Completion report

Project number: MEEF2021010
Project title: Environmental Tolerance of Octocoral *Guaiagorgia* in Hong Kong
Recipient organization: The Chinese University of Hong Kong, School of Life Sciences
Reporting Period: 1 July 2021 – 31 December 2022

<u>(i) Executive Summary (1-2 pages):</u>

Since the last decade, there has been an increased awareness of anthropogenetic pressures on octocoral (including soft coral and gorgonian) populations in Hong Kong, such as coastal development and the abandoned fishing nets that entangled the octocorals. In 2016, with the commencement of construction of the Hong Kong International Airport (HKIA) Third Runway, around 3300 gorgonian *Guaiagorgia* sp. colonies were estimated to have been affected due to reclamation. *Guaiagorgia* sp. is an uncommon gorgonian species with slow growth rate. It is also the only gorgonian species found in western waters of Hong Kong and not anywhere else within Hong Kong. Gorgonian assemblages have a fundamental ecological role as a habitat that provides a complex three-dimensional structure to support high biodiversity. Hence, there is an urgent need for the protection and preservation of these gorgonian populations in Hong Kong through the implementation of restoration strategies and management measures.

While study has recently confirmed it as a gonochoric broadcast spawner, however, the response of this gorgonian species to changes in environmental parameters is virtually unknown. Hong Kong western water is brackish with large fluctuation in salinity due to the influence of Pearl River outflow. Hong Kong marine environment in general experiences large fluctuation in water temperature throughout the year as well. To fill our knowledge gap on our understanding on the tolerances of *Guaiagorgia* sp. in Hong Kong to environmental conditions, laboratory experiments were conducted to identify the salinity and thermotolerance thresholds of this species. The information from this study may provide answers to explain the dominance of *Guaiagorgia* sp. in Hong Kong western water and could further help in the design of its conservation and protection plan.

Salinity threshold experiment was carried out in October 2022. Our results demonstrated that long-term exposure to 8 psu appears to be lethal for *Guaiagorgia* sp.. *Guaiagorgia* sp. was found to perform best under the salinity range of 20-32 psu with a significantly higher buoyant weight change. The best-performing salinity range is wide which matches the range of fluctuating salinity in its natural habitat in western Hong Kong. This result also suggested that *Guaiagorgia* sp. distribution is not limited to low-salinity seawater. It should also have the ability to grow in eastern and northern waters with more oceanic 32-33 psu mean salinity. This led to a new hypothesis that the *Guaiagorgia* sp. distribution in Hong Kong is limited by other factors, but not salinity as previously proposed. Insufficient food availability in eastern and northern waters, and geographical isolation due to ocean current might be some of the factors that contributed to its very localized distribution in western Hong Kong. All these need further studies to confirm.

Temperature threshold experiments were carried out in March - April and July - August 2022. The study shows that *Guaiagorgia* sp. performed best in lower temperature range of 12-24°C, but can

also tolerate higher temperatures below 33°C for up to 28 days of exposure. All branches in the lower-temperature threshold experiment showed positive weight growth, while in the upper-temperature threshold experiment, the branches experienced negative growth (i.e. die-back) at 27°C or above. Long exposure to 33 °C appears to be lethal for *Guaiagorgia* sp.. In general, exposure to elevated temperature resulted in an increase in tissue necrosis and a decrease in both polyp activity and skeletal growth rates. This indicate that *Guaiagorgia* sp. is likely to grow better in winter and become stressed in summer.

All these information will form the baseline for further evaluation of the potential adaptation of this species under the projected global climate change scenarios. These information are also essential in the design of an adaptive strategic plan for the conservation and protection of this octocoral species.

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

Environmental Tolerance of Octocoral *Guaiagorgia* in Hong Kong

Octocoral assemblages have a fundamental ecological role as a habitat that provides a complex three-dimensional structure to support high biodiversity. The octocoral *Guaiagorgia* sp. is found abundantly in Hong Kong western waters but not elsewhere in other parts of Hong Kong marine environment. While study has recently confirmed it as a gonochoric broadcast spawner, however, the response of this gorgonian species to changes in environmental parameters is virtually unknown. Hong Kong western water is brackish with large fluctuation in salinity due to the influence of Pearl River outflow. Hong Kong marine environment in general experiences large fluctuation in water temperature throughout the year as well. Evaluation of how *Guaiagorgia* sp. responds to changes in salinities and temperatures may therefore provide insights to explain its success in Hong Kong western waters. This information is essential in the design of strategic plan for its conservation and protection. This proposed study, therefore, focused mainly on: 1) investigating the biological responses (including linear growth, skeletal growth rate, polyp activity and tissue necrosis) of the gorgonian Guaiagorgia sp. to hyposalinity and changes in seawater temperature; and 2) identifying salinity and temperature thresholds of *Guaiagorgia* sp..

(iii) Completed activities against the proposed Work Schedule:

With the extension of the project period being granted on 20 Jun 2022, the *original work schedule* below is updated according to the project period extension application document submitted on 4 April 2022.

Original Work Schedule	Actual Work Schedule	Proposed Work description
Jul 2021	Jul 2021	Recruitment of RAs (research assistant I)
Jul 2021	Jul 2021	Recruitment of student RAs
Jul 2021	Jul 2021– Jan 2022	Preparation for the field trips, lab culture facilities setup and maintenance
Jul 2021	Feb 2022	1st sampling for Octocoral *Guaiagorgia*
Aug-Oct 2021	Feb - Mar 2022	Acclimation period of the corals in laboratory facilities

Feb – Mar 2022	Mar - Apr 2022	Laboratory experiment on corals (**Experiment 2b** Lower temperature threshold of *Guaiagorgia* sp.)
Apr 2022	May 2022	2nd sampling for Octocoral *Guaiagorgia*
May 2022	May - Jun 2022	Acclimation period of the corals in laboratory facilities
Aug – Sep 2022	Jul – Aug 2022	Laboratory experiment on corals (**Experiment 2a** Upper temperature threshold of *Guaiagorgia* sp.)
/	Jul 2022	3rd sampling for Octocoral *Guaiagorgia*
/	Jul – Aug 2022	Acclimation period of the corals in laboratory facilities
Jun – Jul 2022	Sep – Oct 2022	Laboratory experiment on corals (**Experiment 1** Response of *Guaiagorgia* sp. to a range of salinities)
Mar – Oct 2022	Mar – Dec 2022	Education outreach materials preparation (case study writing and education video production)
Nov – Dec 2022	Nov – Dec 2022	Data analysis; 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 completed from **1 Jul 2021 to 31 Dec 2022**:

1. *The setup and routine maintenance of two temperature controlled self-circulating nursery tanks for keeping the* Guaiagorgia *samples during acclimation period and post-experiment recovery (**Fig. 1**).*

   The nursery tanks were properly aerated, coral branches were fed every morning with a combination of natural food (*Artemia*) and a gorgonian powder food mixture throughout and after the project period. These branches grown in the nursery could provide source materials for other future studies as well as restoration exercises in Hong Kong.

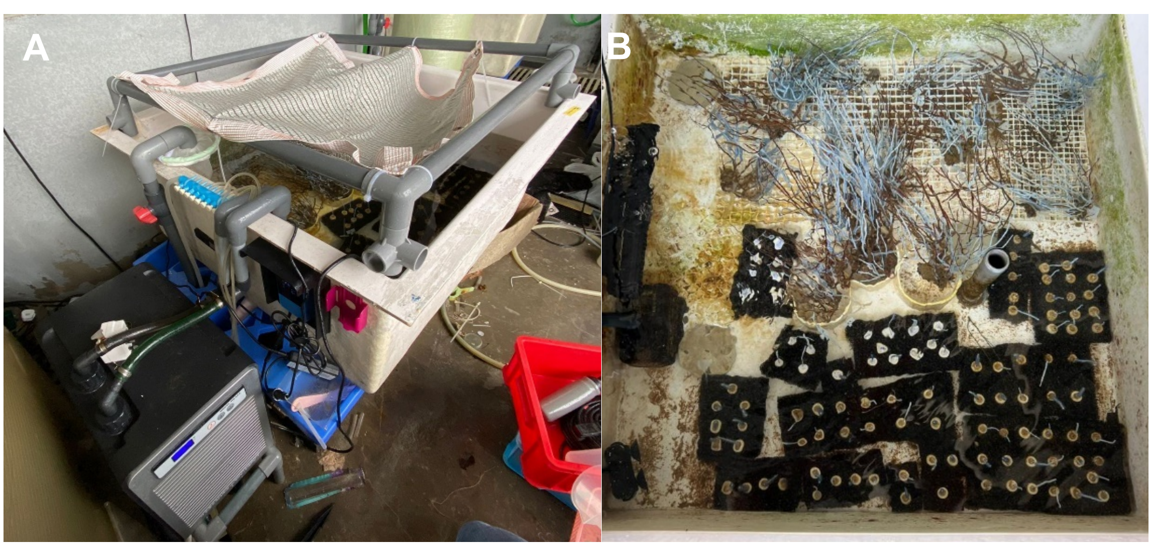


Figure 1 A. Overview of the temperature controlled self-circulating nursery tank setting, with heater and chiller connected to a temperature controller to maintain a constant temperature. Garden shade cloth added

to prevent excessive irradiance as *Guaiagorgia* grows generally in turbid western HK waters. **B.** Top view of the nursery tank showing individual colonies of *Guaiagorgia* in upper part and blocks with individual branches of *Guaiagorgia* ready for use in experiment in lower part of the tank.

2. *Laboratory experiment on corals*

2.1 Methodology

Experiments were carried out with specimens collected in Ma Wan, western Hong Kong. The *Guaiagorgia* population was mainly located in shallow waters of 3-6 m in depth. Branch tips of around 6 cm in length were randomly cut from 20 healthy *Guaiagorgia* colonies in each sample collection. Each branch was mounted on a bio-ring with the base wrapped in filter cloth (**Fig. 2A**).

These coral branches were then kept at 27°C (for experiment 1 *Response of* Guaiagorgia *sp. to a range of salinities* and 2a *Upper temperature threshold of* Guaiagorgia *sp.*) or 21°C (for experiment 2b *Lower temperature threshold of* Guaiagorgia *sp*.) in a temperature controlled self-circulating nursery tank at the Simon F. S. Li Marine Science Laboratory, The Chinese University of Hong Kong (MSL CUHK), for a 3-week acclimation period before each set of experiments.

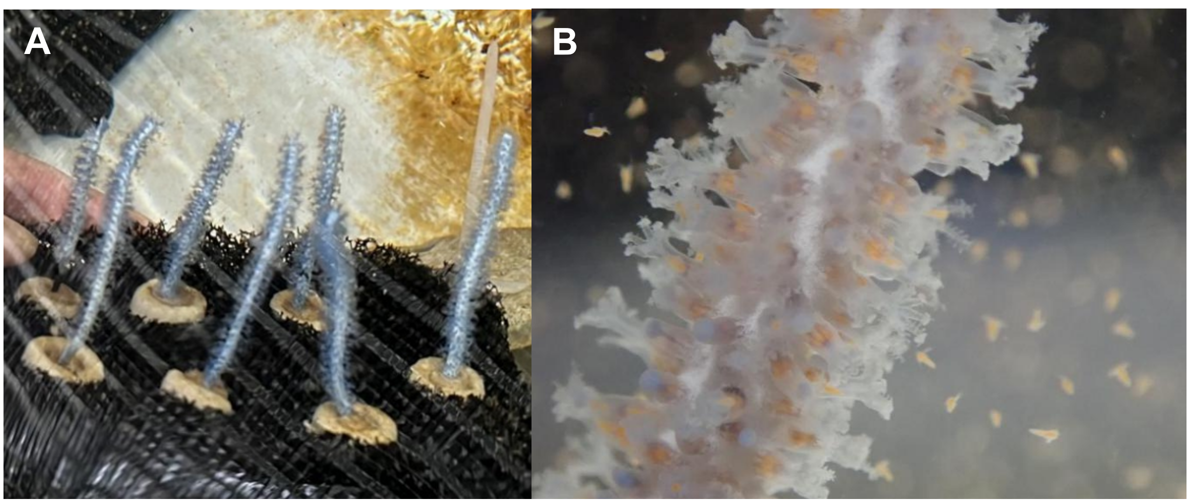


Figure 2 A. Individual branches of *Guaiagorgia* mounted on a bio-ring with the base wrapped in filter cloth. **B.** Close up photo of *Guaiagorgia* with polyps fully extended during feeding.

2.1.1 Experiment 1: Response of *Guaiagorgia* sp. to a range of salinities

Individual water tanks were used in this study for eight salinity treatments (8, 12, 16, 20, 24, 28, control 32, 36 psu). After acclimation, 72 healthy *Guaiagorgia* sp. branches were randomly distributed into individual control and treatments aquaria with 1.2L of 0.45 μm filtered seawater (each with 9 replicates). Aquaria were placed in water baths regulated with chiller and heater to minimize daily temperature fluctuation. A constant temperature of 27°C (± 1°C) was set to mimic the *in situ* environmental temperature. At the start, all aquaria were at 31.5 psu salinity. Salinity of treatment aquaria was then gradually decreased or increased to the desired salinities at 1 psu per hour. Milli-Q water was used to dilute the filtered seawater. Artificial seawater was used to increase the salinity. Once the desired salinity had been reached, the conditions were kept constant for 4 weeks (experiment period) or until 95% of the branches were affected by tissue necrosis. Salinity was checked every day using a salinity tester (Marine Salinity Tester HANNA Instruments, USA). Each aquarium was properly aerated, branches were fed every morning for 2 hours with a combination of freshly hatched *Artemia* (natural food) in concentration of about 50 nauplii per ml and a gorgonian powder food mixture throughout the experiment (**Fig. 2B**). About 50% of seawater in the aquaria was replaced every day with 0.45 µm filtered seawater pre-adjusted to respective salinity. All aquaria were cleaned regularly to minimize algal growth on the walls. After the experiment, the salinity of treatment aquaria was gradually changed back to ambient 32-33 psu for recovery.

2.1.2 Experiment 2a: Upper temperature threshold of *Guaiagorgia* sp.

Individual water tanks were used in this study for five temperature treatments (21,24, control 27, 30, 33°C). After acclimation, 50 healthy *Guaiagorgia* sp. branches were randomly distributed into individual control and treatments aquaria with 1.2L of 0.45 µm filtered seawater seawater (each with 10 replicates). At the start, all water baths were set at 27°C control temperature. Temperature of treatment water baths was then gradually decreased to the desired temperatures at 0.5°C per 12 hours. Once the desired temperatures had been reached, the conditions were kept constant for 4 weeks (experiment period) or until 95% of the branches were affected by tissue necrosis. Salinity was checked every day using a salinity tester (Marine Salinity Tester HANNA Instruments, USA). Salinity values ranged from 29-31 psu during the experiment period. The salinity range was slightly lower than 32-33 psu, the average salinity range in driest months (December-January) in western Hong Kong recorded in water quality monitoring stations NM1-6 by the Hong Kong Environmental Protection Department from 1999-2019 (http://epic.epd.gov.hk/ EPICRIVER/marine/?lang=en). About 50% of seawater in the aquaria was replaced every day with fresh 0.45 µm filtered seawater pre-adjusted to respective temperatures. Each aquarium was properly aerated and the branches were fed as described under Experiment 1.

2.1.3 Experiment 2b: Lower temperature threshold of *Guaiagorgia* sp.

The experiment set-ups and procedures were similar to those described under Experiment 2a except that four temperature treatments (12, 15, 18, control 21°C) were used.

2.1.4 Response variables

Time series measurements were carried out on gorgonian branches throughout the experiments. Polyp activities and sign of necrosis were assessed daily up to the end of the experiment (four weeks) or until tissue necrosis was over 95%. Linear growth was measured once a week. Skeletal growth was measured at the beginning and at the end of the experiment on branches that did not show signs of necrosis. Details of these measurements are given below.

Polyp Activity and Tissue Necrosis

The methodology for evaluating polyp activity and tissue necrosis followed those described in Torrents et al. (2008) and Previati et al. (2010). Polyp activity illustrates the ability of coral to capture food. A high polyp activity indicates that the coral is actively consuming food and gaining energy. Polyp activity was estimated daily after feeding through photography taken throughout the duration of the experiment. Three different states of polyp expansion were recorded: fully expanded polyps, semi-expanded polyps and retracted polyps. The polyp activity (fully (1) /semi-expanded (0.5) / retracted (0) polyps, **Fig. 3**) was then calculated using the formula below:

$$\frac{[(\text{state 1 polyps x 1}) + (\text{state 2 x 0.5})]}{\text{total number of polyps}} \times 100\%.$$

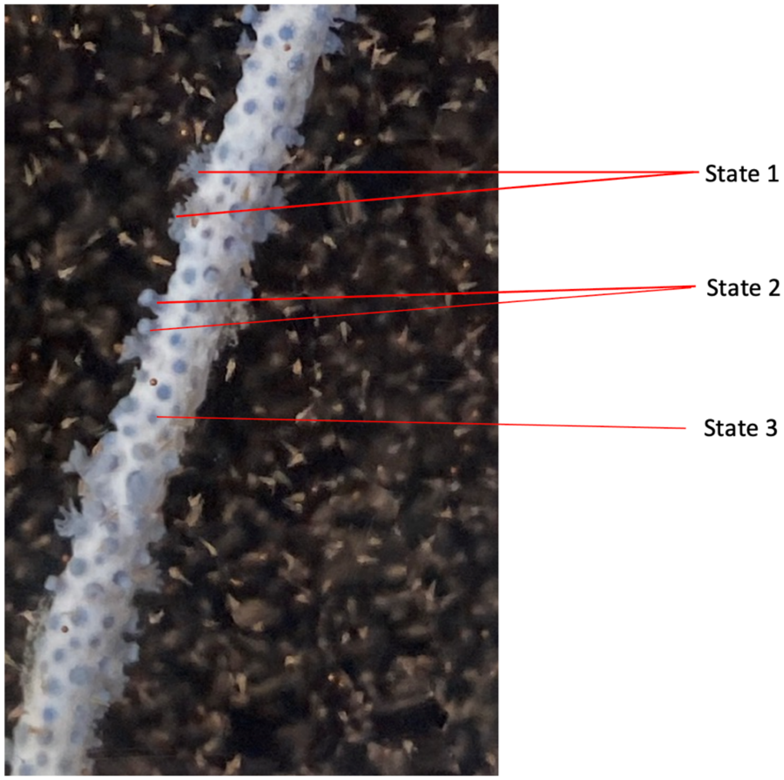


Figure 3. Close up photo of *Guaiagorgia* with polyps showing three states of polyp activities. Stage 1: fully expanded, Stage 2: semi-expanded and, Stage 3: retracted polyp during feeding.

Tissue necrosis represents the damage that is caused by stress. Tissue necrosis was visually monitored daily and assessed as: (1) the change of the coenenchyma colour from clear blue to greyish or whitish; (2) the appearance of denuded axis. When necrosis was observed, photos were taken and the images were analysed using the image analysis program Image-Pro Plus 6.0 (Media Cybernetics, Inc., USA) to measure the surface area of necrosis. The percentage of tissue necrosis was calculated as the proportion of dead tissue over branch length.

Linear Growth

Linear extension was employed to estimate the growth of all the gorgonian branches. In the beginning and weekly thereafter during the experiment, each branch was photographed individually with a scale using a camera. Images were then analysed using the image analysis program Image-Pro Plus 6.0 (Media Cybernetics, Inc., USA) to calculate changes in individual branch length between two time periods to estimate linear extension.

Skeletal Growth

The buoyant weighing technique was employed to estimate the relative change in net skeletal growth in each of the gorgonian branches. This is a technique widely used for determining calcification (skeletal weight) of coral fragments as a result of $CaCO_3$ deposition (Davies 1989). The methodology followed that described in Goméz et al. (2008). Although *Guaiagorgia* is not known to deposit $CaCO_3$ extensively, the principle behind this method may still hold in evaluating changes in the skeletal weight between two time points. Buoyant weighing was performed at the beginning and the end of the experimental period. Skeletal growth was calculated with respect to the initial mass of the gorgonian branch normalized over the 4 weeks experimental period and expressed as the relative monthly percent change in buoyant weight.

2.1.5 Data Analysis

All statistical analyses were done using Jamovi version 2.3.18. The data were first tested for normality using Shapiro-Wilk test. One-way analysis of variance (ANOVA) was used to evaluate significant differences ($p < 0.05$) in changes in buoyant weight and linear growth in all experiments. Follow-up post hoc comparisons were applied using a Tukey post hoc test to distinguish significant groupings among treatments if ANOVA results were significant. A two-way repeated measures ANOVA test was used to test tissue necrosis and polyp activity. Bonferroni correction to the p value was used to adjust the probability of type I error in multiple comparisons. All results are given as mean ± standard error (SE).

2.2 Results

Four response variables were evaluated to measure the performance of *Guaiagorgia* under different treatments: Polyp activity, tissue necrosis, skeletal growth, and linear growth. Data were extracted and analyzed, with results shown below. There were 9 replicates for salinity treatments, and 10 replicates for all the temperature treatments.

2.2.1 Experiment 1: Response of *Guaiagorgia* sp. to a range of salinities

2.2.1.1 Tissue necrosis

Percent tissue necrosis under different salinity treatments differed significantly (Repeated measures ANOVA, $p < 0.001$, Table 1A). There were little to no signs of necrosis observed over a broad range of salinity from 12-32 psu treatments, but this increased significantly to almost 100% at 8 psu (**Fig. 4**). In the highest salinity of 36 psu, the mean (±SE) percentage necrosis was 25.95 ± 10.26% which was not significantly different from those of the other treatments. Results suggested that *Guaiagorgia* sp. is adapted to a broad range of salinities from 12-32 psu, and even 36 psu for up to 28 days of exposure.

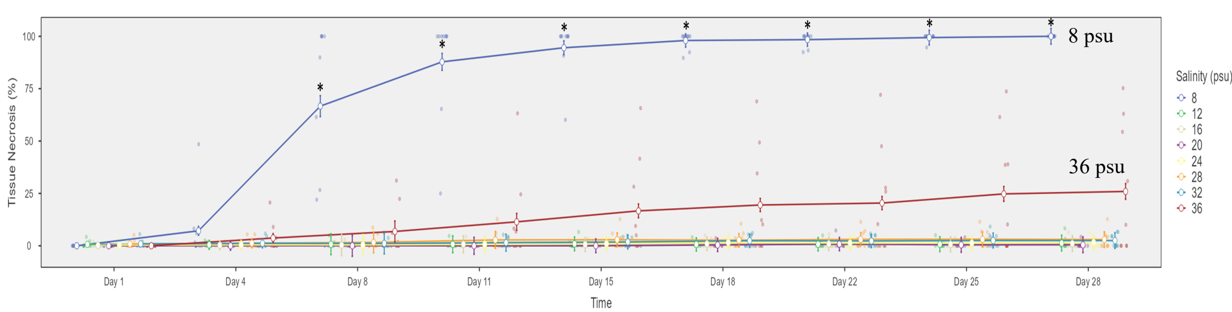


Figure 4. Mean (±SE) percentage (%) of tissue necrosis of *Guaiagorgia* branches under 8, 12, 16, 20, 24, 28, 32, 36 psu salinity treatments over 4 weeks of the experiment period. Significant difference at $p < 0.05$ among treatments at each time point is indicated by an asterisk (*).

2.2.1.2 Polyp activity

Results of repeated measures ANOVA revealed that polyp activity was significantly affected by salinity ($p < 0.005$) and duration of exposure ($p < 0.001$, Table 1A). Polyp activity fluctuated throughout the experiment (**Fig. 5**). Overall, polyp activities in extremely low 8 psu and higher salinity treatments of 32 and 36 psu were lower in general with activities being below 10% in most cases. Polyp activities at 20 psu were significantly higher than those at 8, 32 and 36 psu (Tukey-HSD test, $p < 0.05$, (12=16=20=24=28) > (8=12=16=24=28=32=36 psu)).

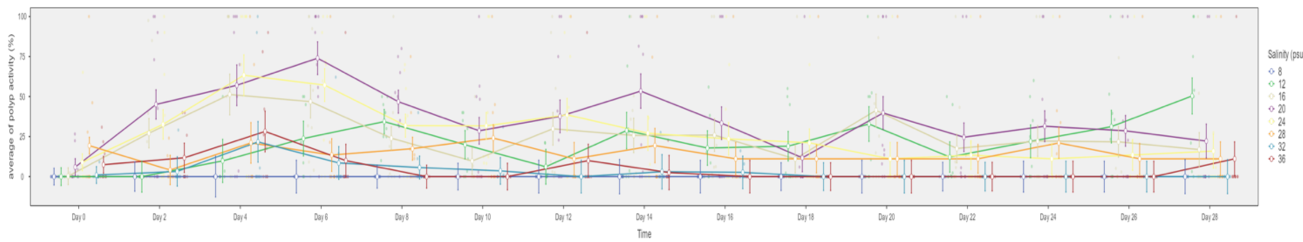


Figure 5. Mean (±SE) polyp activity of *Guaiagorgia* branches under 8, 12, 16, 20, 24, 28, 32, 36 psu salinity treatments over the 4 weeks of experiment period.

2.2.1.3 Skeletal Growth (buoyant weight)

The mean percent changes in buoyant weight for branches of *Guaiagorgia* under different salinity treatments differed significantly (one-way ANOVA, $F_{(7, 27.2)} = 102$, $p < 0.001$), with a net decrease under all treatments. In general, the pattern of the mean (±SE) percent changes in buoyant weight appeared as a parabola with 24 psu treatment showing the least decrease (-12.5 ± 2.40%) and with the decreasing rate increasing towards the two ends, i.e. highest and lowest salinities (**Fig. 6**). Largest mean percentage loss (-76.62 ± 1.70%) was observed under the lowest salinity of 8 psu. This was significantly larger than that of all the other treatments. The change in buoyant weight under 12, 16, and 36 psu also differed significantly from that under 24 psu treatment.

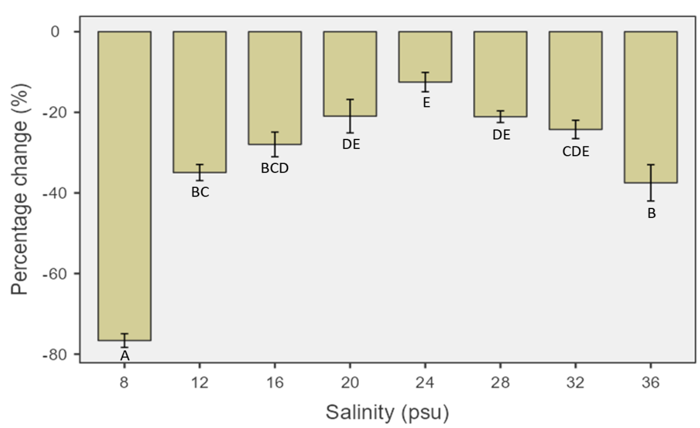


Figure 6. Mean (±SE) percentage change in buoyant weight of *Guaiagorgia* branches under 8, 12, 16, 20, 24, 28, 32, 36 psu salinity treatments over 4 weeks of the experiment period. Significantly different treatment results according to Tukey-HSD post-hoc tests are denoted by different letters.

2.2.1.4 Linear growth

After the 4-week experimental period, the mean percentage changes in linear length of *Guaiagorgia* branches under different salinity treatments differed significantly (one-way ANOVA, $F_{(7, 27.2)} = 5.17$, p <0.001). The overall mean linear growth under 8-36 psu treatments ranged from -4.82 to 2.29% (**Fig. 7**). The largest mean (±SE) percentage reduction appeared at 36 psu with -4.82 ± 0.76% while the highest increase was recorded at 20 psu with 2.29 ± 3.24%. However, except for these two extremes, no significant difference in linear growth was detected among the other treatments.

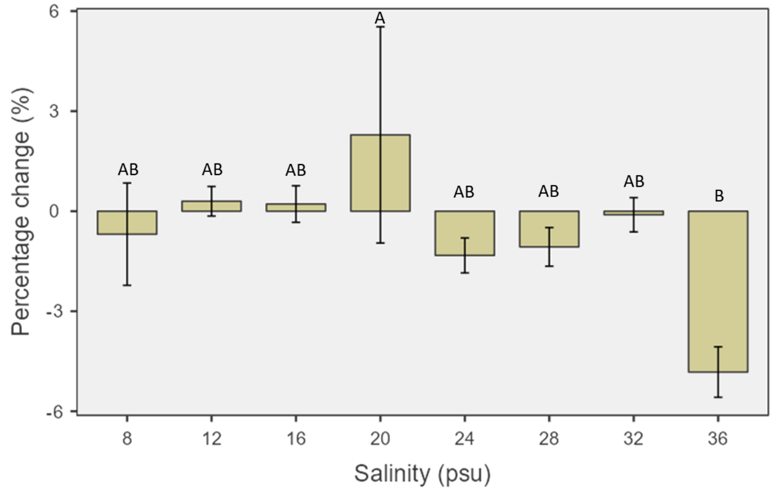


Figure 7. Mean (±SD) percentage change in linear length of *Guaiagorgia* branches under 8, 12, 16, 20, 24, 28, 32, 36 psu salinity treatments over 4 weeks of the experiment period. Significantly different treatment results according to Tukey-HSD post-hoc test are denoted by different letters.

2.2.2 Experiment 2a: Upper temperature threshold of *Guaiagorgia* sp.

2.2.2.1 Tissue necrosis

Percent tissue necrosis under 33°C high temperature treatment differed significantly from the rest of the temperature range from 21°C to 30°C (Repeated measures ANOVA, $p < 0.001$, Table 1B). Under 21 and 24°C treatments, no sign of tissue necrosis was observed with all branches remaining healthy after 4 weeks of experiment (**Fig. 8**). Under 27°C treatment, only 1 out of 10 branches showed minor tissue necrosis of 1.56%. In contrast, under the highest 33°C temperature treatment, six branches (out of ten or 60%) showed severe signs of tissue necrosis of over 95%, with a mean (±SE) of 61.40 ± 15.77%, a significantly higher percentage of tissue necrosis among the five treatments. These results suggest that *Guaiagorgia* sp. is less adaptable to high water temperature.

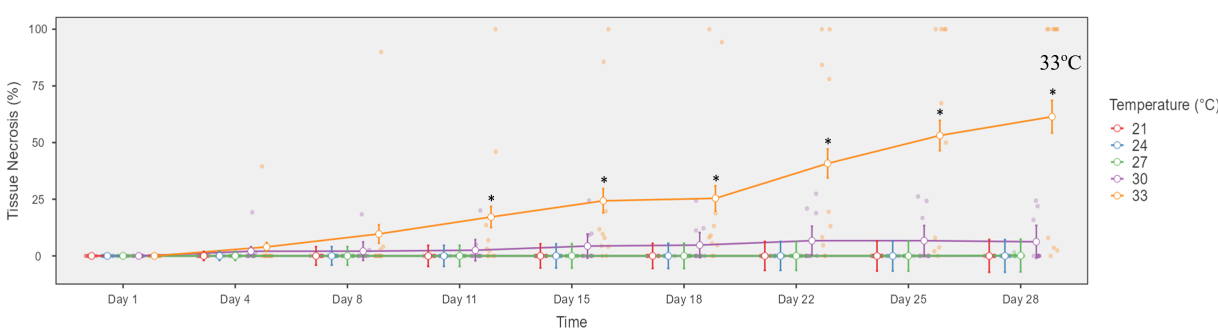


Figure 8. Mean (±SE) percentage (%) of tissue necrosis of *Guaiagorgia* branches under 21, 24, 27, 30, 33°C temperature treatments over 4 weeks of the experiment period. Significant difference at $p < 0.05$ among treatments at each time point is indicated by an asterisk (*).

2.2.2.2 Polyp activity

Results of repeated measures ANOVA revealed that polyp activity was significantly affected by temperature ($p < 0.001$, Table 1B) and duration of exposure ($p < 0.001$). In general, polyp activity was lower under higher temperatures (**Fig. 9**). Polyp activity under the lowest temperature of 21°C was significantly higher than that under 30°C and 33°C treatments. There were no significant differences in polyp activity under 27, 30 and 33 °C treatments. (Tukey-HSD test, $p < 0.05$, (21=24) > (24=27=30) > (27=30=33) °C).

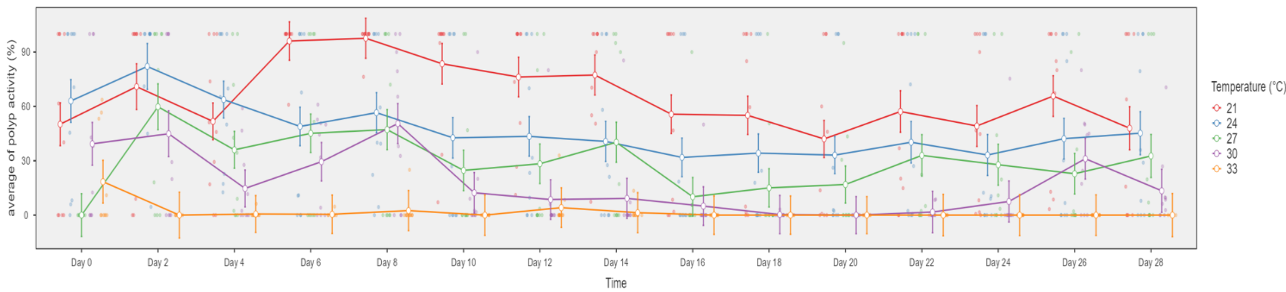


Figure 9. Mean (±SE) polyp activity of *Guaiagorgia* branches under 21, 24, 27, 30, 33°C temperature treatments over 4 weeks of the experiment period.

2.2.2.3 Skeletal growth (buoyant weight)

The mean percent changes in buoyant weight for branches of *Guaiagorgia* under different temperature treatments differed significantly (one-way ANOVA, $F_{(4,20)} = 36.60$, $p < 0.001$), with a net decrease towards elevated temperature treatments (**Fig. 10**). Positive percentage change of buoyant weight was recorded under 21 and 24°C treatments, with a mean (±SE) increase of 8.33± 6.18% and 0.50± 2.53%, respectively. However, treatments under the higher temperatures of 30 and 33°C showed various degrees of percentage loss in buoyant weight, with a mean loss of 39.40 ± 3.92% under 30°C treatment and 72.06% ± 6.79% under 33°C treatment that were significantly higher than those under other treatments.

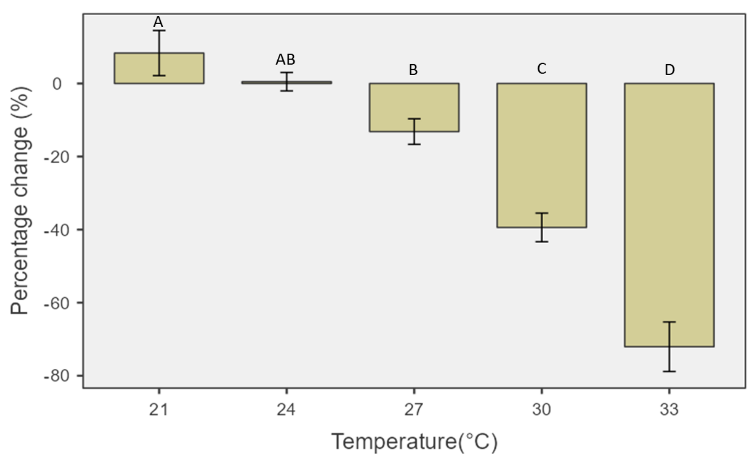


Figure 10. Mean (±SD) percentage change in buoyant weight of *Guaiagorgia* branches under 21, 24, 27, 30, 33°C temperature treatments over 4 weeks of the experiment period. Significantly different treatment results according to Tukey-HSD post-hoc test are denoted by different letters.

2.2.2.4 Linear growth

After the 4-week experimental period, the mean percent changes in linear length of *Guaiagorgia* branches under different temperate treatments differed significantly (one-way ANOVA, $F_{(4, 20.6)} = 6.37$, $p = 0.002$). The overall mean linear growth under all treatments ranged from -1.82 to 5.63 % (**Fig. 11**). Positive percentage linear growth was recorded at 21°C and 24°C treatments, with a high linear growth of up to 30% recorded in two individual branches under 21°C treatments. While 27-33°C treatments showed negative growth, the difference was however not significantly different from those under the other treatments.

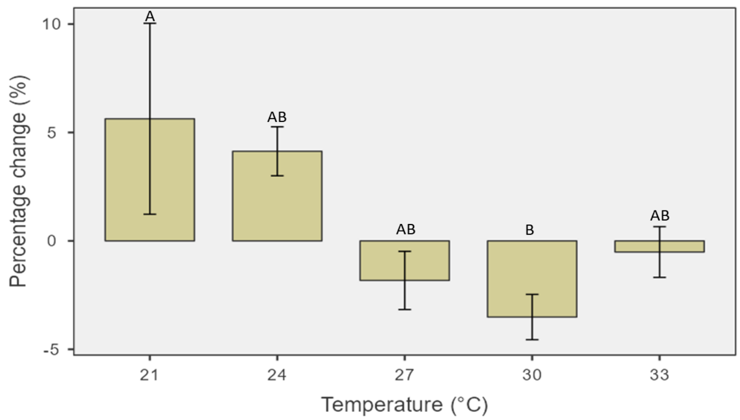


Figure 11. Mean (±SD) percentage change in linear length of *Guaiagorgia* branches under 21, 24, 27, 30, 33°C temperature treatments over the 4 weeks experiment period. Significantly different treatment results according to Tukey-HSD post-hoc test are denoted by different letters.

2.2.3 Experiment 2b: Lower temperature threshold of *Guaiagorgia* sp.

2.2.3.1 Tissue necrosis

No sign of tissue necrosis was observed with all branches remaining healthy after 4 weeks of experiment under lower temperature treatments. This suggests that *Guaiagorgia* sp. is more adapted to cold water temperature.

2.2.3.2 Polyp activity

Results of repeated measures ANOVA revealed that polyp activity was significantly affected by temperature ($p < 0.001$, Table 1C), but not for duration of exposure ($p = 0.066$). Polyp activity fluctuated throughout the experiment (**Fig. 12**). Overall, polyp activity was highest under 12ºC treatment with branches showing >30% of mean polyp activity in most number of days over the 4 weeks of the experimental period. This was significantly higher than polyp activities under 21ºC treatment, where most activities recorded were below 10% (Tukey-HSD test, $p < 0.05$, (12=15=18) > (12=18=21 psu)).

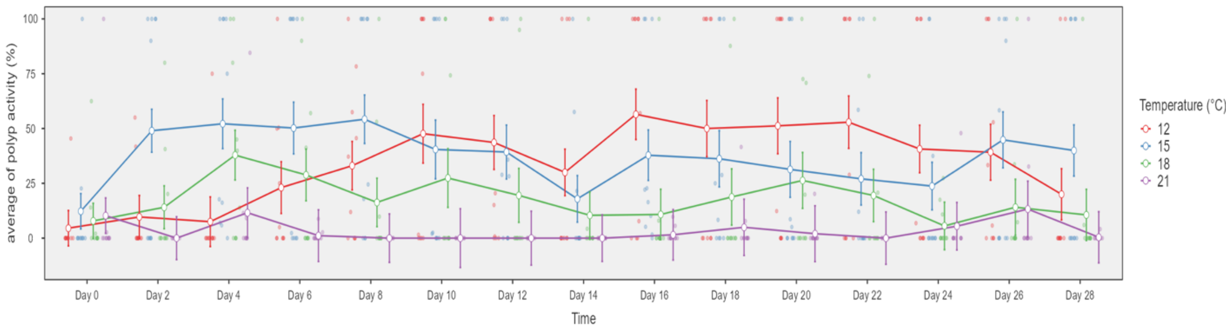


Figure 12. Mean (±SE) polyp activity of *Guaiagorgia* branches under 12, 15, 18, 21°C temperature treatments over 4 weeks of the experiment period.

2.2.3.3 Skeletal growth (buoyant weight)

The mean buoyant weight recorded for branches of *Guaiagorgia* showed a net increase under all temperature treatments (**Fig. 13**). While 12 and 15°C treatments showed higher mean (±SE) percentage growth of 4.53 ± 2.17% and 5.99 ± 3.47%, respectively, these were not significantly different from those of the rest of the treatments (one-way ANOVA, $F_{(3,19.4)}$ =1.43, p =0.265). The ~5% growth at 12°C and 15°C was similar to the ~8% growth recorded at 21°C (the best-performing temperature) from the previous high-temperature range experiment.

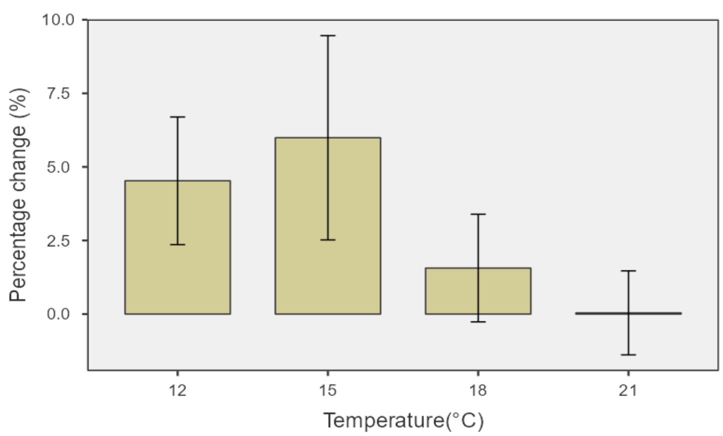


Figure 13. Mean (±SE) percentage change in buoyant weight of *Guaiagorgia* branches under 12, 15, 18, 21°C temperature treatments over 4 weeks of the experiment period. No significant difference was detected among all treatment results (one-way ANOVA, $F_{(3,19.4)}$ =1.43, p =0.265).

2.2.3.4 Linear growth

After the 4-week experimental period, the overall mean linear growth under all temperature treatments ranged from -0.02 to 0.53%. There were however, no significant difference among treatment results (one-way ANOVA, $F_{(3, 17.5)}$ =0.0528, p = 0.983) (**Fig. 14**).

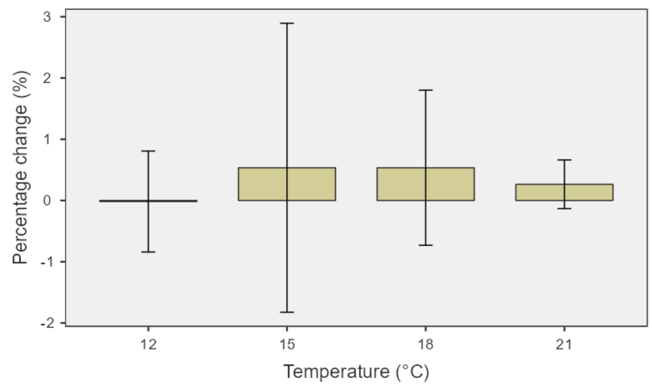


Figure 14. Mean (±SD) percentage change in linear length of *Guaiagorgia* branches under 12, 15, 18, 21°C temperature treatments over the 4 weeks experiment period. No significant difference was detected among all treatments (post-hoc Tukey-HSD, $p>0.05$).

Table 1. Results of two-way repeated measures ANOVA showing the effects of different factors and their interaction on tissue necrosis and polyp activity under (A) salinity (B) high temperature, and (C) low temperature treatments.

(A) Salinity range

	df	MS	*F*	*p*
Tissue Necrosis				
Between-Subjects Effects				
Salinity	7	50579	101	<0.001*
Error	64	500		
Within- Subjects Effects				
Time	2.57	9232	56.8	<0.001*
Time x Salinity	17.96	5592	34.4	< 0.001*
Error	164.23	163		
Polyp activity				
Between-Subjects Effects				
Salinity	7	22045	3.85	0.002*
Error	63	5725		
Within- Subjects Effects				
Time	6.62	7094	7.14	<0.001*
Time x Salinity	46.34	1866	1.88	< 0.001*
Error	417.1	993		

(B) High temperature range

Tissue Necrosis				
Between-Subjects Effects				
Temperature	4	11702	8.44	<0.001*
Error	45	1386		
Within- Subjects Effects				
Time	2.31	3930	9.12	<0.001*
Time x Temperature	9.24	3089	7.17	< 0.001*
Error	103.97	431		
Polyp activity				
Between-Subjects Effects				
Temperature	4	91093	9.88	< 0.001*
Error	45	9220		
Within- Subjects Effects				
Time	5.06	14343	7.8	<0.001*
Time x Temperature	20.24	3722	2.02	0.007*
Error	227.66	1839		

(C) Low temperature range

Polyp activity				
Between-Subjects Effects				
Temperature	3	36548	3.66	0.021*
Error	36	9975		
Within- Subjects Effects				
Time	6.3	3179	1.98	0.066
Time x Temperature	18.91	3126	1.95	0.012*
Error	417.1	993		

2.3 Discussion

2.3.1. The use of linear growth and feeding activities in assessing treatment effects

The linear growth of *Guaiagorgia* was assessed as a change in the total length of each branch measured from the base to the tip of each branch over time. However, over the 4-week experimental period, the linear growth measured is insignificant under most of the experimental conditions. Gorgonian coral has a very slow growth rate. For branches around 6 cm in size, the growth rate is less than 1 millimeter per month (Lasker 2003). A four-month research on other gorgonian corals from the same family as *Guaiagorgia* also showed no significant difference in length over the experimental period (Pereira 2019). The fluctuations in branch length measured are more likely due to angles of photo taken instead of coral growth. Similarly, polyp activity also fluctuated throughout the experimental period under different treatments. Therefore, this parameter might not also be representative in assessing treatment effects. This being the case, the discussion that follows will mainly focus on results obtained on tissue necrosis and changes in buoyant weight.

2.3.2 Salinity thresholds of *Guaiagorgia* sp.

This study provides insights on the lower and upper salinity tolerances of *Guaiagorgia* sp. in Hong Kong, with this species being found thus far only in western Hong Kong waters that generally exhibit an estuarine, highly fluctuating salinity condition.

Long exposure to 8 psu appears lethal for the *Guaiagorgia* sp. populations examined. At 8 psu, most of the branches suffered complete tissue necrosis in less than 2 weeks. For salinity treatments higher than 8 psu (i.e. 12-32 psu treatments), *Guaiagorgia* sp. showed little to no significant tissue necrosis. At the highest salinity (36 psu) tested, an increasing trend of tissue necrosis was detected albeit not significantly different from that of the rest. It is nevertheless possible that this increasing trend of necrosis through time might eventually become significant if the exposure period is prolonged. No salinity higher than 36 psu was evaluated as this higher salinity is unlikely to be encountered by the gorgonian population in the natural environment and the salinity of 36 psu is already close to 30% higher than the mean ambient of 27.8 psu.

Skeletal growth results suggested that *Guaiagorgia* sp. survive best under 24 psu but could nevertheless tolerate salinity ranges from 20 to 32 psu. This is based on the least decrease in percentage buoyant weight than the rest of the salinity treatments, indicating a loss of sclerites. However, one point to be noticed is that no positive changes in buoyant weight were detected in all of the salinity treatments. To start with, individuals of *Guaiagorgia* sp. might have already been under stress when they were collected from the field for the experiment. The mean water temperature recorded in the field was around 28°C in September 2022 (SeaTemperature.org) when the samples were collected and this temperature had already persisted for three months since June 2022. To simulate the field condition, an ambient temperature of 27°C was used as the control for all the salinity experiments. This temperature may prove to be too high, as evident from the results of our subsequent experiments on upper-temperature threshold that also showed a negative or decrease in buoyant weight change at 27°C, compared to positive or increase in buoyant weight change at other, lower temperatures like 12 - 24°C. This may explain why no positive gain in buoyant weight was recorded in the salinity experiment as indivduals of *Guaiagorgia* sp. may already be experiencing thermal stress, with experimental salinity conditions enhancing these stresses even further.

Overall, data from the salinity experiments suggested that *Guaiagorgia* sp. is a euryhaline species that can tolerate a high salinity range of 20-32 psu. This wide range of salinity tolerance is reflected in the ability of *Guaiagorgia* sp. to grow well in Hong Kong western waters which exhibit a salinity range fluctuating from 4.5 to 33.5 psu, with a mean of 27.6 psu (water quality monitoring stations NM1-6 from 2010 to 2021, Hong Kong observatory).

The ability to adapt to a wide range of salinity was also reported in *Leptogorgia virgulata*, a gorgonian coral in south Texas jetties (Williamson et al. 2011). Salinity in south Texas jetties fluctuates from a minimum of 14 psu to a maximum of 42.2 psu. *Leptogorgia virgulate* can tolerate a salinity range from 25 to 40 psu, comparable to the range of *Guaiagorgia* sp. in this study. This is much wider than many stony corals, including those in Hong Kong (Chui et al. 2016, Xie et al. 2020). One mechanism of reaction to salinity stress is suggested to be the increase of heat shock protein Hsp60 (Seveso et al., 2013). The Hsp60 was found to be able to enhance the protein folding ability in coral to cater to environmental changes (Chow et al. 2009). This mechanism may also apply to *Guaiagorgia* sp. and allows it to withstand the wide range of salinity stress.

The wide range of tolerance to salinity suggests that salinity should not be a main factor in limiting the distribution of *Guaiagorgia* sp. in other parts of Hong Kong where salinity ranges from 20 – 32 psu, all within its tolerance limits. Thus, other factors, including insufficient food availability in eastern and northern waters and geographical isolation due to oceanic current might also contribute to its limited distribution. Further studies are needed to address this puzzle and to explore the potential roles of these other factors in affecting the spatial distribution of this gorgonian species.

2.3.3 Upper- and lower-temperature thresholds of *Guaiagorgia* sp.

This study provides insights on the lower and upper temperature tolerances for a gorgonian species, *Guaiagorgia* sp. in Hong Kong. In general, exposure to elevated temperature resulted in an increase in tissue necrosis and a decrease in buoyant skeletal weight. Our data also showed that long term exposure to 33°C appears to be lethal for *Guaiagorgia* sp.. Over half of the branches exhibited complete tissue necrosis after being exposed to 33°C for 3 weeks. Although the species can tolerate a temperature range from 27°C to 30°C, no positive change in buoyant weight was recorded. On the contrary, all lower-temperature treatments (i.e. 12-24°C) recorded zero tissue necrosis and positive weight change. This indicates that *Guaiagorgia* sp. grows better in colder water and that its optimal growing temperature is during winter in Hong Kong where seawater temperature is about 14 to 18°C. Given that the summer mean bottom water temperature recorded in Hong Kong western waters, e.g. at water quality monitoring stations NM1-6 in 2020 was 27.2°C and around 29°C in September and October, this indicates that individuals of *Guaiagorgia* sp. are regularly under stress during summer. In addition, since this octocoral performed well even under the lowest temperature of 12°C used in the present study, it is possible that the lower temperature threshold of *Guaiagorgia* sp. is lower than 12°C. This is likely to be true as this species has also been reported in Fujian Province, mainland China, where winter temperature of the coastal waters could go below 10°C. Additional experiments should be carried out to confirm this.

Overall, our results imply that *Guaiagorgia* sp. is a colder water species susceptible to high temperatures. This also suggests that the future environmental conditions under global warming should become more unfavourable for *Guaiagorgia* sp. with the current summer seawater temperature of 28 to 29°C being just 5 to 4°C below its upper thermotolerance of 33°C. If the warming trend continues, individuals of *Guaiagorgia* sp. might experience increasing tissue necrosis during summer that could lead eventually to their local extinction. This is worrisome as in the face of global warming and the rise of sea water temperature, conservation measures like setting up of marine protected areas will not be able to save the population of this species in Hong Kong waters.

References

Airport Authority Hong Kong (2014). Expansion of Hong Kong International Airport into a Three-Runway System Environmental Impact Assessment Report.

Chow, A.M., Ferriere-Pagès, C., Khalouei, S., Reynaud, S., and Brown, I.R. 2009. Increased light intensity induces heat shock protein Hsp60 in coral species. *Cell Stress Chaperones* 14: 469e476.

Chui, A. P. Y., Yeung, C. W., Tsang, R. H. L., Leung, Y. H., Ng, T. Y., Chai, K. H., and Ang Jr, P. 2016. Lowered temperature and reduced salinity retarded development of early life history stages of *Acropora valida* from the marginal environment. *Regional Studies in Marine Science* 8: 430-438.

Davies SP (1989). Short-term growth measurement of corals using an accurate buoyant weighing technique. Marine Biology 101:389-395.

Goméz CE, Paul VJ, Ritson-Williams R, Muehllehner N, Langdon C, Sanchez JA (2014). Responses of the tropical gorgonian coral *Eunicea fusca* to ocean acidification conditions. Coral Reefs 34:451-460.

International Panel on Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. In: Stocker TF, Qin D, Plattner G-K, Tignor M, Allen SK, Boschung J, et al. editors. Cambridge University Press, Cambridge, UK and New York, NY, USA, 2013.

Lasker, H. R., Boller, M. L., Castanaro, J., and Sanchez, J. A. 2003. Determinate growth and modularity in a gorgonian octocoral. *The Biological Bulletin* 205.3: 319-330.

Pereira, S. M. D. C. 2019. Thermotolerance experiments and transplantation procedures of four gorgonian species from the south coast of Portugal, Algarve.

Previati M, Scinto A, Cerrano C, Osinga R (2010). Oxygen consumption in Mediterranean octocorals under different temperatures. Journal of Experimental Marine Biology and Ecology, 390, 39-48.

Roberts JM, Wheeler A, Freiwald A, Cairns S (2009). Cold-Water Corals: The Biology and Geology of Deep-Sea Coral Habitats. Cambridge University Press, Cambridge.

Torrents, O., Tambutté, E., Caminiti, N., and Garrabou, J. 2008. Upper thermal thresholds of shallow vs. deep populations of the precious Mediterranean red coral *Corallium rubrum* (L.): assessing the potential effects of warming in the NW Mediterranean. *Journal of Experimental Marine Biology and Ecology* 357:7-19.

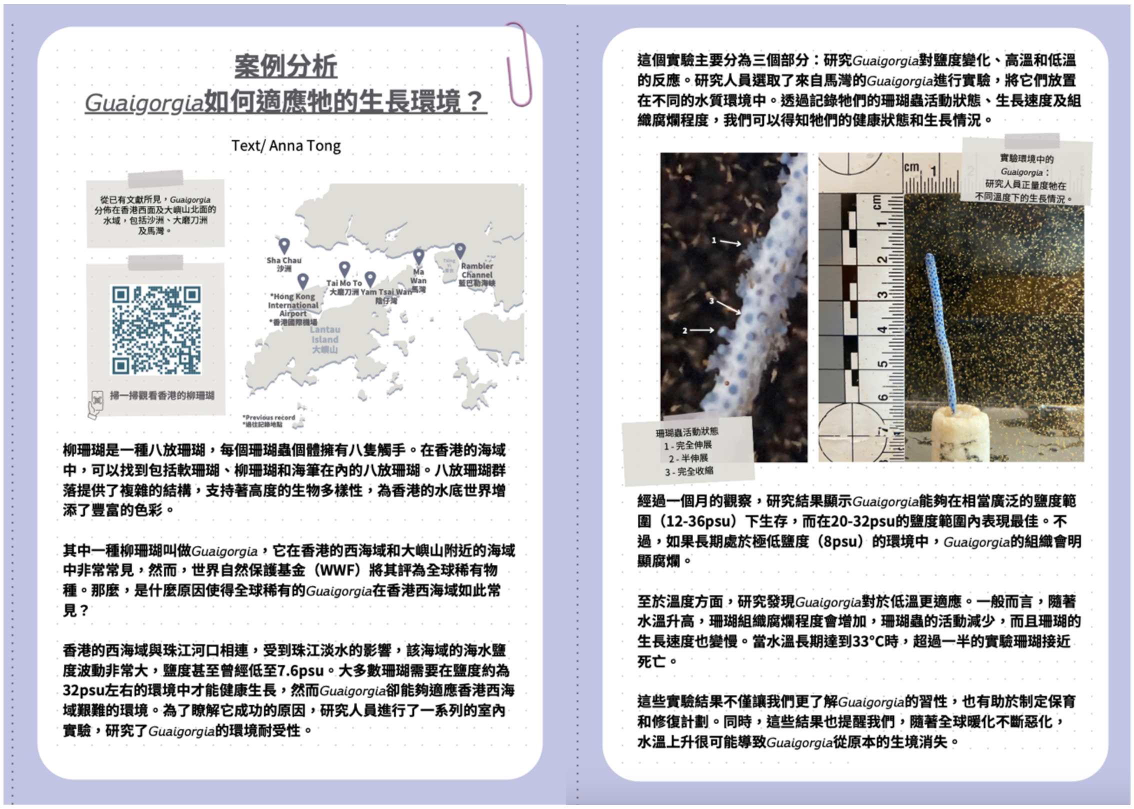
Williamson, E. A., Strychar, K. B., Withers, K., and Sterba-Boatwright, B. 2011. Effects of salinity and sedimentation on the Gorgonian Coral, *Leptogorgia virgulata* (Lamarck 1815). *Journal of experimental marine biology and ecology* 409.1-2: 331-338.

Xie, J. Y., Yeung, Y. H., Kwok, C. K., Kei, K., Ang Jr, P., Chan, L. L., and Qiu, J. W. 2020. Localized bleaching and quick recovery in Hong Kong's coral communities. *Marine Pollution Bulletin* 153: 110950.

3. 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 of this present study and to present the beauty and importance of octocorals in Hong Kong. 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
*Guaigorgia*如何適應牠的生長環境？

Text/ Anna Tong

從已有文獻所見，*Guaigorgia*分佈在香港西面及大嶼山北面的水域，包括沙洲、大磨刀洲及馬灣。

掃一掃觀看香港的柳珊瑚

柳珊瑚是一種八放珊瑚，每個珊瑚蟲個體擁有八隻觸手。在香港的海域中，可以找到包括軟珊瑚、柳珊瑚和海筆在內的八放珊瑚。八放珊瑚群落提供了複雜的結構，支持著高度的生物多樣性，為香港的水底世界增添了豐富的色彩。

其中一種柳珊瑚叫做*Guaigorgia*，它在香港的西海域和大嶼山附近的海域中非常常見，然而，世界自然保護基金（WWF）將其評為全球稀有物種。那麼，是什麼原因使得全球稀有的*Guaigorgia*在香港西海域如此常見？

香港的西海域與珠江河口相連，受到珠江淡水的影響，該海域的海水鹽度波動非常大，鹽度甚至曾經低至7.6psu。大多數珊瑚需要在鹽度約為32psu左右的環境中才能健康生長，然而*Guaigorgia*卻能夠適應香港西海域艱難的環境。為了瞭解它成功的原因，研究人員進行了一系列的室內實驗，研究了*Guaigorgia*的環境耐受性。

這個實驗主要分為三個部分：研究*Guaigorgia*對鹽度變化、高溫和低溫的反應。研究人員選取了來自馬灣的*Guaigorgia*進行實驗，將它們放置在不同的水質環境中。透過記錄牠們的珊瑚蟲活動狀態、生長速度及組織腐爛程度，我們可以得知牠們的健康狀態和生長情況。

珊瑚蟲活動狀態
1 - 完全伸展
2 - 半伸展
3 - 完全收縮

實驗環境中的*Guaigorgia*：研究人員正量度牠在不同溫度下的生長情況。

經過一個月的觀察，研究結果顯示*Guaigorgia*能夠在相當廣泛的鹽度範圍（12-36psu）下生存，而在20-32psu的鹽度範圍內表現最佳。不過，如果長期處於極低鹽度（8psu）的環境中，*Guaigorgia*的組織會明顯腐爛。

至於溫度方面，研究發現*Guaigorgia*對於低溫更適應。一般而言，隨著水溫升高，珊瑚組織腐爛程度會增加，珊瑚蟲的活動減少，而且珊瑚的生長速度也變慢。當水溫長期達到33°C時，超過一半的實驗珊瑚接近死亡。

這些實驗結果不僅讓我們更了解*Guaigorgia*的習性，也有助於制定保育和修復計劃。同時，這些結果也提醒我們，隨著全球暖化不斷惡化，水溫上升很可能導致*Guaigorgia*從原本的生境消失。

Education video

A short video introducing the objectives and activities of this present study has been developed to promote public awareness and education as well as greater appreciation of the efforts by MEEF in conserving Hong Kong marine resources and habitats.

Social media posts

A total of five social media posts have been posted in webpage and Facebook during the project period.

Post 1 – Introducing our *Guaiagorgia* team @Coral Academy Lab		Media reach
Hi everyone! This is Alice and Anna, the final year project students in Coral Academy Lab. This year, our lab received funding support from the Marine Ecology Enhancement Fund (MEEF) to study the reproductive biology and environmental tolerance of a gorgonian coral, *Guaiagorgia*. What is *Guaiagorgia*? What is so special about it? Let's hop on and explore with us.		4391

<table>
<tr><td colspan="2">Post 2 – What is Octocoral?</td><td>Media reach</td></tr>
<tr><td>

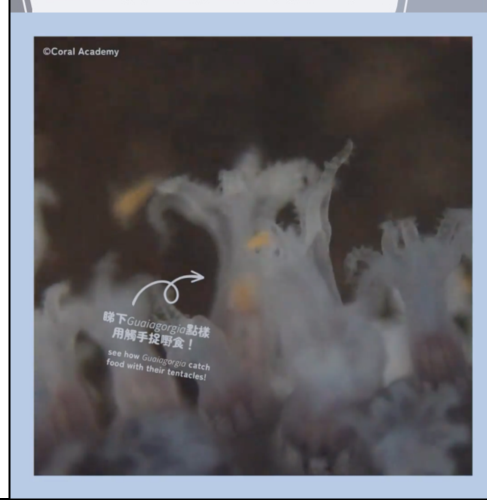
Do you know that corals are divided into two groups? Corals with 6 or multiples of 6 tentacles in each polyp belongs to subclass Hexacorallia, and corals with 8 tentacles in each polyp belongs to subclass Octocorallia. Hard corals and black corals are hexacorals, while soft corals, gorgonian and sea fans are all octocorals. The coral we are studying, *Guaiagorgia*, is a genus of gorgonians. Many octocorals are filter feeders and feed on plankton. Unlike hard corals, octocorals do not possess external calcium carbonate skeleton, nor form coral reefs.

Guaiagorgia is abundant in Hong Kong western water and Lantau Island. Let's learn more about it in the coming post!

</td><td>

</td><td>1572</td></tr>
<tr><td colspan="2">Post 3 – Guaiagorgia, where to find them?</td><td>Media reach</td></tr>
</table>

<table>
<tr><td>According to the present literature review, Guaiagorgia is abundant in western Hong Kong water and Northern Lantau Island. Previous surveys show that Guaiagorgia sp. colonies can also be found near Hong Kong International Airport, Tai Mo To, Ma Wan, and Tsing Yi. They adapt to Hong Kong western water and salinity change better than other corals and become dominant. Hopefully, we will learn more about their distribution as there is more ecological survey on the species. Anna’s experiment will show us how Guaiagorgia response to temperature and salinity change.</td><td>
</td><td>1097</td></tr>
<tr><td colspan="2">Post 4 – What does Guaiagorgia like to eat?</td><td>Media reach</td></tr>
<tr><td>Guaiagorgia is a filter-feeder. Unlike hard corals, Guaiagorgia and many other octocorals do not obtain nutrients from photosynthetic symbionts. Instead, they mainly filter water and capture plankton and tiny particles as food with their tentacles. In the laboratory, they are fed with newly-hatched brine shrimp and powdered marine plankton. Swipe to see how they catch food with their tentacles!</td><td>


</td><td>1485</td></tr>
<tr><td colspan="2">Post 5 – Guaiagorgia in Hong Kong</td><td>Media reach</td></tr>
</table>

Octocoral is a collective term for corals in the sub-class Octocorallia, in which each polyp has exactly eight tentacles. They are filter feeders and they inhabit areas with strong water currents. In Hong Kong, we can find different octocorals including soft coral, gorgonian coral, and sea pen. Octocoral assemblages provides a complex three-dimensional structure to support high biodiversity. Their diversity brings about a colourful underwater world in Hong Kong. *Guaiagorgia* is a genus of gorgonian coral. It is abundant in Hong Kong western water and Lantau Island. It is one of the few corals in Hong Kong that can adapt to environment of low salinity. Being supported by the Marine Ecology Enhancement Fund. We are studying the environmental tolerance of *Guaiagorgia.* Through indoor experimental set-ups, we controlled some water parameters, such as salinity and temperature. To test the response of *Guaiagorgia* on different environment stresses. Meanwhile, we perform coral feeding, tank cleaning and water changing every day. The coral's health status are recorded as well. We then analyse the recorded data to investigate the most suitable growing environment for *Guaiagorgia.* The results may provide insights to explain its success in Hong Kong western waters. This is essential in the design of a strategic conservation and protection plan. This project is funded by the Marine Ecology Enhancement Fund. YouTube link: https://www.youtube.com/watch?v=EptbmK8b88c		1347
Total number of people who see the content via social media posts		9892

(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 biological response (including linear growth, skeletal growth rate, polyp activity and tissue necrosis) of the gorgonian Guaiagorgia sp. to hyposalinity and changes in seawater temperature

All the proposed experiments on salinity range, higher and lower temperature threshold of *Guaiagorgia* sp. had been conducted, all the results of biological response were discussed in this completion report, please refer to the above sessions *2.2. Results* and 2.*3 Discussion*.

Objective 2: To identify salinity and temperature thresholds of Guaiagorgia sp..

Overall, the data suggested that *Guaiagorgia* sp. performed best under the salinity range of 20-32 psu and lower temperature range of 12-24°C, but can also tolerate a broader range of salinity (i.e. 12-36 psu) and higher temperature below 33°C for up to 28 days of exposure. Moreover, since the coral performed well even under the lowest temperature treatment used in this study, it is possible that the lower temperature threshold of *Guaiagorgia* sp. might go below 12°C.

(vi) Summary and Way Forward;

The overall results from this study suggest that *Guaiagorgia* sp. can tolerate a wide range of salinity, thus its distribution should not be limited to low-salinity seawaters in western Hong Kong. Other factors may play a role in limiting its distribution range, including insufficient food availability in eastern and northern waters and its geographical isolation due to oceanic current.

Our results also indicate that *Guaiagorgia* sp. is susceptible to high temperatures, and that the future environment under global warming might be more unfavourable for its survival. This species may regularly be under stress given that the summer mean bottom water temperature recorded in Hong Kong western water in 2020 was 27.2°C and around 29°C in September and October, just 5 to 4°C below its upper thermotolerance of 33°C. If the warming trend continues, *Guaiagorgia* sp. is likely to experience increasing tissue necrosis that could lead to its local extinction. Conservation measures like settling up of marine protected areas would not be able to help preserve the *Guaiagorgia* sp. population in Hong Kong waters.

Further studies are needed to evaluate the physiological responses 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.

(vii) Staff Attendance record

Staff attendance record are not disclosed due to confidentiality reasons.

(ix) Recruitment record

Recruitment record are not disclosed due to confidentiality reasons.

Appendix

List of project asset are not disclosed due to confidentiality reasons.

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.

Any opinions, findings, conclusions or recommendations expressed in this report do not necessarily reflect the views of the Marine Ecology Enhancement Fund or the Trustee.

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