera*, respectively. When oocytes were released, they had irregular shapes and became ovoid.

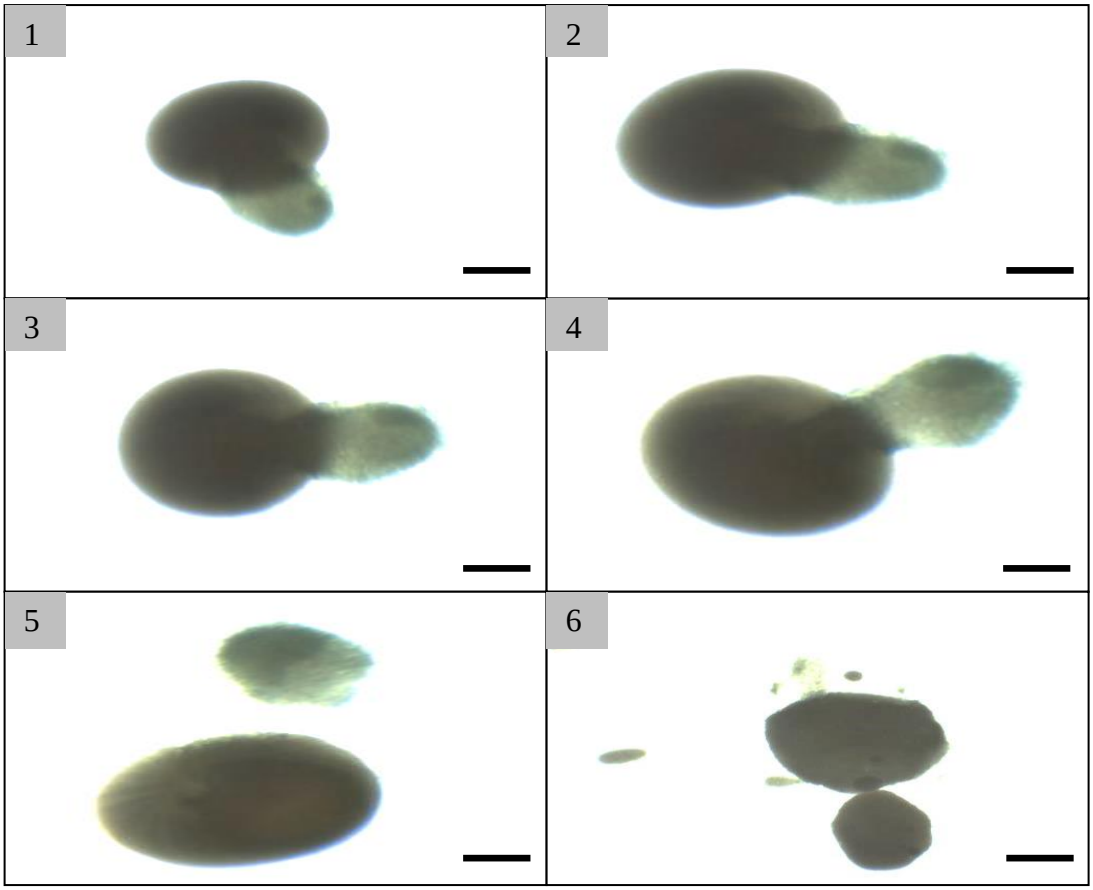

Fig. 6. Images from a light microscope showing the egg-sperm bundle of *A. digitifera* and *A. gemmifera* while separating eggs and sperm from it in the seawater. 1 to 5 show the released spermatozoa from the bundles, and 6 shows that the broken bundle contains eggs. *Scale bar = 200 µm*.

Egg and sperm structure in Acropora digitifera and Acropora gemmifera

After fully dissociating egg-sperm bundles, both oocytes and spermatids were separated; the sperm white cloud got sucked with a syringe for a scanning electron microscope to learn more about the oocytes and sperm structures (Fig. 7). The sperm appeared as an aggregation of a huge number of sperm (sperm mass) (Fig. 7.1, 2) for both *A. digitifera* and *A. gemmifera*, respectively. Through magnification, to get a shot of a single sperm, it consisted of three main parts. The first part was the head, which had an oval shape and measured approximately 1.6µm for *A. digitifera*, while it measured 1.8µm for *A. gemmifera*. The second part was the trunk or (mid-part) of *A. digitifera*, which was measured at 1.3µm, while it was measured at 1.4µm (Fig. 7.3, 4), respectively. The mid-piece contained mitochondria (energy-storing house) which gave the sperm energy

required for its motility, and the last part was the tail (flagellum), which consisted of microtubules surrounded by a flagellar membrane expended laterally (Fig. 7.3, 4). The oocytes under the scanning electron microscope appeared circular and oval in shape (Fig. 7.5, 6). Since sperm structure differs between species, we can use this point as a taxonomy method in coral reef speciation.

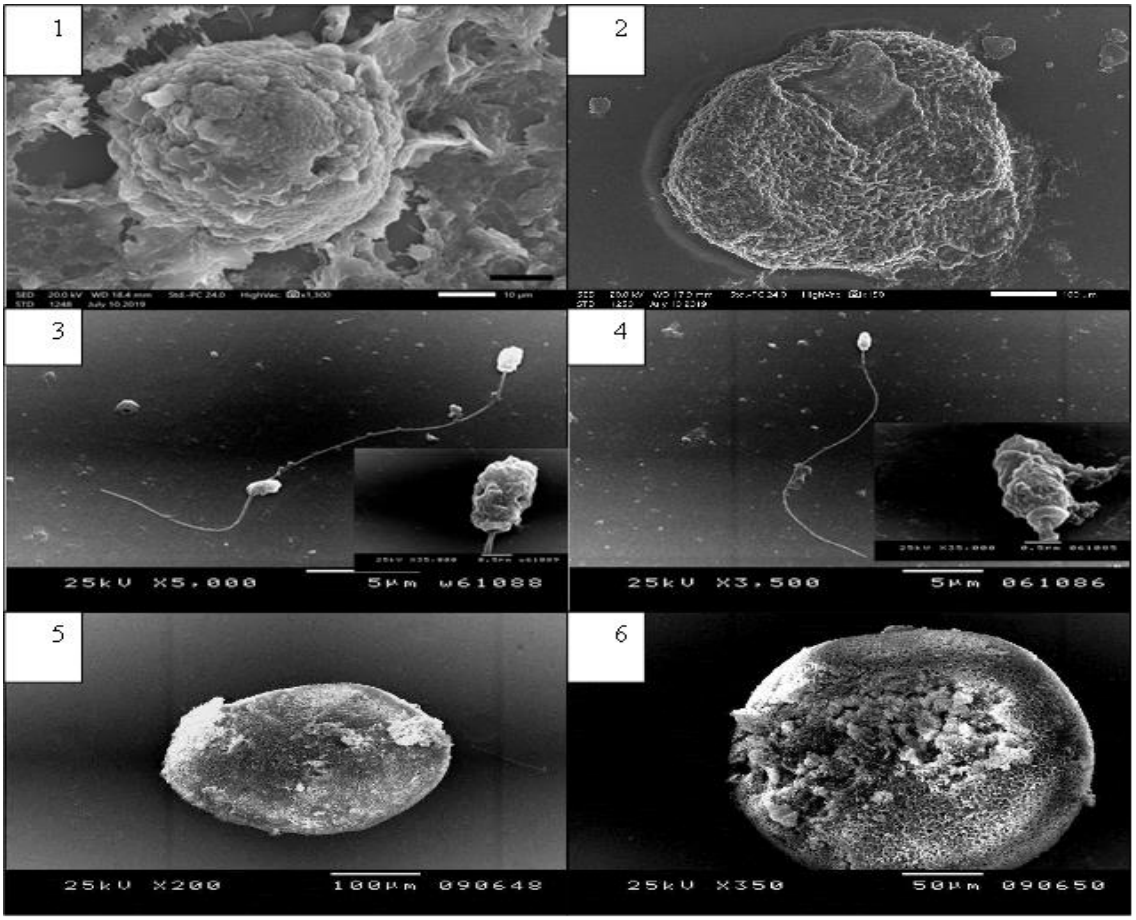

Fig. 7. Image *of A. digitifera* and *A. gemmifera* sperm and egg structure showing that (1) and (2) SEM images show sperm mass. The 3 and 4 SEM images show the sperm structure through different magnification powers that show the 3 parts of the sperm. (5 & 6) SEM images show the egg structure. Scale bar: (1&2 = 100µm), (3&4 = 5µm), (5 = 100 µm) and (6=50µm)

Ultra-structure of *Acropora digitifera* and *Acropora gemmifera* larvae

After 32 hours of the egg-sperm bundles breaking apart, we detected the planulae larvae of *A. digitifera* and *A. gemmifera*. Under the light microscope, it appeared oval under *A. digitifera*, while the planulae of *A. gemmifera* seemed oval with a flattened base. Through the histological sections for both species, planulae larvae were cut into sections about 90 angstroms in thickness to obtain their ultra-structure of them. The larvae of

Planulae had two layers: an ectodermal layer and a gastrodermis layer (Fig. 8.3, 6). Small micro-villi were attached to the surface of the ectodermal layer, which is responsible for the motility of the Planulae larvae (Fig. 8.3, 4, 5, 6). There was no organization in this stage of larvae yet. Lipid material was observed, and we suggested that it helped in the floating of planulae larvae in the water column (Fig. 8.4, 6). Before the spawning of the two studied species, we put three small colonies in plastic aquaria filled with filtered seawater that didn't contain zooxanthellae and another three small colonies in aquaria filled with non-filtered sea water to obtain the means of zooxanthellae transmission. We observed that larvae detected in filtered seawater don't contain zooxanthellae, while those formed in non-filtered seawater contain zooxanthellae. This proved that the zooxanthellae in both *A. digitifera* and *A. gemmifera* were horizontally transmitted from the seawater to the larvae and not from the adult colony genetically.

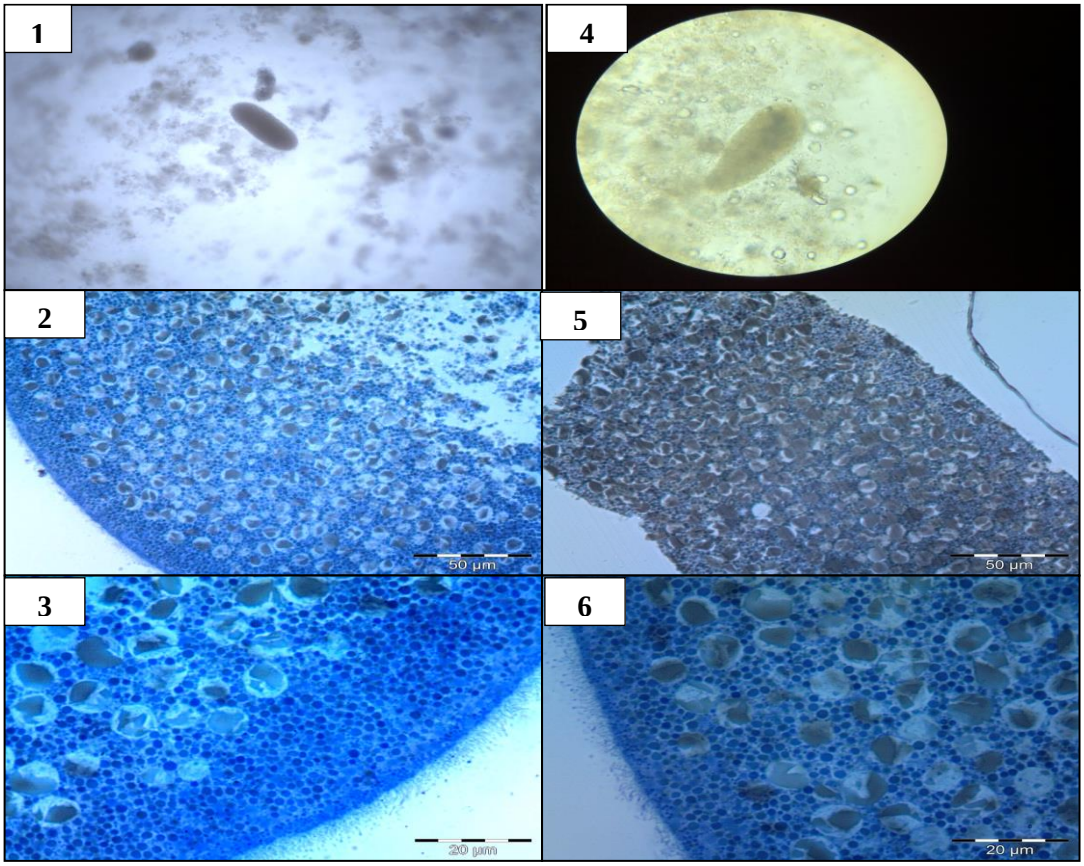

Fig. 8. Transverse histological sections in *A. digitifera* (1, 2, 3) and *A. gemmifer* (4, 5, 6) showing the structural organization of their planula larvae. Abbr: ep, epidermal layer; gs, gastrodermis layer; ci, cilia; fd, fat droplets. Scale bar: (1,4 = 500µm), (2, 5 = 50µm), (3, 6 = 20µm)

Effects of environmental parameters on maturity stages development of gonads

The mean monthly SST during the study period were between 21.95 ± 0.46 in March 2020 to 29.31 ± 0.42 in August 2019, with an annual variation of 7.36ºC (Table 4 & Fig. 9). SST declined in April 2019, 23.38 ±0.45, and there was a visible increase till August 2019; it gradually declined until the second smaller peak in March 2020 (Table 4 & Fig. 9). The pattern for salinity was also distinctly seasonal. Salinity was low in March 2019, measuring 40.21 ± 0.009; then it slightly increased from March 2019 till it reached its highest value in August 2019 (41.79ppt ± 0.334 SD) before gradually declining till it reached its lowest value in November 2019, and increased again gradually from December 2019 till February 2020 before it declined again in March 2020. The dissolved oxygen pattern didn't show the same seasonality as other measured parameters, and there's not much significance between months as in other parameters. In contrast, the photoperiod shows distinct seasonality as the shortest photoperiod was in March 2019 (12.30 ± 0.08), while it increased from March 2019 till it reached the longest period in July 2019 (13.31 ± 0.03), then declined again to the lowest value which was recorded in December 2019 measured (10.42 hr± 0.005 SD), before it increased again till March 2020 (Table 4 & Fig. 9).

Table 4. Water physical parameters: Surface sea water temperature (SST) ^{O}C, photoperiod (hr), salinity (ppt), and dissolved oxygen (DO) (mg/l) during the study period

Date	Temp. (°C)	Photoperiod	Salinity (PPT)	DO (mg/l)
March 2019	23.53 ± 0.32^{f}	12.30 ± 0.08^{f}	40.21 ± 0.009^{ef}	7.17 ± 0.018^{c}
Apr. 2019	23.38 ± 0.45^{fg}	13.07 ± 0.07^{d}	40.26 ± 0.016^{def}	7.11 ± 0.019^{c}
May 2019	25.9 ± 0.56^{d}	13.68 ± 0.04^{b}	40.25 ± 0.010^{def}	7.09 ± 0.008^{c}
Jun. 2019	27.59 ± 0.38^{c}	13.85 ± 0.008^{a}	41.28 ± 0.064^{b}	6.73 ± 0.068^{e}
Jul. 2019	28.46 ± 0.43^{b}	13.31 ± 0.03^{c}	41.67 ± 0.095^{a}	6.49 ± 0.038^{f}
Aug. 2019	29.31 ± 0.42^{a}	12.50 ± 0.05^{d}	41.79 ± 0.334^{a}	6.43 ± 0.038^{g}
Sep. 2019	27.71 ± 0.43^{c}	11.90 ± 0.23^{h}	41.13 ± 0.332^{b}	6.72 ± 0.071^{e}
Oct. 2019	28.09 ± 0.44^{bc}	11.29 ± 0.08^{i}	40.22 ± 0.019^{ef}	6.53 ± 0.018^{f}
Nov. 2019	26.41 ± 0.17^{d}	10.66 ± 0.05^{j}	40.09 ± 0.083^{f}	6.95 ± 0.016^{d}
Dec. 2019	24.16 ± 0.40^{e}	10.42 ± 0.005^{k}	40.45 ± 0.031^{cd}	7.26 ± 0.020^{a}
Jan. 2020	22.85 ± 0.22^{gh}	10.72 ± 0.054^{j}	40.45 ± 0.028^{cd}	7.19 ± 0.015^{b}
Feb. 2020	22.61 ± 0.46^{h}	11.36 ± 0.08^{i}	40.47 ± 0.015^{c}	7.22 ± 0.027^{ab}
March 2020	21.95 ± 0.46^{i}	12.16 ± 0.08^{g}	40.32 ± 0.029^{cde}	7.18 ± 0.012^{b}

Means in a column that do not share a letter are significantly different.

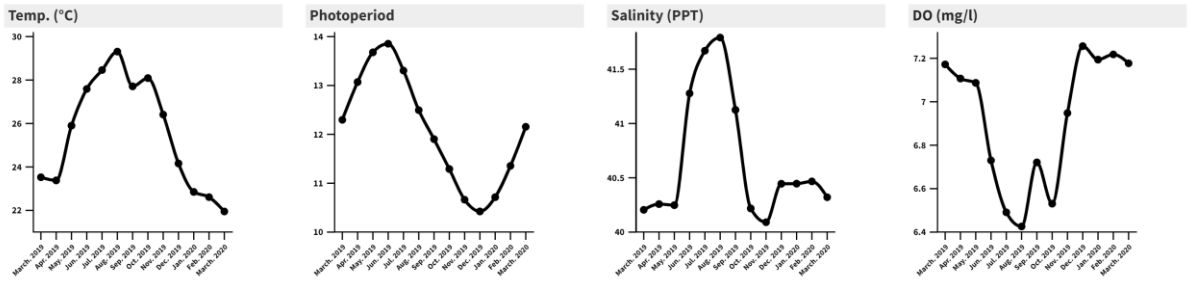

Fig. 9. Monthly changes of water physico-chemical parameters: Temperature (°C), photoperiod (hr), salinity (ppt), and dissolved oxygen (Do) (mg/l) during the study period

The predicted spawning times for both *A. digitifera* and *A. gemmifera* sp. (based on the presence and subsequent disappearance of mature gametes) coincided with a slight decrease in SST and a slight increase in salinity. Between March and April 2019, the mean of SST and salinity showed no significant difference, while there was a significant differentiation in photoperiod between March and April 2019, which coincided with an increase in gametes size for both *A. digitifera* and *A. gemmifera* as mean oocyte diameter increased from 366.7 ± 49.8 to 459.9 ± 31.9µm and from 392.61 ± 24.8 to 479.34 ± 12.37 µm, while the spermary diameters increased from 135.5 ± 33.6 to 183.02 ± 9.77µm, and from 128.45 ± 24.33 to 175.87 ± 11.27µm for both species, respectively.

Through histological examination, we found that the gametogenesis process was controlled by the following water parameters: Temperature and salinity, while the releasing of egg-sperm bundles (spawning process) was controlled by photoperiod. As SST increases, it stimulates Oocyte maturation, while sperms increase in size during winter. Both species were synchronized and spawned on the 19th of April 2019 before April full moon within two days. This was confirmed through a visual examination and histological section by disappearing egg-sperm bundles inside the mesenteries, and no developmental stages appeared in histological sections.

Statistical analysis of environmental data on water physical parameters revealed the correlation between different parameters and the development of the gametogenesis process. There was a clear correlation between the SST, salinity, and dissolved oxygen for both Oogenesis and spermatogenesis for *A. digitifera* and *A. gemmifera* (Fig. 10A, B, respectively), which means the seasonality pattern of physical water parameters didn't happen by accident. However, it happened for some reason, which is the process of gonads maturation. On the other hand, we didn't find any correlation between photoperiod and the gonad's maturation process, which indicates that this parameter didn't have any effect on the maturation process but had a clear, direct relation with the spawning event (releasing of gametes) (Fig. 10A, B).

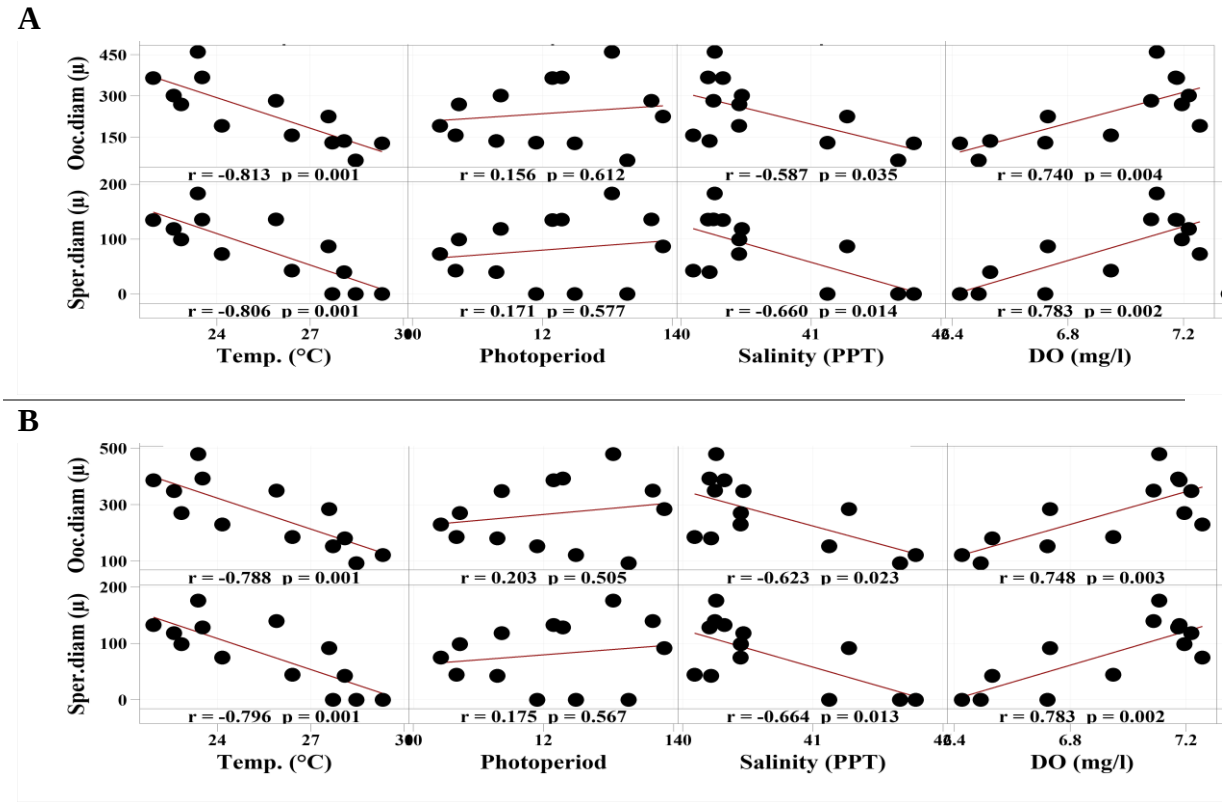

Fig. 10. (A) *A. digitifera* ; **(B)** *A. gemmifera* gametogenesis correlation with the physical water parameters during 13-month study period

DISCUSSION

Acropora species were hermaphroditic, which produced both male and female gametes in the same polyps of the colonies but in different mesenteries as appeared in the (Fig. 3A.1, 3, B1, 3). This result coincides with that of **Nishikawa *et al*., (2003)**, **Wolstenholme (2004)** and **Zakai *et al*. (2006)**. There was no evidence that members of this genus exhibited reproductive suppression during gamete maturation; instead, they underwent a single annual gametogenic cycle **(Szmant *et al.*, 1985; Wallace, 1985; Mangubhai & Harrison, 2008a, b; Rosser & Gilmour, 2008)**.

All the previous studies that studied the prediction of coral spawning (based on available evidence) indicated that reproductive condition could be estimated based on the visibility and color of developing oocytes **(Baird & Marshall., 2002)**. The present study gave details on the gametogenic cycle of scleractinian coral *A. digitifera* and *A. gemmifera* species, determined by histological examination, which provided detailed information on the development of the gonads through the 13-month study period. Although most studies examined reproduction by calculating the proportion of cells at

different stages of development **(Glynn *et al*., 1991; Szmant, 1991; Glynn *et al*., 1996)**, the method used in this study involved measuring the diameter of gonads over the whole gametogenic cycle. This offered a better comparison with the previous local studies, which also directly measured the diameter of gonads and allowed a quantitative analysis of the reproductive abilities between the two selected sites.

The reproductive development of *A. digitifera* demonstrated an Oogenic cycle of 11-12 months with a much shorter spermatogenic cycle of 5-6 months. In **Collinson**'s study **(1997)**, *A. tumida* (previously identified as *A. humilis*) had an Oogenic cycle of 9-10 months and a spermatogenic cycle of 6-7 months. Although the results were similar, the same Oogenic cycle was observed in this study. **Pires *et al*. (1999)** and **Harrison *et al.* (2011)** reported that Oogenesis and spermatogenesis started in different periods, with spermaries appearing in approximately the eighth month of ovary development and lasting about 3 months. There was a synchrony in the maturation of gametes for the hermaphroditic *A. digitifera* and *A. gemmifera* colonies in March - April 2019 (present study), coinciding with the rise in sea temperature (23.38^{o}C). The disappearance of mature gametes from the colonies of *A. digitifera* and *A. gemmifera* in May 2019 indicated that it spawned in April full moon.

In the present histological investigation, testes were observed to be full of sperm during the spring season in the sectioned polyps of *A. digitifera* and *A. gemmifera*, while mature eggs were detected in both winter and spring. The first stage of the egg (oocyte) appeared in July, whereas the first stage of sperm was observed in October. This finding is consistent with the investigation by **Shlesinger and Loya (1985)**, who reported that female gonads require approximately five months (October to February) to mature, whereas male gonads require only two months (January and February) for maturity.

Kongjandtre *et al.* (2010) illustrated a similar finding for *A. digitifera* and *A. gemmifera*, reporting that the male gonads of *A. humilis* began development at the end of January and February, completing development in April. Female gonads began developing in late October, preceding full male gonad development in April. However, **Gylnn and Enochs (2011)** noted that coral reproduction in the Galápagos Islands occurred mainly from March to May, coinciding with seasonally high sea temperatures and rainfall.

In the current study on the spawning of *A. digitifera* and *A. gemmifera*, it was found that ova developed before spermatia. **Rinkevich and Loya (1979a, b)** observed that spermatia were never found alone but always in association with a large number of ova. Breeding seasons for coral reefs varied by locality and species **(Baird & Marshall, 2002; Wolstenholme, 2004; Rosser & Gilmour, 2008; Gilmour *et al.*, 2009; Rosser & Baird, 2009)**. In the northern Red Sea and Gulf of Aqaba, it extended from December to July **(Shlesinger & Loya, 1985)**. In contrast, for *A. humilis* in the Great Barrier Reef, the breeding season lasted from February to June **(Bothwell, 1981)**. **Baird *et al.* (2011)** concluded that the primary spawning season of corals on shallow inshore reefs in the

Dampier Archipelago occurred in autumn, though some taxa also spawned in spring and summer.

Previous studies described male gonads in corals **(Rinkevich & Loya, 1979; Szmant *et al.*, 1980; Fadlallah, 1982; Fadlallah, 1983; Szmant, 1986; Fowler, 1991; Neves & Pires, 2002)**. **Szmant (1986)** provided detailed information on the structure and development of male gonads. Spermatogenesis in *A. digitifera* and *A. gemmifera* was found to be very similar to that described in *A. palmata* by **Szmant (1986)** and in *Balanophyllia elegans* by **Fadlallah and Pearse (1982)**.

In general, development of the male gonads began with stage I inside the mesenteries, first detected in October 2019 (present study). Through successive divisions, the testes expanded from the gonadal periphery toward the centre.

The testes of both species were structurally complex. In *A. digitifera* and *A. gemmifera* they occupied three mesenteries, often forming several patches, each at a different developmental stage. Their shapes varied greatly—an arrangement previously described in in oviparous *Acropora* spp. **(Stimson, 1978; Bothwell, 1982)** and in the brooding species *A. palifera* **(Kojis, 1986)**.

Few studies have examined coral-sperm ultrastructure, but *Montipora capitata* shows a head, neck and tail similar to those documented here **(Padilla-Gamiño *et al.*, 2011)**. The present specimens differ slightly in the dimensions and outlines of these three regions. Fertilisation occurred externally in seawater, as is typical for broadcasting *Acropora* spp. **(Hanafy *et al.*, 2010)**.

Egg morphology in *A. digitifera* and *A. gemmifera* resembled that of other scleractinians —each oocyte contained abundant yolk. Mature-oocyte diameters were 459.9 ± 31.9µm in *A. digitifera* and 479.3 ± 12.4µm in *A. gemmifera*. These values mirror those recorded for *A. digitifera* in Kenya (474.6 ± 84.1 µm) **(Mangubhai & Harrison, 2008)** but exceed measurements from Hong Kong (282.5 ± 57.9 µm) **(Wai Shan, 2010)**, probably reflecting local environmental conditions and methodological differences.

During oogenesis the number of oocytes per gonad declined: more than 120 were counted in six ovaries per polyp early in development, but far fewer remained at maturity. Wallace found no such decline when counting dissected polyps immediately before spawning **(Wallace, 1985; Shakara, 2015)**. Our histological approach, however, captured earlier stages when attrition is still occurring.

Egg–sperm bundles formed a few hours before spawning, required about two hours to complete formation and disaggregated 10–30 minutes after release—comparable to, but slightly longer than, previous reports of <40 minutes **(Wallace, 1985)**. Bundles carry gametes to the surface, enhancing gamete encounter; their mucus matrix keeps the positively buoyant oocytes and denser sperm together during ascent. Variations in gamete buoyancy promote out-crossing **(Harrison & Wallace, 1990; Richmond, 1997)**.

Ultrastructural examination revealed a mucus layer separating oocytes and spermatozoa inside the bundles. This barrier helps prevent self-fertilisation, which is generally rare **(Hodgson, 1990; Knowlton *et al.*, 1997; Padilla-Gamiño *et al.*, 2011)**. In *Platygyra pini*, self-fertilisation is delayed for ≥3 h **(Heyward & Babcock, 1986)**; we observed no self-fertilisation blocks in either study species.

After bundle rupture, planula larvae of *A. digitifera* reached full development within 12h—much faster than the 32h reported by **Rinhevich and Loya (1979)**. Larvae were oval, aseptate and lacked zooxanthellae; *A. gemmifera* larvae were similar but had a broader base. By contrast, *Stylophora pistillata* larvae display segmentation and internal symbionts **(De Putron & Smith, 2011)**. Our larvae comprised epidermal and gastrodermal layers; the epidermis bore motile cilia, whereas the gastrodermis stored abundant lipids that aid buoyancy, corroborating previous findings **(Rinhevich & Loya, 1979; De Putron & Smith, 2011)**.

No significant differences in gametogenic cycles were detected between sites, indicating comparable environmental conditions. Oocyte and spermatium diameters, however, varied significantly among developmental stages, implying size changes throughout gametogenesis. All colonies of both species spawned within two days before the April full moon, matching earlier observations **(Hanafy *et al.*, 2010)**.

Environmental influences on coral reproduction are well documented **(Rinkevich & Loya, 1979b; Wolgast & Zeide, 1983; Shlesinger & Loya, 1985; Rinkevich & Loya, 1987; Kramarsky-Winter & Loya, 1998; Shlesinger *et al.*, 1998; Loya & Sakai, 2008; Harrison, 2011; Eyal-Shaham *et al.*, 2016; Rapuano *et al.*, 2017)**. Seawater temperature is a primary driver, modulating metabolism and hence gametogenesis **(Willis *et al.*, 1985; Babcock *et al.*, 1986)**. Most scleractinian corals spawn in spring or summer as rising temperature and salinity accelerate gonad maturation. Both *A. gemmifera* and *A. digitifera* responded similarly, requiring elevated temperature, salinity and photoperiod to complete maturation.

Comparable temperature–reproduction links have been shown in temperate scleractinians, including *Balanophyllia elegans* **(Fadlallah & Pearse, 1982; Beauchamp, 1993)**, *B. europaea* **(Goffredo *et al.*, 2002; Goffredo *et al.*, 2006)**, *Cladocora caespitosa* **(Kružić *et al.*, 2008)** and *Astroides calycularis* **(Caroselli *et al.*, 2011)**. Collectively, these studies confirm that gametogenesis, gamete release, fertilisation and larval settlement are synchronised to seasonal environmental cues **(Babcock *et al.*, 1986; Harrison & Wallace, 1990; Penland *et al.*, 2004)**.

CONCLUSION

This study highlights the annual gametogenic cycle and synchronized spawning of *A. digitifera* and *A. gemmifera* in the northern Red Sea, driven by environmental cues. These findings enhance our understanding of coral reproductive ecology and provide essential data to support reef restoration and conservation planning in the region.

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