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Photophysiology Response of *Montipora* Sp. to High Temperature and High Nitrate Stress

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Abstract. Coral reef health has declined significantly around the world as a result of anthropogenic activities and natural environment changes, such as sewage pollution and climate change due to global warming. These significant threats can interrupt coral fluorescence yield and also modify zooxanthellae performance, which can lead to bleaching events in case of prolonged stress. Nowadays, other than using zooxanthellae density to determine coral health, studies have started to focus more on observing the effective quantum yield, Y(II) of corals by using a pulse amplitude (PAM) fluorometer, as it is an effective and non-lethal way of determining coral health. The objectives of this study are twofold: (i) to measure the effective quantum yield of *Montipora* sp. before and after nitrate and temperature stress and (ii) to calculate the zooxanthellae density of *Montipora* sp. after nitrate and temperature stress. *Montipora* sp. exposed to high nitrate (HN), high temperature (HT) and high nitrate with high temperature (HNHT) treatment exhibited a decrease in their Y(II) reading and zooxanthellae density after 48 hours of exposure. A major decrease in Y(II) resulted from HN treatment while a major drop in zooxanthellae was caused by HT treatment. To fully understand the photoinhibition process and how it affects the coral body system, future research should include longer term experiment durations, a variety of coral species and molecular studies.

Keywords: Effective quantum yield, PAM fluorometer, Coral photophysiology.

1. Introduction

Healthy coral reefs are the most eye-catching, assorted and economically precious marine ecosystems in the world (El-Naggar, 2021). Coral reefs are very important for biodiversity because they are home to 35,000–60,000 plant and animal species (more than 25% marine life) (El-Naggar, 2021). They are essential for both the coastal population and the country's economy. They serve as nurseries for many commercially important fish species and protect coastal areas from storm waves. As a result of unsustainable overfishing, intensive tourism, urbanisation, sedimentation, water quality decline, pollution and, most vitally, the direct and indirect effects of climate change, coral reef ecosystems have undergone phase shifts to alternate, degraded assemblages (El-Naggar, 2021).

Coral reefs survive in tropical waters with low nutrient levels, low phytoplankton growth and extremely high light penetration. Anthropogenic nutrient enrichment is frequently linked to coral reef decline. As a result, increased nutrient inflow in reef waters has long-term adverse consequences for corals (D'Angelo & Wiedenmann, 2014). Nutrient discharge typically worsens the risk of coral diseases such as aspergillosis, tends to increase coral mortality, minimizes coral reproductive success and reduces recruitment rates of

juvenile corals – the future coral reef seed stock (Bruno *et al*., 2003).

The coral reef degradation due to nutrient enrichment is alarming. There is not much evidence of nutrient enrichment to corals *in situ*, barring the odd laboratory experiments (Koop *et al*., 2001). Data from Thurber *et al*. (2014) provide solid support for the notion that coastal nutrient loading is the biggest contributor to rising levels of coral disease and coral bleaching. Human-induced nutrient accumulation is more likely to harm coral reefs near the shore, within lagoons or embayments, and reefs linked to the larger land masses, particularly those near significant human populations (Tengku, 2018).

In many developing countries, untreated sewage is also commonly discharged into coral reef lagoons (Koop *et al*., 2001). One such location is Malaysia's east coast, where tourism is growing so quickly that there is not enough room for new constructions at popular beaches (Lachs *et al*., 2019). Few islands in Malaysia have sewage treatment facilities, and sewage is pumped and dumped into the sea directly. Unfortunately, improved sewage treatment facilities are still lacking (Lachs *et al*., 2019).

According to Larsen and Webb (2009), alteration to nutrients in coral reef areas can significantly change ecosystems' food web where high nutrient concentrations encourage the spread of plankton, which can then lower transmission of light and delay the zooxanthellae living in coral tissue's activities, which delays coral growth rates. Szmant (2002) mentioned that increased nutrient levels may also affect the physiological interactions between corals and their zooxanthellae by increasing the intracellular symbionts' cell division rates, which could be similar to Muscatine *et al*.'s (1989) findings, who found that increased inorganic nitrogen availability (20 µm ammonium and 2 µm phosphate) can increase the synthesis of protein and number of zooxanthellae. Nordermar *et al*. (2003) concluded that corals exposed to +15M nutrients for 2 weeks may be more sensitive to periods of high temperatures (34ºC), as evidenced by lower primary production rate of zooxanthellae. Surprisingly, nutrient enrichment had no effect on the treatments, but the authors observed that corals with nitrate had decreased primary productivity and smaller zooxanthellae density as a result of the enrichment.

To date, much attention has been given to how sewage from tourism industries has an impact on the marine ecosystems generally but not to how specific-species are affected. Thus, this study aims to understand the effects of elevating nitrate (one of the sewage contents) on the commonly found tropical scleractinian, *Montipora* sp., by measuring its effective quantum yield and zooxanthellae density after a short-term exposure.

2. Materials and Methods

2.1 Sampling Site

The sampling site of this study was Universiti Malaysia Terengganu Research Centre, Bidong Island (Fig. 1). Healthy *Montipora* sp. were collected by SCUBA diving method at a depth of 5–7 m by cutting them from colonies using a tiny hammer and small chisel. *Montipora* sp. in this study was identified as a branching coral. Healthy corals were identified by their vibrant colour and active polyps, or coral colour charts were used to determine the state of coral health in this study. It will be recognised as a healthy coral when the colour level is visible in the dark, and vice versa (Gilmour *et al*., 2006). All samples were put into an ice chest and they were brought to the hatchery for further processing. The corals were put in a sealed zip lock bag.

2.2 Coral Experiment

Coral nubbins of size 2–3 cm were first acclimatised in the preparation tank (Fig. 3).

Acclimatisation is important to ensure that corals are well-adapted to a new environment (Tengku, 2018). Three experimental tanks that mimic the ocean's normal condition were prepared (Fig. 2). The temperature (27°C), salinity (31-32 parts per thousand) (ppt), 5 µmol concentration of nitrate, alkalinity (8 degrees of carbonate hardness) (dKH), calcium (440 part per million) (ppm) and magnesium (1350 ppm) were measured to ensure similarity between the three tanks and coral adaptability.

The corals in this research were subjected to three sets of treatments: (1) high nitrate (HN; 15 µmol), (2) high temperature (HT; 30°C) and (3) combined stress of HN (15 µmol) and HT (30°C; HTHN). Eighteen nubbins of *Montipora* sp. were placed into each experimental tank, as pictured in Fig. 2. The concentration of nitrate was increased to 15 µmol by putting potassium nitrate (KNO_3) into experimental tanks. This concentration was increased to match the projected nitrate concentration expected in 2200 (Nordemar *et al*., 2016). Every 12 h of 72 h of the experiments, three samples for each tank were cut out with a pair of pliers and then the samples were placed in a urine container for the zooxanthellae density counting.

1.3 Quantum Yield Measurement

Fluorescence measurements on coral nubbins were performed using a Diving PAM fluorometer (Walz, Effeltrich, Germany). To obtain effective quantum yield readings, *Montipora* sp. maximum fluorescence (F_m) was measured using the settings of saturating light pulse (0.8 s, >2000 µmol quanta m^{-2} s^{-1}). The effective quantum yield of PSII was determined according to the equation $(F_{m'} - F_t)/F_{m'}$, where $F_{m'}$ is the light-adapted maximum fluorescence and F_t is the fluorescence before a saturating pulse (Genty *et al*., 1989). A 5-mm distance between the fibre optic cable and the sample was set to get a precise measurement of chlorophyll fluorescence (Bhagooli and Hidaka, 2004). Diving-PAM probe was pointed all over *Montipora* sp. nubbin (Fig. 3) to measure its quantum yield before, during and after stress-experiment.

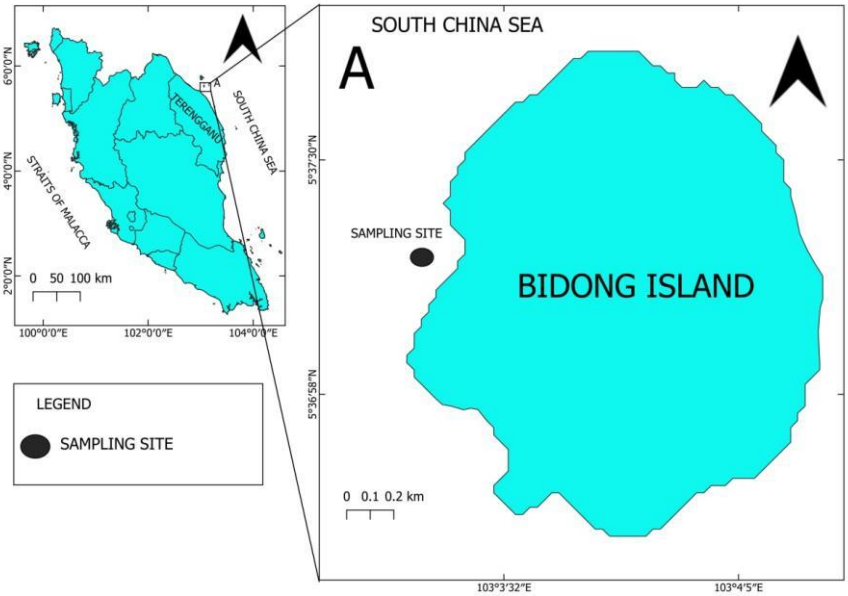

Fig. 1. Map of the sampling site at Bidong Island, Terengganu, Peninsular Malaysia.

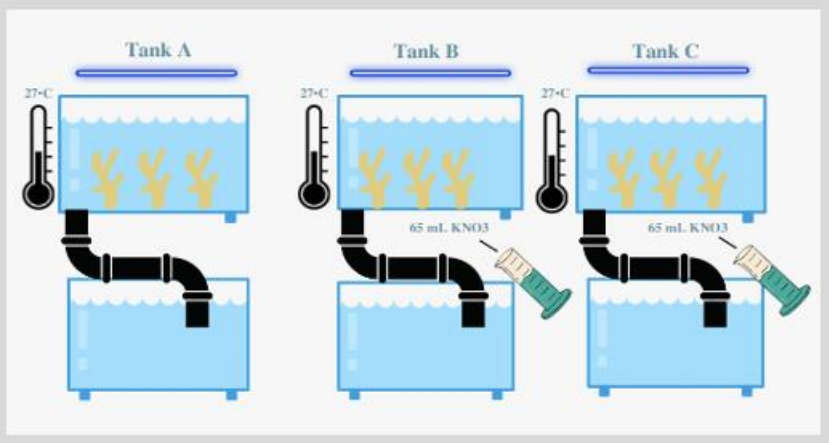

Fig. 2. Diagram of experimental tanks. Tank A: ambient condition; Tanks B and C: high nitrate treatments.

Fig. 3. Montipora sp. in the experimental tanks for short-term stress.

2.4 Zooxanthellae Density Counting

The preserved samples from the treatment experiment were de-calcified using 10% formic acid. The formic acid was refreshed in each container regularly. Then, residual acid de-calcifiers was neutralised by extensive washing in tap water. The tissue sections were cut to a size of 0.5 m² and were placed in a 1.5-mL centrifuge tube with 1 mL of formalin. The samples were mixed in a multi-sample homogeniser for 2 min. The cell density was determined using a Neubauer haemocytometer (Superior, Germany), which has two counting chambers with each consisting of four squares are made up of 16 smaller squares.

2.5 Analysis

One-way and two-way ANOVAs were performed to determine the significant difference between datasets of effective quantum yield and zooxanthellae density for the *Montipora* sp. coral.

3. Results and Discussion

In this study, the coral nubbins that were experimented under HN, HT and HTHN showed a significant decrease in $\Delta F/F_m$ when compared with the control. The effective

quantum yield of zooxanthellae from coral nubbins (n = 15 in each tank) was monitored over 12 h of nitrate enrichment 15 μmol and 30°C. Coral exposed to temperature stress and a combination of nitrate and temperature stress showed greater significant decrement than control corals. The yield showed a 30% decrease in the HT treatment (0.559) and in the HNHT treatment (0.546). Moreover, larger reductions of effective quantum yield were observed only with combined nitrate and temperature stress. The effective quantum yield for *Montipora* sp. was 0.303 after 48 hours of nitrate stress, 45% lower than the controls. Both nitrate and temperature significantly influenced the fluorescence yield of this coral.

Figure 4 showed that short-term exposure to nitrate/temperature under laboratory conditions affects the effective quantum yield of *Montipora* sp. Other corals also experienced a decrease in photosynthetic efficiency after short-term exposure. During 30-h thermal stress in Bhagooli and Yakovleva (2004), *Montipora digitata* showed a significant decrease in PSII functioning (F_v/F_m). Moreover, in the study by Yoshimi *et al*. (2016), *Pocillopora damicornis* was found to be affected by high nitrate (10 μmol) and high temperature (32°C) concerning PSII (F_v/F_m). In Fitt *et al*. (2009), both *Porites cylindrica* and *Stylophora pistillata* were under thermal stress when their quantum yields showed a dramatic increase after heat stress exposure (32°C). A study by Gomez (2015) showed lower effective quantum yield for *A. cervicornis*, *P. furcate* and *Orbicella annularis* after exposed to heat stress of 31°C.

The average effective quantum yield value ($\Delta F/F_m$) in these studies ranged between 0.6 and 0.669, whereas for laboratory-controlled colonies of *Montipora peltiformis*, the quantum yield, ranged between 0.67 and 0.71 (Phillip and Fabricius, 2003). Most studies only focused on the maximum quantum yield (F_v/F_m) of corals. Philipp *et al*. (2002) revealed that healthy *Montipora peltiformis* has a range of F_v/F_m between 0.67 and 0.71. In *Montipora verrucosa*, Torregiani and Lesser (2007) recorded values between 0.767 and 0.898 for *in situ* samples.

Zooxanthellae play an important role in coral growth, reproduction and immune system (Fu *et al*., 2022). In this experiment, zooxanthellae densities in controls averaged 0.13×10^6 cell/ml. On the contrary, densities were between 0.021 and 0.012×10^6 cell/ml after 48 h of nitrate and temperature exposure. There was no significant interaction between zooxanthellae density and HN treatment (p = 0.086) (Fig. 5). However, zooxanthellae density was found to be significant with HT and HNHT treatments (p = 0.03) after 48 h. According to Nitschke *et al*. (2018), coral zooxanthellae are highly susceptible to photochemical destabilisation when exposed to abnormal environmental conditions, which in this study was the high temperature stress. In the study by Higuchi *et al*. (2016), the density of zooxanthellae in *M. digitata* did not change significantly with high nitrate (10 μmol) but was only affected by temperature and light intensity. Furthermore in response to temperature stress (Bhagooli and Yakovleva, 2004), zooxanthellae count in *M. digitata* was significantly reduced from 1.2 to 2×10^6 cells/cm^2. This finding is similar to the finding by Fu *et al*. (2022), where zooxanthellae density of *Acropora hyacinthus* was decreased by 53.1% due to exposure to 30°C of stress. In study of Chumun *et al*. (2013), its zooxanthellae density of coral *P. damicornis* in all stress treatments (high nitrate and high temperature) were significantly lower than control.

This study shows that short-term exposure to nitrate and/or temperature under laboratory conditions affected the effective

quantum yield and zooxanthellae density of *Montipora* sp. According to Chumun *et al*. (2013), a decrement in zooxanthellae numbers leads to a decrement in quantum yield of stressed corals, suggesting that the corals under stress may have released damaged and malfunctioned zooxanthellae.

In Malaysia, studies on the effective quantum photosynthetic yield of scleractinian corals *in situ* and *ex situ*, especially of *Montipora* sp., which is among the common corals in Indo-Pacific waters, are scarce. To the authors' knowledge, this study is the first study on the *Montipora* sp. coral's effective quantum yield in husbandry in Malaysia. Previous studies dealt with only *P. damicornis* (*in situ*; Kee Alfian *et al*., 2009) and massive coral species in Malaysia (*in situ* and laboratory; Abdul Mubin, 2018). The data of the present study might serve as guidelines for coral reef health monitoring in Terengganu state island and Malaysia by researchers and reef managers respectively.

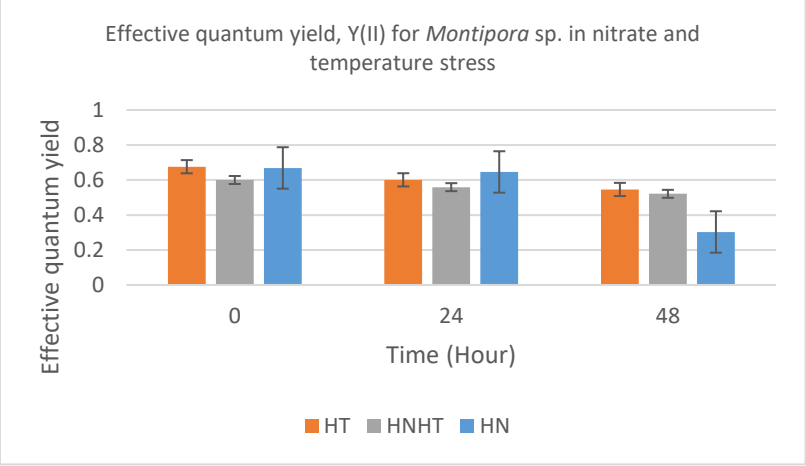

Fig. 4. Effective quantum yield Y(II) of *Montipora* sp. in high nitrate (15 µmol), (2) high temperature (HT) using 30°C and (3) combined stress of high nitrate (15 µmol), and high temperature (30°C) (n=18).

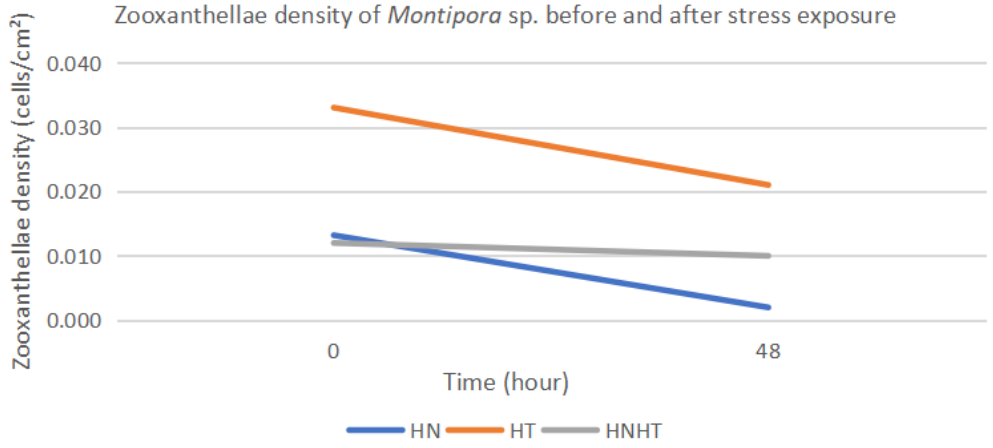

Fig. 5. Zooxanthellae density before and after being exposed to different stressors. Different colours show different type of stressors applied. HN, high nitrate; HT, high temperature; HNHT, high nitrate + high temperature.

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استجابة الفسيولوجيا الضوئية لـ *Montipora* Sp. لدرجات الحرارة المرتفعة والإجهاد الناتج عن النترات

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المستخلص. تدهورت صحة الشعاب المرجانية بشكل كبير في جميع أنحاء العالم نتيجة للأنشطة البشرية والتغيرات البيئية الطبيعية، مثل تلوث مياه الصرف الصحي وتغير المناخ بسبب الانحباس الحراري العالمي. يمكن لهذه التهديدات الكبيرة أن تعطل إنتاج الفلوريسنت المرجاني وتعدل أيضًا أداء zooxanthellae، مما قد يؤدي إلى حدوث تبييض في حالة الإجهاد المطول. في الوقت الحاضر، وبخلاف استخدام كثافة الزوكسانتيللا لتحديد صحة المرجان، بدأت الدراسات في التركيز بشكل أكبر على ملاحظة العائد الكمي الفعال، Y(II) للشعاب المرجانية باستخدام مقياس الفلورسنت بسعة النبضة (PAM)، حيث إنها طريقة فعالة وغير قاتلة لتحديد صحة المرجان. أهداف هذه الدراسة هي: (أ) قياس العائد الكمي الفعال لـ *Montipora* sp. قبل وبعد الإجهاد بالنترات ودرجة الحرارة و(ب) حساب كثافة الزوكسانتيللا لـ *Montipora* sp. بعد الإجهاد بالنترات ودرجة الحرارة. أظهرت *Montipora* sp. المعرضة لمعاملة عالية من النترات (HN) ودرجة حرارة عالية (HT) ومعاملة عالية من النترات مع درجة حرارة عالية (HNHT) انخفاضًا في قراءة Y(II) وكثافة الزوكسانتيللا بعد ٤٨ ساعة من التعرض. نتج انخفاض كبير في Y(II) عن معالجة HN بينما حدث انخفاض كبير في الزوكسانتيللا عن معالجة HT. لفهم عملية تثبيط الضوء بشكل كامل وكيف تؤثر على نظام جسم المرجان، يجب أن تتضمن الأبحاث المستقبلية فترات تجربة أطول أمدًا، ومجموعة متنوعة من أنواع المرجان والدراسات الجزيئية.

الكلمات المفتاحية: العائد الكمي الفعال، مقياس الفلورة PAM، فسيولوجيا الضوء المرجانية.