Completion report

Project number: MEEF2022006

Project title: Population structure and Further Studies on Reproductive Biology of the Octocoral *Guaiagorgia* in Hong Kong Western Waters

Recipient organization: The Chinese University of Hong Kong, School of Life Sciences

Reporting Period: 1 July 2022 – 31 December 2023

(i) Executive Summary (1-2 pages):

The octocoral community in Hong Kong, including soft coral and gorgonian coral, has been receiving increasing attention since the last decade because of the rising concerns about their vulnerability to human disturbance. Coastal development and abandoned fishing nets entanglement are major threats to octocorals. Alongside the commencement of the Hong Kong International Airport (HKIA) Third Runway construction in 2016, 3300 colonies of the octocoral *Guaiagorgia* sp. found growing on HKIA seawall were affected by the reclamation.

Guaiagorgia sp. is an uncommon gorgonian species and is the only gorgonian species found in western Hong Kong waters. To date, this species has not been recorded in other parts of Hong Kong. Hong Kong western water is brackish, with large fluctuation in salinity due to the influence of Pearl River outflow. This water also experiences large seasonal fluctuation in water temperature throughout the year. Gorgonian assemblages play a fundamental ecological role as a habitat that provides a complex three-dimensional structure to support high biodiversity. *Guaiagorgia* sp. is therefore the only gorgonian large enough (> 1m in height) to provide this ecosystem function in Hong Kong western waters. Hence, there is an urgent need to understand this phenomenal species, including its population structure and reproduction, in order to construct effective conservation and restoration strategies to manage and protect this species.

Our previous MEEF supported study has confirmed that *Guaiagorgia* is an euharyline cold water species that has a wide range of salinity tolerance from 12 to 32 psu that prefers a lower ambient temperature. However, field information such as reproductive pattern, colony size and size-frequency distribution of this gorgonian species remain largely unknown. These information are basic and yet critical in enhancing our understanding of ecologically important processes of this species, such as its recruitment and reproductive capacity, to allow us assessment of its population responses to local environmental conditions and disturbances. To address this knowledge gap and to gain insights on the reproductive biology and population dynamics of *Guaiagorgia* sp. in Hong Kong, we conducted histology studies and field surveys to identify the reproductive pattern and colony size-frequency distribution of this species, respectively. The findings from this study provide answers to explain the dominance of *Guaiagorgia* sp. in the western waters of Hong Kong that could further help in the design of effective conservation strategies for this species.

The reproductive study was conducted from March 2022 to February 2023. *Guaiagorgia* sp. was identified as a gonochoric species, with male gonads and female gonads found on separate colonies. Our findings revealed that oocytes were present in female colonies throughout the year, with the maximum oocyte size, polyp fecundity, and with the oocytes at the mature stage (Stage IV) observed in September. A significant decline in these measurements was recorded in October. Meanwhile, spermaries were only observed in August, September, and November, with the maximum size, number of spermaries per polyp, and with proportion of spermaries at the mature stage reaching maxima in September. Based on these observations, we predict that the spawning period of *Guaiagorgia* sp. occurs around the time of the full moon in September.

To confirm the prediction, lab-based aquaria were set up in order to collect reproduction materials released from this species to gain further insights in understanding the reproductive strategies of *Guaiagorgia sp.*. The successful collection of eggs and developing eggs by this set up in late September around full moon served as a validation, confirming that *Guaiagorgia* sp. is a broadcast spawner. Further studies can be conducted to assess the cues that trigger gamete release in this gorgonian species.

Five field surveys were conducted in order to investigate the population structure of *Guaiagorgia* sp. in different sites in western Hong Kong water, including Ma Wan, Tsing Chau Wan, Ha Kok Tsui, Yam Tsai Wan, and Tsin Yue Wan. Ma Wan showed the highest abundance (138 individuals) of this species among these sites, while Yam Tsai Wan exhibited the highest mean colony height ($21.21 \pm 9.61cm^2$). Populations that were closer to the airport appear to show a higher mean height, reflecting a possible dispersal pattern from the airport outwards. Substrate availability may be one of the factors limiting the distribution of *Guaiagorgia* sp. in Hong Kong. It is predicted that recruitment in Yam Tsai Wan and Tsin Yue Wan will be more extensive than the other sites, attributed to a larger proportion of large colonies and hence a higher reproductive potential.

All these information provide important baseline for further evaluation of the potential distribution of this species under future disturbances. These information are also essential in the design of an adaptive strategic plan for the conservation and restoration of this octocoral species.

(ii) Project title and brief description of the Project:

Population structure and Further Studies on Reproductive Biology of the Octocoral *Guaiagorgia* in Hong Kong Western Waters

Octocoral colonies play a vital ecological role in the marine environment by providing intricate three-dimensional structures that support a rich diversity of marine life. In the western waters of Hong Kong, the octocoral species *Guaiagorgia* sp. thrives abundantly and dominates the region, acting as a significant habitat constructor. This species, however, is notably absent in other parts of Hong Kong. There is a possibly that the western waters of Hong Kong provide the best environment for its survival and propagation. Understanding the reproductive biology of this species is thus imperative in gaining insights on its ability to reproduce and propagate.

To shed light on its reproductive behaviour, one of our aims in this project was to quantify the distribution of colony sizes and frequencies of *Guaiagorgia* sp. in the western waters of Hong Kong. This analysis will help to determine how the species recruits new individuals, whether through the mass release of gametes or by nurturing and brooding their own larvae. Typically, species that exhibit larval brooding will show the characteristics of clustering of numerous juveniles around larger, mature individuals. Understanding the mode of recruitment is essential for evaluating the potential of these coral populations to naturally recover after disturbances.

This study should significantly contribute to our overall understanding of the reproductive biology of *Guaiagorgia* sp., while also providing essential insights into the population structure of this octocoral species. The knowledge generated from this research will serve as a cornerstone for developing effective strategies aimed at conserving and safeguarding this remarkable organism. This study focused mainly on the following objectives:

1. To investigate the population size structure of the gorgonian *Guaiagorgia* sp. in Hong Kong western waters
2. To fill in the knowledge gap of the existing pattern of gametogenesis and to verify the reproduction cycle of *Guaiagorgia* sp. in Hong Kong.
3. To establish a lab-based larvae collection protocol for larvae collection and to initiate attempts for larval culturing.

(iii) <u>Completed activities against the proposed Work Schedule:</u>

With the extension of the project period being granted on 9 June 2023, the original work schedule below is updated according to the project period extension application document.

Original Work Schedule	**Actual Work Schedule**	**Proposed Work description**
Mar 2022	Mar 2022	Preparation for the field trips
Mar – Jun 2022	Mar – Jun 2022	Pre-project monthly collection of *Guaiagorgia* samples
Jul – Nov 2022	Jul 2022 – Feb 2023	Monthly collection of *Guaiagorgia* samples
Jul 2022 – Mar 2023	Aug – Nov 2023	Field survey of population structure of *Guaiagorgia* in 5 sites in western waters
Jul 2022 – Apr 2023	Jul 2022 – Apr 2023	Sample processing and evaluation of the gametogenesis of monthly *Guaiagorgia* samples, including standard histological treatment (sectioning, slide mounting and staining), monthly measurement of sizes and stages of oocytes, spermaries, fecundity and density of oocytes using image analysis software.
Jan – Mar 2023	Jun – July 2023	Preparation for a lab-based larvae collection setup
Apr – May 2023	Nov – Dec 2023	Education outreach materials preparation (case study writing and education video production)
Apr – Jun 2023	Aug – Oct 2023	Monthly collection of *Guaiagorgia* samples, laboratory culture of *Guaiagorgia* samples, larvae collection, aquaria maintenance, closely monitoring of presence of brooding larvae and attempt to collect and culture larvae/recruits, if any.
Jan – Jun 2023	Jan – Dec 2023	Data plotting, integration, and analysis
Jun 2023	Dec 2023	Final report writing

(iv) Results/descriptions on the completed activities, with the support of photos, videos, social media platform, etc., if any:

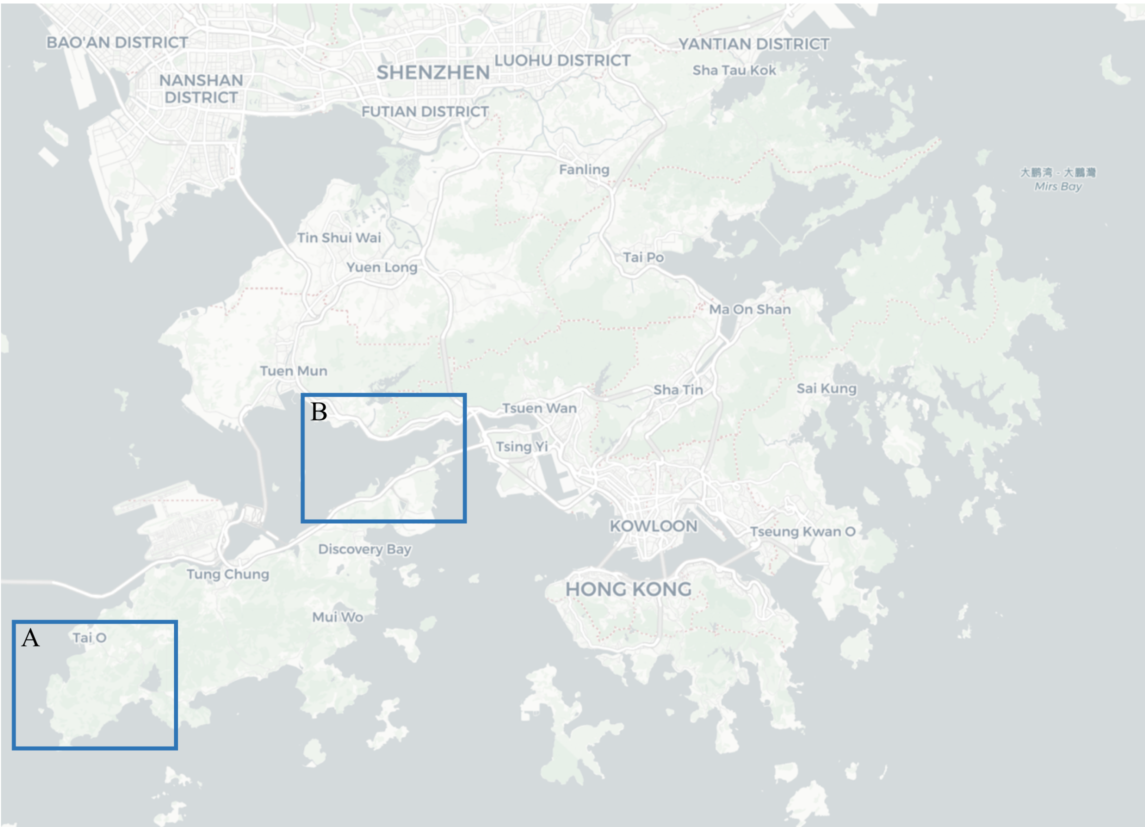
This completion report concluded the work accomplished from **1 Jul 2022 to 31 Dec 2023**:

1. Methodology

1.1 Population structure of Guaiagorgia sp.

1.1.1 Field sites in western Hong Kong

Monthly field survey was conducted at five sites in western Hong Kong water, including Ma Wan, Ha Kok Tsui, Yam Tsai Wan, Tsing Chau Wan, and Tsin Yue Wan from July 2023 to December 2023 (**Figure 1, Table 1**). The area is characterised as turbid with high salinity fluctuation in the wet season, as well as having strong current. These sites were selected due to recorded abundance from EIA or photographic reports. For Ma Wan, Yam Tsai O and Tsing Chau Wan, the dives were guided by Eco-Enviro Consultants Company which was previously involved in *Guaiagorgia* sp. survey and translocation project under the EIA on the Third Runway Expansion of the Hong Kong International Airport.



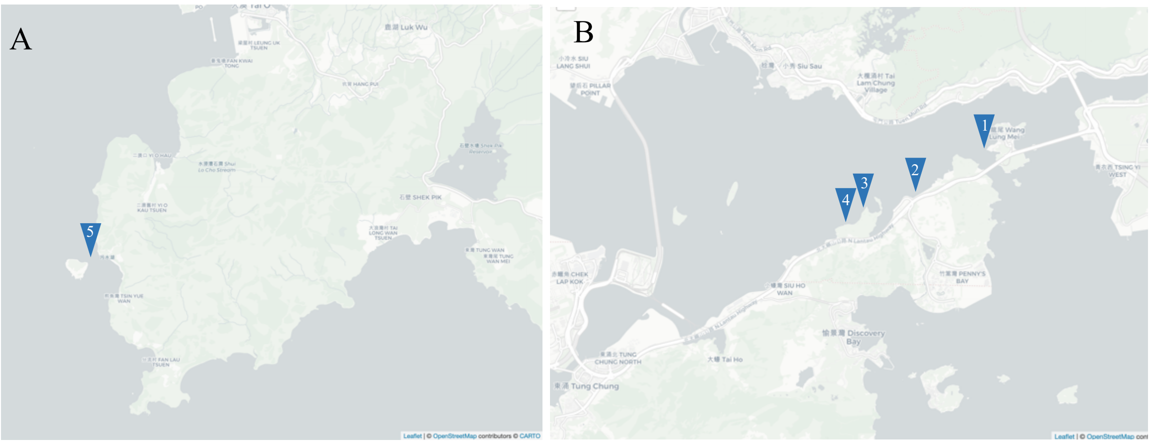


Figure 1. Map of Hong Kong indicating the locations of the five survey sites in (A) Southwestern Lantau. (B) Northern Lantau.

Table 1. GPS coordinates of the five survey sites covered in the present study.

Sites		Coordinates
1	Ma Wan	22.348501, 114.054040
2	Tsing Chau Wan	22.337805, 114.035694
3	Ha Kok Tsui	22.333823, 114.019544
4	Yam Tsai Wan	22.329085, 114.013469
5	Tsin Yue Wan	22.217732, 113.839102

1.1.2 Colony abundance and distribution

Timed swim method was applied for spot check survey. In each site, SCUBA divers swam along shallow water regions at a constant speed (approximately 20-40 m/min) for 20-30min to locate an area with good gorgonian *Guaiagorgia* sp. coral coverage. This area was then surveyed quantitatively by belt transect method.

Using the belt transect method, colony abundance and distribution were determined by laying three 30m transects on the rocky area with gorgonian cover identified in each study site. The starting point of any transect was haphazardly selected. Quadrats 1m^2 in size were then placed at 1 m intervals along each transect. A total area of 60m^2 was therefore surveyed for each transect, and hence 180m^2 for each site. All *Guaiagorgia* individuals inside the quadrats were counted and their heights measured. Individual colonies of *Guaiagorgia* are easily recognizable as each colony has a main branch that grows from a base. Colony height was measured as the maximum distance from the base of the main branch to the tips of the longest lateral branches. The size structure and mean density of *Guaiagorgia* were then calculated and compared among sites.

1.2. Coral gametogenesis and reproductive cycle

1.2.1 Sampling and initial treatments

Samples of *Guaiagorgia* sp. were collected monthly from Ma Wan at least one week before full moon between March 2022 and February 2023. As in many other coral species, it is assumed that *Guaiagorgia* sp. spawns after full moon, hence collection was made before full moon to ensure that oocytes or spermaries were at their maximum sizes right before spawning. As the species is found to be gonochoric (Ang, 2020), at least 10 colonies in each site were collected to ensure that both sexes are collected. These colonies were also longer than 10 cm to ensure maturity (Ang, 2020). These branches were fixed with 10% filtered seawater formalin immediately and left for one week, and further preserved in 70% ethanol before subjecting to the standard histological procedure to examine their oocytes and spermaries (**Figure 2A**). Before the samples were processed into wax blocks, they were decalcified with EDTA pH7.5 solution for 24 hours to remove sclerites in their tissue to avoid tearing from sectioning (**Figure 2B**).

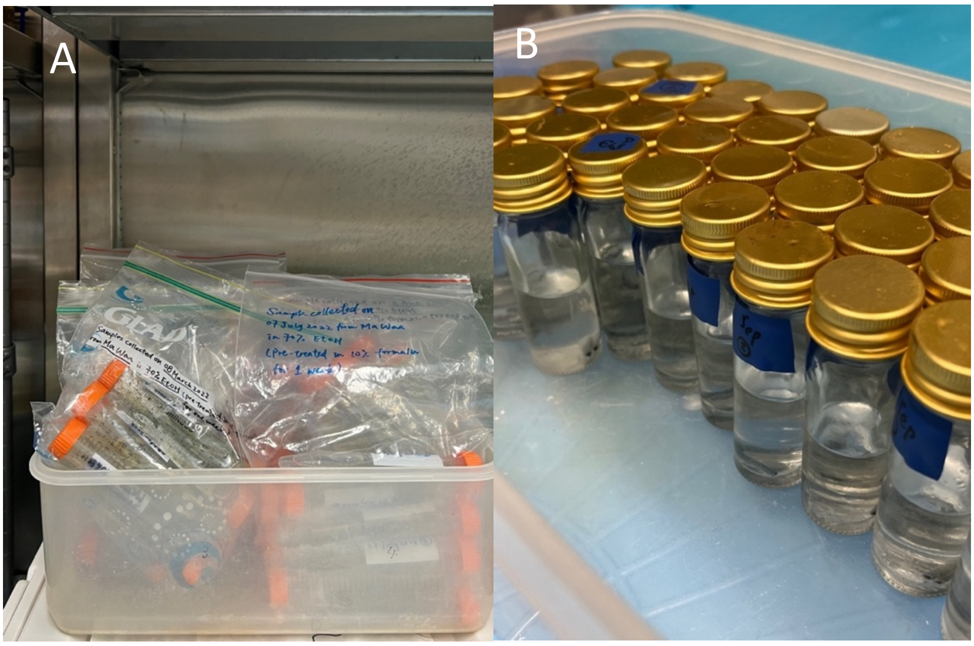


Figure 2. (A) Collected colonies pre-treated with 10% filtered seawater formalin for one week and preserved in 70% ethanol in 15ml falcon tubes. (B) Preserved samples in decalcifying agent EDTA pH7.5.

Polyps collected from different colonies were processed in an Automated Vacuum Tissue Processor (Leica® TP 1050, Leica Instruments GmbH) of the School of Life Sciences, The Chinese University of Hong Kong. A serial dehydration process with increasing ethanol concentration was carried out by the processor to remove any water content in the coral tissues (**Figure 3A**). The processed samples were embedded in wax blocks with paraffin using THERMOLYNE ® histocentre. Blocks were then serially cut at cross-section at a thickness of 7 µm (**Figures 3B-C**).

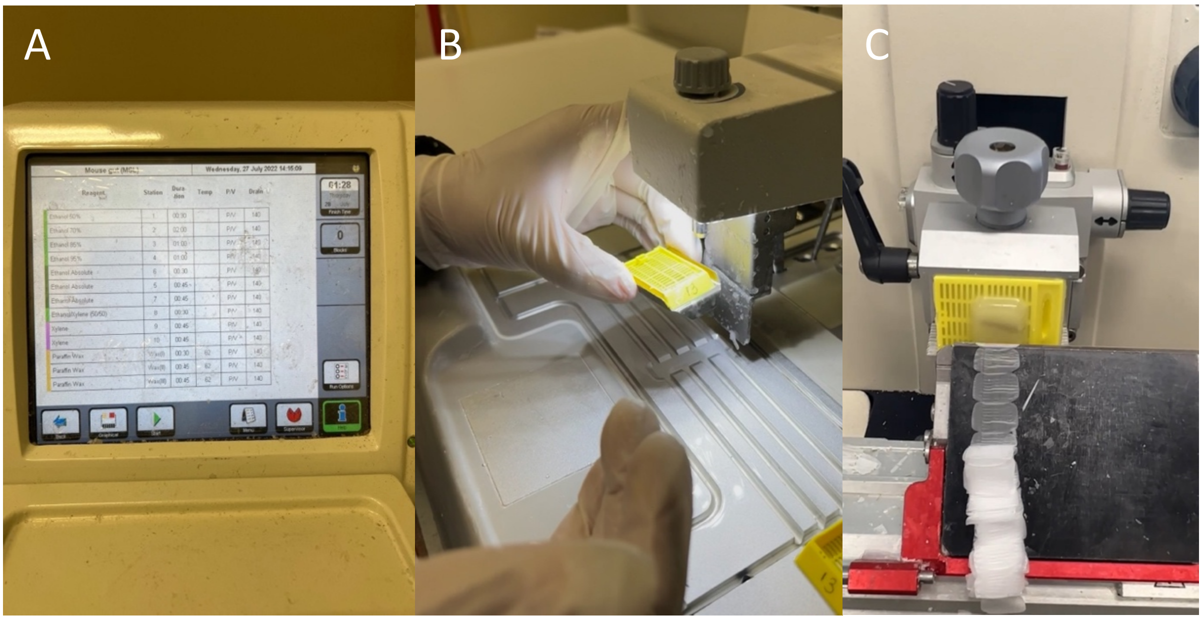


Figure 3. (A) Programme of Automated Vacuum Tissue Processor (Leica® TP 1050, Leica Instruments GmbH). (B) Embedded sample by THERMOLYNE ® histocentre. (C) Blocks being serially cut at cross-section with the thickness of 7 µm.

These serial sections were mounted on glass slides and stained (**Figures 4A-B**). There were four steps in the staining process: 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 stains 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 (**Figure 4C**). Mounted slides were examined under the light microscope for the presence of oocytes and spermaries.

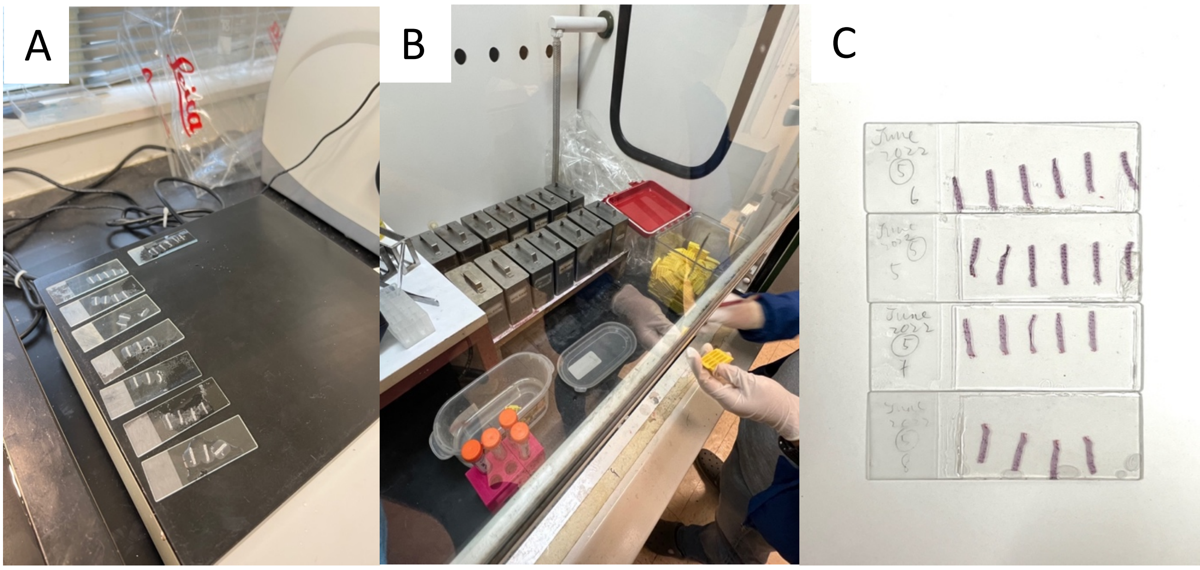


Figure 4. (A) Serial sections fixed on glass slides. (B) Staining station in a fume cupboard located in School of Life Sciences, The Chinese University of Hong Kong. (C) Stained and mounted glass slides.

1.2.2 Light microscopy

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. Developmental stages of the oocytes and spermaries were defined based on Parker (1997) and Chui et al. (2014). Images of both the oocytes (**Figure 5**) and spermaries were taken with light microscope using a digital camera.

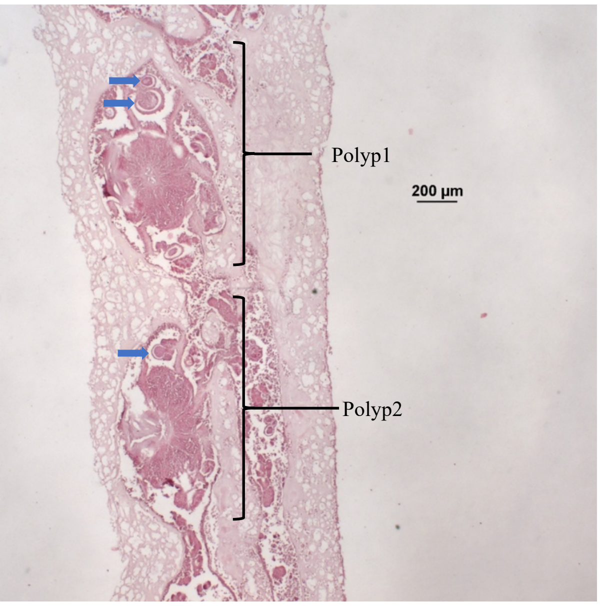


Figure 5. Light microscope image of two polyps with oocytes (indicated by blue arrows) of a female colony.

1.2.3 Gametogenesis

The longest diameter and the corresponding longest diameter perpendicular to it of 6 to 20 largest oocytes and spermaries from each coral colony were measured based on their images captured in a computer using Image-Pro Plus 5.0 (Media Cybernetics, Inc., Bethesda, MD, USA). The geometric diameter of each gamete was then calculated using the following equation (Wallace 1985):

$$\text{Geometric diameter} = \sqrt{\text{Maximum oocyte/spermary diameter x Perpendicular diameter}}$$

Gametes of *Guaiagorgia* were classified into different developmental stages and the number of gametes in each stage was expressed as a percentage of all the gametes encountered in the mounted slides for each colony. Changes in the proportion of different developmental stages of gametes over time were followed. Synchrony of gamete development between colonies was assessed.

1.2.4 Other reproductive traits

Other reproductive traits of *Guaiagorgia* were also determined based on the histology and microscopic study, including sexuality, mode of reproduction, maximum oocyte size, length of oogenic cycle, polyp fecundity, as well as breeding period following Kahng et al. (2011).

1.3 Set-up of aquaria for lab-based larvae collection and collection attempts

Many octocorals are reported to be brooders. To provide a closer examination of the reproductive mode of *Guaiagorgia* sp., as well as to confirm its predicted spawning period based on the gametogenesis results examined above in Section 1.2, branches of *Guaiagorgia* were collected near the predicted spawning period and maintained in a lab-based collection set-up at the Marine Science Laboratory of the Chinese University of Hong Kong (**Figure 6**). Any sign of spawning or presence of floating eggs, egg bundles or larvae was checked daily for seven days prior and after 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 tank to find any traces of larvae that could have settled.

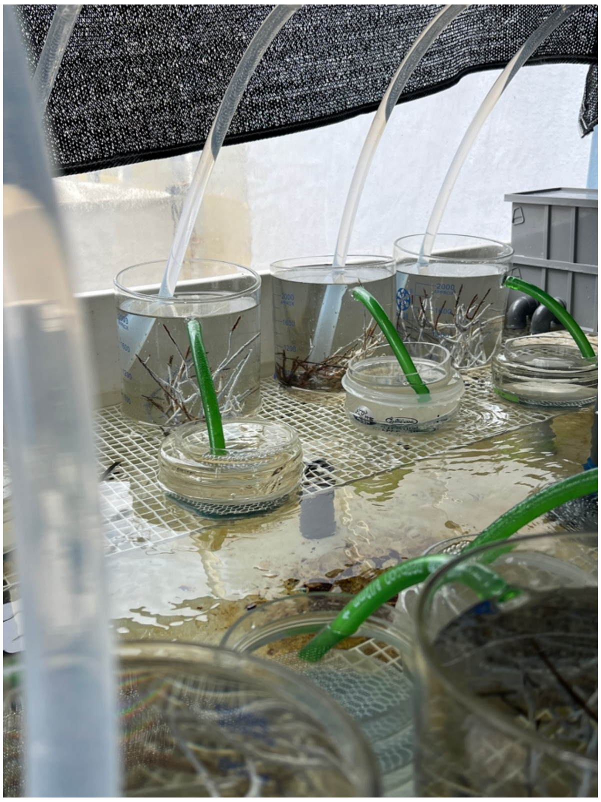


Figure 6. A flow-through system for eggs collection at the Simon F.S. Li Marine Science Laboratory, the Chinese University of Hong Kong.

2. Results

2.1 Population structure of Guaiagorgia *sp.*

2.1.1 Colony density and abundance

The distribution of *Guaiagorgia* sp. was greatly restricted to shallow water (0.5-2m), and was only abundant on hard substrata, including rocks and boulder (**Table 2**). Colonies were commonly seen growing from cracks and cervices between rocks and boulders. *Guaiagorgia* sp. was visually the dominant octocoral species at all the survey sites.

Table 2. Depth range, substrate type, colony type and measured colonies at five survey sites.

Sites		Depth range (m)	Substrate type	Colony type	Number of colonies measured
1	Ma Wan	0.5-1.5	Natural boulder	Natural colony	138
2	Tsing Chau Wan	1.0-2.0	Artificial shoreline	Natural colony	29
3	Ha Kok Tsui	0.5-1.5	Natural boulder	Natural and Transplanted colony	2
4	Yam Tsai Wan	1.0-2.0	Artificial shoreline	Natural colony	82
5	Tsin Yue Wan	0.5-2.0	Natural boulder	Natural colony	28

Most number of colonies was recorded in Ma Wan, followed by Yam Tsai Wan, with least number recorded in Ha Kok Tsui (**Table 2**). The average colony density was 0.32 ± 0.25 ind/m^2 (mean ± SD). As shown in **Figure 7**, the colony density was highest in Ma Wan (0.77 ± 0.57 ind/m^2) and Yam Tsai Wan (0.45 ± 0.44 ind/m^2) and lowest in Ha Kok Tsui (0.011 ± 0.01 m^2).

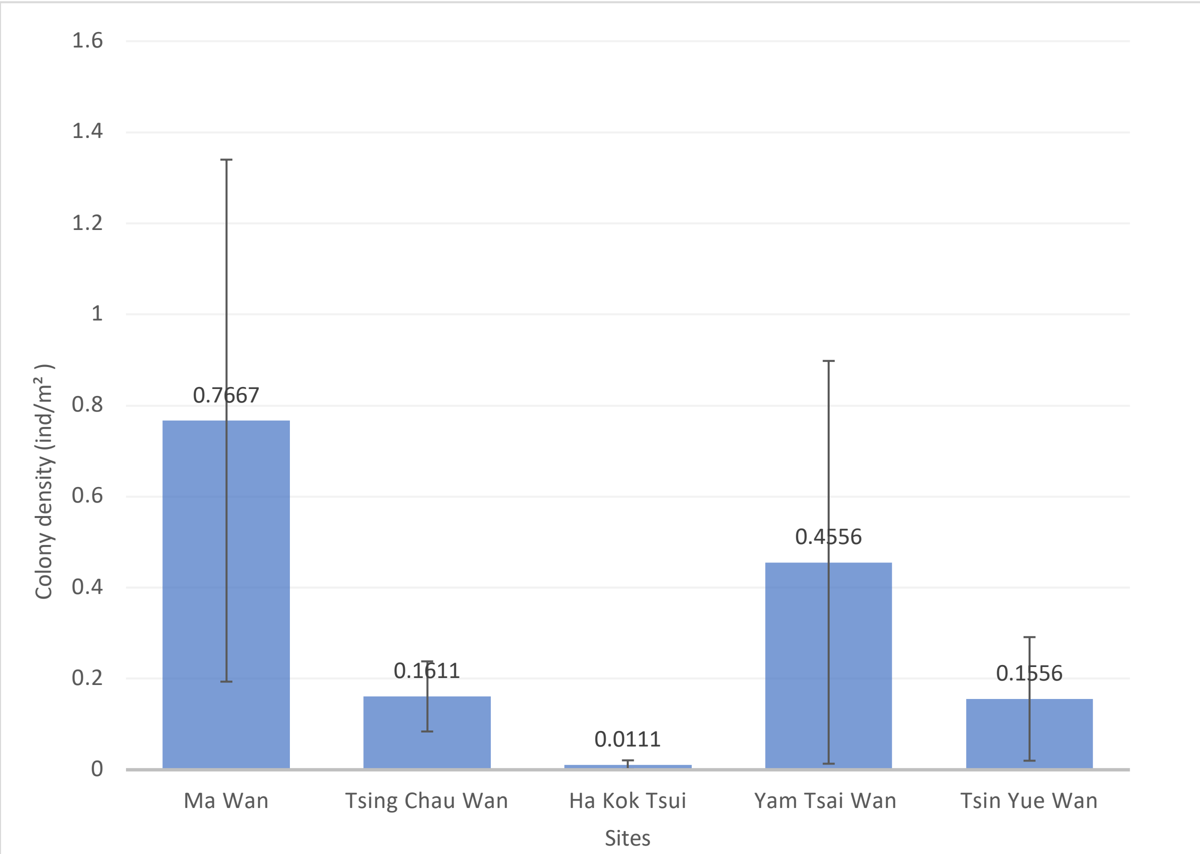


Figure 7. Mean (± SD) density (ind / m^2) of *Guaiagorgia* colonies recorded at the five surveyed sites.

2.1.2 Colony height

Among populations of *Guaiagorgia* sp. measured, that of Yam Tsai Wan exhibited the highest mean (± SD) colony height at 21.21 ± 9.61 cm, followed by that from Tsin Yue Wan (17.46 ± 9.02 cm) (**Figure 8)**. The population in Ha Kok Tsui has the lowest mean height (5.5 ± 0.71 cm).

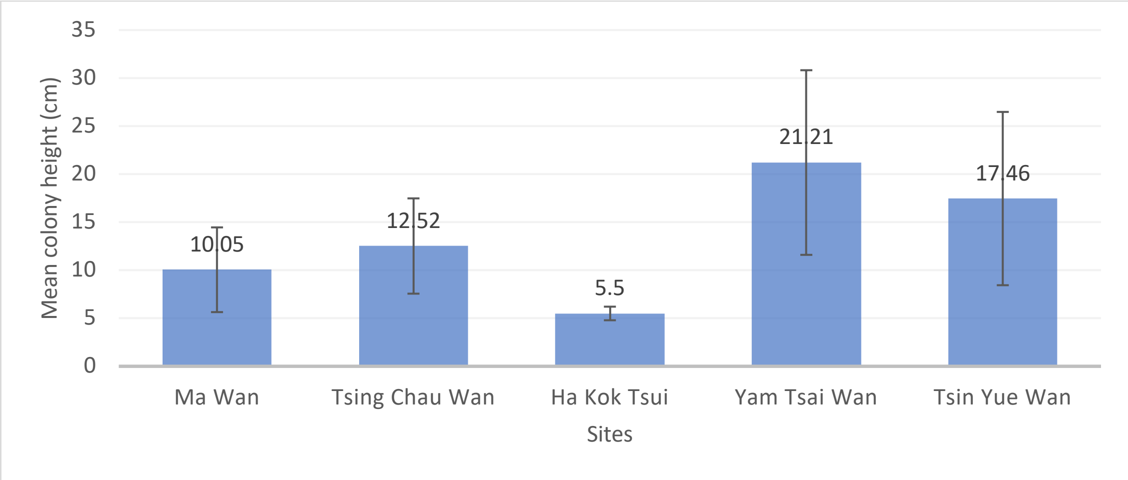


Figure 8. Mean (± SD) colony height (cm) of *Guaiagorgia* in the five sites covered in the present study.

2.1.3. Frequency distribution

The frequency distributions of size classes of the colony height were described as in **figure 9**.

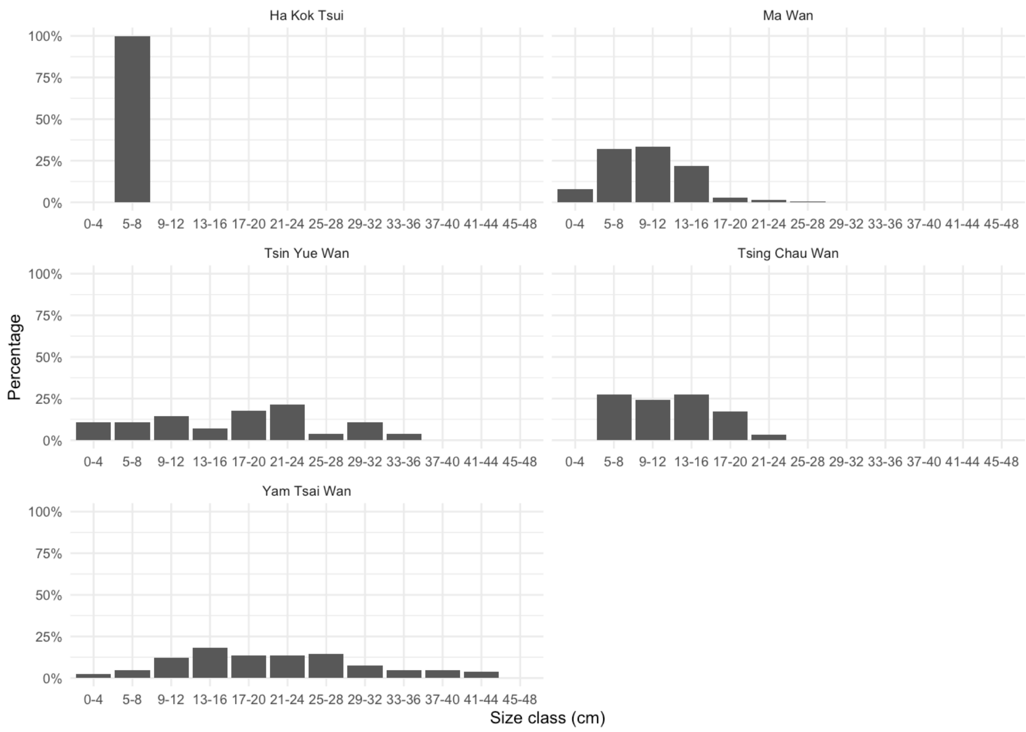


Figure 9. Frequency (%) distribution of the colony height of *Guaiagorgia* colonies at five survey sites.

Size is a major determinant of first reproduction, reproductive output, and survival in gorgonians (Yoshioka, 1994; Coma et al., 1995). Ang (2020) described that the smallest reproductive *Guaiagorgia* colony was 12.9 cm long. This provides an important reference in evaluating the abundance of mature, reproductive colonies in each site, and hence the potential of each of these sites in serving as the source of coral larvae. Thus far among the five sites, Yam Tsai Wan and Tsin Yue Wan are likely to be more important in offering a higher reproductive output given that these sites support a larger proportion of large, i.e. mature colonies. Future studies on the recruitment rate of *Guaiagorgia* in these different sites would help to confirm or validate this suggestion.

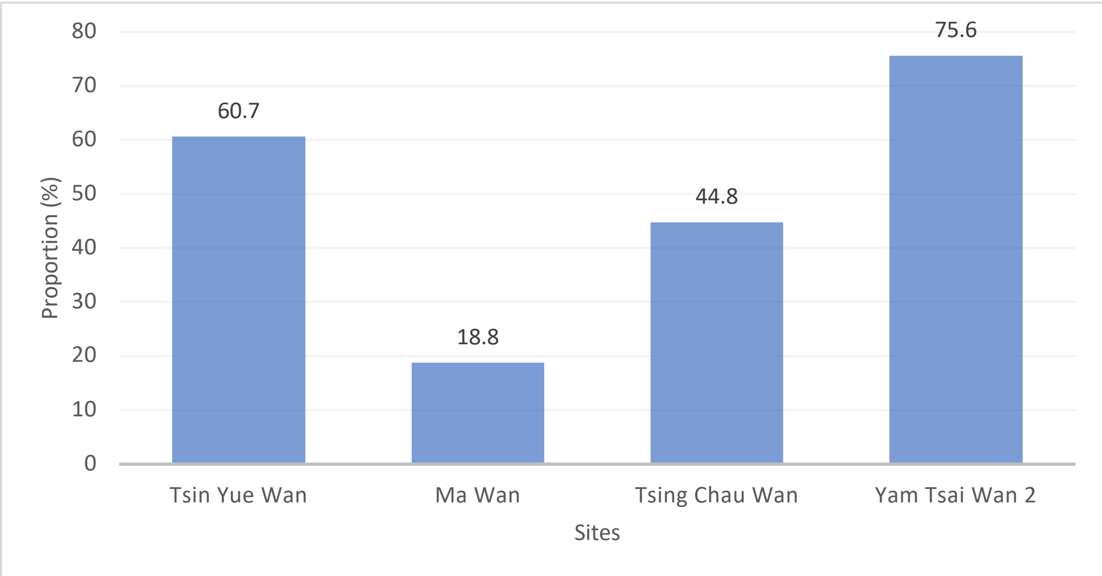


Figure 10. Proportions (%) of mature colonies (> 12.9 cm height) of *Guaiagorgia* sp. in the four sites covered in the present study. No mature colony was found in Ha Kok Tsui.

2.2. Coral gametogenesis and reproductive cycle

2.2.1 Sexuality

Guaiagorgia sp. is a gonochoric species in which the mature colonies are either male or female. The difference between sexes cannot be distinguished by external characteristic. Among the 120 colonies examined over the project period, 38 colonies were female, 12 colonies were male. Neither spermaries or oocytes were found in the remaining 70 sampled colonies. Male colonies were only present in August, September and November, i.e. spermaries were only found in August, September, and November 2022, while female colonies were observed throughout the year with oocytes of different developmental stages present throughout the sampling period.

2.2.2 Gametogenic cycle and fecundity

The smallest mean (± SD) diameter of oocytes over the sampling period was 37.0 ±6.9 µm recorded in May. The maximum mean diameter was 193.1 ± 13.2 µm recorded in September. The other two peaks were in December (114.6 ± 37.6 um) and March (108.8 ± 17.1 um). The most rapid decrease in oocytes diameter was between September and October (**Figure 11A**). For spermaries, the maximum mean (± SD) diameter was 166.8 ± 28.0 µm recorded in

September with the most rapid decrease in spermaries size also recorded between September and October (**Figure 11B**).

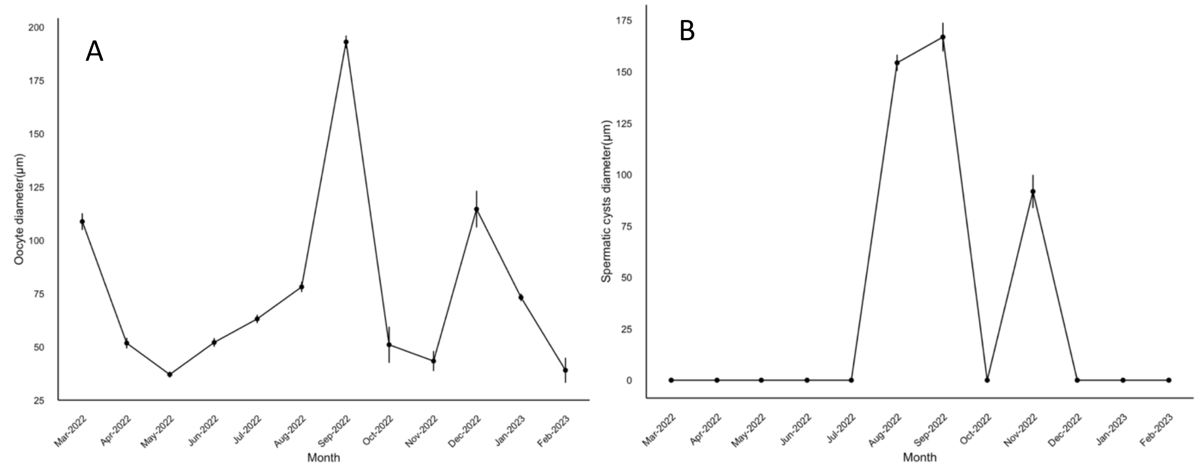


Figure 11. Mean (± SE) diameter of (A) oocytes and (B) spermaries in samples of *Guaiagorgia* sp. collected during 2022 and 2023.

The mean (± SD) number of oocytes per polyp was mostly below 6 except from July to September, with the lowest recorded in February (2.33 ± 1.53) and a maximum reached in August (18.8 ± 5.75) and September (18.2 ± 7.47) (**Figure 12)**. The most rapid drop in mean oocyte count over the year was between September and October. A similar decreasing trend was also observed in male colonies in the same period, with the mean (± SD) number of spermaries per polyp reaching the maxima in August (5.2 ±3.9) and September (5.5 ±3.5).

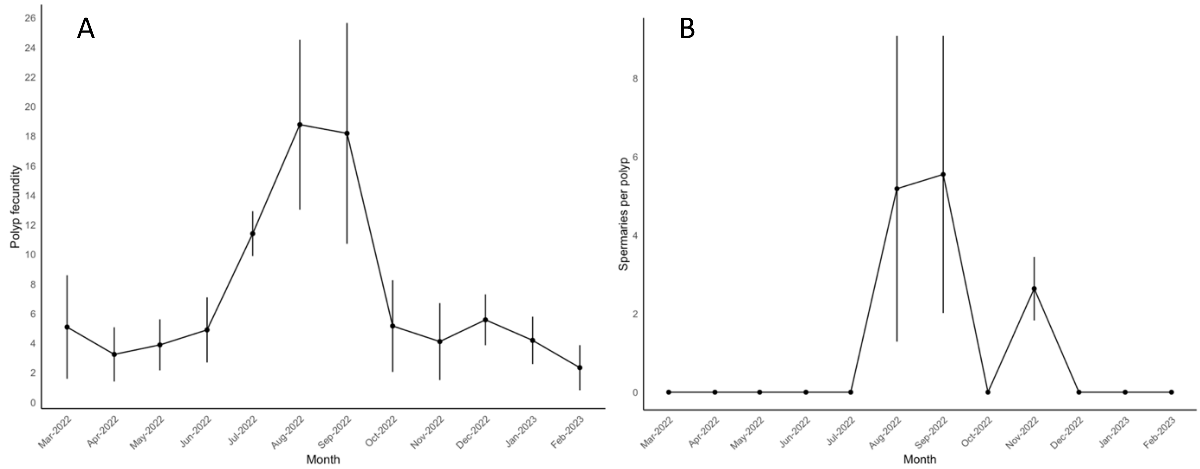


Figure 12. Mean (± SD) number of gonads per polyp in (A) female and (B) male colonies of *Guaiagorgia* sp. collected during 2022 and 2023.

Furthermore, the proportion of gonads at different developmental stages (stage I to IV) in each month was also recorded over the year. As shown in **Figure 13**, stage I and stage II oocytes were generally dominant throughout the sampling period. Stage III oocytes, the maturing oocytes, were present in March and August to December 2022; and Stage IV oocytes, the mature oocytes, were found only in March (very small proportion of 1.6%), September and December with the highest proportion (52.4%) recorded in September. There was an increasing trend of having more stages of oocyte observed from May to September 2022. For male colonies, stages II to IV spermaries appeared abruptly in August and September. All four stages of spermaries were recorded in November samples. The highest proportion of stage IV spermaries was recorded in September (33.3%).

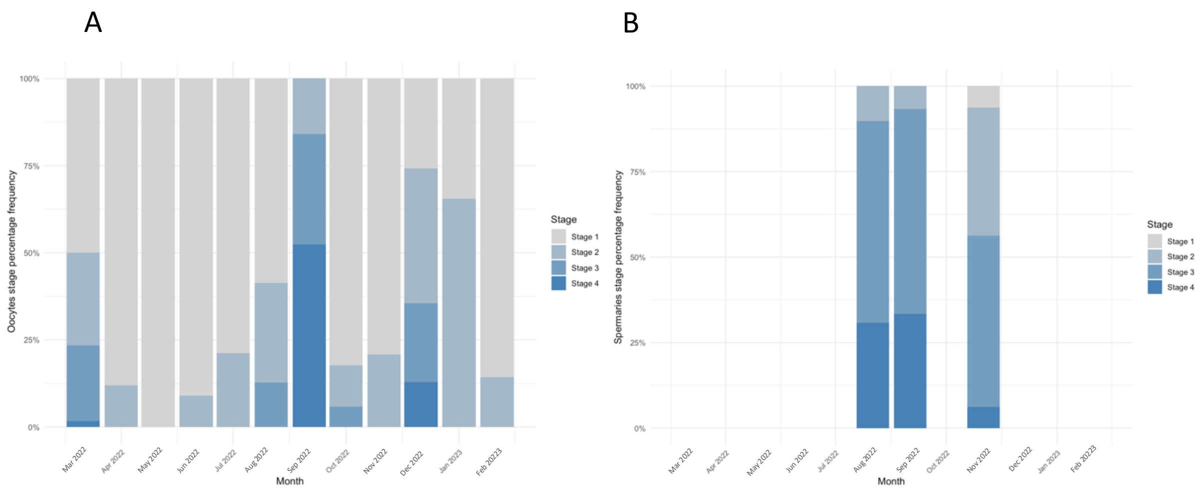


Figure 13. Percentage frequency of reproductive material at 4 stages in (A) female and (B) male colonies collected during 2022 and 2023.

2.3 Set-up of aquaria for lab-based larvae collection and collection attempts

Eggs and developing eggs were collected near full moon by the lab-based collection setup at the Marine Science Laboratory of the Chinese University of Hong Kong. The eggs are blue in colour, at roughly 0.2mm in size (**Figure 14**). The presence of floating cleaving eggs indicated that the species is a broadcast spawner, which releases gametes into the water column.

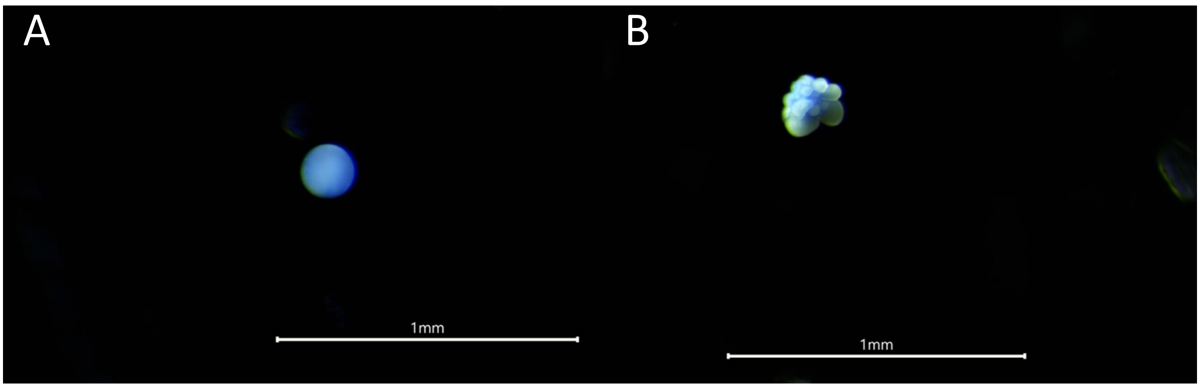


Figure 14. (A). Egg of *Guaiagorgia* sp.. (B). Developing embryo of *Guaiagorgia* sp.. The images were captured under the microscope using a digital camera.

3. Discussion

3.1 Population structure

Octocoral species in Hong Kong are typically found in deeper water, as noted by Yeung et al. (2014). However, the distribution of *Guaiagorgia* sp. is limited to shallow water. There have been reports of *Guaiagorgia* sp. colonies being exposed to air during extreme low tides in Ma Wan and Tsin Yuen Wan. Although the tolerance of *Guaiagorgia* sp. to desiccation is unknown, the population does not appear to be significantly affected by these low tide events. Some other octocoral species have developed the ability to withstand emersion (Williams 2011). Another study, Teixeira et al. 2013, investigated the physiological tolerance of octocoral, *Veretillum cynomorium*, to the intertidal environment showed significant increase in the expression of two molecular chaperones, heat shock cognate 70 (HSC70) and heat shock protein 70 (HSP70) It is proposed that the CAT and GST activities allow the coral to withstand post-hypoxic free radical damage and hence air exposure (Teixeira et al. 2013).

It is still uncertain whether *Guaiagorgia* sp. is zooxanthellate, and thus, whether light conditions limit its vertical range of distribution. However, even in shallow water in Hong Kong western waters where this species is abundant, visibility is poor due to high turbidity. During one of the surveys conducted in Tsin Yue Wan, the visibility was less than 10cm. Additionally, *Guaiagorgia* sp. was observed to be rooted in the overhang of rocks and/or between boulders where light penetration is limited. This suggests that light availability may not be the reason for its restricted distribution in shallow water, but rather the availability of the certain substratum type may be more critical. In shallow western Hong Kong waters, *Guaiagorgia* sp. occupies a specific ecological niche on hard substratum with layers of boulders and rocks. In deeper waters, the substratum is reported to be mostly sandy or muddy with fine sediment (Yeung et al. 2014).

The survey sites where this octocoral species is found are characterized by having strong water currents and exposure to strong waves, which apparently favor individuals of *Guaiagorgia* sp. as filter feeders. Intermediate flow rates of current can greatly influence and benefit the feeding efficiency of suspension feeders like octocorals and black corals (Patterson 1991, Dai and Lin 1993, Fabricius et al. 1995a, b), particularly the azooxanthellate ones. Furthermore, food concentration in terms of suspended particulate matter (SPM) is another important factor that likely determines the abundance and distribution of these two groups of corals. Their diets consist of small-sized organisms such as phytoplankton, dinoflagellates, and weakly swimming or damaged zooplankton (Fabricius et al., 1995a, b; Fabricius and Dommisse, 2000). They have a passive heterotrophic feeding mode and depend on water current to transport food particles through their filter structures for capture, fulfilling their daily energetic requirements. Thus, in addition to food concentration, the strength of the current could also be a key factor influencing the distribution and growth of octocorals.

Compared to other sites in Hong Kong (Yeung et al., 2014), *Guaiagorgia* sp. is the only octocoral found at the survey sites. The western waters of Hong Kong are characterised by high salinity fluctuations due to the influence of the Pearl River. Salinity levels as low as 1.3 psu have been recorded during the wet monsoon season (Morton and Wu 1975). Although low salinity levels have been shown to be lethal to hard corals (Berkelmans et al. 2012) and possibly also to octocorals (Yeung et al. 2014), a recent study by Tong (2023) shows that *Guaiagorgia* sp. is highly tolerant to a wide range of salinity fluctuation, allowing the species to thrive in Hong Kong western waters. With its high tolerance to salinity changes, the distribution of this species is unlikely to be limited by salinity alone.

Among the sites covered in the present study, Ma Wan has the highest density and abundance of *Guaiagorgia* colonies, followed by Yam Tsai Wan, Tsin Yue Wan, Tsing Chau Wan, and finally Ha Kok Tsui. The abundance of *Guaiagorgia* in Ha Kok Tsui was extremely low, with only 2 alive colonies. Ha Kok Tsui was selected as a destination for coral translocation as part of the TM-CLKL project, owing to its naturally rich coral population, which included more than 200 colonies as noted in the coral translocation report of the project (ERM 2014). In 2013, 23 corals were relocated to this site. However, our survey found only two surviving coral colonies at Ha Kok Tsui, suggesting a significant decline in both the translocated and naturally occurring coral populations. Similarly, another potential mortality event was observed in Tsin Yue Wan. Historical photographic evidence from 2021 (**Figure 15**) depicted a previously abundant and clustered population. However, during our 2023 survey, only 28 colonies were encountered at the same location, marking a stark reduction in population size.

Figure 15. Photography of extensive *Guaiagorgia anas* colonies in Tsin Yue Wan, taken in 2021.

Artificial shorelines at Yam Tsai Wan and Tsing Chau Wan appears to be generally more preferred by the octocorals. This is also supported by the extensive abundance (717 colonies per 100m transect on average) of this octocoral along the HKIA seawall prior to reclamation (Airport Authority Hong Kong, 2016). Apparently, artificial shoreline provides a much more suitable substratum for the recruitment of *Guaiagorgia* than natural boulder rocks which are often covered with biofouling organisms. This observation, if supported by more evidences, could be significant as this provides a new insight in developing conservation strategy of using the appropriate substrata to attract natural recruitment of this octocoral. Continuous study on these sites would be crucial to reveal whether the populations are in the midst of expansion, or are experiencing a decline in general.

It was also observed that in Northern Lantau, sites that are closer to the HKIA seawall have a higher mean height of *Guaiagorgia* colonies, except again for Ha Kok Tsui. Further study on the connectivity of the species could provide more insight on the recruitment patterns of the species.

Guaiagorgia sp. can grow to at least 59 cm in height (Mott McDonald, 2017) but the maximum colony size measured in the present study was only 44 cm. As this study was the first attempt to measure the size class structure of *Guaiagorgia* sp. in these sites, it is yet unclear whether this smaller maximum size class recorded was limited by natural mortality from wave actions or anthropogenic stresses that exhibit selective impact on larger sized colonies, or simply because the populations in these sites were only recruited recently and have not had the chance to grow into their full size. Nonetheless, it may be of interest to note that among the five sites surveyed, Yam Tsai Wan and Tsin Yue Wan showed a wider spread of size class distribution of *Guaiagorgia* sp. but the largest colonies of this coral were found in Yam Tsai Wan, a site with artificial shoreline. On the other hand, smaller individuals were found in all sites except Ha Kok Tsui, suggesting that active recruitments are taking place in all these sites.

To summarize, the distribution of *Guaiagorgia* sp. in Hong Kong is limited to shallow water with hard substrata. Natural recruitment was observed especially in Tsin Yue Wan, Ma Wan, and Tsing Chau Wan. The reproductive potential is higher in Yam Tsai Wan and Tsin Yue Wan given that these sites support a larger proportion of larger, mature colonies. The findings could provide valuable insights for constructing a more effective management plan. Conservation efforts should focus on protecting the species' specific habitats, monitoring and maintaining water quality, managing human activities, conducting further research, and raising awareness about the importance of conservation. By implementing these measures, a more comprehensive management plan can be developed to ensure the long-term survival and well-being of *Guaiagorgia* sp. and other related species.

3.2 Reproductive biology

3.2.1 Gametogenesis

The presence of stage I and stage II oocytes in most months throughout the sampling period suggests that female reproductive cells are always available and may stay in a dormant state until triggered by some external or internal factors to develop further into more advanced stages. The presence of different other stages, i.e. stage III and stage IV oocytes in March, then again in September and December within the same polyp and colony clearly indicates that oogenesis is asynchronous. Stages III and IV oocytes are the largest in size, hence largest sizes of oocytes were recorded in the months when these stages of oocytes are most abundant. Nonetheless, were at different stage. It reflected an asynchronous oogenesis that oocytes are produced all year round and have multiple and overlapping oogenesis, resulting in oocytes with various sizes and stages. The mature oocytes in December and March could be resulted from the continuous oogenesis from the previous months. Hence, the result above did not reveal the onset of oogenesis.

Spermaries were found only in August, September and November in different stages. As mentioned above, it was difficult to recognize the early developmental stages of spermaries. Failure to recognize them may partly explain their absence in the period before August, not until when their geometric diameters reached above 100 µm in size. Hence, by the time they were observed, they were mostly at the maturing (Stage III) or mature (Stage IV) stages already. The onset of spermatogenesis remains unknown. However, similar to that observed in oogenesis, the presence of different stages of spermaries within the same polyp and colony indicates that spermatogenesis is also asynchronous.

The observations made in the present study agree with those of earlier studies showing that oocytes require longer time (> 10 months) to develop than spermaries (2–3 months) in octocorals (Benayahu & Loya 1983, 1984; Benayahu 1989, 1991; Braxeau & Lasker 1989; Coma et al. 1995). Male colonies tended to have a lower fecundity (i.e. lower number of spermaries per polyp) than the female colonies.

3.2.2 Mode of Reproduction

The presence of clusters of high density of *Guaiagorgia* sp. on the seawall of HKIA (Airport Authority Hong Kong, 2014) led to the suspicion that this species may be a brooder as brooders tend to have their larvae settled very close to the mother colony. However, no brooding larvae were found in the present study in the aquaria, on the surface of the colonies, or in histology sections. Instead, eggs and developing embryos (cleaving eggs) were found in the laboratory culturing tank, albeit only in a few numbers. This suggests that *Guaiagorgia* sp. is likely to be a broadcast spawner. Broadcast spawning is not an unusual reproductive mode of octocorals. In fact, octocorals, particularly tropical species, are most commonly gonochoric broadcast spawners (Kahng 2011). It was observed that the eggs released from *Guaiagorgia* in the present study were negatively buoyant. This could result in their limited dispersal as larvae that developed would settle near the mother colony. This may explain the presence of clusters of colonies of *Guaiagorgia* sp. in the HKIA seawall observed earlier.

3.2.3 Spawning time of *Guaiagorgia* sp.

Continuous gametogenesis and year-round breeding have been documented in several broadcast spawning octocorals, including *Dendronephthya hemprichi*, *Pennatula aculeata*, and *Carijoa riisei* (Dahan & Benayahu 1997, Eckelbarger et al. in 1998, Kahng et al. in 2008). Similarly, *Leptogorgia virgulata* and *Lobophytum crissum* have been reported to have an extended spawning season (Adams 1980, Fan et al. 2005). Reports on the spawning behavior of Caribbean gorgoniids *Pseudopterogia acerosa*, *Montastraea faveolata* and *Eusmilia fastigiata P. americana* (Yoshioka 1979, Bastidas et al. 2005) exhibit high variability, suggesting that these broadcast spawners may engage in year-round or nearly year-round breeding activities.

Despite the apparent asynchrony in their development, both sperms (spermaries) and especially the eggs (oocytes) were observed to have maturing (Stage III) and/or mature (Stage IV) gametes from August to December, with peaks in September. Variabilities do exist and some missing observation (e.g. absence of spermaries in October and December) could be an artifact. This synchronized development to reach maturity in the same period especially in September is a common strategy of broadcast-spawning to ensure a higher chance of fertilization success (e.g. Baird et al. 2009). The presence of highest proportion of stage IV gametes (both spermaries and oocystes) was also supported by having the largest geometric diameters and the highest fecundity of both the male and female reproductive cells recorded in September. The drastic drop in all these features in October clearly points to the highest likelihood that spawning occurred between September and October, as similarly observed in other studies (e.g. Fabricius et al., 2008). This was further verified by the collection of eggs and developing embryos in September released from the cultured *Guaiagorgia* individuals in the aquarium. The presence of stage IV oocytes in December and March, albeit at a much lower number or proportion, suggests that there may also be other, minor spawning events in these months. However, fertilization success of a species is determined by the male colonies and sperm availability (Brazeau and Lasker 1989) with the amount of sperms being the limiting factor. In the absence of any spermaries observed in these months, the chances of any fertilization success in these minor spawning events are doubtful. To further verify if this species spawns multiple times in a year, ex-situ gamete collection in the laboratory during November to December and March to April can be conducted with tagged colonies.

3.2.4 Effects of environmental factors on gametogenesis

As in most marine organisms, gorgonians show seasonal reproductive cycle and environmental cues like increase (or decrease) in seawater temperature could serve to trigger gametogenesis (Baker et al. 2008). Research on the gorgonian *Leptogorgia virgulata* revealed that gametogenesis began with an increase in water temperature throughout the spring and summer, with gamete discharge taking place in the late summer and early fall (Bayer et al. 2013). Similarly, gametogenesis in another gorgonian *Swiftia exserta* was shown to be triggered by a rise in sea water temperature and that gamete discharge took place in the summer (Slattery et al., 2014). It is generally believed that winter water temperatures is too cold for reproduction (Tanner, 1996). Comparing the trend of fecundity, changes in mean gamete sizes and the proportion of reproductive stages in *Guaiagorgia*, it would appear that increase in the size and number of oocytes started after May, and the appearance of stage III in August coinciding with the increase in seawater temperature from around 25℃ in late spring to around 28℃ in mid-summer (**Table 3**). Spermatogenesis occurred more rapidly after July. All these parameters in both sexes reached their maxima in late summer. However, oogenesis persisted through the winter and spring. Tong (2023) found that *Guaiagorgia* sp. grew better under lower temperature (below 21℃) whereas higher temperature (above 27℃) was stressful and inhibited the growth of its tissue. It is possible that reproductive effort may be reduced to facilitate colonial growth in winter as observed in other coral species (Guest et al. 2008; Baird et al. 2009). This trade-off between resource allocation to growth or to reproduction needs further evaluation to understand the reproductive strategy of this species, especially the continuous allocation to produce mature reproductive cells in winter when there is a very low chance of reproductive success.

Table 3. Monthly average of water temperature (℃) during 2012-2022 at Environmental Protection Department (EPD) North Western Water Control Zone. Data are from EPD (2024).

Month	Temperature (℃)
Jan	18.50
Feb	17.91
Mar	19.12
Apr	22.33
May	25.41
Jun	27.47
Jul	28.22
Aug	27.85
Sep	27.88
Oct	28.03
Nov	25.13
Dec	21.59

Further verification on the linkage between water temperature and gametogenesis of *Guaiagorgia* sp. can help to understand the potential effects of climate change, especially the occurrence of heatwaves and extreme cold events, on the reproductive behaviour of *Guaiagorgia.* For instance, an octocoral inhabitant in the Red Sea, *Rhytisma fulvum*, showed varied timing of onset of brooding and synchronicity under 5-week exposure of Representative Concentration Pathway conditions, as well as significant reduction in its planula survival and polyp metamorphosis rate (Liberman et al. 2021). One study specifically focused on the impact of climate change on the reproductive physiology of a gorgonian coral, *Paramuricea clavate*, found that elevated water temperatures resulted in a significant decrease in their reproductive output, and that female colonies are more susceptible than male colonies. Besides, shallow water populations that are originally long-lived may be jeopardized in the future because of their greater exposure to temperature change (Arizmendi-Mejía et al. 2015) Would asynchronous oogenesis and spermatogenesis of the species help resist the effect of climate change? Understanding the reproductive cycle and cues that trigger this cycle in *Guaiagorgia* sp. is essential to predict its response to climate change pressures in a changing environment. Other environmental factors and biological drivers, such as water quality, salinity and current, may also play a role in triggering or maintaining gametogenesis in this species, especially in winter. These need to be further explored. It is also worth looking at the asexual reproduction of *Guaiagorgia* sp. as it was suggested that fragmentation was a successful asexual propagating strategy for gorgonians with high survival rate (Brazeau et al, 1998).

4. Summary and future prospects

The reproductive biology of *Guaiagorgia* sp. is consistent with the prevailing patterns observed in other octocorals. The evidences available point to this species as a broadcast spawner. More specifically, gametogenesis is asynchronous and oocytes appear to be present year long. However, judging from information on reproductive fecundity, changes in the size of the gametes (both oocytes and spermaries) and on the presence of mature stages of oocytes and spermaries, population of this species should exhibit one major spawning event between September and October, but several minor events in the period from November to March may also be possible. Presence of eggs and embryos from laboratory based cultured individuals of *Guaiagorgia* sp. lent support to this prediction on the major event of spawning. Nonetheless, spermaries were not always detected except between August to December, suggesting that the presence of sperms may be a limiting factor in determining the reproductive success of this species. The information obtained from the present study contributed valuable insights to the growing body of knowledge essential for understanding the patterns of gorgonian reproduction as a gonochoric broadcast spawner. Moreover, our investigation on this ecologically significant species yielded crucial data regarding the size at first reproduction and the reproductive cycle, which should be duly incorporated into the management strategy for the conservation of this main structural species in Hong Kong western waters.

Changes in seawater temperature may be closely related to the reproductive phenology, with rising temperature in late spring and early summer triggering the rapid maturation of the oocytes and spermaries. The roles of other environmental factors and biological drivers in triggering or maintaining gametogenesis in this species should also be further explored to provide insights on the reproductive preference of the species as well as to help inform conservation and management efforts.

References

Airport Authority Hong Kong (2016). Expansion of Hong Kong International Airport into a Three-Runway System Coral Translocation Plan.

Airport Authority Hong Kong (2017). Expansion of Hong Kong International Airport into a Three-Runway System. Detailed Coral Translocation Report.

Adams R.O. (1980). Investigations of color, morphology, and development of the sea whip, Leptogorgia virgulata, (Lamarck) (Cnidaria: Octocorallia: Gorgonacea). PhD thesis, Florida State University

Ang P.O. (2020). Reproductive Biology of the Dominant Octocoral *Guaiagorgia* in Hong Kong Western Waters. Final Report, November 2020. Final Report submitted to the Marine Ecological Enhancement Fund.

Arizmendi-Mejía, R., Ledoux, J.-B., Civit, S., Antunes, A., Thanopoulou, Z., Garrabou, J., & Linares, C. (2015). Demographic responses to warming: Reproductive maturity and sex influence vulnerability in an octocoral. *Coral Reefs*, *34*(4), 1207–1216. https://doi.org/10.1007/s00338-015-1332-9

Baird, A. H., Guest, J. R., & Willis, B. L. (2009). Systematic and biogeographical patterns in the reproductive biology of Scleractinian corals. *Annual Review of Ecology, Evolution, and Systematics*, *40*(1), 551–571. https://doi.org/10.1146/annurev.ecolsys.110308.120220

Bayer, F. M., Grasshoff, M., & Verseveldt, J. (2013). Illustrated trilingual glossary of morphological and anatomical terms applied to Octocorallia. Brill.

Baker, A. C., Glynn, P. W., & Riegl, B. (2008). Climate change and coral reef bleaching: An ecological assessment of long-term impacts, recovery trends and future outlook. *Estuarine, coastal and shelf science*, *80*(4), 435-471.

Bastidas, C., Cróquer, A., Zubillaga, A. L., Ramos, R., Kortnik, V., Weinberger, C., & Márquez, L. M. (2005). Coral mass- and split-spawning at a coastal and an offshore Venezuelan reefs, Southern Caribbean. *Hydrobiologia*, *541*(1), 101–106. https://doi.org/10.1007/s10750-004-4672-y

Benayahu Y. (1997) Developmental episodes in reef soft corals: Ecological and cellular determinants. Proc 8th Int Coral Reef Symp 2:1213−1218

Benayahu Y. (1989) Reproductive cycle and developmental processes during embryogenesis of *Clavularia hamra* (Cnidaria, Octocorallia). Acta Zool 70:29−36

Benayahu Y. (1991) Reproduction and developmental pathways of Red Sea Xeniidae (Octocorallia, Alcyonacea). Hydrobiologia 216-217:125−130

Benayahu Y, Loya Y (1983) Surface brooding in the Red Sea soft coral *Parerythropodium fulvum fulvum* (Forskal, 1775). Biol Bull 165:353−369

Benayahu Y, Loya Y (1984) Life history studies on the Red Sea soft coral *Xenia macrospiculata* Gohar, 1940. I. Annual dynamics of gonadal development. Biol Bull 166: 32−43

Benayahu Y, Weil D, Kleinman M (1990) Radiation of broadcasting and brooding patterns in coral reef alcyonanceans. In: Hoshi M, Yamashita O (eds) Advances in invertebrate reproduction 5. Elsevier, Amsterdam, p 323−328

Brazeau D.A., Lasker H.R. (1989) The reproductive cycle and spawning in a Caribbean gorgonian. Biol Bull 176:1−7 (Woods Hole)

Brazeau, D.A., Lasker, H. R., & Winget, D. M. (1998). Sexual and asexual reproduction in a gorgonian coral: implications for clonal persistence and evolutionary potential. Marine Ecology Progress Series, 169, 97-108.

Chui P.Y., Wong M.C., Liu S.H., Lee K.W., Chan S.W., Lau P.L., Leung S.M., Ang P.O. (2014). Gametogenesis, embryogenesis and fertilization ecology of Platygyra acuta in marginal non-reefal coral communities in Hong Kong. Journal of Marine Science. Article ID 953587.

Coma R., Ribes M., Zabala M., Gili J.M. (1995) Reproduction and cycle of gonadal development in the Mediterranean gorgonian Paramuricea clavata. Mar Ecol Prog Ser 117:173–183

Dahan M., Benayahu Y. (1997). Reproduction of Dendronephythya hemprichi (Cnidaria: Octocorallia): Year-round spawning in an azooxanthellate soft coral. Mar Biol 129: 573−579

Dai, C.F., Lin, M.C. (1993). The effects of flow on feeding of three gorgonians from southern Taiwan. J. Exp. Mar. Biol. Ecol. 173, 57–69.

Eckelbarger KJ, Tyler PA, Langton RW (1998). Gonadal morphology and gametogenesis in the sea pen *Pennatula aculeata* (Anthozoa: Pennatulacea) from the Gulf of Maine. Mar Biol 132:677−690

Environmental Protection Interactive Centre (2024). *Marine Water Quality Data* [Data set]. Environmental Protection Department. https://cd.epic.epd.gov.hk/EPICRIVER/marine/download/

Environmental Resources Management (ERM) (2014). *Detailed coral translocation report: Tuen Mun - Chek Lap Kok Link Northern Connection Sub-sea Tunnel Section (Contract No. HY/2012/08).* https://www.hzmbenpo.com/EMnA_Report/TMCLKL_HY201208/coral_detailed/pdf/detailed_coral_translocation_report_northern_v0_20140226.pdf

Fabricius, K. E., Alderslade, P., & Williams, G. A. (2008). Gorgonian corals (Order Gorgonacea) in the Great Barrier Reef Marine Park: A review of their diversity and ecology. Cairns, Australia: Great Barrier Reef Marine Park Authority.

Fabricius, K.E., Benayahu, Y., Genin, A., 1995a. Herbivory in asymbiotic soft corals. Science 268, 90–92.

Fabricius, K.E., Dommisse, M., 2000. Depletion of suspended particulate matter over coastal reef communities dominated by zooxanthellate soft corals. Mar. Ecol. Progr. Ser. 196, 157–167.

Fabricius, K.E., Genin, A., Benayahu, Y., 1995b. Flow-dependent herbivory and growth in zooxanthellae-free soft corals. Limnol. Oceanogr. 40, 12901301.

Fan T.Y., Chou Y.H., Dai C.F. (2005) Sexual reproduction of the alcyonacean coral *Lobophytum pauciflorum* in southern Taiwan. Bull Mar Sci 76:143−154

Guest, J. R., Baird, A. H., Clifton, K. E., & Heyward, A. J. (2008). From molecules to moonbeams: Spawning synchrony in coral reef organisms. *Invertebrate Reproduction & Development*, *51*(3), 145–149. https://doi.org/10.1080/07924259.2008.9652264

Harrison P.L. (2011) Sexual reproduction of scleractinian corals. In: Dubinsky Z, Stambler N (eds) Coral reefs: An ecosystem in transition. Springer, Dordrecht, p 59−85

Kahng, S. E., Benayahu, Y., Lasker, H.R. (2011). Sexual reproduction in octocorals. *Marine Ecology Progress Series* 443: 265-283.

Kahng S.E., Benayahu Y., Wagner D., Rothe N. (2008) Sexual reproduction in the invasive octocoral, *Carijoa riisei* (Duchassaing & Michelotti, 1860), in Hawaii. Bull Mar Sci 82:1−17

Liberman R., Fine, M., & Benayahu, Y. (2021). Simulated climate change scenarios impact the reproduction and early life stages of a soft coral. *Marine Environmental Research*, *163*, 105215. https://doi.org/10.1016/j.marenvres.2020.105215

Morton, B., Wu, S.S. (1975). The hydrology of the coastal waters of Hong Kong. Environ. Res. 10, 319–347.

Parker, N.R., Mladenov, P.V., Grange, K.R. (1997). Reproductive Biology of the Antipatharian Black Coral *Antipathes fiordensis* in Doubtful Sound, Fiordland, New Zealand. *Marine Biology* 130:11-22.

Patterson, M.R. (1991). The effects of flow on polyp-level prey capture in an octocoral, *Alcyonium siderium*. Biol. Bull. 180, 93–102.

R Core Team (2021). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/

Tanner, J. E. (1996). Seasonality and lunar periodicity in the reproduction of Pocilloporid corals. *Coral Reefs* 15.1: 59–66.

Teixeira, T., Diniz, M., Calado, R., & Rosa, R. (2013). Coral physiological adaptations to air exposure: Heat shock and oxidative stress responses in *Veretillum Cynomorium*. *Journal of Experimental Marine Biology and Ecology*, *439*, 35–41. https://doi.org/10.1016/j.jembe.2012.10.010

Tong Y.Y. (2023). Environmental Tolerance of Octocoral *Guaiagorgia* in Hong Kong. Undergraduate Final Year Project, The Chinese University of Hong Kong.

Wallace C.C. (1985). Reproduction, recruitment and fragmentation in nine sympatric species of the coral genus *Acropora*. Marine Biology 88:217-233.

Yeung, C. W., Cheang, C. C., Lee, M. W., Fung, H. L., Chow, W. K., & Ang, P.O. (2014). Environmental variabilities and the distribution of octocorals and black corals in Hong Kong. *Marine Pollution Bulletin* 85: 774-782.

Yoshioka B.B. (1979). Aspects of the ecology of *Pseudopterogorgia americana* and *Pseudopterogorgia acerosa*. MSc thesis, University of Puerto Rico, Mayaguez

Yoshioka P.M. (1994). Size specific life history pattern of a shallow-water gorgonian. J Exp Mar Biol Ecol 184:111–122

\

5. Education Outreach Materials

Case study

A case study with graphic illustrations has been produced as education materials for secondary school students, with aims to introduce the objectives and present key findings of this present study. This case study will be released to the PI's education outreach programme secondary school network after the manuscript has been published in international referred journal.

案例分析 - 八放珊瑚的繁殖

Text/ Alice Lau

2014年的一份報告顯示，在香港國際機場的海堤上，生長了超過6000棵柳珊瑚 *Guaiagorgia sp.*。柳珊瑚是一種八放珊瑚，每個珊瑚蟲個體擁有八隻觸手。要知道，八放珊瑚的生長速度一般較緩慢，但單單在香港國際機場成立的二十年間，便長成如此龐大的群落，可謂非常驚人。那麼，是什麼原因使得 *Guaiagorgia sp.* 生長得如此蓬勃？科學家希望從牠的繁殖方式中找到線索。

不同品種的珊瑚的繁殖方法都不盡同。每個珊瑚品種的性別可以分為兩類，分別是雌雄異體和雌雄同體。在雌雄同體的品種中，成熟的珊瑚蟲體內，同時有雄性及雌性生殖腺，可以同時製造精子及卵子。而雌雄異體，則是珊瑚群體分為雄性或雌性，同一個群體的珊瑚蟲只有雄性或雌性的生殖腺，分別製造精子或卵子。

而珊瑚孕育生命的方法亦可以分為兩種，分別是排卵型和孵育型。排卵型珊瑚會同步將精子和卵子排出體外，精子和卵子會在海中受精并發育成幼蟲。而孵育型珊瑚的卵子會在珊瑚體內授精，受精卵會被留在體內或體表發育，直到長成幼蟲才釋放出去。

但是，我們無法單靠外表來分辨珊瑚的性別及生育方式，而需要使用組織學技術，分析珊瑚體內的生殖腺。組織學亦能揭曉珊瑚的繁殖時間和間隔。

研究人員將每個月收集到的珊瑚樣本切片及染色，以便用顯微鏡辨認出珊瑚蟲體內的生殖腺，然後分析生殖腺的每月大小變化。珊瑚的卵子一般會用數月時間製造及成熟。因此，科學家可以透過觀察珊瑚每個月卵子的成熟程度，推斷出珊瑚產卵日子。當卵母細胞轉趨成熟并於下個月突然消失時，該月便是珊瑚的「預產期」。

組織學的結果顯示，*Guaiagorgia sp.* 是雌雄異體的品種，大概率會於夏末時生產。以往的假設認為，由於 *Guaiagorgia sp.* 的繁殖成功率機相對較高，他們採用孵育型策略繁殖的機會較大。但是，組織學切片上面并沒有幼蟲的蹤影。為了進一步揭開 *Guaiagorgia sp.* 的生育秘密，研究人員在實驗室設立收集儀，希望收集到珊瑚的精子、卵子或幼蟲，從而證實他們的繁殖時間和策略。

Coral academy

Coral academy

1mm

於2023年夏天，我們的收集儀成功收集到 *Guaiagorgia sp.* 的卵子，其中一些卵子甚至發育中。收集到卵子，代表 *Guaiagorgia sp.* 屬於會將精子和卵子排出體外的排卵型珊瑚。

這些研究結果深化了我們對於 *Guaiagorgia sp.* 特性的了解。科學家們可以利用這些關於 *Guaiagorgia sp.* 繁殖的基礎資訊，進一步揭開更多他們生長的奧秘，同時也能幫助制定更具體的保育和修復計劃。柳珊瑚及其他八放珊瑚正面臨著來自水質污染和全球暖化等各種不同的威脅。在環境快速變化的情況下，珊瑚是否能夠成功地繁殖下一代，將對海洋生態系統產生深遠的影響。

Social media posts

A total of five social media posts have been posted in Coral Academy webpage (https://www.coralacademy.hk/guaiagorgia), Facebook, and IG during the project period.

Post 1 –【 #珊瑚學院研究室 - 如何分辨八放珊瑚品種？】		Media reach
目前為止，香港已記錄嘅八放珊瑚一共有 67 個品種。究竟科學家係點分唔同品種嘅八放珊瑚嘅呢？ 仔細啲睇，每個品種都細節多多！因此，科學家可以透過分析同比較呢啲特徵, 將珊瑚分類及命名。係海底入面，我哋可以睇到唔同八放珊瑚品種嘅群體同珊瑚蟲嘅特徵嘅唔同。但要比較骨針的話，就需要係實驗室用特定嘅儀器觀察。 以我哋研究嘅*Guaiagorgia* sp. 為例，佢哋嘅珊瑚蟲係伸展嘅時候係呈長管狀，可以完全收縮入共肉組織。成個群體咁睇，佢哋嘅分枝比其他八放珊瑚零散同雜亂，枝條亦較幼而長。至於骨針，佢哋嘅骨針較小，一般少於 0.3mm。佢哋表層骨針嘅形狀為多刺嘅紡綞形；珊瑚蟲入面嘅骨針就呈棒狀，表面有突起物。而隨著分子生物技術越來越進步，科學家亦會比較基因組 DNA 及粒線體 DNA 的序列，為八放珊瑚繪製親緣關係樹，進行品種鑑定及調整分類。 FB: https://www.facebook.com/share/p/1D4J3scBk8/?mibextid=wwXIfr		1899

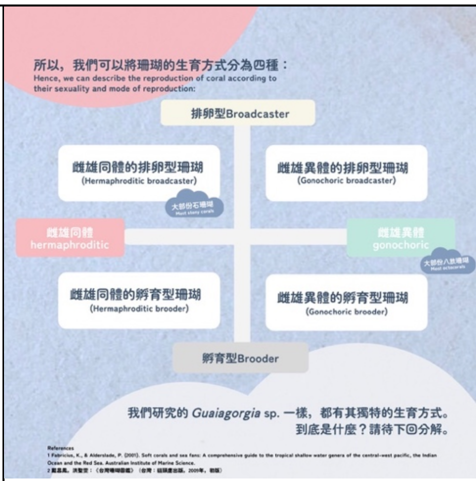
<table>
<tr>
<td>IG: https://www.instagram.com/p/C4YGV1-x9vC/?igsh=N3ZkbzltbGhtbm1j</td>
<td>
</td>
<td></td>
</tr>
<tr>
<td colspan="2">Post 2 – 【 #珊瑚學院研究室 - Guaiagorgia如何繁殖？】</td>
<td>Media reach</td>
</tr>
<tr>
<td>珊瑚既然係動物，究竟佢哋係點生育下一代架呢？

珊瑚嘅性別可以分兩種，有啲品種嘅珊瑚係雌雄同體，有啲則係雌雄異體。珊瑚亦有兩種生殖模式，分別係排卵型同孵育型。唔同品種嘅珊瑚係會採取唔同嘅生育方式架！
但值得留意嘅係，我哋無辦法從珊瑚嘅外表判斷佢哋嘅性別，需要用到織學切片，睇到佢哋嘅生殖腺先決定到佢哋嘅性別。

咁我哋研究嘅Guaiagorgia sp. 又係點繁殖呢？敬請留意下一集！

FB: https://www.facebook.com/share/p/18HTt1rqcT/?mibextid=wwXIfr
IG: https://www.instagram.com/p/C4ncPhfI3Jf/?igsh=Z3hkZG84dGFmZHBm</td>
<td>


</td>
<td>1418</td>
</tr>
</table>

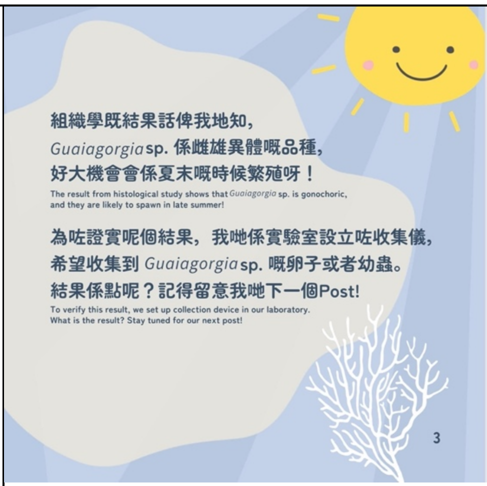
<table>
<tr><td></td><td>
</td><td></td></tr>
<tr><td colspan="2">Post 3 –【 #珊瑚學院研究室 - Guaiagorgia如何繁殖？（中）】</td><td>Media reach</td></tr>
<tr><td>除咗性別同生殖模式之外，科學家仲想知道珊瑚產卵嘅時間同頻率 ，呢個時候就要用到組織學嚟研究珊瑚嘅生殖部分。組織學切片結果話俾我哋知，Guaiagorgia sp. 係雌雄異體嘅品種，而且好大機會係夏末產卵 。

而為咗進一步證實Guaiagorgia sp. 嘅生殖方式，研究人員係實驗室設立咗收集儀，希望收集到Guaiagorgia sp. 嘅卵子或者幼蟲 。

究竟我哋嘅收集儀能唔能夠成功收集卵子或幼蟲呢？敬請留意下一集！

FB: https://www.facebook.com/share/p/18TZuovJm8/?mibextid=wwXIfr
IG: https://www.instagram.com/p/C5SZCyIPo3L/?igsh=a3premloY2FnZnJ4</td><td>


</td><td>1261</td></tr>
</table>

<table>
<tr>
<td></td>
<td>
</td>
<td></td>
</tr>
<tr>
<td colspan="2">Post 4 – 【 #珊瑚學院研究室 - Guaiagorgia如何繁殖？（下）】</td>
<td>Media reach</td>
</tr>
<tr>
<td>為咗進一步證實Guaiagorgia sp. 嘅生殖方式，研究人員係實驗室設立咗收集儀，希望收集到Guaiagorgia sp. 嘅卵子或者幼蟲 。

係上年夏天，我哋嘅收集儀成功收集到Guaiagorgia sp. 嘅卵子呀！收集到卵子，就代表Guaiagorgia sp. 係屬於排卵型嘅珊瑚。

如是者，我哋探索到Guaiagorgia sp. 嘅性別、生殖模式同產卵時間 —— 佢哋係係雌雄異體嘅排卵型珊瑚，一般係夏末產卵 。希望未來嘅研究可以更深入咁了解唔同因素對Guaiagorgia sp. 繁殖嘅影響。

FB: https://www.facebook.com/share/p/14rpVupzsz/?mibextid=wwXIfr
IG: https://www.instagram.com/p/C5dnyu9Ccas/?igsh=ZjY5Y3p1aGRjMWk4</td>
<td>
</td>
<td>1097</td>
</tr>
</table>

Post 5 –【 #珊瑚學院研究室 - *Guaiagorgia*繁殖調查實錄】 Facebook link: FB: https://www.facebook.com/share/v/15mns9T48k/?mibextid=wwXIfr IG: https://www.instagram.com/reel/C7Td8ajPK-V/?igsh=NmRjM21xb28wMzk5		2303
Total number of people who see the content via social media posts		7978

(v) Evaluation of the project effectiveness in achieving the proposed objectives as well as the impact (benefits) of the Project:

Objective 1*: To investigate the population size structure of the gorgonian Guaiagorgia sp. in Hong Kong western waters*

The proposed study on population size structure of the species were conducted and discussed in the previous sessions.

Objective 2*: To fill in the knowledge gap of the existing pattern of gametogenesis and to verify the reproduction cycle of Guaiagorgia sp. in Hong Kong.*

The proposed study on gametogenesis and reproductive cycle of the species were conducted and discussed in the previous sessions.

Objective 3*: To establish a lab-based larvae collection protocol for larvae collection and to initiate attempts for larval culturing.*

The lab-based collection successfully collected egg near full moon in September to confirm the gametogenesis of the gorgonian species. However, the number of fertilised and developing eggs was low and none of these eggs grew into larvae. It is suspected that the number of male colonies in the aquaria is insufficient, and the ex-situ environment was not resembling the in-situ environment, causing stress to the egg.

(vi) Summary and Way Forward:

The overall results from this study suggest that *Guaiagorgia* sp. is gonochoric and exhibits asynchronous gametogenesis, with oogenesis starting in June and reaching maximum size and maturity in September. The presence of spermaries in August, September, and November suggests that the species is most likely to spawn in September, and it is confirmed by the successful egg collection. Nonetheless, our study contributes to the understanding of the reproductive biology and population dynamics of this ecologically important foundation species in the local ecosystem. These findings can inform future restoration projects and raise public awareness of the importance of conserving and protecting this rare and valuable species in Hong Kong waters.

Further studies are needed to evaluate the physiological trigger for spawning of Guaiagorgia sp. under additional scenarios of climate change conditions, including the interactive effects of salinity and temperature and factors that limit its distribution beyond western Hong Kong waters.

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.

____

Project leader
Apple PY Chui
Research Assistant Professor,
School of Life Sciences,
The Chinese University of Hong Kong

(vii) Financial statement

Financial statement is not disclosed due to confidentiality reasons.

(viii) Staff Attendance record

Staff attendance record is not disclosed due to confidentiality reasons.

(ix) Recruitment record

Staff recruitment record is not disclosed due to confidentiality reasons