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Coral Bleaching Experiment Systems

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ABSTRACT

The declining of coral reef ecosystem has been observed over the last 30 years and projected to continue declining in the future because corals are sensitive animals towards any changes in their surrounding; temperature, nutrient and water clarity. Coral bleaching is one of disaster affected by natural or human impact towards coral reefs. Mass coral bleaching events has been going on since 1998 and getting worse in 2010 and 2016 throughout the whole world, including in Malaysia. Increase of slightly higher temperature can interrupt the coral-zooxanthellae relationship, and leading to coral whitening, aka coral bleaching. Coral bleaching experiments has been done through the years from various countries to aid in coral conservation and restoration to identify the degree of bleaching towards stressors. In this study, coral triangle in South-east Asia region is the focused area because of the high diversity of coral species growth. The main species in Pulau Bidong, Terengganu, Malaysia, *Acropora digitifera* was chosen for this study, studied its bleaching criteria by examined the coral polyps and colour changes after synergistic effect of temperature and nitrates, the stressors. The coral nubbins were experimented in three sets of optimum, high and higher of temperature and nitrate levels in laboratory for 3 days. Its physiological changes were recorded every 12 hours. Differentiate this study with other coral bleaching experiment systems; their highest temperature been applied to the corals, the duration of the elevated temperature that corals can withstand, and the species that have been used in the coral bleaching experiment. Thus, this study can contribute knowledge to identifying *A. digitifera* as super coral for its resilience and resistance in husbandry. However, more studies need to be done on identify the genes that make them more tolerant and can be used in enhanced coral breeding.

1. Introduction

Coral reefs (CR) are known as one of the most diverse and productive ecosystems on earth. Most of them are limited to the euphotic zone and are found in tropical and subtropical waters. According to Tuwo and Tresnati [1], the growth of corals is limited due to their sensitivity to many environmental parameters including salinity, turbidity, depth, temperature, and humidity. In addition, hard corals are one of the main forms of corals that reside in colonies and they form their

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skeletons from the intake of calcium carbonate in water column [6]. Corals rely on the zooxanthellae that live inside of them to flourish.

Corals and zooxanthellae have a symbiotic relationship in which the coral give shelter to the zooxanthellae and the zooxanthellae give food to the coral for growth [14]. CR are crucial as they support the habitats of countless marine creatures and offer various benefits to millions of people globally, such as food, coastal protection, economic opportunities, and medical advantages [14]. CR face significant threats from both human activities and natural factors. According to the same studies, human-made risks encompass climate change, pollution, poor resource management, and nutrient enrichment, while natural challenges involve diseases, storms, and predators. Among these, climate change, with its temperature rise and nutrient enrichment effects, stands out as a major peril to coral reefs, notably leading to coral bleaching.

Coral reef ecosystems are highly sensitive to various factors, like temperature and nutrient levels. [9] stated that the increase of temperature can disturb the corals-zooxanthellae relationship leading to coral bleaching. Additionally, land-based sources, such as chemical pollutants, fertilizer runoff, and sedimentation, can harm corals by reducing the light available for coral growth [8]. These factors lead to coral bleaching, which is when vibrant coral loses its colour and become pale due to the loss of their symbiotic algae [8]. Coral bleaching has far-reaching effects on coral reef ecosystems, including reduced biodiversity, changes in the food chain, economic consequences, increased susceptibility to disease, and diminished coastal protection [10,14]. Since these corals rarely regenerate after they die and their impacts on marine life and humans are significant, addressing this issue is of utmost importance.

Hence, Pulau Bidong was selected as the research site for this study due to itsstatus as a research station for the University Malaysia Terengganu and its position as one of the riches coral reef islands located in the South China Sea (SCS) off the coast of Terengganu. The study focuses on *Acropora digitifera* due to its significance as the most dominant coral species responsible for building reefs in Pulau Bidong. Understanding this species is crucial as it plays a big role in the overall reef ecosystem of the area and *A. digitifera* is highly sensitive to stress, and any disturbances can have rapid and easily observable impacts, making it an important species to study for conservation efforts [4].

Furthermore, the limited number of studies examining the effects of temperature and nitrate on coral colouration and polyps underscores the need for further research in this area. Given the potential significance of temperature and nitrate levels in influencing the health and vitality of coral, particularly *A. digitifera*, conducting comprehensive studies on their effects becomes important for better understanding and preserving these delicate ecosystems. By delving into the impacts of various stressors like climate change, pollution, ocean acidification, and human activities on coral, researchers can unravel the mechanisms that contribute to their ability to withstand and recover from disturbances. Studying the effects on coral is imperative due to its profound impact on understanding the resistance and resilience of these magnificent organisms, thereby informing crucial strategies for effective coral reef management.

2. Methodology

The sampling was done at Pantai Pasir Tenggara, Pulau Bidong at 5°36'40.377''N, 103°03'38.883''E on 7th of September 2023. Pulau Bidong is a small island located around 14km away from the mainland, east coast of Peninsular Malaysia. The chosen coral species used in this study is *A.digitifera* species which is a common and ecological important coral species in many coral reef ecosystems. The SCUBA diving method was used to collect the sample of healthy and high-density corals under the water of the sampling site. The corals colonies were collected by using hammer and chisel by divers [4]. The collected coral colonies were kept submerged in ziplock bag filled with seawater. After a week of acclimatization process, the experiment was set up involving three tanks. Back to university, experimental tanks were designed as Figure 1. The involving tanks were: one control tank (TANK 1) and two experimental tanks (TANK 2 & TANK 3), each was supplied with natural seawater from nearby university beach.

After that, the water was circulated in that three tanks for 7 days [17] before starting the experiment. The set up was include the instalment of heater for each tank to adjust the temperature changes, the stress of high temperature (30–32 °C) was set up for experimental tanks for 5 days [15]. In each tank, five coral nubbins were added to the experimental setup using tweezers. The water parameter including salinity, pH, Kh, magnesium, calcium and temperature (using thermometer) was regularly assessed and managed. Additionally, an underwater camera was used to capture images of each nubbin for every 24 hours during the duration of the study [17]. The stress of high nitrate concentration (15 µmol) was set up for another set of experimental tanks for 5 days [15]. The quantity of nubbins utilized was match those used in the temperature experiment. Water parameters, such as salinity, pH, Kh, magnesium, calcium, and nitrate (using Handheld Colorimeter Marine Nitrate HR), were undergo routine monitoring and maintenance.

An underwater camera (Olympus TG-6) was used for this data collection; capture the image of each nubbin. Pictures of each coral nubbin for every 24 hours were taken from each side of the nubbin. Then, ImageJ software was used to count the active polyp's number [7]. The image analysis was done by extract the desired coral image from the underwater camera and open in that software. For the colour observation of the coral nubbins, colour references card of coral watch which is the Coral Health Chart (Coral Watch) [18] was used to assess colour changes in the coral.

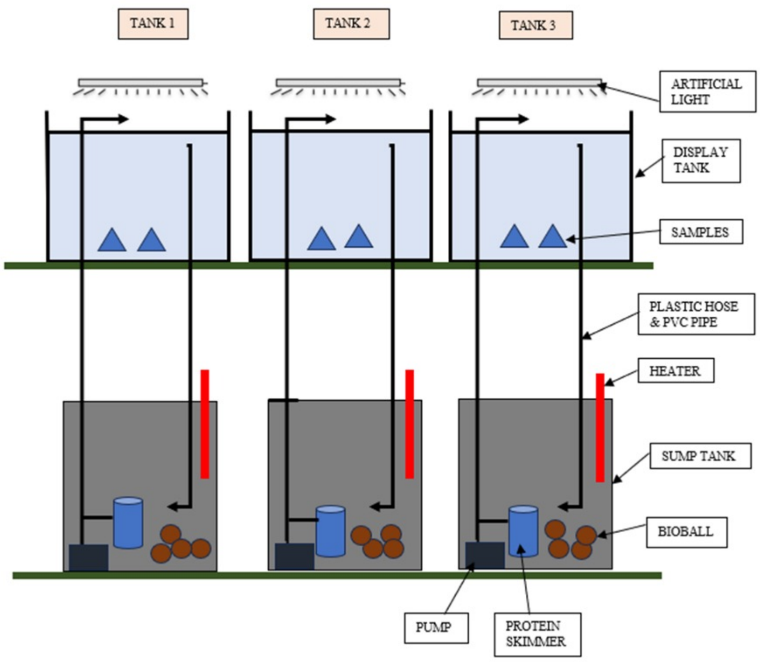

Fig. 1. Coral bleaching experimental design for temperature and nitrate stress of *Acropora*

3. Results and Discussion

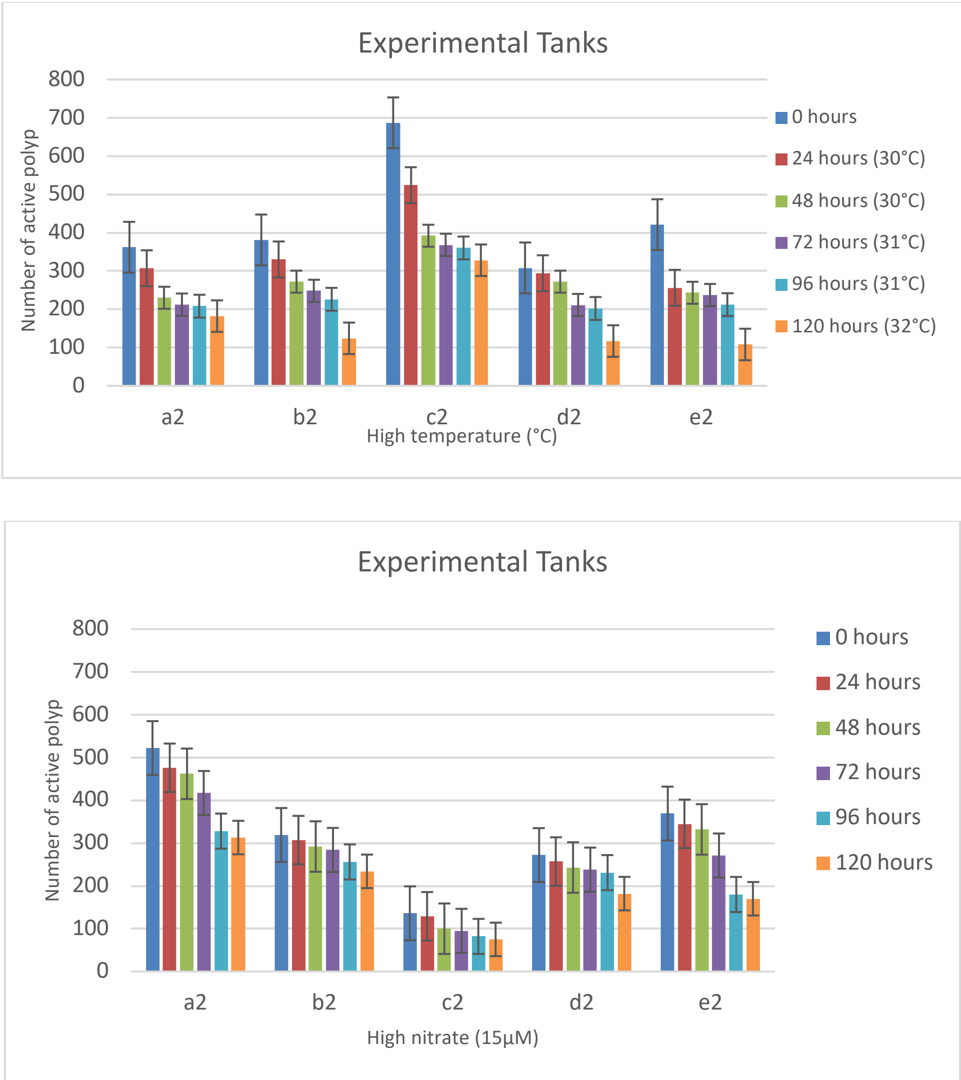

Fig. 2. The number of active polyps of *A.digitifera* for temperature (Graph A) and nitrate (Graph B) stress experiments

The results of this study confirmed that the variation in active polyp counts between normal temperature (28°C) and higher temperatures (30-32°C) are not significant. The results show that temperature stress did not significantly affect the polyps' survivability, with the F-values and p-values for each tank (Tank 1: F=1.248, p=0.323; Tank 2: F=2.691, p=0.061; Tank 3: F=1.408, p=0.2 67) indicating that the variations in active polyp counts are likely due to random chance rather than a direct effect of temperature.

The decline rates for all nubbins were consistent, indicating similar responses to the high temperature conditions (30-32°C). This shows that high temperatures produce unfavourable development conditions that cause a notable and continuous decline in the number of active polyps in all nubbins, a factor that has been repeatedly found in other studies to be harmful to coral health. High temperatures damage the zooxanthellae, the microscopic algae that live inside corals and are responsible for coral bleaching [16]. The discharge of zooxanthellae in coral polyps causes the polyp to begin to disintegrate under changes in high temperature, which causes the coral nubbins to lose their pigment. Corals are stressed by high water temperatures, which causes them to expels vibrant, symbiotic algae that are essential to their survival, growth, and reproduction [11]. Through photosynthesis, zooplankton produce vital nutrients for the coral. When they are stressed by heat or acidity, however, the coral's energy supply is diminished, resulting in polyp and coral death. Subsequently, the stress of elevated temperatures impairs the photosynthesis system of symbionts, resulting in an excess of oxygen radicals that cause harm to both the symbionts and their hosts [12]. This led to damage to the tissue of the corals because of the higher metabolic demands brought on by the high temperatures, which made it harder for the corals to satisfy their energy needs because of their reduced photosynthetic efficiency [13].

The one-way ANOVA findings show that there is no significant relationship between the number of active polyps and excessive nitrate. The findings show that the number of active polyps in the control tank's normal nitrate (existed nitrate) and the high nitrate (15µM) do not differ statistically significantly. Every nubbin displayed consistent decline rates, indicating that they all reacted to the high nitrate conditions in a comparable way. It also suggests that high nitrate levels create unfavourable development conditions that cause a discernible and persistent reduction in the number of active polyps in each nubbin—a characteristic that has been repeatedly associated with negative impacts on coral health in previous studies. High nitrate levels have the potential to disturb the symbiotic connection and cause nutrient enrichment [10]. Additionally, Higuchi *et al.*, [15] pointed out that too many nitrates might result in algae blooms, which put corals in competition for light and space and may cause a decrease in polyp activity. Corals may become choked and suffocated by this eutrophication process, which will impair their ability to develop and reproduce. Additionally, corals may experience physiological stress as a result of high nitrate levels, which may impact their metabolic functions and lower the number of active polyps.

D'Angelo [3] also emphasised that nutrient enrichment causes bleaching and a decrease in coral health by upsetting the symbiotic interaction between corals and their zooxanthellae. These investigations support the reported decrease in active polyp counts in high nitrate environments, suggesting that high nitrates are a common stressor that might harm coral health in various research contexts and environments. Although nitrates had a substantial effect, it was not as strong as temperature, indicating that corals may be more resilient to nutritional stress than to heat stress. Prolonged exposure to elevated nitrate levels, however, may still be harmful to coral health and the stability of the ecosystem. Furthermore, studies conducted by Szmant [2] revealed that not all coral species would be adversely affected by mild nitrate loading. Their research revealed that although several species exhibited stress reactions, others did not demonstrate appreciable variations in their behaviour or capacity to survive under comparable circumstances. This highlights even more how intricate coral responses to nitrate enrichment are and how important it is to do species-specific research.

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4. Conclusion

The combined observations of reduced polyp activity and progressive loss of pigmentation confirm that these two indicators are reliable and complementary metrics for assessing coral health under controlled experimental conditions. Such measurements provide critical insights into the timing, severity, and potential reversibility of bleaching events. The findings also underscore the importance of early detection and monitoring, as even moderate stress-induced changes in these parameters can precede irreversible coral mortality.

Long-term monitoring is essential to capture not only the onset and progression of bleaching but also the potential for polyp reactivation and pigment restoration during recovery phases. Comparative studies across multiple coral species and morphologies would provide valuable insight into species-specific tolerance thresholds and resilience patternsThe observed decreases and colouration changes highlight the significance of addressing climate change and lowering nutrient pollution to preserve coral health and reef resilience, even in the absence of statistically significant connections in active polyp counts. Creating successful conservation plans to protect coral reef ecosystems requires long-term monitoring and multidisciplinary research partnerships. Coral reefs and the marine life that depends on them must also be protected by lowering greenhouse gas emissions and minimising nutrient contamination.

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