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A study on the spawning season of 3 *Acropora* species in Nha Trang bay, Southern waters of Vietnam

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ABSTRACT

Specimens for the study on coral spawning of three species were collected at two sites in Nha Trang bay, South Vietnam, in 2014, 2015, 2016, 2018 and 2019. The determination of spawning seasons was based on observations of gonadogenesis development of *A. florida* and *A. robusta* and variation of egg average sizes of these two species and *A. hyacinthus*. Data analysis and comparative discussions allow us to assume that the 3 *Acropora* species exhibited single cycle spawning annually and their spawning period occurred in March/April between the full moon and crescent moon. However, their maturity periods were not the same, starting before and ending after the full moon for *A. florida* and *A. robusta* but starting after the full moon and lasting until the crescent moon for *A. hyacinthus*. Further studies are needed to improve the understanding of coral spawning and support coral restoration using sexual reproduction.

Keywords: Maturity, spawning season, moon cycle, *Acropora*, Nha Trang bay.

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INTRODUCTION

Acropora genus was the most diverse among genera of reef corals in the world, with 113 species described in 1999 [1] and 163 species listed in the web-based data [2] updated by Veron et al., (2016). This genus is also the most diverse in Bien Dong, with records of 98 species among 571 species of reef corals in the total [3]. The studies on the reproduction of *Acropora* corals were conducted in many areas, for example, in Western Samoa for *A. hyacinthus*, *A. gemmifera* and *A. humilis* [4], Papua New Guinea for *A. formosa* [5], Western Australia for *A. samoensis* and *A. cytherea* [6], in Malaysia for *A. millepora* and *A. nasuta* [7], in the Philippines for *A. tenuis* [8] and Singapore for 22 *Acropora* species [9]. In Viet Nam, *Acropora* species were also very diverse, with nearly 17% species among more than 400 species belonging to 79 genera of reef corals

[10]. This genus exhibited diversity and dominance in many areas in Van Phong and Nha Trang bays, Ninh Hai coastal reefs and Con Dao islands [10]. However, the studies on reproduction had not been conducted yet for reef corals and for *Acropora* species particularly in Vietnam. This initial research focused on determining the spawning seasons based on observations of gonadogenesis development of *A. florida* and *A. robusta* and variation of egg average sizes of these two species and *A. hyacinthus*.

MATERIALS AND METHODOLOGY

Specimens for the study on coral spawning of three species were collected mainly at Hon Mieu island ($12^{\circ}11'37.31''\text{N}$ and $109^{\circ}14'1.82''\text{E}$) from 2014 to 2019. One additional site for specimen collection in 2019 was at Bich Dam nearby (Figure 1).

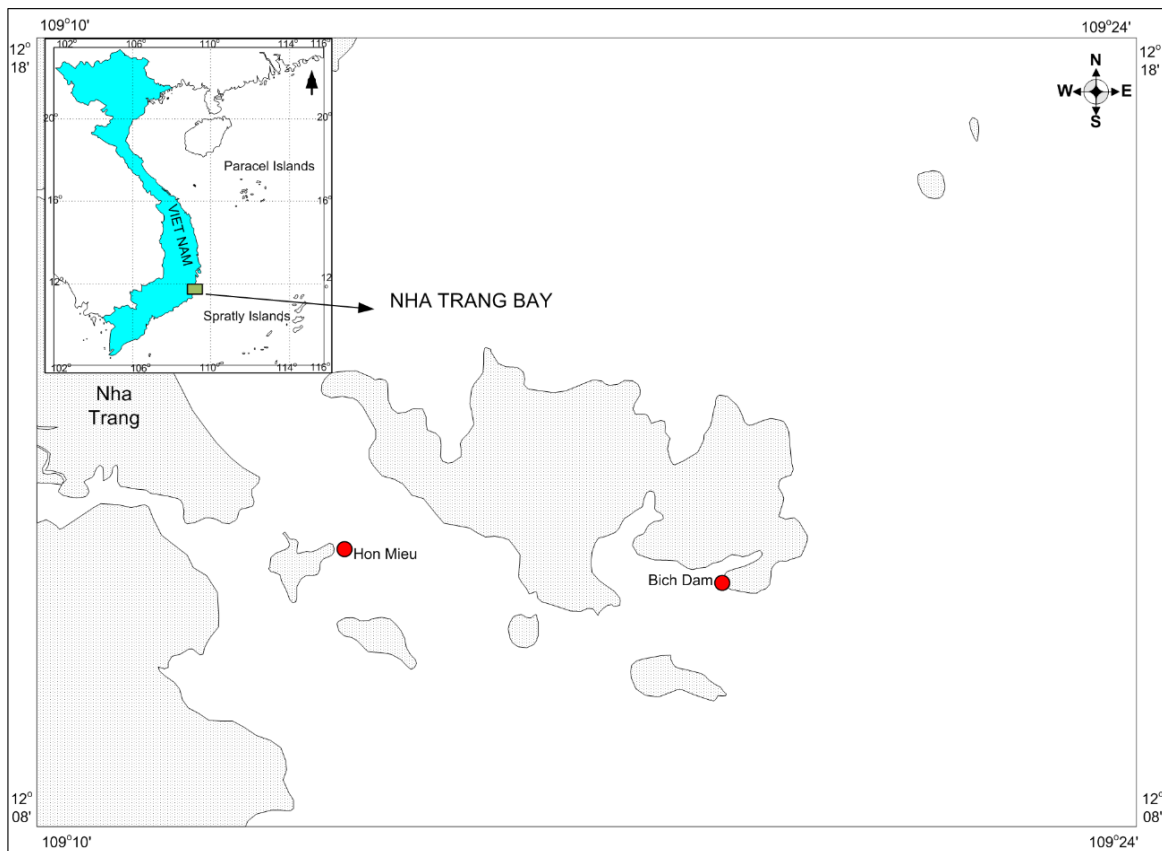


Figure 1. Map of Nha Trang bay indicating the sites of collecting specimens

Specimens of 2 species of *Acropora florida* and *A. robusta* were collected monthly, from May to September and December 2014, March to July 2015, to determine if their egg appeared or not. During 2016 to 2019, specimens of two species were collected in March and April more frequently and in other months when specimens had not yet been available in the past, except in November due to unfavorable conditions. The specimens included: 72, 128 in 2016 and 2018, respectively, for *A. florida*; 108, 189, 84 in 2016, 2018, and 2019, respectively, for *A. robusta*.

Acropora hyacinthus was selected as a tabulate coral to study its spawning season in 2019. The specimens were 6 in January (once), 9 in February (once), 108 in March (9 times) and 12 in April (once), 135 specimens in total. In addition, the ratio of samples with eggs per total was calculated for the collections of 2016, 2018 and 2019.

Specimen collection and fixation were conducted for the spawning study following Mangubhai (2007) [11]. Every specimen was a single branch cut from its colony, measuring 3–5 cm below the tip. Tissue samples were then fixed, decalcified and preserved in 70% ethanol. The specimens then were operated vertically from the tips and observed to classify stages of oogenesis using a stereomicroscope (Olympus SZ61-RT) with a magnification * 40. Mature oocytes (stage IV) were determined differently from other stages by having a larger size and their nucleus moving nearby peripheric membranes (Figure 2), as described by Harrison & Wallace (1990) [12]. Photographs were made for all specimens in which oocytes existed. Oocyte sizes were measured using the ImageJ software.

Some histology slides were prepared to observe oogenesis stages of the specimens. Number of slides ranged between 2 to 7 for *A. florida* and between 1 to 4 for *A. robusta*.

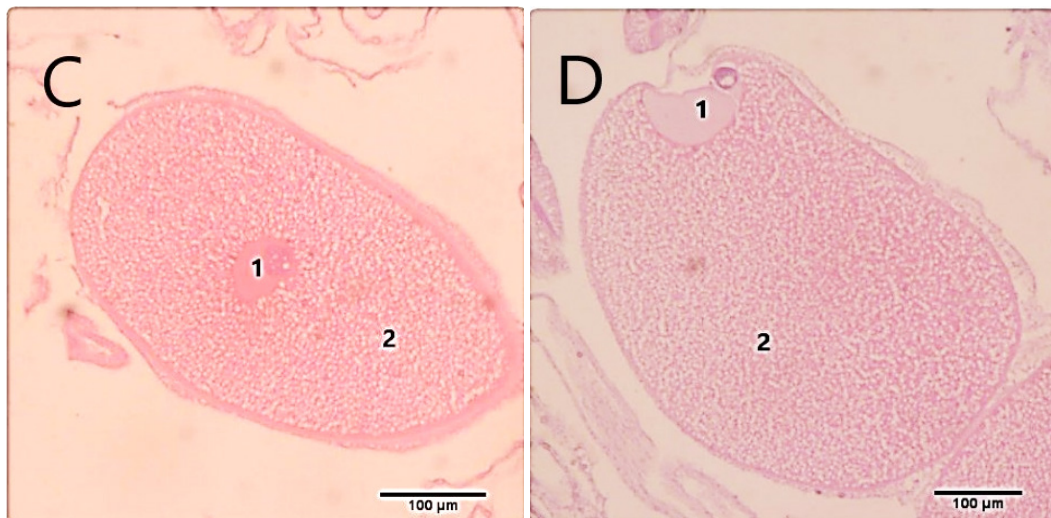


Figure 2. The photo of an oocyte at the stage IV of *A. robusta* with nucleus allocated nearby peripheric membrane (D, the specimen collected on March 26, 2018), comparing with another at the stage III with its nucleus at the centre (C, the specimen on March 6, 2018)

Note: 1 = nucleus; 2 = cytoplasm

RESULTS

Times of egg appearance and gonadogenesis

Regarding gonadogenesis by months, analysis of the specimens collected in 2014, 2015, 2016 and 2018 (Table 1) indicated that *A. florida*'s eggs were found from September

to April. Meanwhile, the appearance of *A. robusta* eggs was observed from October to April. The gonadogenesis had been observed from May to August for *A. florida* and from May to September for *A. robusta*.

Table 1. Observation of egg appearance of 3 *Acropora* species (*n*: specimen amount; -: no specimen collected, Y/N: egg recorded or not)

Year		Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Dec.
<i>A. florida</i>												
2014	<i>n</i>	-	-	-	-	6	6	6	6	6	-	6
	Y/N					N	N	N	N	N		Y
2015	<i>n</i>	-	-	6	6	6	6	6	-	-	-	-
	Y/N			Y	N	N	N	N				
2016	<i>n</i>	6	6	48	12	-	-	-	-	-	-	-
	%	Y	Y	Y	Y							
2018	<i>n</i>	-		31	97		-	-	-	7	7	-
	%			Y	Y					Y	Y	-
<i>A. robusta</i>												
2014	<i>n</i>	-		-	-	9	9	9	9	9	-	9
	Y/N					N	N	N	N	N		Y
2015	<i>n</i>	-	-	9	9	9	9	9	-	-	-	-
	Y/N			Y	N	N	N	N				
2016	<i>n</i>	9	9	72	18	-	-	-	-	-	-	-
	Y/N	Y	Y	Y	Y							
2018	<i>n</i>	-	-	41	111	-	-	-	-	12	13	-
	Y/N			Y	Y					N	Y	
2019	<i>n</i>			84								
	Y/N			Y								
<i>A. hyacinthus</i>												
2019	<i>n</i>	-	5	179	18	-	-	-	-	-	-	-
	Y/N	-	Y	Y	Y							

Calculation of the ratio of specimens with eggs for corals collected in 2016, 2018 and 2019 provided a figure on the frequency of egg appearance by months. *A. Florida* had borne eggs with high frequency in most months (less

in April in both years). *A. Robusta* exhibited lower ratios of specimens with eggs, ranking between 33–68% (Table 2). Meanwhile, the egg frequency of *A. Hyacinthus* reached a high value in February but was relatively low in April.

Table 2. Egg frequency (%) of 3 *Acropora* species by months (see number of specimens at Table 1)

Year	Jan.	Feb.	Mar.	Apr.	Sept.	Oct.
<i>A. florida</i>						
2016	83	83	75	42		
2018			87	56	86	86
<i>A. robusta</i>						
2016	44	56	13	28		
2018			63	33	0	46
2019			68			
<i>A. hyacinthus</i>						
2019		100	65	6		

Observation of histology slides of specimens of two species (*A. florida* and *A. robusta*) collected in March, April, September and October of 2018 (Table 3) indicated that

mature eggs (stage IV) existed only in late March and early April. The appearance of immature eggs simultaneously with mature eggs suggested a multiple spawning of each

coral colony. No egg existed on April 9, 2018, evidence of the spawning end in early April. This histology observation allowed us to suggest the spawning season of both species

from late March to early April of 2018. The appearance of immature eggs at the same time with mature eggs suggested a multiple spawning of each coral colony.

Table 3. Ratio of immature and mature eggs collected in 2018 based on the observation from histology specimens

Day	<i>A. florida</i>			<i>A. robusta</i>		
	Slide number	Immature eggs (%)	Mature eggs (%)	Slide number	Immature eggs (%)	Mature eggs (%)
Mar. 1	2	100	0	3	100	0
Mar. 6	2	100	0	1	100	0
Mar. 26	2	19	71	2	50	50
Apr. 2	2	17	73	2	20	80
Apr. 4	7	22	78	4	25	75
Apr. 6	4	5	95	3	22	78
Apr. 9	No eggs existd anymore			No egg existed anymore		
Sept. 25	3	100	0	2	0	0
Oct. 26	3	100	0	3	100	0

Variation of egg size by time

Following the recorded periods of egg appearance as above mentioned and based on egg diameters measured in 2016 and 2018 (Table 4 & 5), the gonadogenesis of the 2 *Apropora* species may start in the late time of a

certain year, having eggs around 120–150 µm in September or October. Their eggs reached an average size of less than 400 µm (*A. florida*) and around 300 µm (*A. robusta*) in early March and maximum size (400–500 µm) in late March or early April of the following year.

Table 4. Variation of average egg diameter (µm) of *A. florida* collected in 2016 and 2018

2016	Egg size	n	2018	Egg size	n
18/3	384 ± 82	243	1/3	369 ± 70	1,386
19/3	347 ± 71	240	6/3	367 ± 76	2,551
21/3	399 ± 90	452	26/3	456 ± 91	947
22/3	369 ± 71	455	2/4	519 ± 99	2,371
24/3	373 ± 61	114	4/4	535 ± 100	3,290
25/3	413 ± 84	382	6/4	516 ± 97	1,913
27/3	421 ± 83	513	9/4		No egg recorded
28/3	432 ± 89	272	11/4		No egg recorded
3/4	470 ± 79	494	25/9	138 ± 22	642
7/4	313	2 (few eggs left)	26/10	145 ± 27	649

Eggs of both species reached the most significant average size from March 25 to April 3 in 2016 and from March 26 to April 6 in 2018. Based on the trend of variation of egg size by time, *A. florida* and *A. robusta* exhibited their spawning season in the period from late March and early April every year.

In the case of *A. hyacinthus*, there existed the same trend with egg average sizes around

200 µm in January, 300 µm in February, 400 µm in early March and more than 450 µm in late March of 2019 (Table 6). The data analysis on egg sizes of *A. Robusta* collected in 2019 recorded the specimens bearing big eggs (the average > 400 µm) from March 13 to 23 and no eggs were observed on March 25. It meant that the spawning time of *A. hyacinthus* was later than that of *A. robusta* in 2019 by a week.

Table 5. Variation of average egg diameter (µm) of *A. robusta* collected in 2016 and 2018

2016	Egg diameter	n	2018	Egg diameter	n
19/3	250 ± 49	51	1/3	325 ± 63	720
21/3	292 ± 60	84	6/3	351 ± 69	508
22/3	329 ± 57	167	26/3	465 ± 83	1,233
25/3	409 ± 78	382	2/4	490 ± 80	1,346
27/3	404 ± 71	32	4/4	488 ± 81	1,474
3/4	464 ± 98	258	6/4	505 ± 91	2,179
7/4	378 ± 65	170	9/4		No egg recorded
			11/4		No egg recorded
			25/9		No egg recorded
			26/10	120 ± 16	80

Table 6. Variation of average egg diameter (µm) of *A. robusta* and *A. hyacinthus* collected in 2019

Time	22/1	18/2	13/3	15/3	17/3	19/3	21/3	23/3	25/3	27/3	29/3	31/3
	<i>A. robusta</i>											
Egg size			411	410	421	425	424	429	No more egg			
SD			47	53	49	52	53	56				
n			80	80	80	80	80	80				
	<i>A. hyacinthus</i>											
Egg size	211	304	404	428	435	458	467	473	463	473	471	470
SD	10	18	49	37	52	29	31	28	29	23	22	23
n	40	60	80	80	80	80	80	80	80	80	80	80

DISCUSSIONS

Based on egg appearance and variation of average egg size of 3 species, the 3 *Acropora* species exhibited single cycle spawning annually. The observation in Nha Trang bay was consistent with a review by Harrison and Wallace (1990) [12] that a single cycle pattern was commonly recorded for broadcast spawning corals with the periods for gonadogenesis lasting for 3–10 months and followed by resting for 3–4 months before a new stage of gonadogenesis. However, it is needed to have further studies to determine if the biannual cycle of breeding during the year occurred in Nha Trang bay, particularly and in the western East Vietnam Sea, generally. Every six months, the cycle was recorded rarely for *A. hyacinthus*, *A. gemmifera* in West Samoa [4] as well as *A. formosa* and *A. hyacinthus* on the northern reefs of Papua New Guinea [5].

Moonlight was considered as an important factor that influenced coral spawning patterns. Many invertebrates, including corals with broadcast and brooding reproduction, had their spawning depending on the moon cycle [12, 13]. Multispecific spawning occurred

synchronously between the full moon and crescent moon, similarly during the years in central Great Barrier Reefs and Akajima island, Japan [13–16]. Gametogenesis releases of broadcast spawning corals during the full moon were observed rarely but recorded in Great Barrier Reefs and Hawaii [13, 15, 17].

According to the lunar calendars, the full moons occurred on March 23, 2016 and March 31, 2018. Meanwhile, *A. florida* and *A. robusta* eggs reached the biggest average size and mature stage from March 25 to April 3 in 2016, and from March 26 to April 6 in 2018. Therefore, their maturity started before and lasted until after the full moon in March/April annually. As mentioned above, *A. hyacinthus* was bearing big eggs during the late March, following the full moon (on March 20, 2019) and its spawning time was later than that of *A. robusta* by a week. This explanation allowed us to conclude the spawning time of the 3 species on Mar/April annually, considering their relation with the full moon. Comparing with a study in Singapore [9], which described the maturity of 22 different *Acropora* species starting before and spawning after the full

moon, indicated the similarity with the observations for *A. florida* and *A. robusta* but the difference from the record for *A. hyacinthus* in Nha Trang bay.

CONCLUSION

This initial study on reef coral spawning provided information on the seasonal spawning of the 3 *Acropora* species. They exhibited the single cycle of annual reproduction and their spawning period occurred in March/April between the full moon and crescent moon. Further studies are needed to widely understand the breeding patterns of more *Acropora* species and other reef corals and apply scientific knowledge for reef conservation and restoration using sexual reproduction.

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