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First observations of coral spawning at the Banda Islands, Maluku, Indonesia

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Abstract. *Novriansyah A, Huhn M, Wicaksono H, Senen B, Subhan B, Fenner D, Madduooa H, Dias PJ. 2023. First observations of coral spawning at the Banda Islands, Maluku, Indonesia. Biodiversitas 24: 6082-6091.* Due to their high diversity and productivity, their socio-economic value to coastal communities and their crucial role in coastal protection, coral reefs belong to the most important marine ecosystems in the world. Understanding and protecting coral reefs has never been so important, given the unprecedented anthropogenic pressures they face. Indonesia holds 18% of the world's coral reefs and is home to some of the richest marine biodiversity on the planet. However, with over 17,000 islands scattered across 86,700 km^2, marine monitoring and conservation remains challenging. The Banda Sea (Maluku, Indonesia) ecoregion ranks as a high priority for marine conservation. The present study reports on the first observations of coral spawning at the Banda Islands from 2016 to 2019. Ad-hoc opportunistic monitoring was carried out to identify patterns of coral spawning. Our results suggest the sharpest temperature increases and full moon timing have the biggest influence on the month and day of coral spawning, whereas the tidal cycle influences the hour when gametes are released. Coral spawning peaks were observed to occur in two seasons, April and October-November, 5-7 days after full moon and 2-3 hours after sunset during falling tides. The presence of two spawning seasons may provide coral reefs at the Banda Islands with an advantage to recover from potential impacts, such as storms, bleaching or destructive fishing.

Keywords: Banda Sea, coral conservation, spawning cues, spawning monitoring methods

INTRODUCTION

Coral reefs are one of the most important marine ecosystems in the world due to their high diversity, productivity, and socio-economic value to coastal communities worldwide (Baird et al. 2021). They play a crucial role in supporting fisheries resources, dive tourism, coastal protection, and fixing carbon through associated algae, seagrass meadows and mangroves (Kinsey and Hopley 1991; Alongi 2014; Guerra-Vargas et al. 2020). Understanding and protecting coral reefs has never been so important, given the unprecedented anthropogenic pressures they face today worldwide, at both global and local levels (van Hooidonk et al. 2016; Hughes et al. 2017; IPCC 2019).

Coral reproduction studies were mainly developed in the Great Barrier Reef in Australia, the Red Sea, and the Caribbean in the 1980s and 1990s (Harrison and Wallace 1990; Richmond 1997). In this period, the phenomenon of synchronous coral species mass spawning was discovered in the Great Barrier Reef (Willis et al. 1985). Since then, the same phenomenon has been observed and recorded in other reefs, including the Red Sea (Shlesinger and Loya 1985), Japan (Loya et al. 2009) and only more recently throughout the Indo-Pacific (Baird et al. 2021).

Scleractinian corals' two modes of reproduction are brooding, where eggs are fertilized and embryos kept internally until they reach the larvae stage, and broadcast spawning, where corals release their gametes for external fertilization (Harrison and Wallace 1990). Many coral species (around 83%) use a broadcast reproduction strategy in synchronized mass spawning events that generally happen once a year. Synchronously timing spawning within one species is crucial as eggs and sperm are viable for a limited time (about 2-3 hours) and fertilization, therefore, needs to take place soon after gametes are released (dela Cruz and Harrison 2020). It is further thought that, by having many species spawning thousands of eggs at the same time, their chance of survival may increase as predators can only feed on a small amount of the huge number of fertilized eggs that currents can then disperse longer until the larvae are developed and settle (Harrison and Wallace 1990).

Coral reproduction is complex, and its success is dependent on timing, which varies with factors such as latitude, geographical isolation, season, and oceanographic conditions (Keith et al. 2016). A wide range of

environmental factors is thought to underlie this success and trigger corals into the optimal timing for synchronized mass spawning over fine temporal scales, such as sea surface temperature, moon phases and time after sunset (Gilmour et al. 2016b, Lin and Nozawa 2023). Although fundamental findings on coral reproduction have accumulated in recent years, base background knowledge, such as the timing of coral mass spawning events, is still limited in most areas and countries, including Indonesia (Baird et al. 2021).

Indonesia's coastal ecosystems contain 18% of the world's coral reefs, which are home to some of the richest marine biodiversity on the planet (Asian Development Bank 2014). However, with over 17.000 islands scattered across 86,700 km2 of coral reef area, it remains a challenge for marine monitoring and conservation. This is particularly true for remote areas, far from central government and education hubs. In an assessment of geographic priorities for marine conservation, the Banda Sea ecoregion has been ranked a high marine conservation priority in Indonesia based on attributes such as high diversity of coral reef species and nearshore reef habitats (Asaad et al. 2018).

In adult corals, morphological identification can be done by macroscopic observation of species-specific characters, but it is difficult, time-consuming, and by non-experts, often only possible up to the genus level. Molecular DNA-based tools have gained popularity in the last two decades as a tool to support morphological observations (Neigel et al. 2007). They have been applied and refined to many marine organisms to provide increasingly reliable identifications at genus and species level, including from coral tissue and their gametes (Schweinsberg et al. 2014).

The present study reports on the first observations of coral spawning at the Banda Islands between 2016 and 2019. Ad-hoc opportunistic monitoring was carried out by researchers from IPB in collaboration with a local conservation team and the local university UBN to identify patterns of coral spawning and relate them to environmental cues.

MATERIALS AND METHODS

Location and timeline of ad-hoc observations

Coral spawning was observed at the Banda Islands (4.50 S, 129.88 E), Maluku (Moluccas) Province, Indonesia (Figure 1), for the first time in November 2016 during a night dive on the 6th day following the full moon (Table 1). In October 2017, after observing coral mass spawning again on three consecutive days (5th, 6th, and 7th) following the full moon, a project was established by the Institut Pertanian Bogor (IPB) Marine Biodiversity and Biosystematics Laboratory to investigate the phenomenon. Coral spawning surveys were further performed in November and December 2017, April and October 2018, and October, November, and December 2019. The main study site was a hard coral reef growing on an old lava flow on the north side of the island of Banda Api. This reef was chosen for its high coral cover and extraordinary diversity in *Acropora* species and other Scleractinian corals (Tomascik et al. 1996; Welly et al. 2012).

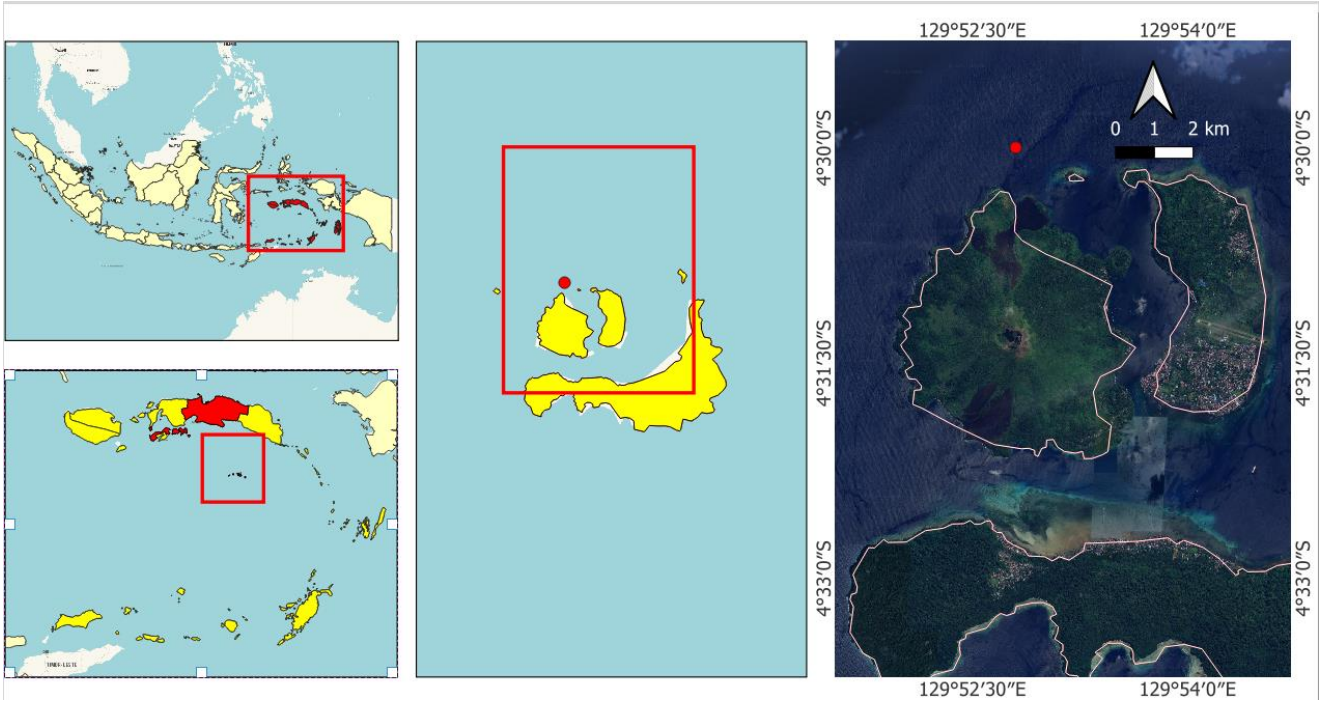

Figure 1. Map of the study location (Lava flow reef) at the Banda Islands, Maluku, Indonesia. The red circle marks the position of the studied reef area (Source: googlemaps.com & googleearth.com)

Coral spawning monitoring approach

Fieldwork consisted of scuba diving for visual observation and egg trap sampling (egg trap fabrication, installation, monitoring, and trap retrieval). Egg traps were built from household funnels 20 cm in diameter, which were placed upside down on top of a coral colony and tied to the colony or nearby rocks with nylon ropes. A PET bottle (600 mL) was attached to the small opening of the funnel (also upside down) by stitching and gluing the bottle lid to the funnel opening and screwing the bottle into the lid. The lid contained a hole so that gametes that would float up from the parent colony would first enter the funnel and then rise through the hole inside the lid into the PET bottle. The trap was kept afloat above the colony by a 2cm thick layer of air inside the bottles, which was added through the alternative air sources of the scuba diving regulators. The PET bottle could be unscrewed and covered with an intact lid whenever spawning was detected, and gametes were sampled. Traps were placed on 12-22 different colonies and 5-10 different species per sampling event. Using egg traps allowed for identifying whether spawning took place later during the night when divers were not in the water for direct observations. The area where traps were placed covered 100 m in length and was located between 3 m and 15 m in depth. After trap installation, traps were visually inspected for spawning daily in the mornings. If spawning occurred in colonies monitored with traps, eggs were collected for further analysis (attempt at species identification using DNA barcoding from eggs). Traps were used in November and December 2017, April 2018, and November 2019 to supplement direct diver observations.

Direct diver observations to identify coral spawning were conducted in the same reef area where the traps were installed by taking videos and photos of coral colonies spawning during night dives. Observations were done by 3-4 buddy teams that spread out across the reef and divers remained in the water for 60-70 min.

We monitored with either, or a combination of, traps and night diving on day 2-8 after full moon (AFM) in October 2017, day 1-8 AFM in November 2017, day 2-7 AFM in December 2017, day 3-8 AFM in April 2018, day 6 & 7 AFM in October 2018, day 2-7 AFM in Oct 2019, day 4-7 AFM in November 2019 and day 5 & 6 AFM in December 2019 (Table 2).

Data on environmental cues were obtained from online databases for sea surface temperature and moon phases. Daily mean SST were downloaded from Copernicus Marine Service (https://cds.climate.copernicus.eu) for position E129.8, S4.55 to investigate whether a shift in temperature could be a trigger for the corals to spawn. Tide tables for the nearest available position, S4.3332 E129.4763 (26 nm northwest of the study site) were freely available and downloaded from Badan Informasi Geospesial (BIG) Indonesia. Minimum and maximum monthly tidal means were calculated and plotted from the data. SST, daylight length and tidal means were plotted in MS Excel.

Coral identification

Identification of the colonies that spawned and of which samples were taken for DNA barcoding was conducted visually based on close-up photos and videos using 'Coral Finder Indo Pacific' (Kelley 2016) and the online database 'Corals of the World' (www.coralsoftheworld.org). Only those colonies that could confidentially be identified to species level were recorded as species. For the rest, the genus was recorded. The eggs collected using the traps were preserved in 96% ethanol and taken to the molecular marine biodiversity laboratory at IPB for DNA extraction and barcoding. DNA extraction was conducted using a Geneaid Tissue Genomic DNA Extraction Kit (Geneaid Biotech Ltd., New Taipei City, Taiwan), following the manufacturer's protocol. Polymerase Chain Reaction (PCR) amplification of the mtDNA COI gene region was performed using the primers available at the molecular lab in Bogor and respective published PCR conditions [universal primers LCO1490 and HCO2198 by Folmer et al. (1994); ITSZF forward and ITSZR reverse (Hardja 2009). PCR reactions were conducted in a peqStar 96 Universal (peqLab) thermal cycler. A negative control, with no template DNA added, was included in all PCR assays. PCR products were separated by electrophoresis using 1.5% agarose gels stained with ethidium bromide alongside 100 base pairs (bp) molecular weight markers and visualized under UV light.

Sequencing of impurified PCR products was performed in one direction, using the BigDye® Terminator v3.1 cycle sequencing kit chemistry and analyzed on an ABI 3730 at First Base, Malaysia. All sequences and the original chromatographs were manually checked for correct base calls and edited using the MEGA 6.0.5 (Molecular Evolutionary Genetic Analysis) program. Sequences were uploaded individually online to the NCBI website and matched against the GeneBank database using the Basic Local Alignment Search Tool (BLAST).

RESULTS AND DISCUSSION

In this study, we report the first records of coral spawning at the Banda Islands, Moluccas, Indonesia. It was important to us not to use any invasive methods to place an emphasis on good biological collection practices and education. We, for example, did not break any corals to determine egg maturation but relied entirely on direct observation of spawning and collection of gamete bundles using traps. Relying only on these methods makes it more labor-intensive to cover larger time frames with sampling, which is why we could not monitor throughout the entire year. With the sampling techniques available, a total of 35 coral spawning observations were made following the full moons in April, May, October, November, and December (Table 2). We identified four events where four or more species spawned at the same time (Oct and Nov 2017, April 2018, and Nov 2019). These are the only events that could potentially be termed 'mass spawning' because several colonies of each species spawned synchronously. However, our observations did not come near the 134 species observed to spawn following the full moon on the Great

Barrier Reef (GBR), within hours on the same night in the same area (Harrison and Wallace 1990). Apart from that, spawning events, at first sight, seemed more irregular and involved rather few species. When inspecting the data in detail and comparing the observations with sea surface temperature (SST), tidal and lunar patterns, we could identify patterns that will hopefully help predict future spawning events for a wider range of coral genera and species.

Spawning months

We obtained valuable first information on the months when spawning occurs and peaks. October, November, and April were the months during which we recorded most events of *Acropora* spawning (Figures 2A and 2B). Other genera (*Cyphastrea, Echinopora, Fungia, Galaxea, Goniopora, Montipora, Mycedium* and *Pectinia*) spawned in October, November, December, and April (Figures 2C-I). These months were also those with a change in mean tidal differences from rather low to rather high (Figure 3C) and a rapid increase in SST (Figure 3A). All except two spawning events occurred when SST had increased by at least 0.3°C compared to the previous month (Figure 3A). One exception was the spawning observation of one *Pectinia lactuca* Pallas 1766 colony in December 2017, detected using egg traps, where spawning occurred when mean monthly SST had stayed the same as the previous month. The other exception was one *Acropora* table coral in May 2018, when SST had dropped by 0.9°C (Figure 3A). The observation of the *Acropora* table spawning in May 2018 was coincidental, but in December 2017, when *P. lactuca* was found spawning, we monitored 24 colonies from several genera *(Acropora, Favia, Turbinaria, Galaxea, Pectinia, Fungia, Montipora, Goniastra, Stylophora*) with egg traps and none of them spawned. This leads us to conclude that December is not a peak month of spawning across genera and a reason could be that the sharpest increase in SST usually occurs in October and November (Figure 3A). The presence of two annual spawning seasons in low-latitudinal regions is not unusual. Wijayanti et al. (2019) reported spawning events from Karimunjawa Archipelago in March-April and September-October. Biannual spawning has also been reported from Scott Reef, Western Australia (Gilmour et al. 2016b). From South Vietnam, however, only one spawning season per year has been reported (Vo et al. 2022).

Annual SST averages at the Banda Islands (4.55 S, 129.8 E) during the study period were 29.86°C in 2017, 28.84°C in 2018 and 28.39°C in 2019 (CMEMS 2018). The lowest SST between Jan 2017 and Dec 2019 was measured on Aug 13th, 2019 (25.76°C), and the highest was on Dec 27th, 2019 (31.04°C).

SST plays a central role in triggering gamete maturation in corals of the Great Barrier Reef, determining the spawning season (Harrison and Wallace 1990). A correlation between gamete maturation and SST was also found for two species of *Acropora* corals in Palau (Gouezo et al. 2020) and a review of spawning seasons at 34 different reefs in the Indian and Pacific oceans showed that an increase in SST predicts spawning better than absolute high SSTs (Keith et al. 2016). Paxton et al. (2016) showed that an unusual increase in temperature can cause spawning to begin a day earlier than expected. Our observations, therefore, confirm previous findings, but are-to the best of our knowledge-the first ones to show the correlation between SST increase and timing of spawning in coral reefs in Indonesia.

Only a few studies have investigated coral spawning in the Indonesian Archipelago and most studies reported did not determine general synchronization patterns in reproduction. The latest and most comprehensive study of coral spawning patterns in Indonesia reports observations from Karimunjawa, Java Sea, from 2008 until 2013 (Wijayanti et al. 2019). This study observed 21 different species of *Acropora* corals and found an association between rising water temperature and spawning month and an association between a short drop in temperature and spawning time (Wijayanti et al. 2019).

Spawning days and time

In terms of the day of spawning, we could identify a coherency with the day after full moon. Even though we monitored once on day 1 after full moon (1 DAF), 4 times 2 DAF, 5 times 3 DAF and 3 times 8 DAF during the months when spawning was most common, we never recorded spawning on those days. Spawning always occurred between 4 DAF (1 observation) and 7 DAF (5 observations) with most colonies spawning 5 DAF (11 observations) and 6 DAF (18 observations, Table 2). This trend contradicts the days of spawning found by Wijayanti et al. (2019), where very irregular spawning days from 7 days before full moon (DBF) to 9 DAF were observed. Wijayanti et al. (2019) found *Acropora muricata* spawning 3, 6 and 7 DBF, whereas we found *A. muricata* spawning 5-7 DAF. Both studies (Wijayanti et al. 2019 and the present study) found incidences of *A. muricata* spawning on two consecutive days. We do not know about the local levels of artificial light pollution at the sites observed by Wijayanti et al. (2019), but if light pollution was present, it might be an explanation for the earlier or more irregular day of spawning in *A. muricata* than at the site observed in the present study, where no artificial light was present. The influence of artificial light at night (ALAN) on the day of spawning was recently shown in a study that analyzed Indo-Pacific spawning observations (Baird et al. 2021) combined with data from the 'Global atlas of artificial light at night under the sea' (Smyth et al. 2021) and found that ALAN-impacted corals spawned 1-3 days earlier (Davies et al. 2023).

Table 1. Nights of full moon at the Banda Islands, which were counted as day 0 for the calculation of spawning day after full moon (DAF)

2017		12 Jan	11 Feb	12 Mar	11 Apr	11 May	09 Jun	09 Jul	08 Aug	06 Sep	05 Oct	04 Nov	04 Dec
2018	02 Jan	31 Jan	02 Mar	31 Mar	30 Apr	29 May	28 Jun	28 Jul	26 Aug	25 Sep	24 Oct	23 Nov	23 Dec
2019		21 Jan	20 Feb	21 Mar	19 Apr	19 May	17 Jun	17 Jul	15 Aug	14 Sep	13 Oct	12 Nov	12 Dec

Table 2. Coral spawning observations at the Banda Islands from 2016 until 2019. Rows without observation number indicate days with sampling (diving or gamete traps) when no spawning was observed. DAF = days after full moon

Spawning observation number	Year	Species or lifeform	Genus	Date	DAF	Sunset time	Time spawning	Moonrise time	Method
1	2016	*Acropora* (table)	*Acropora*	20/11/2016	+6	18:21	20:52	23:58	Diving
2	2016	*Acropora* (table)	*Acropora*	20/11/2016	+6	18:21	20:58	23:58	Diving
	2017	no spawning (diving, 19:30, 70 min)		06/10/2017	+1	18:20		-	Diving
	2017	no spawning (diving, 19:30, 65 min)		07/10/2017	+2	18:19		00:05	Diving
	2017	no spawning (diving, 19:35, 70 min)		08/10/2017	+3	18:19		00:59	Diving
	2017	no spawning (diving, 19:40, 60 min)		09/10/2017	+4	18:19		01:53	Diving
3	2017	*Acropora subglabra*	*Acropora*	10/10/2017	+5	18:19	20:29	22:40	Diving
4	2017	*Acropora* sp. *(digitate)*	*Acropora*	11/10/2017	+6	18:19	20:58	23:39	Diving
5	2017	*Acropora* sp. *(table)*	*Acropora*	11/10/2017	+6	18:19	20:49	23:39	Diving
6	2017	*Acropora muricata*	*Acropora*	11/10/2017	+6	18:19	20:55	23:39	Diving
7	2017	*Acropora muricata*	*Acropora*	12/10/2017	+7	18:19	20:58	-	Diving
	2017	no spawning (diving, 20:15, 65 min)		13/10/2017	+8	18:18		00:37	Diving
	2017	no spawning		05/11/2017	+1	18:18		19:28	Traps
	2017	no spawning		06/11/2017	+2	18:18		20:28	Traps
	2017	no spawning		07/11/2017	+3	18:18		21:30	Traps
	2017	no spawning		08/11/2017	+4	18:18		22:30	Traps
8	2017	*Montipora* sp.	*Montipora*	09/11/2017	+5	18:19	20:30	23:29	Diving
9	2017	*Mycedium* sp.	*Mycedium*	09/11/2017	+5	18:19	20:30	23:29	Diving
10	2017	*Acropora muricata*	*Acropora*	09/11/2017	+5	18:19	-	23:29	Traps
11	2017	*Acropora* sp. *(table)*	*Acropora*	09/11/2017	+5	18:19	-	23:29	Traps
12	2017	*Acropora* sp.	*Acropora*	10/11/2017	+6	18:19	20:59	-	Diving
13	2017	*Acropora* sp.	*Acropora*	10/11/2017	+6	18:19	20:59	-	Diving
14	2017	*Acropora muricata*	*Acropora*	10/11/2017	+6	18:19	20:59	-	Diving
15	2017	*Fungia* sp.	*Fungia*	10/11/2017	+6	18:19	20:59	.	Diving
	2017	no spawning		11/11/2017	+7	18:19		00:24	Traps
	2017	no spawning		12/11/2017	+8	18:19		-	Traps
	2017	no spawning		06/12/2017	+2	18:28		21:17	Traps
	2017	no spawning		07/12/2017	+3	18:28		22:15	Traps
16	2017	*Pectinia lactuca*	*Pectinia*	08/12/2017	+4	18:29	-	23:10	Traps
	2017	no spawning		09/12/2017	+5	18:29		-	Traps
	2017	no spawning		10/12/2017	+6	18:30		00:01	Traps
	2017	no spawning		11/12/2017	+7	18:30		00:49	Traps
	2018	no spawning		03/04/2018	+3	18:32		20:53	Traps
	2018	no spawning		04/04/2018	+4	18:32		21:39	Traps
17	2018	*Acropora* sp. (table)	*Acropora*	05/04/2018	+5	18:31	21:30	22:27	Diving
18	2018	thick slicks on surface	*mass spawning*	05/04/2018	+5	18:31	21:39	22:27	Diving
19	2018	*Acropora* sp. (table)	*Acropora*	05/04/2018	+5	18:31	-	22:27	Traps
20	2018	*Montipora* sp. *(massive)*	*Montipora*	06/04/2018	+6	18:31	21:04	23:15	Diving
	2018	no spawning		07/04/2018	+7	18:31		-	Traps
	2018	no spawning		08/04/2018	+8	18:30		00:03	Traps
21	2018	*Acropora* sp. *(table)*	*Acropora*	06/05/2018	+6	18:22	20:47	23:32	Diving
22	2018	*Cyphastrea microphthalma*	*Cyphastrea*	30/10/2018	+6	18:18	20:40	23:36	Diving
23	2018	*Cyphastrea microphthalma*	*Cyphastrea*	31/10/2018	+7	18:18	20:44	-	Diving
24	2018	*Pectinia lactuca*	*Pectinia*	31/10/2018	+7	18:18	20:00	-	Diving
	2019	no spawning		15/10/2019	+2	18:18		19:28	Traps
	2019	no spawning		16/10/2019	+3	18:18		20:15	Traps
	2019	no spawning		17/10/2019	+4	18:18		21:04	Traps
	2019	no spawning		18/10/2019	+5	18:18		21:56	Traps
	2019	no spawning		19/10/2019	+6	18:18		22:50	Traps
25	2019	*Echinopora lamellosa*	*Echinopora*	20/10/2019	+7	18:18	20:13	23:46	Diving
26	2019	*Goniastrea retiformis*	*Goniastrea*	20/10/2019	+7	18:18	20:04	23:46	Diving
27	2019	*Montipora* sp.	*Montipora*	20/10/2019	+7	18:18	19:49	23:46	Diving
	2019	no spawning		16/11/2019	+4	18:20		21:42	Traps
28	2019	*Acropora florida*	*Acropora*	17/11/2019	+5	18:20	20:48	22:38	Diving
29	2019	*Acropora* sp. *(table)*	*Acropora*	17/11/2019	+5	18:20	-	22:38	Traps
30	2019	*Favites (halicora* or *abdita)*	*Favites*	18/11/2019	+6	18:21	21:37	23:34	Diving
31	2019	*Galaxea astreata*	*Galaxea*	18/11/2019	+6	18:21	21:35	23:34	Diving
32	2019	*Montipora* sp.	*Montipora*	18/11/2019	+6	18:21	20:57	23:34	Diving
33	2019	*Echinopora lamellosa*	*Echinopora*	17/12/2019	+5	18:33	20:20	23:18	Diving
34	2019	*Galaxea* sp.	*Galaxea*	18/12/2019	+6	18:33	20:48	-	Diving
35	2019	*Goniastrea minuta*	*Goniastrea*	18/12/2019	+6	18:33	21:01	-	Diving

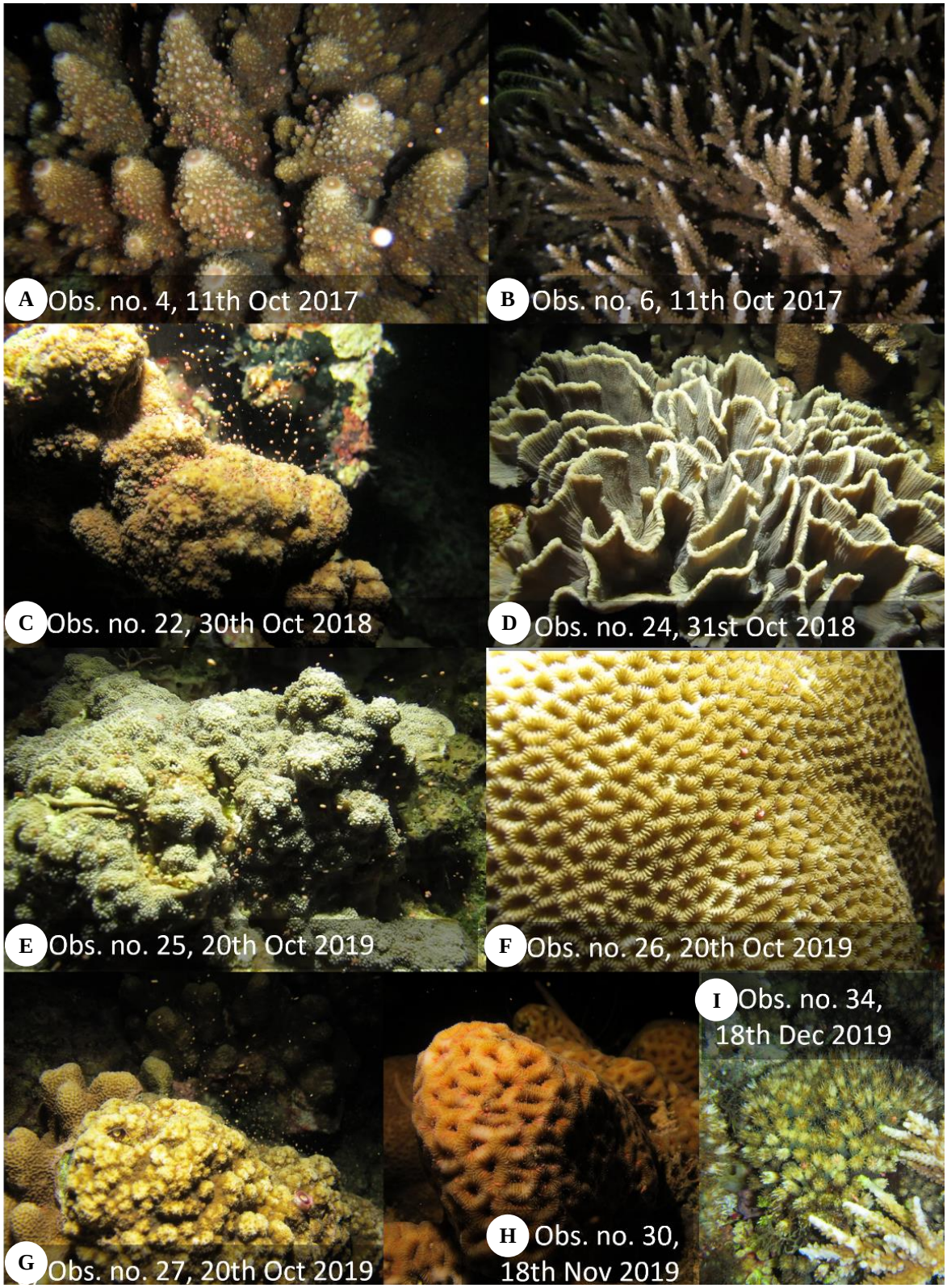

Figure 2. Examples of the different coral genera found spawning at the Banda Islands 2016-2019. Observation number (Obs. No.) and spawning date are shown. A. *Acropora* (digitate); B. *Acropora muricata;* C. *Cyphastrea microphthalma*

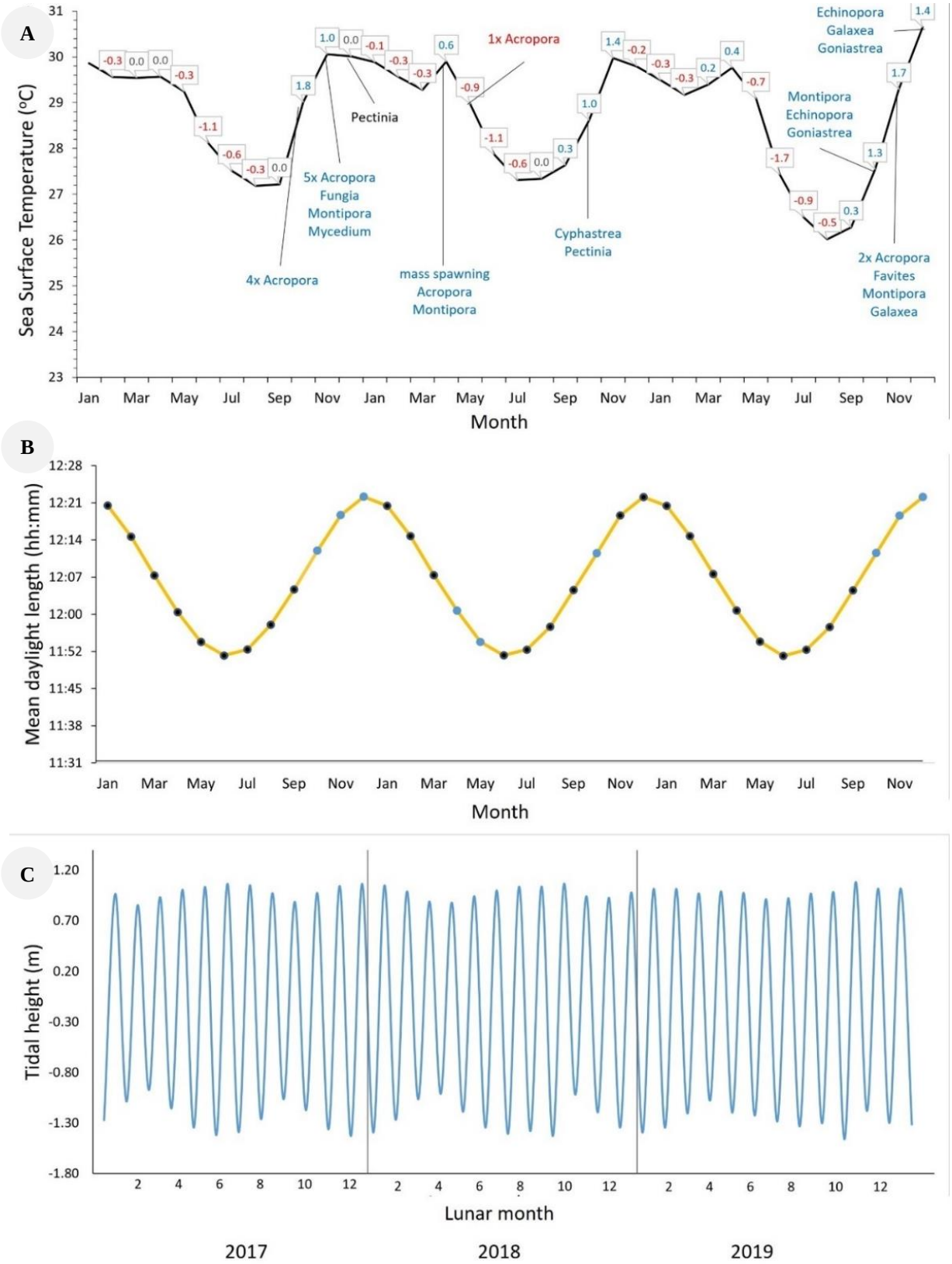

Figure. 3 A. Sea Surface Temperature (SST) and spawning events at the Banda Islands, Moluccas, Indonesia (January 2017 - December 2019). Colored numbers show the change in SST relative to the previous month. Red indicates a drop, blue indicates a rise, and black indicates no change in SST. Coral genera that spawned are shown i) in blue when spawning occurred after rising SST, ii) in red when spawning occurred after a SST drop, and ii) in black when SST had stayed the same from the previous to the month spawning was detected. B. Monthly average daylight length at the Banda Islands from January 2017 until December 2019. Months when spawning occurred are indicated with light blue data points. C. Minimum and maximum monthly tides at the Banda Islands from January 2017 until December 2019. Data from Badan Informasi Geospesial (BIG) Indonesia

Our data matches the overall picture of spawning day data around 5 DAF (Figure 5), extracted from the publicly available Indo-Pacific coral spawning database (Baird et al. 2021), downloaded on 09.11.2022. All 6153 observations (3569 in situ and 2584 ex-situ) available in the database at that date were included. These observations comprised 291 species and came from 38 different ecoregions across 29 countries in the Indian and Pacific Oceans. A peak of spawning on days 3-5 after full moon was also recently reported from Singapore (Ip et al. 2022)

In low-latitude regions, the day of full moon matches the day when moonrise occurs nearly at the same time as sunset. In the days after a full moon, the moonrise shifts back by almost one hour per day, meaning that the time of complete darkness between sunset and moonrise extends daily. This period of darkness was suggested to be the trigger for the coral *Dipsastrea speciosa* for the initiation of gamete maturation and subsequent gamete release 4-5 days later (Lin et al. 2021). This hypothesis is supported by our data, as we found the day after full moon to be the most reliable predictor of spawning day. Spawning at the Banda Islands occurred when the time between sunset and moonrise was at least 4 hours (Figure 4, Table 2). We suggest that this aspect should be investigated with manipulative field experiments for different species of coral. By installing simple setups to shade colonies in situ from moonlight and varying shading initiation and length, Lin et al. (2021) demonstrated a very suitable and non-invasive approach that could be adapted to match local field conditions at the Banda Islands.

Figure 4. Tidal patterns at the Banda Islands during the respective lunar months when coral spawning was observed (for 10 days starting from day of full moon). Dates and times above the curve indicate dates and times of spawning. The colored horizontal bar on top shows the daylight (yellow), darkness (black) and moonlight (blue) periods

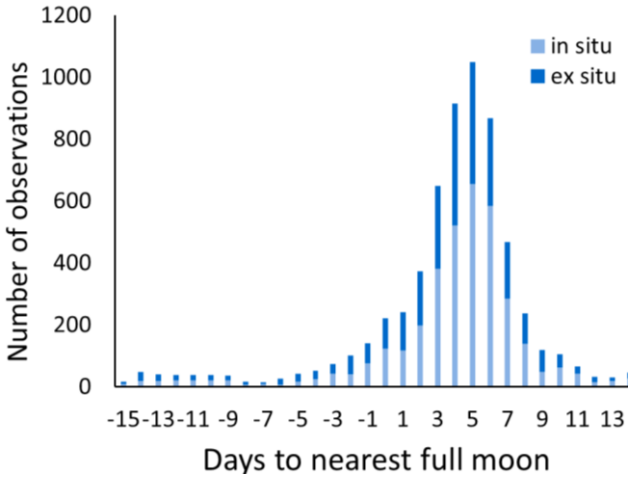

Figure 5. Distribution of coral spawning observations in the Indian and Pacific Oceans with respect to the nearest day of full moon (-15 until 14). Data was obtained from the publicly available Indo-Pacific coral spawning database, accessed on Nov 9th, 2022 (Baird et al. 2021)

We also looked at the potential influence of daylight length on the day of spawning, as published by Gouezo et al. (2020), but could not find any connection for our study site. Daylight length does not vary greatly at the Banda Islands over the course of a year as the islands are located near the equator (4°S). The shortest days of the year are in June (monthly mean = 11:52 hh:mm) and the longest days are in December (12:22 hh:mm). The spawning observations occurred on some days with relatively shorter and others with relatively longer daylengths (11:56 - 12:22, Figure 3B). We, therefore, suggest that in very low-latitude regions, such as the Banda Islands, spawning month and day are rather determined by SST fluctuations and the lunar cycle.

Time of spawning

Spawning always occurred during falling tides, about halfway between high and low tides (Figure 4). Furthermore, the time of spawning for all observations was at least 2 hours after sunset and 1 hour before moonrise, which was the darkest period of the night (Table 2, Figure 4). Spawning was reported during high slack from Okinawa, Japan (Hayashibara et al. 1993) and during neap tides following a full moon from the Great Barrier Reef, Australia (reviewed in Harrison and Wallace 1990) and Western Australia (Gilmour et al. 2016a). Hayashibara et al. (1993) also reported *Acropora* mass spawning occurring during falling tides, which matches our observations. However, the total number of our observations was relatively small, and our sampling was not continuous. Continuous monitoring over an entire year would be an ideal scenario that could reveal patterns of spawning in relation to lunar and tidal cycles in more detail. It would also shed light on reproductive patterns of other, less abundant and/or less common species, of which spawning times are still unknown.

Identification of corals

We were only able to identify one gamete sample to species level with the primers readily available, as genera like Acropora need dedicated primer sets to achieve species identification (Alexander et al. 2020). Also, given the high diversity of coral species in Banda, DNA metabarcoding of environmental DNA (eDNA) could prove more efficient for broader genus-level monitoring in the long run. Identification based on close-up photographs worked well for several species but not for all and depended on specialized skills and experience. As expert consultation for photo ID will probably not always be available, putting effort into training students at the Banda Islands species identification and DNA methods and data analysis should be high priority. In conclusion, the monitoring methods presented here, while useful for identifying two distinct spawning seasons in the Banda Islands for the first time, are unsuited to a much-needed seamless survey in this high biodiversity hotspot. Crucial patterns were identified within each season, but questions remain, especially about the spawning days and times of all those species we have not observed spawning yet. Achieving a more detailed picture could greatly benefit coral conservation, restoration and management efforts at the Banda Islands and motivate similar studies in the region. Nevertheless, during this project, we were able to gather strong support and educate recreational divers, students, and scientists alike. We established collaborations with educators locally and taxonomy and molecular experts in the USA. This is highly relevant for future work, as effective conservation will rely on local capacity to correctly identify genera and species.

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