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# Gametogenic development and synchronous spawning of the acroporid corals *Acropora cytherea* and *Acropora tenuis* in the Red Sea

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**ABSTRACT**

Sexual reproduction of corals is one of the essential processes for the stability and integrity of coral populations. Hence, this study was conducted to assess the reproductive and spawning patterns of two reef-building scleractinian corals belonging to genus *Acropora*, namely *Acropora cytherea* and *Acropora tenuis* inhabiting Hurghada, northern Red Sea. Histological and in situ examinations were carried out on 16 tagged colonies from the two coral species (8 for each species) in two selected shallow reef sites located in the north and south of Hurghada. Coral fragments were collected monthly from March 2018 to June 2018 and from October 2018 till May 2019 for dissection and histological analyses. The present results indicated that *A. cytherea* and *A. tenuis* were hermaphroditic broadcast spawners with a single annual gametogenic cycle (7 months) that began in October 2018 and terminated in April 2019, and both oogenesis and spermatogenesis were occurring for 7 and 6 months, respectively. Both species exhibited conspicuous spawning synchrony amongst colonies of the same species, and the time of spawning was mostly correlated with lunar cycle and photoperiod (day length). They spawned around the full moon in April 2018 and 2019. *A. Cytherea* spawned two days before the full moon (30 Apr) in April 2018 and one day before the full moon (19 Apr) in April 2019. *A. tenuis* spawned one day before the April full moon in 2018, while in 2019 it spawned in the full moon day (19 Apr) and one day after the full moon. This study provided information that will aid in future coral reef restoration and management. Further research on reproduction of additional coral species is required in order to provide a better perspective of reproductive patterns in Red Sea scleractinian corals.

## INTRODUCTION

Coral reefs are among the most diverse and productive ecosystems on the planet, providing habitats with almost one quarter of the world's marine species **(Fisher *et al.*, 2015)**. They are also one of the most economically important marine ecosystems because of the various services and goods they provide to human populations in coastal regions

comprising food, fisheries, tourism, recuperation and coastal preservation **(Woodhead *et al*., 2019)**.

Information on coral reproduction is fundamental for ecological studies of coral populations and communities **(Harrison & Wallace, 1990)**, which in turn are significant for coral reef management and conservation **(Richmond, 1997)**. The constancy and conservation of coral communities depend on the existence of coral species that reproduce sexually **(Kaniewska *et al*., 2015)**. Reef-building corals essentially possess two modes of sexual reproduction—broadcast spawning and brooding **(Baird *et al*., 2009)**. Individual colonies produce either one type of gametes (gonochoric) or both types (hermaphroditic), while fertilization of eggs and subsequent embryonic development can occur either inside or outside the parent colony (i.e. brooding or broadcast spawning, respectively). Broadcast spawning corals can be either gonochoric or hermaphroditic and can release their gametes separately or concurrently. The wide plurality of scleractinian corals, comprising all acroporid corals, are hermaphroditic broadcast spawners **(Guest *et al*., 2008; Baird *et al*., 2009)**, and the majority of these corals release their gametes as egg–sperm bundles **(Kinzie, 1996)**. The egg–sperm bundle is an effective method of transporting gametes to the ocean surface where fertilization occur, while decreasing sperm dilution and increasing the opportunity for gamete encounters during a spawning episode **(Padilla-Gamiño *et al*., 2011)**. The fertilized eggs float on the surface for ~4-7 days and continue to develop into coral larvae upon which the ciliated larvae are able to swim and actively search for appropriate substrate to settle on. The brooding corals can also be gonochoric or hermaphroditic, but the oocytes are fertilized inside the coral polyp and well-developed larvae are released **(Harrison & Wallace, 1990)**.

Coral spawning is an apparent feature in some coral reef populations. In the Great Barrier Reef, this phenomenon was first observed and recorded, where over 30 species spawn and release gametes within hours on one reef, and over 150 species were spawning in October and/or November during the full moon in the succeeding week **(Willis *et al*., 2006)**. Broadcast spawners usually have annual reproductive cycles and often participate in multi-specific spawning events **(Guest *et al*., 2008)**. On the other side, brooder species ordinarily possess multiple reproductive cycles within a year (e.g., monthly) and several species at one site may exhibit asynchronous release of larvae within a lunar cycle **(Villanueva *et al*., 2008)**. The spawning events recorded in most regions are seasonal, focused on a specific time of the year **(Baird *et al*., 2009)**.

Scleractinian corals often reproduce in synchronized spawning episodes to enhance gamete fertilization and production of coral larvae (planulae). The extent of spawning synchrony and its timing vary amongst coral species and amongst biogeographic areas. The majority of corals in the Great Barrier Reef (GBR) and Western Australia (WA) simultaneously reproduce in a mass spawning phenomena that occurs annually, but at different months **(Babcock *et al*., 1994; Wilson & Harrison, 2003)**. Many dominant species, found in the northern Red Sea, spawn in variable times, months

and seasons with lunar cycle's relation **(Bouwmeester *et al.*, 2011)**. However, studies related to spawning timing and synchronization of scleractinian corals in Egyptian Red Sea water are still limited **(Rashad *et al*., 2020)**.

Reproductive strategies of *Acropora* species have been studied in different geographical areas **(Baird *et al.*, 2002; Guest *et al.*, 2005; Hanafy *et al*., 2010; Bouwmeester *et al*., 2015; Gan *et al*., 2021)**. These acroporid species usually participate in a synchronous mass spawning event **(Hanafy *et al*., 2010; Bouwmeester and Berumen, 2015; Chelliah *et al*., 2015; Jamodiong *et al*., 2018a)**, despite extended spawning characteristics **(Baird *et al*., 2009)**. Extended spawning takes place when coral colonies spawn over a series of successive lunar cycles or months **(Baird *et al*., 2009)**. On the other hand, asynchronous spawning was recorded in 20 species of *Acropora* on equatorial reefs of Kenya **(Mangubhai & Harrison, 2008a)**. In the Indo-Pacific region, the spawning of many *Acropora* species takes place mainly according to the lunar phase **(Harrison *et al.*, 1984; Wilson & Harrison, 2003; Baird *et al*., 2010)**. Environmental factors, such as temperature and photoperiod, have been proposed to be motivating factors for corals to spawn simultaneously **(Babcock *et al*., 1994)**. *Acropora* corals inhabiting seven reefs in Australia and Japan have the capacity to adjust their development and physiology in response to environmental factors for fine-tuning the timing of synchronous spawning, thereby maximizing reproductive success and post-fertilization survival **(Sakai *et al*., 2020)**.

Although genus *Acropora* is one of the most abundant coral genera in the northern Egyptian Red Sea region **(Ghallab *et al*., 2020)**, the reproductive biology of its species is still poorly covered in the research, specifically to determine their gametogenesis, spawning synchrony among colonies, and spawning time. Therefore, the main objectives of this study were to determine the reproductive modes, gametogenesis, and spawning pattern and timing of two common acroporid corals; *Acropora cytherea* and *Acropora tenuis* ,inhabiting the shallow reefs at Hurghada, northern Egyptian Red Sea.

## MATERIALS AND METHODS

### 1. Study sites:

The present study was conducted at two selected sites in Hurghada region, Egyptian Red Sea coast (Figure 1) during the years 2018 and 2019 (from March 2018 to June 2018, and from October 2018 to May 2019). The first site (SI) is located in the north of Hurghada city at coordinates 27°17`01"N and 33°46`20"E, next to the Red Sea branch of the National Institute of Oceanography and Fisheries (NIOF-Red Sea). This location is portrayed with fringing reefs and reef patches, the intended *Acropora* colonies in this site are found in the shallow reef lagoon within a depth range of 1-2 m. On the other hand, the second site (SII) is situated in the south of Hurghada city at coordinates 27°07`55"N and 33°50`02"E, next to the beach of Remevera recreational resort. This area is characterized

by moderate density of recreational activities including snorkelling, surfing and yachting. The *Acropora* colonies in this site are found at depth range 2-3 m. The distance between the two selected reef sites is approximately 15 km.

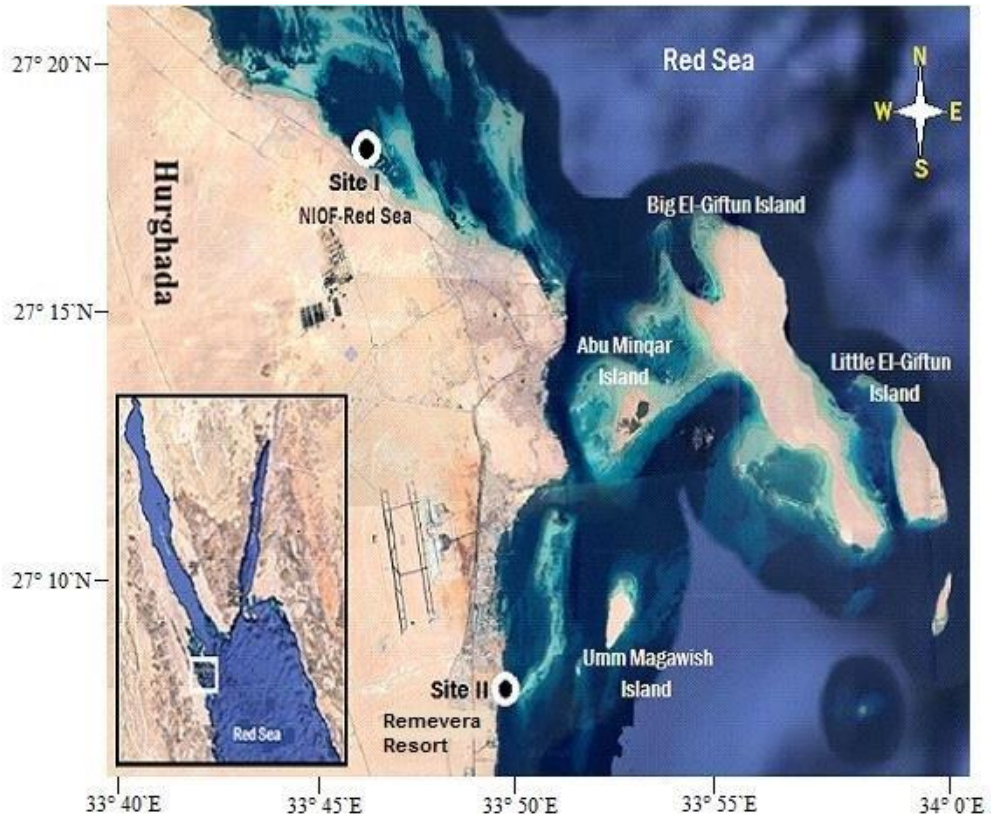


**Figure 1:** A map showing location of the two study sites in Hurghada region (White Square). Inset: Map of the northern Red Sea showing location of Hurghada in the Red Sea. NIOF-Red Sea = National Institute of Oceanography and Fisheries, Red Sea branch.

## 2. Environmental parameters at the study sites:

The following environmental variables were determined to elucidate the environmental conditions in the sampling sites: sea surface temperature (SST, °C), day length (hours), lunar cycle, and seawater salinity (ppt) and pH (Table 1). From these parameters, three factors were reported correlating with the timing of coral gamete maturation and spawning, including lunar cycle **(Guest *et al*., 2002; Levy *et al*., 2007; Baird *et al*., 2010; Lin & Nozawa, 2017)**, seawater temperature (**Nozawa, 2012; Sakai *et al*., 2020)** and day length (**Gouezo *et al*., 2020).**

Surface seawater temperature, pH and salinity of the water mass in the study sites were measured during the full moon week of each month throughout the study period (with the exception of July, August and September 2018) using a Hydro lab device of HANNA (HI 9892) multi-parameter meter" with data recorder type that was deployed at 2-m depth.

**Table 1:** Dates of sampling and full moon; average values of water pH, salinity (ppt or ‰s), temperature (°C); and day length (Hours) recorded from March-2018 to May-2019 at the two sampling sites.

| Month-year | Sampling date | Full moon date | pH | Salinity (ppt) | Temperature (°C) | Day length (H) |
|---|---|---|---|---|---|---|
| Mar-2018 | 27-30 | 31 | 7.96 | 40.12 | 22.5 | 11.73 |
| Apr-2018 | 27-30 | 30 | 7.96 | 39.85 | 23.5 | 12.91 |
| May-2018 | 26-28 | 29 | 7.88 | 39.74 | 28 | 13.36 |
| Jun-2018 | 25-27 | 28 | 7.90 | 39.59 | 29 | 13.50 |
| Oct-2018 | 21-23 | 24 | 7.97 | 39.44 | 28.5 | 11.05 |
| Nov-2018 | 20-22 | 23 | 7.82 | 39.48 | 22 | 10.36 |
| Dec-2018 | 19-21 | 22 | 7.89 | 39.18 | 20 | 10.13 |
| Jan-2019 | 18-20 | 21 | 7.87 | 38.95 | 17.5 | 10.41 |
| Feb-2019 | 16-18 | 19 | 7.85 | 39.8 | 18.5 | 11.06 |
| Mar-2019 | 18-20 | 21 | 7.85 | 40.11 | 24 | 11.83 |
| Apr-2019 | 16-18 | 19 | 7.86 | 40.21 | 26 | 12.65 |
| May-2019 | 15-17 | 18 | 7.89 | 40.23 | 28.5 | 13.23 |

The day length was calculated from sunrise to sunset, and it was obtained accurately from the calendar of the city. However, full moon timing and status were observed matching with the Hijri calendar where the nights of day $14^{th}$ (in the Arabic months) is the determined full moon time, while the other days were calculated by counting the days number prior (-) or after (+) the full moon time.

Naturally, the seasonal variations of sea surface temperatures (SST) in the study sites follow those of the prevailing climate conditions, higher in the summer season and lower in winter. The highest SST (29 °C) was recorded in June 2018, while the lowest degree (17.5 °C) was recorded in January 2019 (Table 1). The seawater salinity in the area of study varied between 38.95‰s in January 2019 and 40.23‰s in May 2019, with an average value (±SD) of 39.73±0.41‰s. Levels of pH in the area of investigation varied within a narrow limit, ranging from 7.82 in November 2018 to 7.97 in October 2018 (Table 1). The average value (±SD) of pH calculated throughout the study period was 7.89±0.048‰s. The average day length (DL) varied among months (Table 1). The largest average day length (13.50 hours) was reported in June 2018, whilst the smallest one (10.13 hours) was reported in December 2018.

**3. Coral sampling and identification:**

Coral sampling, tagging and monitoring were performed using snorkeling. In every study site, 4 colonies were tagged and followed considering each studied species. In order to follow the gametogenesis conveniently, both study sites were checked every morning during the full moon week of each month from March 2018 to May 2019, with the exception of the following three months: July, August and September 2018 (Table 1). The studied coral species, *Acropora cytherea and Acropora tenuis* were identified to species level according the identification guides of **Wood (1983)**, **Veron (1986, 2000)**, and **Sheppard and Sheppard (1991)**. Corals were identified in the field and in the laboratory using underwater photos and dissecting microscope.

**4. Determination of reproductive and spawning patterns:**

According to the previous studies, colonies with mature oocytes are expected to spawn around the full moon of the same month or of the next month **(Mangubhai & Harrison, 2008a; Kongjandtre *et al*., 2010)**, but colonies that have immature gametes may spawn within 1–3 months, and the empty ones are unlikely to spawn at least after 7 months **( Harrison *et al.*, 1984)**. Thus, samples of 2-3 branches (>5 cm length) were taken from the tagged colonies of the two *Acropora* species during the full moon week and preserved for histological examination to investigate the gametogenic development. In addition, 1-3 branches of each tagged colony were covered with a plankton net to collect the gametes before complete releasing. During sampling, the surface area of the chosen colonies were calculated using a specific equation [max. length x min. length (=width)] in centimetres to ensure that they were larger than the minimum reproductive size (20 cm) **(Kojis & Quinn, 1981; Harrison & Wallace, 1990)**.

*Acropora* colonies were ranked as gravid when the coloration of fractured branches ranged from yellowish brown to pink **(Babcock *et al*., 1986)**. Because not all branch fractures could show the polyp content, some branches appeared to be empty. Consequently, if a coral branch appeared to be empty at first, it was re-broken to confirm the absence of oocytes before a colony was recorded as empty.

Gametes maturation throughout the study period were accurately monitored using histological technique and in situ examination for the two studied *Acropora* species (n=16 colony). During the field trips, the changes in coloration of fractured branches was reported to emphasize the sequence of gametogenic development progress. In the laboratory, selected histological sections (stained with Hematoxylin and Eosin Protocol) that contained clear views of oocytes or spermaries were photographed to determine the onset and the end of gametes formation. The photographs were taken using an Olympus camera connected to a digital compound microscope. Maximum oocytes and spermaries diameters were determined regarding the photos. To differentiate the different stages of gamete development, the previous gametogenesis histological studies were followed **(Vargas-Ángel *et al*., 2006; Morita *et al*., 2019)**.

Spawning was inferred from the existence of egg-sperm bundles around the oral disks of covered polyps and the disappearance of mature oocytes and spermaries from sequential samples **(Mangubhai & Harrison, 2008a).** The coral branches were re-checked for 3 days after the full moon of each month during the study period.

**5. Statistical analysis:**

Statistical analysis was carried out using the computer software Excel package. Two-way analysis of variance (Two-way ANOVA) was utilized to compare the gametes diameters of both *Acropora cytherea* and *Acropora tenuis* between the two sampling reef sites, as well as among the maturation dates. Two-way ANOVA was used also to test the effect of interaction between dates and sites (dates × sites) on the gametes sizes of both studied species. The level of significance for the analysis was determined at $P \leq 0.05$.

## RESULTS

### 1. Gametes maturation:

Monthly observations and histological examination of the tagged colonies of *Acropora cytherea* and *Acropora tenuis* during the study period (from March 2018 to June 2018 and from October 2018 to May 2019) revealed the existence of four gametogenic developmental stages (I, II, III and IV), and the developmental stage of each oocyte extended for 2-3 months (Table 2 & Figure 2). The present histological investigation confirmed the hermaphroditism of the two studied acroporid corals, where their male and female gametes developed in separate mesenteries in the same polyp (Figure 2). While oocytes migrate to the mesoglea of the mesenteries from the gastrodermis, formation of sperms occurs firstly in the mesenterial endoderm before their early stages being captured within mesoglea gonadal cyst.

Histological and field investigations demonstrated that the oocytes stage (I) in the two *Acropora* species firstly appeared in the middle of autumn (October and November, 2018), where the formation peak of this stage was recorded in November 2018 (Table 2, n=15, 93.75%). In only 3 colonies of *Acropora cytherea*, the oocytes continued in stage (I) through December 2018 (Table 2). In this stage, the oocytes had a creamy colour as seen in all fractured branches (Table 2). The mean (±SD) diameters of oocytes stage (I) ranged between 272±2.68 and 310.60±5.15 µm (Table 3), and their nucleus were not obvious during development (Figure 2). Furthermore, the histological observations revealed that the number of oocytes for each polyp during stage (I) varied from 1-3 oocytes dispersed from each other in different mesenteries (Figure 2A, E), and sometimes they are accumulated near each other. During this developmental stage, the oocytes were observed usually to have an irregular shape and a rough-edged structure, but sometimes appeared with an oval to an oblong shape (Figure 2A, E).

Oocytes of the two studied species continued their development through stage (II), which dominated in December 2018 and January 2019 with percentages 81.25% and 87.5% of all observed colonies, respectively (n=13,14 colonies, respectively) (Table 2). Though the peak of oocytes stage (II) was recorded in January 2019, the lowest percentage (31.25%) was registered in February 2019. The oocytes in that stage were round to oval in shape with mean (±SD) diameter, ranging from 322.40±3.24 to 358.70±3.77 µm (Table 3). The oocytes in stage (II) appeared with coloration gradient from yellow to orange (Table 2). In this stage the oocytes were seen to have obvious nucleus with fine or coarse granular cytoplasm, and are found sometimes very close to each other, and other times they are occasionally found widely separated from each other (Figure 2B, F).

Through stage (III) the oocytes sizes increased, the mean (±SD) diameter ranged from 373.20±3.77 to 391.90±4.65 µm (Table 3). In this stage, the nucleus appeared with

smooth structure containing a bright cranberry nucleolus, and the nucleus membrane was evident (Figure 2C). In February 2019 the percentage of colonies contained oocytes. In stage (III) it was 68.75%, whereas the peak of this stage formation was observed in March 2019 with 75% of colonies (n=12 colonies, Table 2). During stage (III), the in situ chipped off coral branches showed pale pink coloured oocytes. In med- to late-stage (III) oocytes, the nucleus began to shift their position from the centre towards periphery, and the nucleolus was found to move towards the pole of nucleus in the same stage (Figure 2C).

Gonad development was completed in April 2019 when the maturity stage (IV) was noted in 100% of the investigated colonies in both species (Table 2, n=16), and the mean (±SD) oocyte diameter varied between 415.60±2.30 and 419.70±2.62 µm (Table 3). Observations on oocyte maturation revealed no marked differences between stage (III) and stage (IV) except that the nucleus were seen positioned towards one pole of the oocytes, the nucleolus were less and often appeared to migrate towards the pole of nucleus, and the oocyte colour turned from pale pink to pink (Table 2).

Regarding the spermaries development of *Acropora cytherea* and *Acropora tenuis,* Stage (I) began when enlargement of the middle portion of the mesenteries was observed in its peak early in November 2018 (Table 2) with 75% colonies (n=12 colonies) containing spermaries in stage (I), whereas only 2 colonies from each species completed stage (I) during December 2018 (Table 2 and Figure 2E). The mean (±SD) diameters of this stage ranged from 61.57±0.50 to 83.06±1.08 µm (Table 3).

Stage (II) of spermaries was commonly observed in December 2018 reaching its maximum level (81%, n=13 colonies) in January 2019 (Table 2), with moderate increase in number and size (Table 3). Stage (III) of spermaries appeared in February 2019 in 62.5% colonies and reached its ceiling (68.75%, n=11 colonies) in March 2019 (Table 2 & Figure 2C, G), with mean (±SD) diameters varying between 141.90±2.10 and 163.30±2.90 µm (Table 3). Stage (IV) of spermaries development was recorded in March 2019 with 31.25% colonies (n=5 colonies) and spiked to its highest level in April 2019 as 100% of colonies (Table 2). At the end of stage (IV), spermaries were found fully mature with no further progression as early as 7 days before April full moon.

Finally, the present results concluded that female and male gametogenesis of *A. cytherea* and *A. tenuis* started in October and November 2018, respectively and ended in April 2019 (Figure 2 & Table 2). Consequently, such coral species proved to possess only one annual gametogenic cycle, and assured that the gametes reach their peak of maturity during March-April period every year.

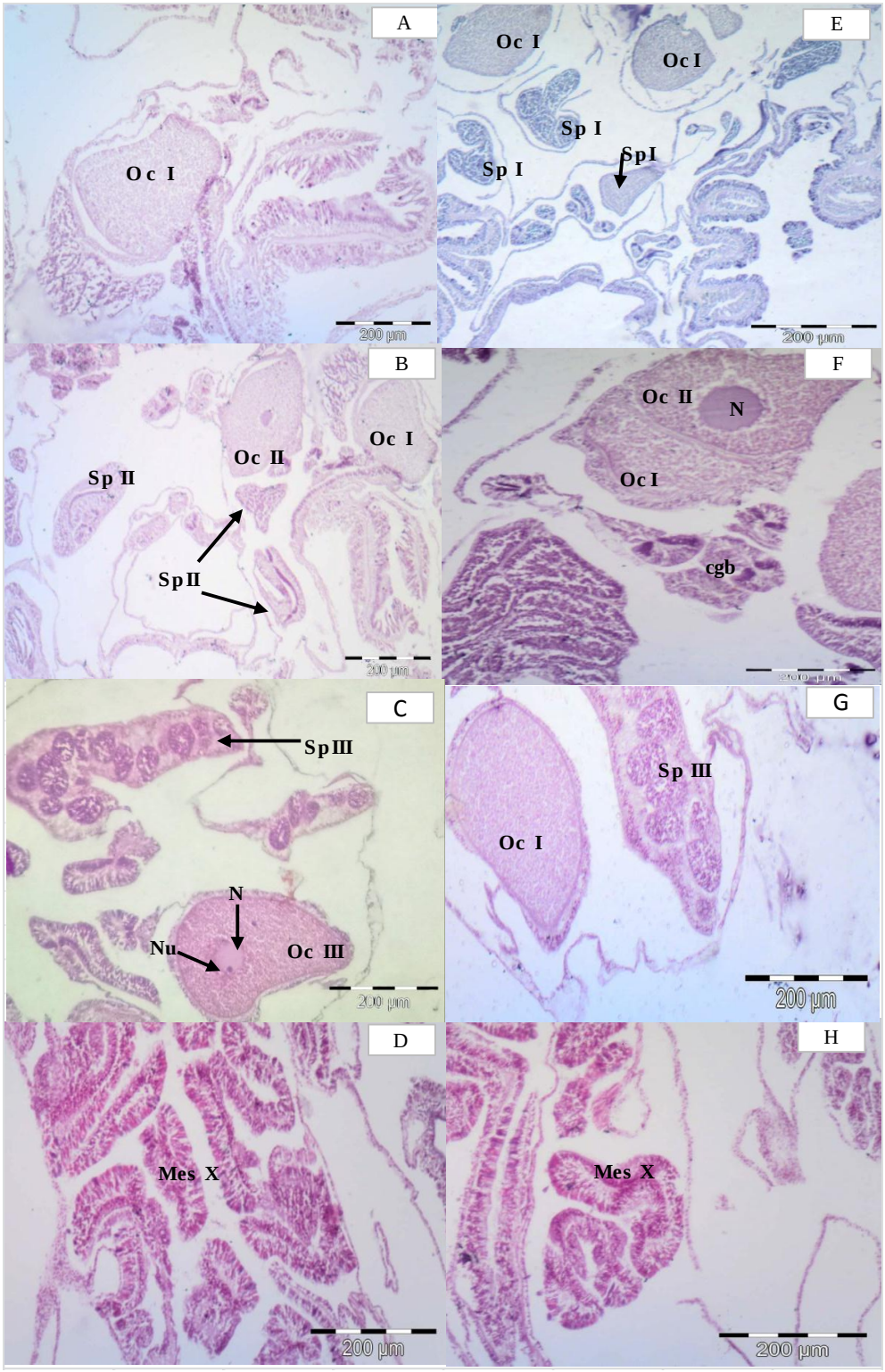


**Figure 2:** Photomicrographs showing various stages of oogenic and spermatogenic development of *Acropora cytherea* (A-D) and *Acropora tenuis* (E-H). Oc I, Oc II and Oc III represent oocyte stages, while Sp I, Sp II and Sp III indicate spermary stages. N, nucleus; Nu, nucleolus; Mes X, spawned mesenteries; cgb, cnidoglandular band. Section dates: A, E (Nov-2018); B, F (Jan-2019); C, G (Mar-2019); D, H (End of Apr-2019). Scale bar: 200µm.

**Table 2:** Dates of development stages and spawning of *Acropora cytherea* and *Acropora tenuis* gametes during successive two years (2018 and 2019) of observations in the two study sites. Number of colonies (n) contained each gametogenic development stage is shown in parenthesis. I, II, III and IV represent various stages of oocytes and spermaries, while 0 represent the spawned colonies. NA (not appeared) refers to the absence of gametes. Asterisks mark the spawning months.

| Date | *Acropora cytherea* | | | | *Acropora tenuis* | | | | 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-2018 | III(1), IV(3) | IV(4) | IV(4) | III(1), IV(3) | IV(4) | IV(4) | IV(4) | IV(4) | Pale pink |
| Apr-2018***** | IV(4) | IV(4) | IV(4) | IV(4) | IV(4) | IV(4) | IV(4) | IV(4) | Pink |
| May-2018 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | NA |
| Jun-2018 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | NA |
| Oct-2018 | I(4) | I(3), NA(1) | I(4) | I(1), NA(3) | I(4) | I(2), NA(2) | I(2), NA(2) | I(1), NA(3) | Creamy |
| Nov-2018 | I(4) | I(3), NA(1) | I(4) | I(2), NA(2) | I (4) | I(3), NA(1) | I(3), NA(1) | I(4) | Creamy |
| Dec-2018 | I(1), II(3) | I(1), II(3) | I(2), II(2) | I(1), II(3) | II(4) | II(4) | II(4) | I(2), II(2) | Yellow |
| Jan-2019 | II(4) | II(4) | I(1), II(3) | I(1), II(3) | II(4) | II(2), III(2) | II(3), I(1) | II(4) | Yellow -orange |
| Feb-2019 | III(4) | II(1), III(3) | III(4) | III(4) | II(2), III(2) | II(2), III(2) | II(3), III(1) | II(3), III(1) | Orange |
| Mar-2019 | III(1), IV(3) | III(4) | III(4) | III(1), IV(3) | III(4) | III(4) | III(3), IV(1) | III(2), IV(2) | Pale pink |
| Apr-2019***** | IV(4) | IV(4) | IV(4) | IV(4) | IV(4) | IV(4) | IV(4) | IV(4) | Pink |
| May-2019 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | NA |

**Table 3:** Ranges of mean (±SD) oocytes and spermaries diameters recorded for the different developmental stages of the two studied coral species.

| Development stage | I | II | III | IV |
|---|---|---|---|---|
| Oocyte diameter (µm) | 272.00±2.68 – 310.60±5.15 | 322.40±3.24 – 358.70±3.77 | 373.20±3.77 – 391.90±4.65 | 415.60±2.30 – 419.70±2.62 |
| Spermary diameter (µm) | 61.57±0.50 – 83.06±1.08 | 104.40±4.20 – 126.20±4.00 | 141.90±2.10 – 163.30±2.90 | 166.70±3.90 – 175.35±3.10 |

## 2. Statistical comparison of gametes diameters among dates and reef sites:

Gametes diameters of *Acropora cytherea* and *Acropora tenuis* were compared among dates, as well as between the two studied reef sites using two-way ANOVA analysis. The results of this statistical analysis demonstrated that the oocytes and spermaries diameters of both species were significantly varied ($P \leq 0.01$) not only among months but between the two study sites as well (Table 4). In addition, the interaction

between dates and sites (dates × sites) was significantly (P≤0.01) affected on the oocytes' sizes of *Acropora tenuis* and spermaries sizes of *Acropora cytherea* (Table 4). This reflects the effect of changes in lunar cycle and photoperiod (day length) on the gametes maturation and spawning timing.

**Table 4:** Summary of two-way ANOVA for oocytes and spermaries diameters of *Acropora cytherea* and *Acropora tenuis* colonies collected from the two sampling sites on nine dates. At every site, the data on gametes diameters were gathered from 4 colonies of each species. Marked differences are significant at p≤0.05.

**A- Oocytes diameters**

| Variables | Df | *Acropora cytherea* | | | *Acropora tenuis* | | |
|---|---|---|---|---|---|---|---|
| | | SS | MS | F | SS | MS | F |
| Dates | 8 | 156776.58 | 19597.07 | 1526.35** | 117901.94 | 14737.74 | 1128.10** |
| Sites | 1 | 155.06 | 155.06 | 12.08** | 4151.35 | 4151.35 | 317.77** |
| Dates×sites | 8 | 1.15 | 1.43 | 1.12 | 3806.34 | 475.79 | 36.42** |
| Error | 54 | 693.31 | 12.84 | | 705.47 | 13.06 | |
| Total | 71 | 157624.95 | | | 126565.10 | | |

**B- Spermaries diameters**

| Variables | Df | *Acropora cytherea* | | | *Acropora tenuis* | | |
|---|---|---|---|---|---|---|---|
| | | SS | MS | F | SS | MS | F |
| Dates | 7 | 96795.7 | 13828 | 1190** | 98010 | 14001 | 1343.04** |
| Sites | 1 | 178.5 | 178.5 | 15.36** | 178.5 | 178.5 | 17.99** |
| Dates×sites | 7 | 4.67 | 6.67 | 5.77** | 0.1922 | 0.03 | 0.003 |
| Error | 48 | 557.76 | 11.6 | | 529.40 | 10.43 | |
| Total | 63 | 97532 | | | 143015.68 | | |

* = Significant at P≤0.05, **= Significant at P≤0.01

### 3. Spawning timing and synchronization:

According to the present results, the two studied species *Acropora cytherea* and *Acropora tenuis* reached their maturity peak during March-April period every year and spawned only once around April full moon during 2018 and 2019 (Table 2 & Figure 2). In the year 2018, the spawning of *A. Cytherea* occurred in 28 April, two days before the full moon (30 April); while *A. tenuis* spawned in 29 April, one day before the full moon (Table 5). The colonies were found empty through the rechecking that occurred after 3 days following the full moon date (Table 1).

In the year 2019, *A. Cytherea* spawned in 18 April, one day before the full moon (19 April); whereas *A. tenuis* spawned in the full moon day and one day after the full moon (Table 5). On 21st of April 2019 and in the next summer months, all colonies of both species in the both study sites were empty of oocytes (Figure 2 D, H). Therefore, it can be concluded that all tagged colonies of *Acropora cytherea* and *Acropora tenuis* in the two study sites released their gametes to the seawater around the full moon of the spawning months (Tables 1, 2 & 5).

Generally, our results revealed high similarity in gametes maturation between the two studied species, where these acroporid species released bundles of sperms and eggs in a highly synchronized fashion with all colonies of the same species on the study reefs

spawning concurrently. Despite that the oocyte development typically began in October and preceded the development of spermaries by one month, the two types of gametes were released synchronously together as egg-sperm bundles.

**Table 5:** Spawning timing of *Acropora cytherea and Acropora tenuis* in relation to the full moon in the years2018 and 2019 at the study sites.

| Species | 2018 | | 2019 | |
|---|---|---|---|---|
| | Spawning time | Full moon date | Spawning time | Full moon date |
| *Acropora cytherea* | 28 April | 30 April | 18 April | 19 April |
| *Acropora tenuis* | 29 April | 30 April | 19 and 20 April | 19 April |

## DISCUSSION

This study was presented to provide data on the reproductive characteristics and spawning pattern of two reef-building acroporid coral species, *Acropora cytherea* and *Acropora tenuis* (Scleractinia: Acroporidae) in Hurghada, Egyptian Red Sea coast during the years 2018 and 2019. Despite the highly abundance and diversity of *Acropora* species in the Red Sea reefs **(Ghallab *et al*., 2020)** along with their ease of sampling and study, only very few studies have described gametogenic development of this genus in the Red Sea (**Shlesinger & Loya, 1985; Bouwmeester *et al.*, 2011; Rashad *et al*., 2020**). In a long-term study (10 years), **Shlesinger *et al.* (1998**) examined the reproductive characteristics of 23 species of scleractinian Red Sea corals (seven families) comprising six species that belong to genus *Acropora* at Eilat, northern Red Sea. This previous study is one of the very few studies that investigated the reproductive traits of hermatypic scleractinian corals using laboratory and histological examinations, in addition to the field observations, in the Red Sea. The present study also investigated the coral reproductive mode and spawning pattern in the northern Red Sea using histological examinations and field observations. The current results revealed that both studied acroporid species were simultaneous hermaphroditic broadcast spawners **(Shlesinger & Loya, 1985; Wallace, 1985; Morita *et al.*, 2019)**. This is the dominant mode of sexual reproduction in scleractinian corals (**Baird *et al*. 2009**), encompassing *Acropora* species **(Howells *et al*., 2014; Prasetia *et al*., 2016; Jamodiong *et al*., 2018b; Gan *et al*., 2021).**

Concerning the gametogenesis and the spawning timing of *Acropora cytherea* and *Acropora tenuis*, the present study demonstrated that both species exhibited a single annual gametogenic cycle (7 months), that started in October 2018 and ended in April 2019. Oocyte development preceded the development of spermaries by one month, the oogenesis occurred for 7 months from October 2018 to April 2019, whereas spermatogenesis occurred for 6 months from November 2018 to April 2019. Both female and male gametes became fully matured in different mesenteries by the end of March and are ready to be released before April full moon for the two consecutive years (2018-2019). No overlapping gametogenic cycle was recorded in both investigated

*Acropora* species during their reproductive period. Thus, it is suggested that the single annual gametogenic cycles are traits of the major colonies of *Acropora cytherea* and *Acropora tenuis* in the northern Red Sea. Similar findings were reported in Singapore for *Acropora humilis* **(Guest *et al.,* 2005)**; and north-western Philippines for *Acropora hyacinthus* (**Jamodiong*et al.,* 2018b**). Furthermore, the Indo-Pacific mesophotic coral, *Acropora tenella* showed a single annual gametogenic cycle, and both oogenesis and spermatogenesis occurred for 11–12 and 5–6 months, respectively in an upper mesophotic reef (40 m depth) in Okinawa, Japan **(Prasetia *et al.,* 2016).**

The spawning events reported in most areas are seasonal and limited to a certain time of the year (**Baird *et al.,* 2009**). It was reported that, the day of spawning is generally in tune with lunar periodicity, and assumed to be determined by variations associated with the lunar cycle, e.g. in moonlight, pressure or water motion **(Mangubhai & Harrison, 2008a; Baird *et al.,* 2010; Howells *et al.,* 2014; Lin & Nozawa, 2017**). To determin the spawning hour, the duration of darkness after sunset is reportedly a potential proximate clue **(Hayashibara *et al.,* 2004; Brady *et al.,* 2009).** The present data highlighted the important role of lunar phase and photoperiod (day length) to assess the timing of spawning of *A. cytherea and A. tenuis.* Furthermore, results indicated that the spawning timing of both *Acropora* species was fairly related to the changes in seawater temperature in the study sites. Based on the disappearance of gametes within coral colonies and direct field observations, the spawning of both studied species predominantly occurred within the week of April full moon in 2018 and 2019. In 2018, *A. Cytherea* was found to spawn in the 28$^{th}$ of April; two days before the full moon (30 April); while *A. tenuis* was observed to spawn in th 29$^{th}$ of April, one day before the full moon. The relatively same trend was noticed in 2019, the spawning of *A. Cytherea* occurred in the 18$^{th}$ of April, one day before the full moon (the 19$^{th}$ of April); whereas *A. tenuis* spawned in the full moon day and one day after the full moon. It is worth mentioning that in the Indo-Pacific region, the spawning of many *Acropora* species takes place mainly according to the lunar phase **(Harrison *et al.,* 1984; Babcock *et al.,* 1986; Wilson & Harrison, 2003; Baird *et al.,* 2010**). Several *Acropora* species in other regions of the world were reported to spawn between March and May in each year **(Howells *et al.,* 2014; Gouezo *et al.,* 2020; Gan *et al.,* 2021).** In the Kuantan coastal region (Peninsular Malaysia), the two *Acropora* species; *Acropora cytherea* and *Acropora clathrata*, spawned between 10-11 nights after the full moon during the interval from April until May 2018 **(Hanapiah *et al.,* 2020).** As in the majority of hermaphroditic broadcast spawning scleractinian corals, *A. cytherea and A. tenuis* released their reproductive material (including female and male gametes) into the water column separately or packaged as positively buoyant egg–sperm bundles during spawning events **(Kinzie, 1996**; **Padilla-Gamiño *et al.,* 2011).** The egg–sperm bundle is an efficient method of transfering gametes to the sea surface where fertilization occurs, while

decreasing sperm dilution and increasing the chance for gamete encounters during a spawning episode **(Padilla-Gamiño *et al*., 2011).**

Synchrony of spawning within populations of sessile species like corals is essential for successful fertilization and larval production. However, the extent and timing of synchronous spawning episodes differ amongst and within taxa and biogeographic areas **(Shlesinger & Loya, 1985, 2019; Penland *et al*., 2004**). Consequently, synchrony of coral reproduction has significant consequences for the biodiversity and conservation of reefs **(Guest *et al*., 2008)**. The present observations revealed synchronous spawning events among colonies of both *Acropora cytherea* and *Acropora tenuis* around the full moon of the spawning month (April 2018 and 2019). These acroporid coral species released bundles of sperms and eggs in a highly synchronized fashion with all colonies of the same species on the reef sites spawned concurrently. This spawning pattern is consistent with several previous observations in other locations in the Indo-pacific region (**Howells *et al*., 2014; Baird *et al*., 2015**).

In the Gulf of Oman ( that connects the Arabian Sea with the strait of Hurmuz), the appearance of mature gametes within the same month for *Acropora downingi*, *Acropora hemprichii*, *Cyphastrea microphthalma* and *Platygyra daedalea* (≥75% of colonies, n = 848) elucidated a synchronous and multi-specific spawning time **(Howells *et al*., 2014)**. In contrast, asynchronous spawning pattern was reported in other regions for diverse scleractinian coral species **(Prasetia *et al*., 2016; Gouezo *et al*., 2020)**. Asynchronous spawning events were registered in 20 acroporid coral species and some Merulinidae species on equatorial reefs of Kenya **(Mangubhai & Harrison, 2008a, b).** In addition, *Acropora hyacinthus* in north-western Philippines exhibits an extended release of gametes within 2 to 3 months (from February to April), and the two types of extended spawning patterns that are unique in this region were observed in this species (*i.e.*, asynchronous spawning amongst colonies and split spawning of individual colonies) **(Jamodiong *et al*., 2018b)**.

## Conclusion

In conclusion, the present study documented the reproductive conditions of the hermatypic scleractinian corals, *Acropora cytherea* and *Acropora tenuis* in Hurghada, northern Red Sea. The current results showed that both species are hermaphroditic broadcast spawners with an annual gametogenic cycle (7 months) that began in October 2018 and terminated in April 2019. Moreover, both oogenesis and spermatogenesis occurred for 7 and 6 months, respectively. Both *A. cytherea* and *A. tenuis* displayed synchronous spawning patterns among colonies of the same species, and the timing of spawning was closely related to the lunar cycle and photoperiod (day length). Accordingly, colonies of both species were recorded to spawn around the full moon in April 2018 and 2019. Since data on the reproductive biology of Red Sea corals is still poorly covered, a need for further research on large number of species is recommended.

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