Marine Ecology Enhancement Fund (MEEF)

Declaration

To: The Secretariat of the MEEF

Reference No.: MEEF2017003

Project Title: Reproductive Biology of the Dominant Octocoral *Guaiagorgia* in Hong Kong Western Waters

Name of Project Leader: Put Ang Jr.

I hereby irrevocably declare to the MEEF Management Committee and the Steering Committee of the relevant Funds including the Top-up Fund, that all the dataset and information included in the completion report has been properly referenced, and necessary authorisation has been obtained in respect of information owned by third parties.

Signature：

Project Leader, Put Ang Jr.

Date：Oct 22, 2020

*This report presents the findings of the project (Ref No.: MEEF2017003) up to the end of the reporting period in June 2018, and this project has not completed all of the proposed components as per the project proposal due to project termination by the Recipient Organisation.

Completion Report on MEEF2017003

Title: Reproductive Biology of the Dominant Octocoral *Guaiagorgia* in Hong Kong Western Waters

By Put Ang, Jr. The Chinese University of Hong Kong

A. Report

INTRODUCTION

The octocoral *Guaiagorgia* sp. was first reported from Sha Chau Lung Kwo Chau Marine Park in Hong Kong waters by Ang et al. (2010). It attracted significant public attention in recent years because of its abundance found along the seawall of the Hong Kong International Airport (HKIA) (Airport Authority Hong Kong 2014). Issues were raised regarding its conservation, especially given that a large part of the existing seawall of HKIA would eventually be covered by the new reclamation project in connection with the construction of the HKIA Third Runway.

Populations of *Guaiagorgia* sp. were reported to be present not just along the seawall of HKIA, but also in other rocky shores within Hong Kong western waters (Airport Authority Hong Kong 2014). With the HKIA seawall being built only less than 20 years ago, these populations present on the seawall have undoubtedly been a result of recent (< 20 years) recruitment. This pattern of recruitment is in sharp contrast with that of the hard corals reported in eastern waters of Hong Kong where recruitment rates are very low (<1 ind m^2 y^{-1}) (Chui 2011, Yeung 2014). One explanation for such rapid recruitment and spread of *Guaiagorgia* sp. lies in the understanding of its reproductive biology. It would appear that such pattern of recruitment is characteristic of brooding corals, where fertilized eggs are incubated inside the polyps and released as larvae. Larvae released in this way would generally settle near their parent, forming a high density of recruits and juveniles within a small area (Fadlalah 1983, Harrison and Wallace 1990, Harrison 2011).

While reproductive biology of important hard corals (Collinson 1997, Lin 2003, Chui et al. 2014), soft coral (Yeung and Ang 2010) and black coral (Lau and Ang, 2017) species

have been examined in Hong Kong, there is no information on the reproductive biology of the erect octocoral (i.e. the gorgonians). A detailed study on the reproductive biology of *Guaiagorgia* sp. was therefore initiated to fill the gap on our understanding of the reproductive biology of this group of octocorals. This study has the following two objectives: To understand the pattern of gametogenesis in the octocoral *Guaiagorgia* in western Hong Kong waters and to evaluate the mode of reproduction in this octocoral species. Information generated from this study were essential in understanding the population dynamics of this species, hence in the design of strategic plan for its conservation and protection. This information will also contribute to our general understanding of the reproductive biology of octocorals.

MATERIALS AND METHODS

Sample collection

Samples were collected from three sites along Siu Ho Wan to Yam Tsai Wan starting from November 2017 to September 2018. The collection was conducted monthly within one week before full moon to ensure gametes reaching their maximum size before spawning could be captured.

Sample treatment and preparation

Five samples of different branches were taken from each of 10 random colonies collected. Pieces of 1cm long samples were cut from the colonies at least 3cm from the tip of the branches to avoid the active growing regions (Fan et al., 2005). The samples were fixed in 10% formalin immediately for one week and preserved in 75% ethanol afterwards. They were then decalcified with decalcifying agent (containing 1500ml 32% concentrated hydrochloric acid, 3.5g ethylenediamin-tetraacetic acid, 0.04g sodium potassium tartrate, 0.7g sodium tartrate dehydrate, and made up to 5L final volume). During decalcification, the presence of gas bubbles evolved was an indication to show the extent in which the coral samples had been completely decalcified. It usually required a few hours to decalcify.

Polyps collected from different colonies were then processed in an Automated Vacuum Tissue Processor (Leica® TP 1050, Leica Instruments GmbH). A serial dehydration process with increasing ethanol concentration was carried out by the processor to remove any water content in the coral tissues. The processed samples were embedded in wax blocks with paraffin using THERMOLYNE ® histocentre of the School of Life Sciences, The Chinese University of Hong Kong. Blocks were then serially cut at cross-section at a thickness of 7 μm. Since gonads are mainly located near the base of the polyp, more sections were cut at that region.

These serial sections were subsequently mounted on glass slides and stained. Four steps in the staining process were involved: dewax, hydration, staining and dehydration. In the dewax process, slides were immersed in xylene to remove all the wax infiltrated into the coral tissues. This was followed by submerging the slides in a series of solutions with descending ethanol concentrations from 100% to 30%. This allowed hydration to take place. Water-soluble stains, i.e. haematoxylin and eosin, were used to stain those hydrated slides. Scott's tap water, a blueing agent, was used after haematoxylin to help stain the nuclei blue, while eosin stained the cytoplasm red. After staining, slides were put in a series of solutions with increasing ethanol concentrations from 75% to 100% to remove any water. All slides were then mounted with Permount® and placed horizontally overnight for complete drying. Mounted slides were examined under the light microscope for the presence of oocytes and spermaries. The presence of oocyte and spermary in polyps from the same colony or from different colonies was compared to determine the sexuality of the species.

Light microscopy and developmental stages of gametogenesis

Samples collected from each month were treated with the same histological procedure described above and examined under the light microscope for the presence of oocytes and spermaries, as well as to identify their developmental stages. Images of both the oocytes and spermaries were taken with a calibrated eyepiece micrometer on a light microscope using a digital camera. Their longest diameter and the corresponding perpendicular diameter were measured using a computer program, the Image-Pro Plus

5.0 (Media Cybernetics, Inc., Bethesda, MD, USA). The geometric diameter of each oocyte or spermary was calculated by the square root of the product of the two diameters. The monthly variation in the oocyte or spermary size was plotted to determine the seasonal pattern of gametogenesis and the time of spawning of *Guaiagorgia*. Developmental stages of gametogenesis were also determined according to Parker (1997) and Chui et al. (2014) and the frequency of each stage in each month expressed as a percentage based on all the oocytes or spermaries being sectioned and examined.

Ex situ observation of possible coral spawning

Colonies of *Guaiagorgia* sp. were also collected during or close to the predicted spawning period (November 2017, May and June 2018) and grown in the flow through tanks at the Chinese University of Hong Kong. Any sign of spawning or presence of floating eggs, egg bundles or larvae was checked daily for seven days following full moon. As this species is suspected to be a brooder where larvae would be incubated and not a broadcaster where egg bundles are released into the water, closer observation was also made on the branches and on pre-conditioned ceramic tiles left at the bottom of the flow through tanks to find any traces of larvae that could have settled.

RESULTS

Sexuality of Guaiagorgia

Among the samples of *Guaiagorgia* examined, no brooded larvae were found in the coelenteron or on the colony surface. There were also no oocytes and spermaries observed simultaneously within the same branch or the same colony. This suggests that *Guaiagorgia* is gonochronic, i.e. sexes are separate in different colonies. This is consistent with that found in other species under its family *Gorgoniidae* (Kahng et al., 2011).

Gametogenic development: size changes

Following Parker (1997) and Chui et al. (2014), four developmental stages of oocytes (Figure 1) and spermaries (Figure 2) can be identified. There was a clear pattern of development for both oocytes and spermaries (Figure 3). There were no oocytes found

from November to March. Many large oocytes were then observed in April (mean ± SD = 73 ± 39μm) while the smallest colony with oocyte is 171 cm long. The size dropped in the following months. The size and number of oocytes decreased to 23 ± 15 μm in June. The smallest colony with oocyte is 129 cm long.

Large spermaries were also found in April (mean ± SD = 146 ± 38 μm) while the smallest colony with spermaries was 174 cm long. However, no spermaries were observed in the remaining months over the sampling period.

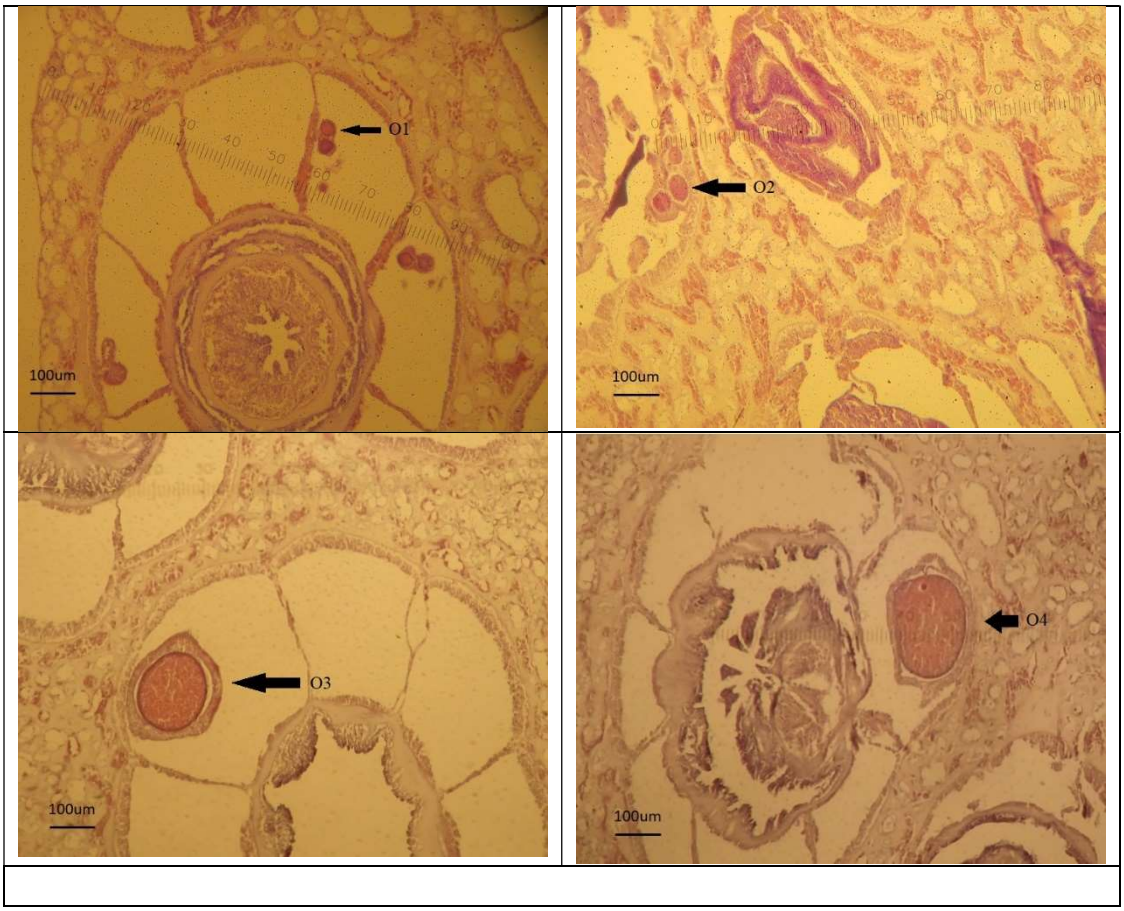

Figure 1. Oogenic developmental stages in *Guaiagorgia*: (**A**) Stage 1 oocytes (O1) in the mesentery; (**B**) Stage 2 oocytes (O2) connected to the mesentery by pedicle; (**C**) Stage 3 oocyte (O3) with cytoplasm accumulated around the nucleus; (**D**) Stage 4 oocytes (O4) with nucleus at the periphery.

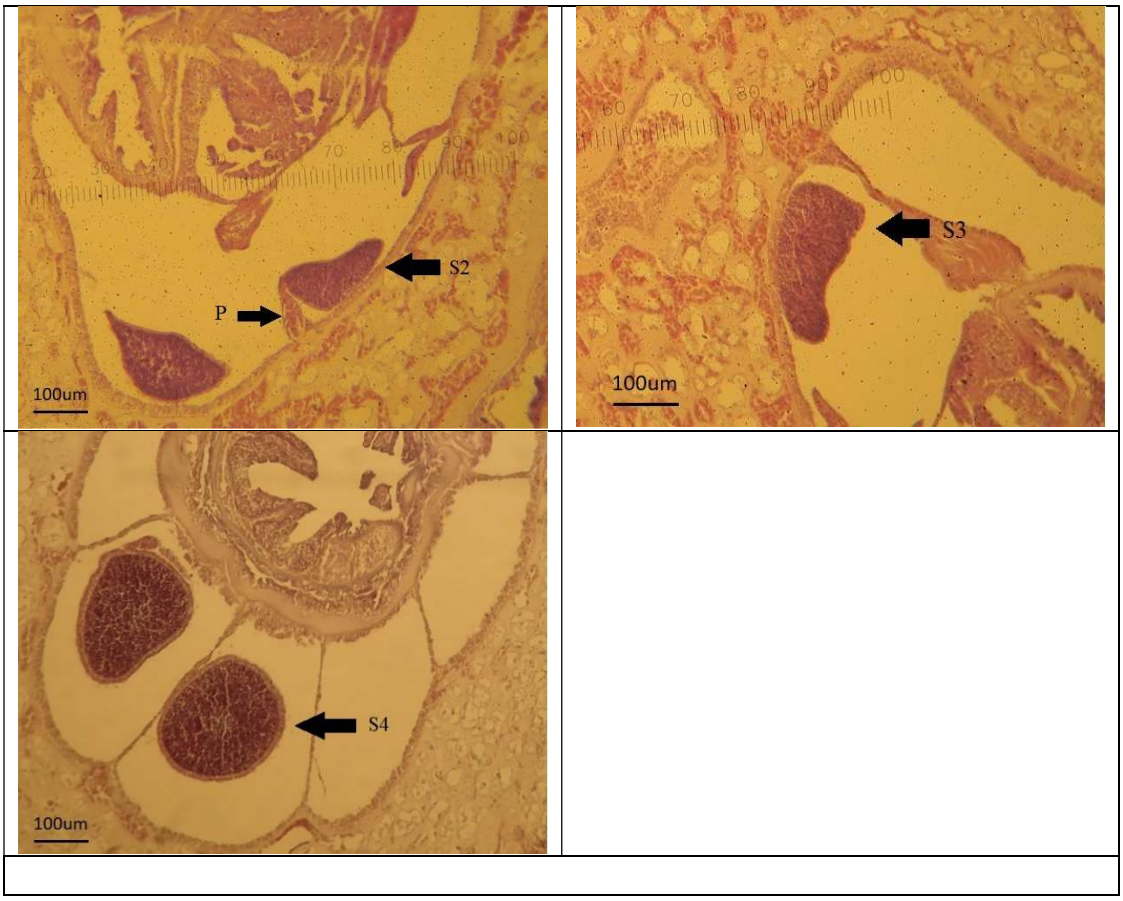

Figure 2. Spermatogenic developmental stages in *Guaiagorgia.* (**A**) Stage 2 spermary (S2) connected to the mesentery by pedicle (P); (**B**) Stage 3 spermary (S3) with spermatocytes arranged at the periphery; (**C**) Stage 4 spermary (S4). Picture was not captured for Stage 1 spermary as the structure was too small.

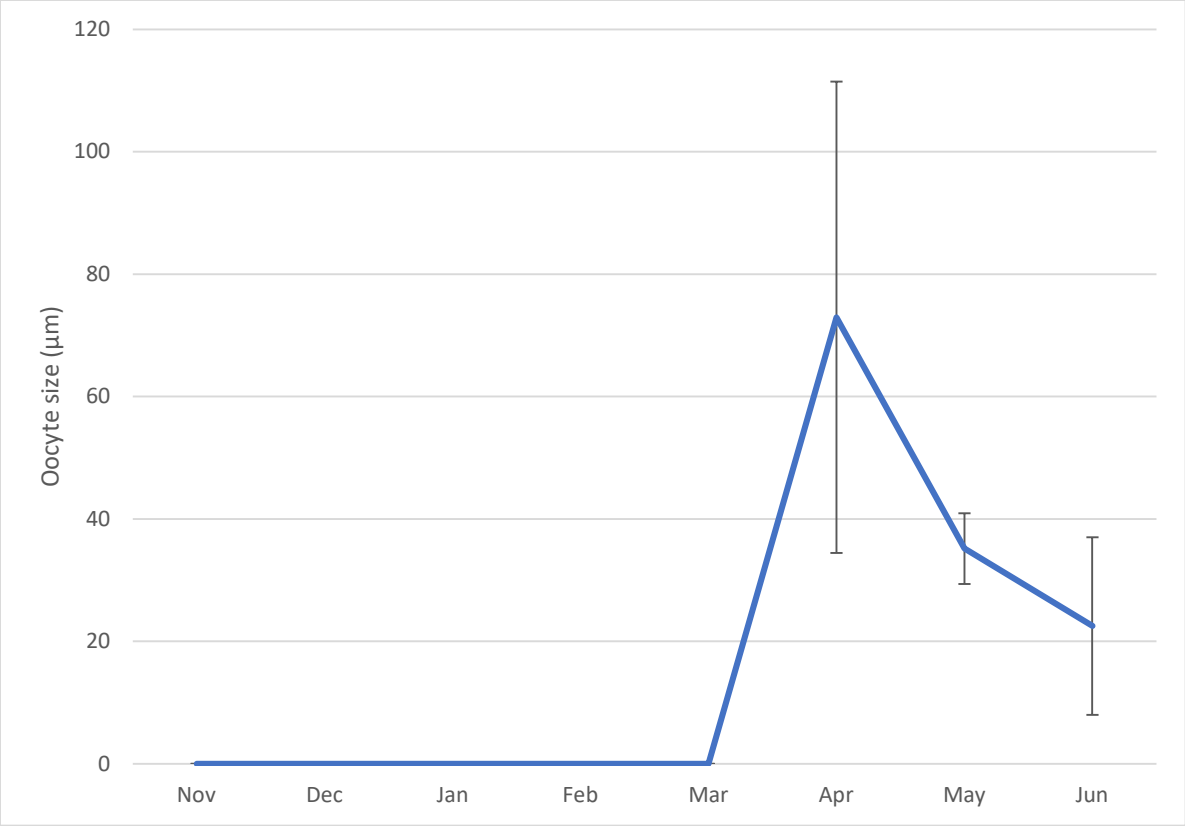

Figure 3. Changes in the mean ± SD size of oocytes over the sampling period

Frequency of different developmental stages

The frequency of appearance of different developmental stages of oocytes and spermaries between April and June is expressed as a percentage of the total number of gametocytes observed in each histological section. Oocytes were found in the samples from April to June, but the percentage frequencies of occurrence of different stages were not consistent over time (Figure 4). Stage 2 appeared to be most dominant among these three months but only stage 4 oocytes were found in April. Stage 3 was found in both May and June.

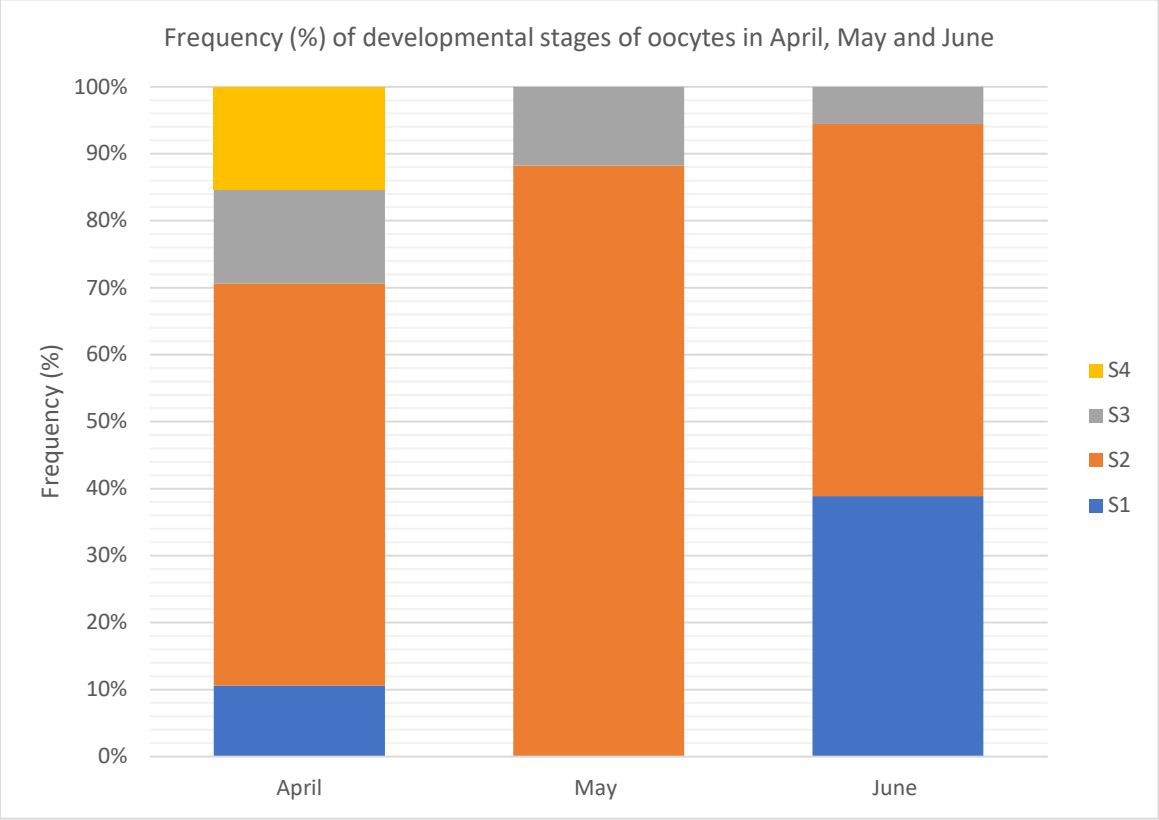

Figure 4. Mean frequency (%) of occurrence of developmental stages of oocytes in samples collected in April, May and June 2018.

As spermaries were only found in April samples, the percentage frequencies of occurrence of different stages were presented with respect to the three sampling sites (Figure 5). As shown, stage 2 appeared to be the most dominant stage among samples collected from different sites. Stage 4 spermaries were present in samples from two of the three sites and stage 3 spermaries were present in all sites.

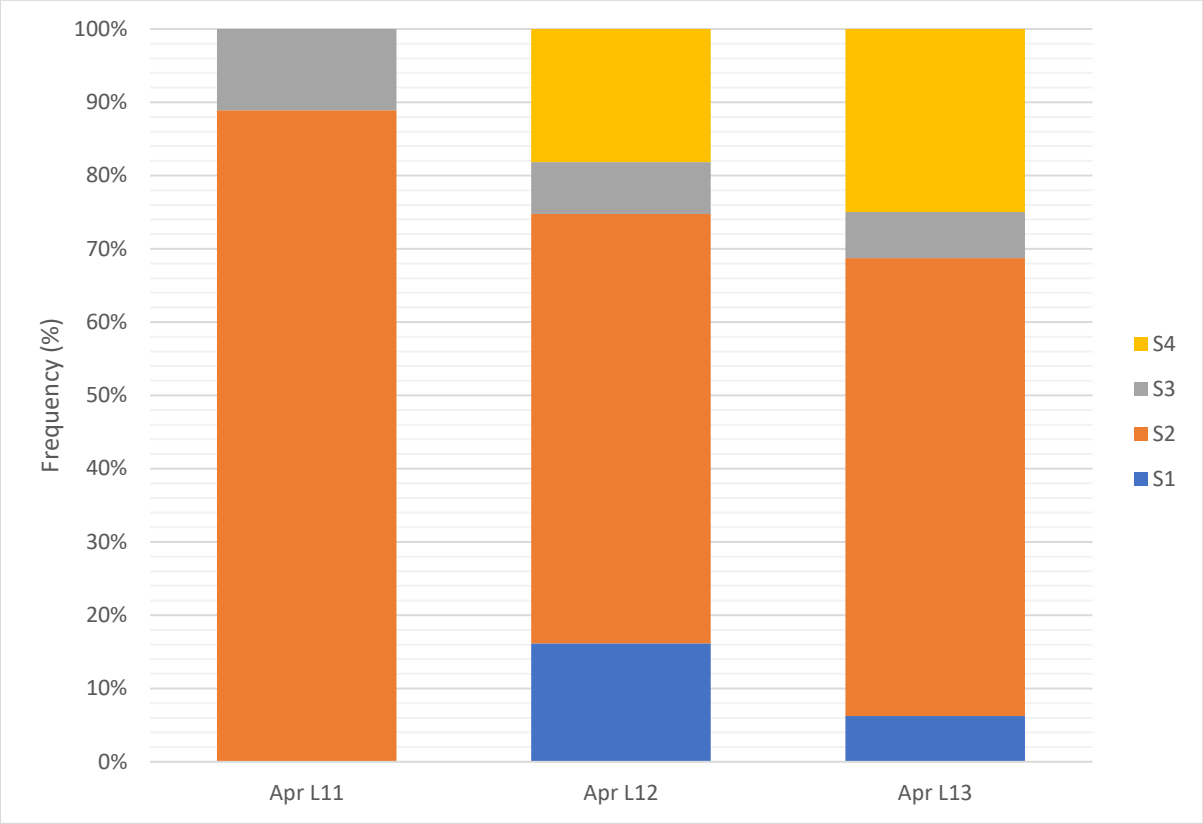

Figure 5. Frequency (%) occurrence of developmental stages of spermaries in April 2018 among samples collected from three study sites.

To compare with the spatial variability of oocytes present in different sites, frequency distribution of stages of oocytes found in different sites was also examined (Figure 6). The pattern was similar to that observed for spermaries wherein stage 4 oocytes were found only in two of the three sites, and stage 2 oocytes were the most dominant. However, in contrast to that for spermaries. Stage 3 oocytes were not present in all sites in all times.

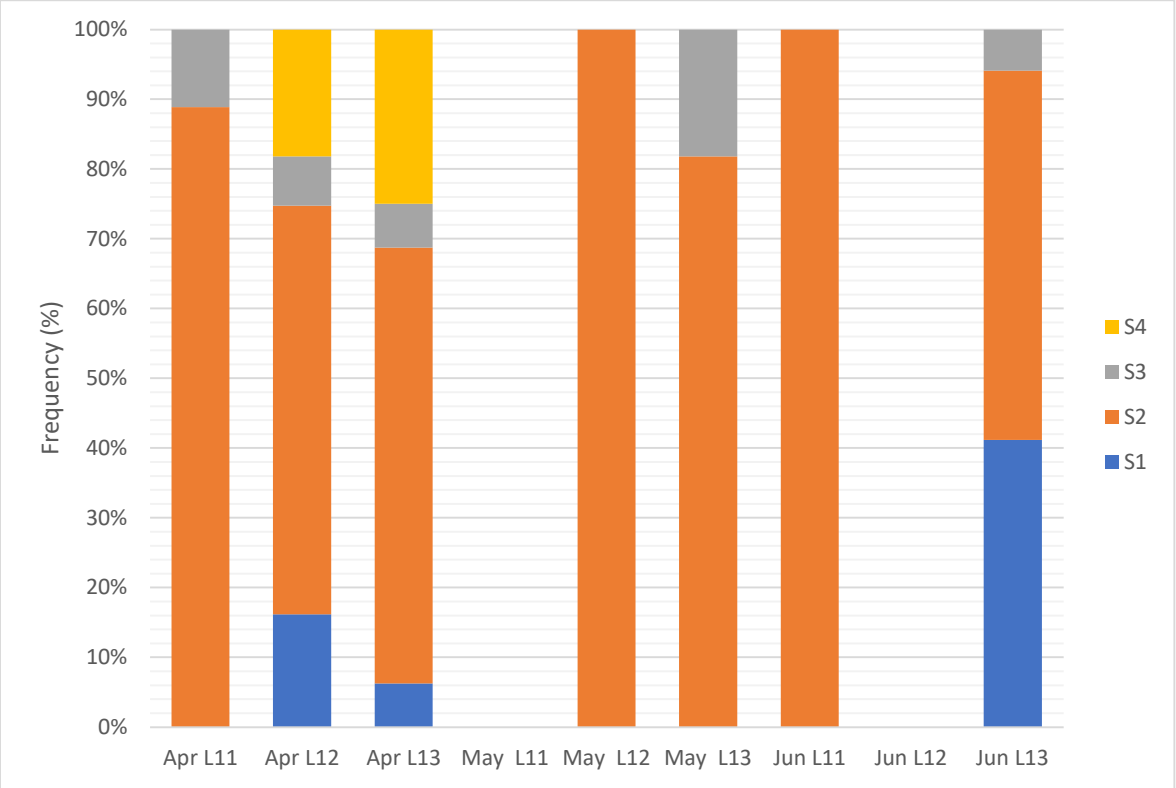

Figure 6. Frequency (%) occurrence of developmental stages of oocytes in April, May and June 2018 among samples collected from three study sites.

Ex situ observation of possible coral spawning

No larvae or eggs were found in the water surface where colonies of *Guaiagorgia* sp. were grown. There were also no traces of larvae on the branches or on the ceramic tiles left at the bottom of the tanks.

DISCUSSION

This species of *Guaiagorgia* is gonochronic, with sexes being found in separate colonies. There appears to be a minimum size for these colonies to start gametogenesis, being around 130 cm for female colony and around 175 cm for male colony. As the growth rate of this species is not known, this size information cannot be translated into age information for now.

Based on the pattern of gametogenesis, colonies of *Guaiagorgia* appeared to spawn only once a year, which would most likely be between April to May. However, other gametogenetic patterns observed pointed to several interesting features. First, gametogenesis appears not synchronous among individual colonies, and also among colonies from different sites collected in the same day. It remains a puzzle that no spermaries were found in samples collected in May and June. Also, it is important to note that stage 3 oocytes were found in May and June, but no stage 4; while stage 1 oocytes continued to appear in June. This suggests that these oocytes could still develop into stage 4 and release thereafter. But in the absence of any mature spermaries, these oocytes would have been wasted, i.e. not fertilized.

It should be noted the samples were also collected in July to September 2018. But these samples were not processed because the project had to be terminated prematurely. These unprocessed samples may hold some key to address the question on the extent of spawning period of this species and also whether stage 4 oocytes or other developing spermaries would have been found. There remains a possibility that spawning is continuous within the summer months from April to September.

Differences in the timing and pattern of gametogenesis among colonies of *Guaiagorgia* found within and between sampling sites suggest that these populations are not effective in their reproductive strategy. Mass spawning and synchronized gametogenic development observed in many other marine organisms, including the hard corals, have been suggested as an optimal strategy to ensure higher success rate of fertilization. Further environmental information of the different sites would be needed to evaluate how this asynchronous pattern of gametogenesis among colonies could have been affected by factors like wave exposure, current and depth. These factors may be critical in affecting the plankton food supply to these colonies, hence their growth, reproduction and other physiological performance.

With this asynchronous pattern of gametogenesis, an alternative reproductive strategy to ensure more successful larval development would be the brooding of larvae. Although brooding of larvae was suspected for this species, no brooded larvae were observed inside the polyp of any samples examined. Again, there remains some hope that these larvae may be found in the unprocessed samples of July to September.

The *ex situ* experiment also failed to observe any larvae or eggs from colonies of *Guaiagorgia* grown in the flow through tanks. However, it is possible that the culture condition of the tanks may not be suitable for these gorgonian colonies such that their biological rhythm was disturbed. On the other hand, it is also possible that the actual spawning period was missed as only colonies collected in November and in May and June were incubated for this experiment.

Although the study was cut short for some technical reason, it was nevertheless able to address the objectives set out. There is at least a better understanding of the reproductive pattern and mode of reproduction of this gorgonian species as detailed above but additional works would be needed to further confirm these patterns. Additional samples in April to September may be collected in more sites in greater frequency (or shorter intervals) to reveal a clearer picture of changes in stage structures over time. Samples collected previously from July to September should also be processed. In addition, *ex situ*

observation may continue to be followed. Colonies should be collected especially in April and May for similar *ex situ* observation of the possible presence of any brooded larvae or larval settlement on ceramic tiles. Furthermore, size structure of *Guaiagorgia* populations found in different sites may also be measured to lend some idea on the potential pattern of recruitment of this species through brooding larvae. The presence of many small colonies with clear cohorts near bigger colonies could usually be a good indication of the pattern of dispersal by brooding larvae. As mentioned earlier, environmental factors like wave exposure, current patterns and water depth among sites should also be monitored to assess the potential impact of these factors on the asynchronous reproductive patterns observed among colonies from different sampling sites.

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B. Completed Activities against the Proposed Work Schedule

The completed activities followed basically the proposed scheduled except for the delay at the beginning due to a need to make arrangement to obtain the gorgonian samples. Currents in western waters of Hong Kong are strong and the water visibility very poor. Initial plan to collect samples ourselves was aborted. An arrangement was finally made to contract more professional and experienced divers to collect the samples for us. Based on our experience with black corals and other hard and soft corals, two periods could be critical. Black corals were found to spawn in November and most hard and soft corals in May to July. We were able to obtain gorgonian samples in November and did not find any evidence of developing eggs or spermaries. So this delay should not have missed the potential spawning period of this species, should the spawning period be in November. Besides, the extension of the project was later applied for and approved to further the collection of samples till November of the following year. This, however, was not done because the project was terminated prematurely.

C. Evaluation of the project effectiveness in achieving the proposed objectives as well as the impact (benefits) of the project

The objectives of the project were basically achieved although more could have been done, these are given in more details in the Discussion section. This is the first project to assess the reproductive biology of a gorgonian coral in Hong Kong and also the first for this general region in the northern South China Sea. Studies on the reproductive biology of gorgonian corals have not received much attention, compared to those on hard or even soft corals. Whatever data generated from this project are important addition to this limited pool of information about the biology of gorgonian corals. This type of information is essential for the development of a strategic plan for its conservation.

D. Summary and Way Forward

Populations of the gorgonian *Guaiagorgia* sp. were found in western waters of Hong Kong. As many of these were also found to be growing on the seawall of the Hong Kong Airport Runway and thus could be affected by the construction of the third runway, a project was initiated to understand the reproductive biology of this gorgonian coral as part of an effort to evaluate the population dynamics of this species.

Samples were collected from three sites along Siu Ho Wan to Yam Tsai Wan starting from November 2017 to September 2018. The collection was conducted monthly within one week before full moon to ensure gametes reaching their maximum size before spawning could be captured. Standard histological protocols were followed to assess the pattern of oogenesis and spermatogenesis. Results indicate no oocytes and spermaries were observed simultaneously within the same branch or the same colony, suggesting that *Guaiagorgia* is gonochronic, i.e. sexes are separate in different colonies. A clear pattern of development for both oocytes and spermaries was observed with no oocytes found from November to March. Many large oocytes were then observed in April (mean ± SD = 73 ± 39μm) while the smallest colony with oocyte was 171 cm long. The size and

number of oocytes decreased to 23 ± 15 μm in June. Large spermaries were also found in April (mean ± SD = 146 ± 38 μm), however, no spermaries were observed in the remaining months over the sampling period. Patterns of frequency occurrence of different developmental stages of oocytes and spermaries were less distinct, with different stages appearing in different frequencies from April to June. Based on these results, it is predicted that this gorgonian species is most likely to spawn between April and May.

Additional samples were collected from July to September but were not processed due to premature termination of the project. It is likely that some of these samples may still contain developing gametes, at least the oocytes. But given that spermaries were found only in April, this presented a biological puzzle as to why both sexes were not developing in synchrony to ensure greater reproductive success. There was also observed site difference in the reproductive patterns. From a biological perspective, it would be a challenge to continue to monitor the reproductive biology of this species to address this apparent biological paradox of the absence of synchronous patterns of development between the two sexes.

Understanding the reproductive biology of this species will also address the question of how its population dynamics is being maintained. As a way forward, additional round of studies should be carried out to clarify the reproductive pattern thus far observed with the possibility of filling up some remaining gaps in our understanding of the reproductive biology of this species. Samples from more sites should also be compared to address the question of site dependent differences. Environmental factors like wave exposure, current patterns and water depth among sites should also be monitored to assess the potential impact of these factors on the asynchronous reproductive patterns observed among colonies from different sampling sites. A strategic plan for the conservation of this species could then be drawn up that could also serve as the blue print for further development of conservation strategies for other related coral species.

E. Project Asset List:

Project Assets*	Quantity	Date of Purchase	Receipt Reference no.	Location of Item	Person-in-Charge (Name and post)	Photo is / is not Provided
N/A						

*No equipment or any other asset was purchased under this project.

Marine Ecology Enhancement Fund (MEEF)

Declaration

"I hereby irrevocably declare, warrant and undertake to the MEEF Management Committee and the Steering Committee of the relevant Funds including the Top-up Fund, that I myself, and the Organisation:

1. do not deal with, and are not in any way associated with, any country or organisation or activity which is or may potentially be relevant to, or targeted by, sanctions administered by the United Nations Security Council, the European Union, Her Majesty's Treasury-United Kingdom, the United States Department of the Treasury's Office of Foreign Assets Control, or the Hong Kong Monetary Authority, or any sanctions law applicable;

2. have not used any money obtained from the Marine Ecology Enhancement Fund or the related Top-up Fund (and any derived surplus), in any unlawful manner, whether involving bribery, money-laundering, terrorism or infringement of any international or local law; and

3. have used the funds received (and any derived surplus) solely for the studies or projects which further the MEEF Objectives and have not distributed any portion of such funds (including any derived surplus) to members of the Recipient Organisation or the public."

Signature：_____

Project Leader, Put Ang Jr.

Date：Oct 22, 2020