Observations of synchronized spawning, larval survival and settlement in corals of the genus *Acropora* in Lakshadweep, India

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Reports of synchronous spawning in corals of Lakshadweep reefs, India have been purely anecdotal. To understand factors that underlie coral spawning in Lakshadweep, we studied four *Acropora* species (*A. muricata*, *A. cytherea*, *A. hemprichii* and *A. nobilis*) in Kavaratti atoll from February to March 2018. These species were observed *in situ* for oocyte maturation, and the larvae of species *A. muricata* and *A. cytherea* were assessed for survival and settlement rates. Observations on oocyte maturation indicated possible spawning closer to the immediate full moon of species in which 7–28% of the colonies had mature oocytes. Thus, *A. muricata*, *A. cytherea* and *A. hemprichii* spawned on 25 and 26 February 2018, demonstrating multi-specific synchronous spawning, whereas *A. nobilis* spawned on 21 March 2018. Larval survival rates revealed a steep die-off beginning on day 12 post-spawning and maximum survival up to 24–27 days. Settlement occurred between 12 and 20 days for *A. muricata* and between 12 and 22 days for *A. cytherea*. The comparatively low survival duration and fewer larvae surviving for settlement show that maximum settlement could be heavily compromised. However, the study reveals that the comparatively late onset of settlement (from day-12, post-spawning) and the broader settlement window of 8–10 days could allow room for long-distance dispersal of larvae. Further studies on this front are required to gather a better picture.

**Keywords:** *Acropora* genus, coral reproduction, larval survival, oocyte maturation, synchronized spawning.

**Synchronized** spawning has been observed in corals during sexual reproduction, which occurs either through broadcasting or brooding¹². Broadcasting is the process where coral polyps release bundles of sperm and eggs into the water column, and thus fertilization is external; brooders release hatched planula developed from internally fertilized eggs. Brooding and broadcasting gametes are found in both gonochoristic or hermaphroditic corals⁴. Broadcasting and synchronized spawning in corals are vital to accomplishing high fertilization rates, and increasing the probability of genetic mixing between colonies¹⁴. Spawning also indicates the presence of adult colonies capable of maintaining sustainable and self-seeding populations on a reef⁵. Most scleractinian corals are simultaneous broadcasting hermaphrodites that spawn annually, usually during the warmest months in the year, although the exact duration differs from location to location based on latitudinal and temperature variations⁶–¹¹. Since spawning is influenced on a regional scale, depending on factors like time of the year, lunar phase, seawater temperature and availability of settlement cues, it is necessary to make observations on a regional scale³,⁷,¹². Besides, it is also critical to assess survival and settlement rates of coral larvae to understand spawning success.

We undertook the present study given the lack of observations of coral spawning in Lakshadweep reefs, and the importance of such knowledge to the conservation of coral species. We observed four species of the coral genus *Acropora*, viz. *A. muricata*, *A. hemprichii*, *A. cytherea* and *A. nobilis*, for their spawning patterns. Experiments to study rates of survival of larvae were executed for two species, *A. muricata* and *A. cytherea*, the results of which are presented here.

**Methods**

**Preliminary observations**

Kavaratti atoll (10°34′22.25″N; 072°37′49.15″E) in the Lakshadweep Islands was selected as the study site. The atoll, elliptical in shape, is aligned in a northeast direction and has a broader lagoon to the west, and the island to the east (Figure 1). The total extent of reef is about 3.3 sq. km (ref. 13). Although the live coral cover estimated at the reef slopes was only 5–9.2%, thickets of *Acropora* spp. of ~76% cover were found in the lagoon near the reef crest. We inferred the approximate time of coral spawning for the Lakshadweep atolls from spawning occurrences reported from neighbouring reef regions and reports of spawn slick for the study site reported from Agatti atoll, Lakshadweep⁸,¹⁴,¹⁵. We carried out preliminary observations between January and March 2017,
observing oocyte maturation in randomly selected colonies. Colonies with pigmented oocytes were scored as 'reproductively mature', whereas those with unpigmented oocytes and empty mesenteries were scored as 'reproductively immature' as these were considered to have already spawned or highly unlikely to spawn within the next lunar cycle\(^2,6-8,11,14,16\). The observations showed matured oocytes from 11 February 2017 in *A. muricata*, and the spawn slicks on 7 March 2017. Taking these as inference, we planned the study to record spawning in selected *Acropora* species the following year (2018).

**Observation of maturation in oocytes**

A sampling station about 500 sq. m was marked (10°34′22.25″ N; 72°37′49.15″ E) in the lagoon, which suited the criteria of having the highest abundance and area cover of *Acropora* spp. (Figure 1). We chose *A. muricata, A. cytherea, A. hemprichii* and *A. nobilis* as candidate species given their sufficient colonies for sampling within 500 sq. m of the marked study site. The colonies were sampled from 11 February 2018 onwards – the date decided based on observation of mature oocytes in *A. muricata* colonies in the preliminary observations during January–March 2017. Fifteen random colonies of each species with >30 cm of colony diameter were sampled to observe the maturation of oocytes\(^14\). One or two fragments were broken from each colony, 5–15 cm away from the tip of the sterile axial corallite\(^14,17\). The oocyte maturation was scored as noted above.

**Collection of spawn**

Spawn traps were used for the collection of egg–sperm bundles. The traps were designed to be conical in shape (made using 100 \(\mu\)m plankton nets that measured 147 cm in height and had a square base that covered an area of 90 cm\(^2\)) with the tapered end opening into a sleeve provided with a drawstring to attach a plastic collection container of 1 litre volume (Figure 2). The base was anchored using small weights in such a way to cover the colony, and the sleeve of the net was fitted with floats.

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**Figure 1.** The study site in Lakshadweep, India (marked here by a darker green shade) was selected as it had an abundance cover of the targeted *Acropora* spp. The site has an extent of 500 × 500 sq. m, and was located on the northwestern side of the Kavaratti atoll inside the lagoon (10°34′22.25″ N; 72°37′49.15″ E).

**Figure 2.** Diagrammatic representation of the spawn-trap fabricated to collect coral spawn. It consists of weights made of concrete anchors, and 100 \(\mu\)m plankton net stitched conically and tapering towards the tail, where a collection chamber could be easily attached using a draw string. The collection chamber is attached to a float to make it stand perpendicular.
(in order to gather and collect the buoyant spawn) that maintained the conical shape of the net and kept the collection container upright. Two gravid colonies for each of the individual species were set up with nets every evening at 1730 h. The nets were removed the next morning at 0630 h. Any spawning activity was monitored by keeping overnight vigils in a boat, and the collection containers checked for spawn every 30 min throughout the period of deployment. This operation was followed from 11 February 2018 until the spawning night. Observers either snorkelled or SCUBA-dived during deployment and monitoring. Once spawning was complete, the containers possessing spawn were carefully removed to be transported to the laboratory within 2 h for effecting fertilization. The event of spawning was videographed.

Fertilizing the eggs

Spawns from different colonies of each species were transferred to circular polyvinyl culture tanks, maintained at a sheltered enclosure containing 100 litres of filtered seawater, stirred gently to aid fertilization, and left undisturbed for 30 min to avoid polyspermy. Thereafter the tanks were topped up to 200 litres and maintained. The water was replaced periodically (once in every 6 h on the first two days, and every 24 h, for the following 10 days, and every 48 h thereafter) by siphoning out approximately half the volume of water through a 60-μm filter cloth and replacing it with fresh filtered seawater to cope with rising nitrate levels due to decaying unfertilized eggs. Lipid accumulation along the sides of the tank due to dead larvae and unfertilized eggs was removed by wiping with a sponge and skimming the surface foam whenever observed. The water was aerated continually, and the water quality tested daily using a reef-aquarium water quality test kit (API marine reef master test kit). The temperature of water was maintained between 28°C and 30°C by providing proper shading. The approximate stocking density of planula larvae obtained was 372 and 328 larvae/l for A. muricata and A. cytherea, respectively. Only a few eggs/larvae were obtained from A. nobilis and A. hemprichii were therefore not considered for the experiments to assess larval survival and settlement rates.

Estimation of larval survival rates

The survival rates were estimated for the larvae of A. cytherea and A. muricata. The number of larvae surviving in the culture throughout the duration (28 days) was enumerated from 50 ml aliquots (n = 5) taken every 48 h from the culture tanks. Care was taken to ensure a homogenous distribution of larvae in the water column by gently stirring the culture using a spatula during sampling. It may be noted that the larvae in the culture tanks did not settle throughout the study duration, for which inducement by introducing crustose coralline algae was required.

Estimation of larval settlement rates

The experiments for larval settlement rates were carried out from the sixth day of culture (day 2 of the larval life), when the larvae of A. muricata and A. cytherea, obtained fully formed pear shapes. Ten randomly sampled individuals were introduced into shallow polyethylene cups (n = 3) containing 10 ml of filtered seawater with 10 mg of crustose coralline algae (CCA) slurry to induce them to settle. Figure 3. A broken fragment of Acropora muricata showing pigmented oocytes in the mesenteries, sampled on 20 February 2018. An estimate of the percentage of colonies with mature oocytes was arrived at by breaking a coral fragment to note the presence or absence of pigmented oocytes.

Figure 4. Percentage of colonies with mature oocytes as of 20 February 2018, in Acropora muricata (27%), A. cytherea (20%) and A. hemprichii (7%). Acropora nobilis colonies were not observed with pigmented oocytes, indicating a later spawning date for this species.
to settle. The number of larvae settled and free-floating was counted after 48 h (on day 8 of the culture) under a dissection microscope following Pollock et al. The experiment due for observation on day 10 was begun immediately after completing the first observation, i.e. on day 8, following the same protocol. The control (n = 3) consisted of the same set-up without the introduction of CCA. The experiment was repeated ten times for 18 days until day 26 of culture.

Results

Maturation of oocytes

Three of the four species of Acropora studied were observed with mature pigmented oocytes, beginning 11 February 2018 (Figure 3). Figure 4 shows the score as on 20 February 2018 for each species (n = 15). A. muricata, A. cytherea and A. hemprichii exhibited pigmented oocytes at the rate of 7–28%, indicating possible spawning of these species within the next full moon, as deduced from earlier studies, falling on 2 March 2018. A. nobilis, on the other hand, did not exhibit pigmented oocytes at this time, but was observed with mature oocytes the following month.

Occurrence of spawning

We reported synchronous spawning during 25–26 February 2018 for the three species of Acropora, which were predicted to spawn closer to the full moon night on 2 March 2018 (Table 1; Figure 5). A. cytherea and A. hemprichii spawned at 2130 h on 25 February 2018. However, out of two colonies per species laid with spawn-traps, spawn (egg–sperm bundles) could be observed on one each, in these species (Figure 5). All three species (including A. muricata) spawned on the next day (26 February 2018), four days before full moon, and the egg–sperm bundles were collected on all the six (two each for three species) traps placed. Spawn–slick was observed across the lagoon in the night and along the southwestern shores of the island on the next morning (Figure 6). Observation of coral fragments of the species spawned showed empty mesenteries until the first week of April 2018, confirming the absence of split spawning pattern in these species (Figure 7). A. nobilis spawned on
Table 1. Species studied, date and time of spawning, particulars of spawn-traps, temperature and moon-visibility data at the time of spawning

<table>
<thead>
<tr>
<th>Species</th>
<th>Date of spawning</th>
<th>Time</th>
<th>Number of traps laid (two for each species)</th>
<th>Temperature (°C)</th>
<th>Moon visibility (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acropora cytherea</td>
<td>25 February 2018</td>
<td>9.00–10.00 pm</td>
<td>One full, one empty</td>
<td>31</td>
<td>69</td>
</tr>
<tr>
<td></td>
<td>26 February 2018</td>
<td>9.30–10.00 pm</td>
<td>Two full</td>
<td>30</td>
<td>79</td>
</tr>
<tr>
<td>Acropora hemprichii</td>
<td>25 February 2018</td>
<td>9.00–10.00 pm</td>
<td>One full, one empty</td>
<td>31</td>
<td>69</td>
</tr>
<tr>
<td></td>
<td>26 February 2018</td>
<td>9.30–10.00 pm</td>
<td>Two full</td>
<td>30</td>
<td>79</td>
</tr>
<tr>
<td>Acropora muricata</td>
<td>26 February 2018</td>
<td>9.30–10.00 pm</td>
<td>Two full</td>
<td>30</td>
<td>79</td>
</tr>
<tr>
<td>Acropora nobilis</td>
<td>21 March 2018</td>
<td>9.30–10.00 pm</td>
<td>Two full</td>
<td>31</td>
<td>18</td>
</tr>
</tbody>
</table>

Figure 6. Spawn slick observed on 27 February 2018 at 8:30 am on the southwestern shores of Kavaratti Island, Lakshadweep.

Figure 7. A broken coral fragment sampled after the spawning event on 28 February 2018. The fragments had mesenteries empty of oocytes.

21 March 2018. However, oocyte maturation rates in this species could not determined due to logistic constraints.

Larvae survival experiment

The larvae were noticed in the water column on the fourth day after effecting fertilization (Figure 5d). The number of eggs that developed into planula larvae was highest on day 6 of the culture for A. cytherea (average 17.8 larvae/50 ml, n = 5), and day 10 for A. muricata (average 9.4 larvae/50 ml, n = 5). The two species showed similar survival patterns (a one-way ANOVA performed showed P values = 0.22, alpha value = 0.05), with a steep decrease in larval numbers after the 12th day of culture, followed by a gradual decline (Figure 8). The
larvae of *A. muricata* and *A. cytherea* survived up to days 25 and 27 respectively.

**Larval settlement experiment**

The larvae of *A. muricata* settled between 12 and 20 days and those of *A. cytherea* between 12 and 22 days. The peak larval settlement was on day 16 for *A. muricata* and day 18 for *A. cytherea* (Figure 9). Both the species followed a similar pattern of settlement. None of the larvae in the control setups settled, proving that CCA is essential to induce settlement.

**Discussion**

Until 1970 scientists did not realize that certain coral species were capable of fertilization externally, apart from the general assumption that corals reproduced internally by brooding larvae. This discovery led to further research on the sexual reproduction of corals that reproduced externally, also known as broadcast spawners. The findings that corals which broadcast egg–sperm bundles could have long dispersal ranges and multi-specific synchronous spawning enables genetic mixing underscore the importance of broadcast spawning to the well-being of reefs and hence the need for studies to understand spawning patterns of coral species in reefs.

There is little information on coral spawning from Indian reefs, and studies on behaviour of coral larvae are unavailable. Previous reports in Indian reefs show spawning occurring in March, except for a report from the Andaman Islands, where it is in February. For Lakshadweep reefs, a previous report of spawn-slick was on 18 March 2013 (ref. 15). The review of coral spawning in the Arabian Sea and western Indian Ocean by Howells et al. in 2014, which also covers previous spawning reports from Lakshadweep and the Maldives, points to the general occurrence in March.

We observed pigmented oocytes in February (11 February 2018 onwards) in three of the *Acropora* species (*A. muricata*, *A. hemprichii* and *A. cytherea*) with 7–28% of the colonies exhibiting pigmented oocytes on 20 February (Figure 3). These species were predicted to spawn during or closer to the approaching full moon and spawned five days prior to the full moon on 25–26 February. *A. nobilis*, which did not exhibit pigmented oocytes in February, spawned on 21 March 2018, i.e. five days after new moon. The moon visibility graph shows the two spawn timings of the species observed in Lakshadweep (Figure 10).

Coral spawning before full moon is a rare occurrence; most reported spawning occurrences were 4–7 nights after full moon across the Great Barrier reef, Taiwan, Japan, Red Sea, Hawaii and Florida. However, as observed by Hayashibara et al. during 1989–91 in Akajima, Japan, and by Babcock et al. in the Great Barrier Reef, Australia in 1985, where the lunar phases of mass spawning were not consistent, the peak temperature could trigger spawning before a full moon. The annual rise in sea-surface temperature has been shown to predict synchronous spawning in corals. The spawning occurrence in the present study coincides with the initial summer-time rise in temperature, which falls in February–March (Figure 11), and not during the peak in May, suggesting that the spawning is triggered by initial warming of seawater. Nonetheless, all the spawning events occurring at night from 2100 to 2130 h indicate the influence of sunset time and diel cycles, as seen in previous studies over lunar periodicity and tidal influence.

Scleractinian corals have been observed to spawn synchronously in all parts of the world’s reefs, which
increases the probability of genetic mixing through cross-fertilization and reduces overall predation\textsuperscript{3,4}. Multi-specific synchronous spawning has been reported from \textit{Acropora} species in the Indo-Pacific\textsuperscript{6}. Our observations confirm synchronous spawning between colonies of \textit{Acropora} sp. in Lakshadweep, thus confirming the possibility for genetic mixing between colonies, and hence the long-term survival and adaptability of this species in these reefs.

Earlier studies on larval survival and settlement show the larvae of \textit{A. gemmifera}, \textit{A. millepora} and \textit{A. valida} surviving for a maximum duration of 60, 110, 130 days respectively\textsuperscript{5} – a longer survival duration compared to our estimates. However, Baird\textsuperscript{5} observed that these species could face limited dispersal due to low percentage of survival and limited settlement times. Studies have also reported that the larvae that show peak settlement between 7 and 8 days post-fertilization had lower chances of long-distance dispersal\textsuperscript{28}. Our study showed maximum survival duration of 25 and 27 days respectively, for the larvae of \textit{A. cytherea} and \textit{A. muricata} (Figure 8). Further, we observed a steep die-off beginning from day 12 of the culture, where the rate of survival on day 16 dropped to 21.4% and 26.0% for \textit{A. cytherea} and \textit{A. muricata} respectively (Table 2; Figure 8). This indicates that few larvae may be available for settlement during the peak settlement time, i.e. between day 16 and 18 of culture (Figure 9). It is clear that the larvae of \textit{Acropora} in the present study had comparatively low survival duration than that observed by Baird\textsuperscript{5}. The larvae also exhibited low survival rates. These factors could heavily compromise maximum settlement\textsuperscript{28}. However, the study reveals that the late onset of settlement time, i.e. from day 12 post-spawning and the relatively broader settlement window of 8–10 days (between 12 and 20 days for \textit{A. muricata}, between 12 and 22 days for \textit{A. cytherea}) could allow room for long-distance dispersal of larvae. Further studies are necessary in this regard.


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