Response of scleractinian corals to nitrate enrichment in high and ambient seawater temperatures

Tengku-Mohd-Kamil, T F K; Turner, J R

IOP Conference Series: Earth and Environmental Science

DOI:
10.1088/1755-1315/736/1/012068

Published: 01/04/2021

Publisher's PDF, also known as Version of record

Response of scleractinian corals to nitrate enrichment in high and ambient seawater temperatures

To cite this article: T F K Tengku-Mohd-Kamil and J R Turner 2021 *IOP Conf. Ser.: Earth Environ. Sci.* **736** 012068

Response of scleractinian corals to nitrate enrichment in high and ambient seawater temperatures

T F K Tengku-Mohd-Kamil[1], J R Turner[2]

[1]Faculty of Science and Marine Environment, Universiti Malaysia Terengganu (UMT), 21030 Kuala Terengganu, Terengganu, Malaysia.
[2] School of Ocean Sciences, College of Natural Sciences, Bangor University, Westbury Mount, Menai Bridge, Anglesey, United Kingdom.

***** Corresponding author: tg.fara@umt.edu.my

Abstract. Coral bleaching and mortality events have recently increased in frequency and severity in the whole world. Combined effects of natural and anthropogenic impacts were assumed to be the cause for coral's health degradation. Sedimentation, urban waste, sewage discharge and agricultural activities are among the nutrient input for Malaysian waters, which can affect the coral reefs indirectly. In this study, photosynthetic performance of tropical corals under stresses were studied by exposing three common tropical scleractinian corals, *Stylophora pistillata*, *Montipora digitata* and *Seriatopora hystrix* to combination of different levels of high and ambient temperature and in a laboratory condition. Quantum yield fluorescence before, after stress and after recovery stage were recorded using a dark-adapted photochemical efficiency (F*v*/F*m*) methodology with a pulse-amplitude-modulation (PAM) chlorophyll fluorometer (WATER-PAM, Walz, Germany). Physical appearance of the corals were after the post-stress stages. The results showed that nutrient enrichment not have a synergistic effect, and that high temperatures alone significantly impact F*v*/F*m* values (three-way ANOVA, $p>0.05$) for all coral species. Slow growing corals (*S. pistillata*) appeared to cope better with the high temperatures than the fast-growing corals (*M. digitata* and *S. hystrix*). Hence, that a nitrate concentration contributed as an intial response of the symbiont's physiological changes, which can give more understanding on studying specific coral species resistance towards coral bleaching issues.

1. Introduction

Coral-zooxanthellae symbioses have the ability to survive in a low-nutrient environment, unlike any other organisms [1]. This is because they have an effective way of recycling the dissolved nitrogen in the symbiotic association [2] [3] . The low concentration of inorganic nutrients in the reef ecosystem means low primary production by symbiotic algae [4]. The productivity of a coastal ecosystem is very dependent on nutrient distribution and behaviour and the nutrient concentration is in large part due to the entry of domestic and industrial waste, urban drainage and agricultural effluents from the terrestrial ecosystem [5]. Elevated nutrient concentrations surrounding coral reef ecosystems can affect the function and composition of the zooxanthellae photosynthesis process [6] and it can cause the photosynthetic algae to alter its protein composition, increase in chlorophyll a specific absorption coefficients and reduce maximum quantum yields of PSII [6].

A study of coastline pollution in West Malaysia, identified oil palm plantations as the cause of nitrate and phosphorus enrichment in the sediment and nutrient loads of rivers and coastal waters [7]. Combined nitrification, global warming and loss of top members of the food chain occurred over the last 65 million

years [8]. Human activities increase the flux of nutrient into coastal waters causing a complex process of organic production and accumulation and change in the nutritional status of the community [9] [10]. Coastal regions often receive large anthropogenic inputs of nitrogen that cause eutrophication [11] by human activities such as agriculture and aquaculture [12]. The study by Marubini and Davies [13] found that increasing nitrate concentration in the water (1, 5 and 20 μM) caused an increase in the population density of zooxanthellae, chlorophyll a, and protein (c2) per cell. Marubini and Atkinson [14] showed that an increase in nitrate did not affect the corals' growth at all, in term of calcification rates, after being exposed to 0.5 – 5.0 μM to corals in five weeks. In a study of nitrate limitation in symbiotic dinoflagellates, photochemical efficiency of PSII (F*v*/F*m*) values were negatively correlated with external nitrogen concentrations in the culture of *Symbiodium kawagutii* and *S. pilosum* [6].

Malaysia is situated in Southeast Asia, comprising of 30% of the world's coral reef, but in 1992 (ASEAN-Australia Living Coastal Resources project) 60% of it was destroyed [15]. One of the causes of reef destruction in Southeast Asia is organic and inorganic pollution [16], with concentrations of particulate organic nitrogen (PON), ranging from <10 to 131 μM found in Malaysian creek waters, while dissolved organic nitrogen (DON) were ranging between 20-50 μM [9]. Nutrient input in Malaysian waters caused by coastal development, sewage discharge and sedimentation, need to be managed to let the corals reefs survive in Malaysian waters [17]. Somchit et al. [18] believed that the organic chemicals pollution that occurs in Malaysia originated from urbanization (sewage waters, industrial), agricultural activities (nutrients, pesticides and sediment) and transporting (boating and shipping). Bong and Lee [19] reported that dissolved inorganic nutrients (ammonium, nitrite, nitrate, phosphorus and silicate) were high in nearshore areas of West Coast, Peninsular Malaysia, due to anthropogenic activities affecting the marine water quality threatening the recreational industry, tourism, fisheries and biodiversity. In Malaysia, there are no studies relating to the nitrate levels with coral bleaching and fluorescent measurement (using PAM fluorometer). There are only studies on PAM fluorometer measurements with before-after tsunami and coral bleaching events [20]. This study is specifically designed to examine the synergistic effects of ambient/high temperature levels combined with ambient/high nitrate levels on three common Indo-Pacific coral species, *S. pistillata, M. digitata* and *S. hystrix* by data collection on maximum quantum yield (F*v*/F*m*) using PAM fluorometer. Maximum quantum yield of chlorophyll fluorescence (F*v*/F*m*) of the coral species were measured to calculate the differences between different levels of temperature and nitrate stress.

2. Experimental protocol for photochemical efficiency measurement

Experiments were performed in the laboratory using nubbins (4-5 cm) of three species of scleractinian corals, *S. pistillata*, *M. digitata* and *S. hystrix*. They were chosen because of their high and moderate susceptibility to bleaching, fast growth and abundance in Malaysian waters [21] [22]. The corals were maintained in a 100 L seawater tank in the laboratory at 27°C, under a light intensity of 200 μmol quanta $m^{-2}s^{-1}$, on a 10h light /14h dark cycle [23]. The tank was heated by a heater control unit using two metal halide lamps. The coral colonies were sourced from a sustainable lab and were acclimatized to the maintenance conditions in the laboratory aquaria. Small fragments, 4-5 cm in size, were mounted on cement plugs providing stability for the fragile organisms to be moved from and to experimental tanks.

Before and after treatments, the mounted coral nubbins were put in a separate black container box, covered with black canvas, for 20 minutes dark-adapted photochemical efficiency measurement (Fv/Fm) [24] using a pulse-amplitude-modulation (PAM) chlorophyll fluorometer (WATER-PAM, Walz, Effeltrich, Germany). The black container box was filled with seawater high enough to allow the fluorometer probe to orientate between the corals. All the coral nubbins were mounted on their cement plugs in a vertical position. Measurements were conducted on surface point of each branch of the nubbins.

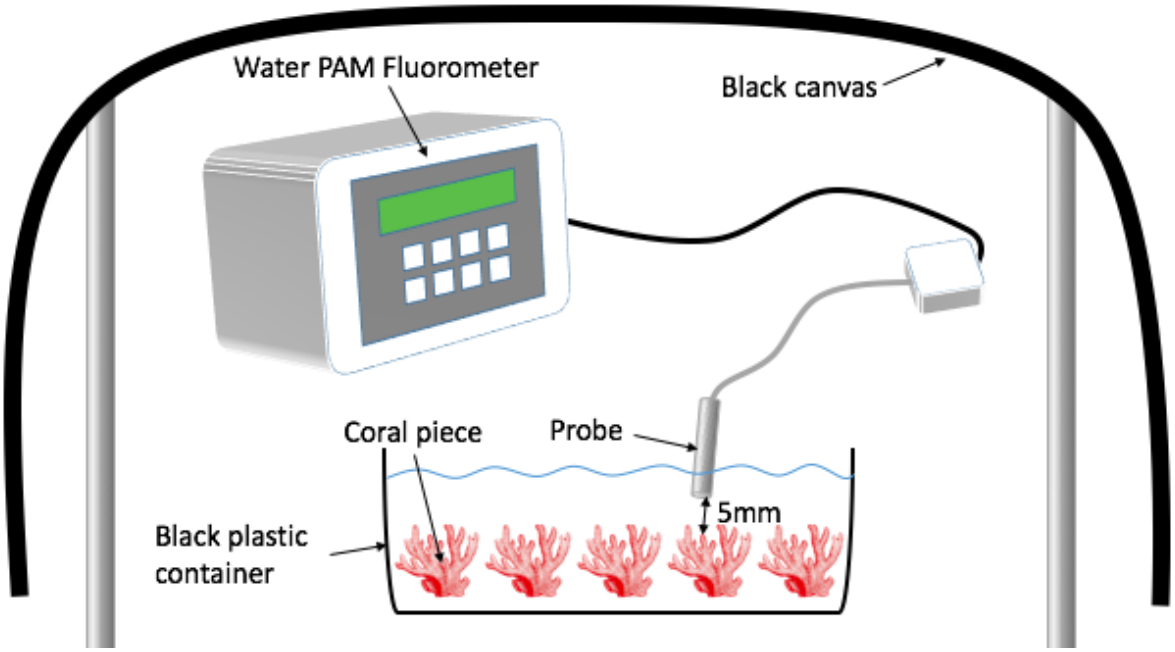

Figure 1. Measurement of Fv/Fm onto corals by Water-PAM fluorometer under a black canvas for a dark adaptation measurement, recording maximum quantum yield before stress, after stress and after 24-hr post-treatment stage.

Healthy coral nubbins were transferred from controlled conditions into experimental tanks. They were collected and straightaway put into a small cup underwater in the control tanks and moved to the experimental tank. Temperatures in the experimental tanks were stabilized for 3 h prior to experiments using a heater control unit. For the experiment, 3 aquaria (50 l each) were used for each treatment as replicates. All experimental aquaria were aerated by spreader bars to keep the water evenly heated.

In this experiment, nitrate was added to the nutrient enriched treatments as NaNO3. Nitrate was used in this study because it is the main form of nitrogen available in tropical waters besides ammonium and phosphate [13]. Ambient laboratory concentration was maintained between 1.0 - 2.0 µM as per other studies (eg. Nordermar et al. [25] recorded 1.4 – 2.1 µM is ambient nitrate concentration in a laboratory condition). The corals were exposed to 3 treatments of normal and elevated temperature and nitrates. In total, 4 treatments of normal and elevated temperature and irradiance for 3 hours. In total, 5 coral pieces were used in each of three replicates of the 4 treatments (n=5). The treatments were:

(a) ambient control (27ºC, 2 µM $NO3^-$)
(b) high nitrate (27ºC, 15 µM $NO3^-$)
(c) high temperature (30ºC, 2 µM $NO3^-$)
(d) high temperature + high nitrate (30ºC, 15 µM NO3)

After the stress-experiments, corals were brought to a shallow black water-filled container under a black canvas. Both black container and canvas allowed the corals to dark-adapt and made successive measurements of the F*v*/F*m* parameter of the samples [26]. Corals were placed 2-3 cm below the surface of the water in a shallow tank because the Water-PAM only has 10cm of waterproof probe. The probe was placed within 5mm of the corals in the water, so as to avoid air-water signal dispersion on a surface parallel to the water surface, as recommended by Hennige et al. [27]. After 20 minutes of dark-adaptation, the ratio of variable to maximal fluorescence (F*v*/F*m*) was measured on each coral. All measurements were taken on the vertical sides of tissues, 3 cm along from the branch tip [28]. This also minimized within-branch variability in photosynthetic pigments [29]. The initial fluorescence (F*o*) was measured by applying a weak pulsed red light (LED 650 nm, 0.6 kHz, 3µs). A saturating pulse of bright actinic light (8000 µmol photons m-2 s-1, width 800 ms) was then applied to give the maximal

fluorescence value (F*m*). Variable fluorescence (F*v*) was calculated as F*m* – F*o* and maximal quantum yield as F*v*/F*m* [30].

The F*v*/F*m* of each nubbin was measured in the control tank just before the stress, at the end of the 5-h stress, and after 24 h of recovery. For the following (24 h) stress, paling and mortality of the corals were also recorded. Coral colours were compared using a Coral Health Card by CoralWatch.

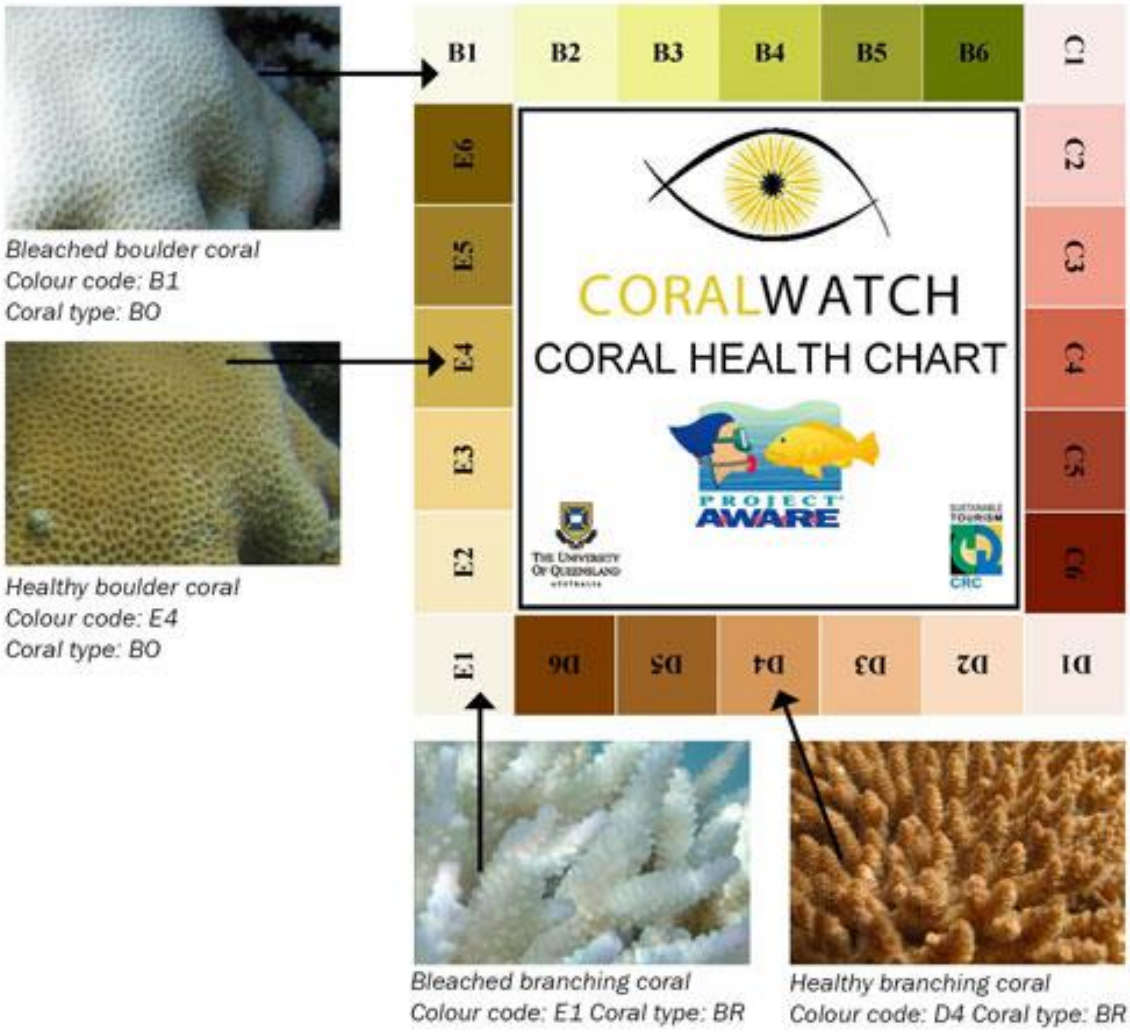

Figure 2. Coral colour before and after stress were compared with CoralWatch Coral Health Chart by CoralWatch.org.

2.1 Data Analysis

Three-way analysis of variance (3-way ANOVA) was used for comparisons of F*v*/F*m* before stress, after stress and after recovery for each species and among treatments. If the results of the experiments were not significant, the data was used in one-way ANOVA to increase the power to detect treatment effects. The post-hoc Tukey is also used for multiple comparison of means at $P<0.05$.

3. Results

3.1 Effects of different temperature and nitrate levels on the corals species

Figure 3 shows the data of F*v*/F*m* for ambient temperature and high nitrate of stress (27ºC, 15 µM NO_3^-) for the three corals, *S. pistillata*, *M. digitata* and *S. hystrix* before stress, after 5-h stress and after 24-h of recovery period. *S. pistillata* and *S. hystrix* did not show any recovery after the 5-h stress. Only *M. digitata* showed a recovery after ambient temperature level and high nitrate level of stress. This Figure 3 shows the coral species displayed a lower level of maximum quantum yield after a dark adaptation soon after 5 hours in stress experiments.

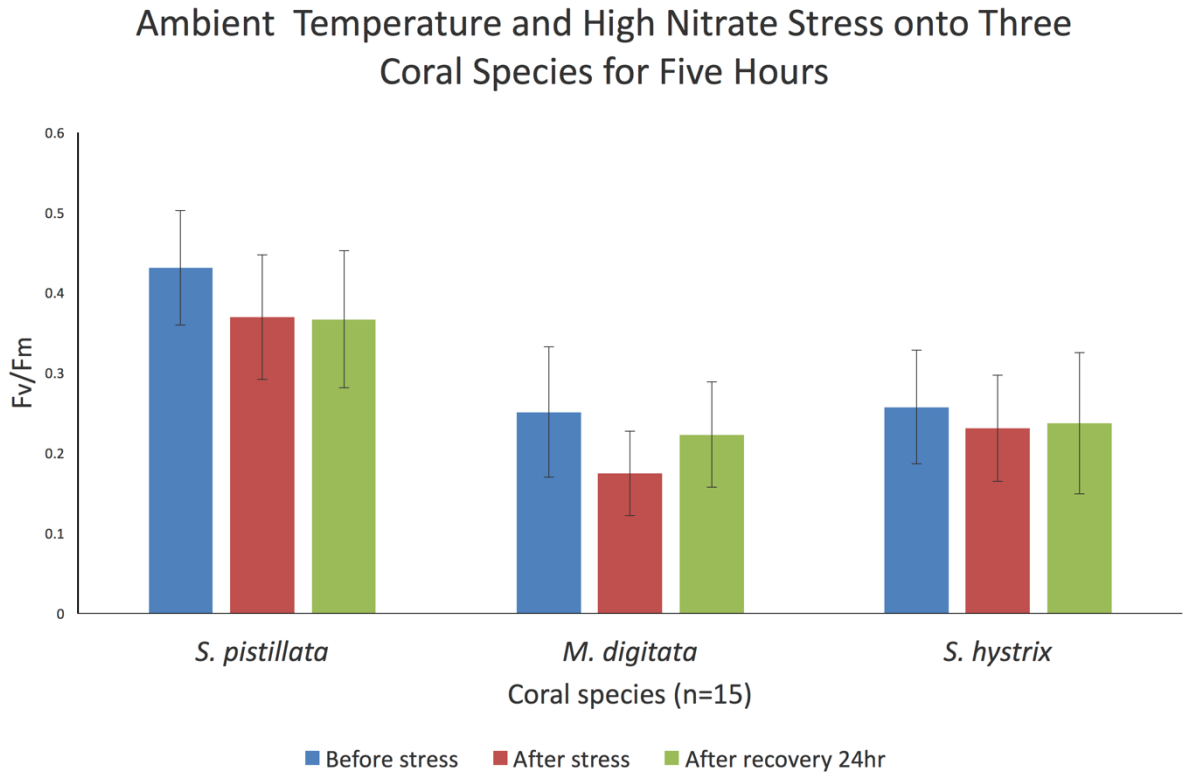

Figure 3. Maximum quantum yield measurements for three coral species before stress, after 5-h stress and after 24-h recovery stage during ambient temperature and high nitrate stress treatments.

For the second experiment (Figure 4), high temperature (30ºC) and ambient nitrate level (2 µM NO_3^-), all corals species were affected by the stress by showing low level of F*v*/F*m* measurement soon after 5-hr stress. However, after a 24-hr recovery period, all the corals species showed an increased level of maximum quantum yield compared to the after-stress data before stress condition. For after stress and after recovery, all of the coral species were not significant with each other.

In the 3rd experiment (Figure 5), high temperature and high nitrate level stress treatments (30ºC, 15 µM NO_3^-), the yield increased back after 24-hour recovery, although still lower that before exposure to stress (Figure 5).

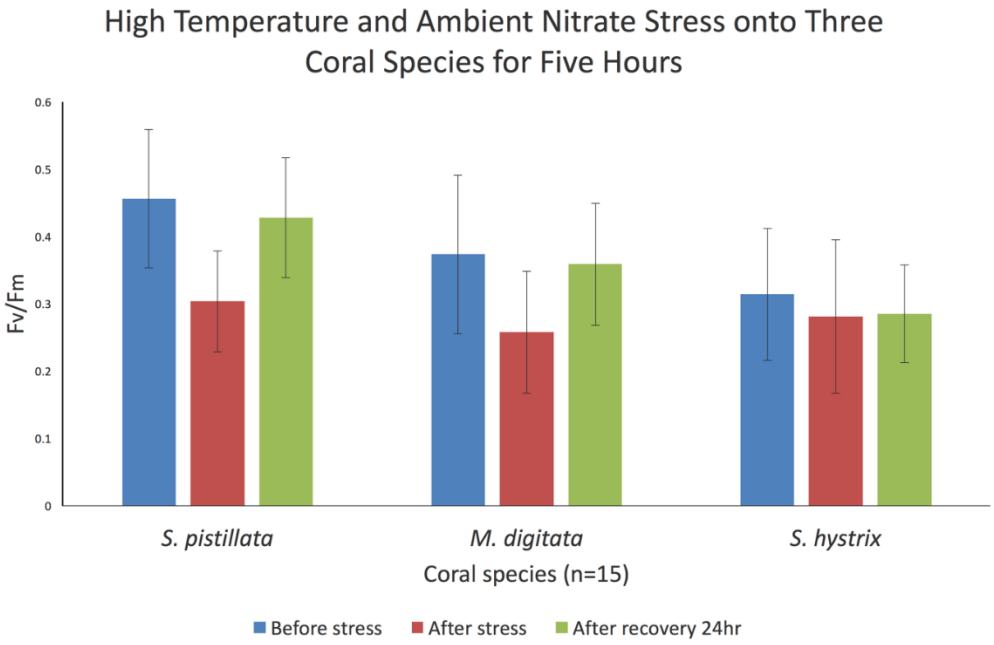

Figure 4. Graph of maximum quantum yield measurements for three coral species before stress, after 5-h stress and after 24-h recovery stage during high temperature and ambient nitrate stress treatments.

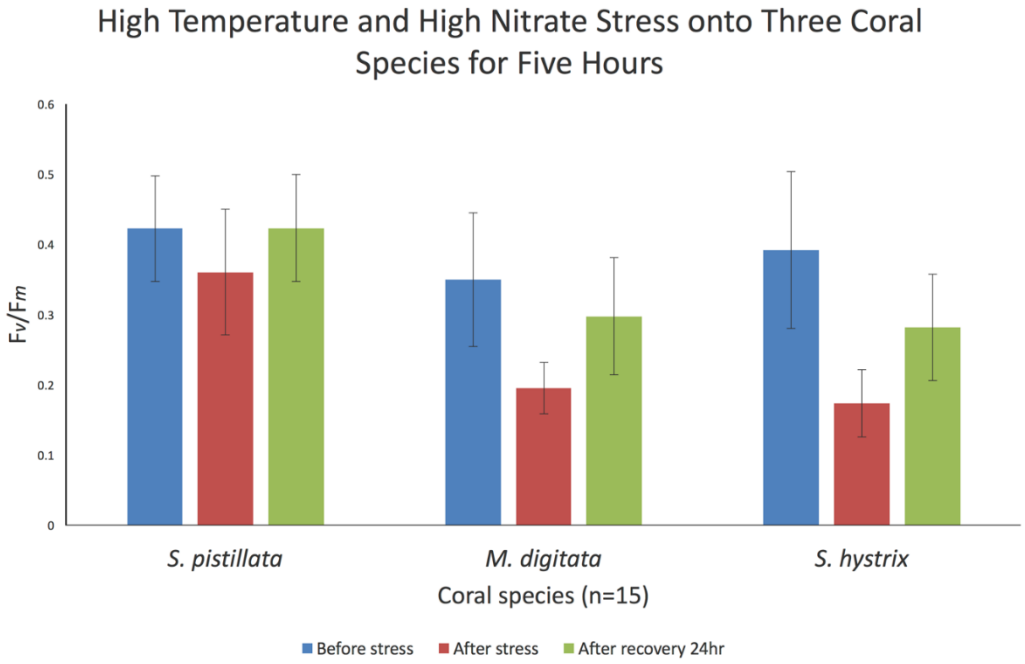

Figure 5. Graph of maximum quantum yield measurements for three coral species before stress, after 5-h stress and after 24-h recovery stage during high temperature and high nitrate stress treatments.

3.2 Maximum quantum yield ration after 5-h treatments

Table 1: Multiple comparison ANOVA for F_v/F_m mean values of *S. pistillata*, *M. digitata* and *S. hystrix* during before stress, after 5-hr stress and after 24-hr recovery period for high temperature and high nitrate stress treatments.

Dependent Variable	(I) SPC	(J) SPC	Std. Error	Sig.
beforestress	*M. digitata*	*S. hystrix*	.066154	.510
		S. pistillata	.067711	1.000
	S. hystrix	*M. digitata*	.066154	.510
		S. pistillata	.062925	.458
	S. pistillata	*M. digitata*	.067711	1.000
		S. hystrix	.062925	.458
afterstress	*M. digitata*	*S. hystrix*	.065003	.018
		S. pistillata	.066533	.019
	S. hystrix	*M. digitata*	.065003	.018
		S. pistillata	.061830	.999
	S. pistillata	*M. digitata*	.066533	.019
		S. hystrix	.061830	.999
post-treatment	*M. digitata*	*S. hystrix*	.065223	.797
		S. pistillata	.066758	.276
	S. hystrix	*M. digitata*	.065223	.797
		S. pistillata	.062040	.584
	S. pistillata	*M. digitata*	.066758	.276
		S. hystrix	.062040	.584

Maximum quantum yield for all species after stress and after recovery were analysed for each species for all treatments. Ambient temperature and ambient nitrate is the control experiment. Table 1 shows that after stress treatment, all three coral species were significantly affected by high temperature and high nitrate stress experiments.

3.3 Visual bleaching and mortality after stress treatments

In the stress experiment of ambient temperature and high nitrate, coral species responded differently in terms of paling and bleaching by comparing coral colours with CoralWatch Coral Health Chart before and after stress. For high temperature and high nitrate stress experiments (Figure 6), all coral nubbins of *M. digitata* and *S. hystrix* showed retracted polyps and pale colours immediately after the 5-h experiments where pale colour score 4 for *M. digitata* and colour score 3 for *S. hystrix*. But *M. digitata* and *S. hystrix* are fully recovered to normal conditions (after 24-h recovery stage). For high temperature and ambient nitrate stress experiments (Figure 7), 7 of the *S. hystrix* coral nubbins (out of 15) showed paling condition (the most paling colours which is colour score 2), but recovered after the 24-hour recovery stage. While three coral nubbins of *M. digitata* had retracted polyps after the stress experiment, they returned to normal condition after the recovery stage. *S. pistillata* were normal in colour throughout all the stress treatment experiments. There was no obvious "outward" physiological effect after recovery period.

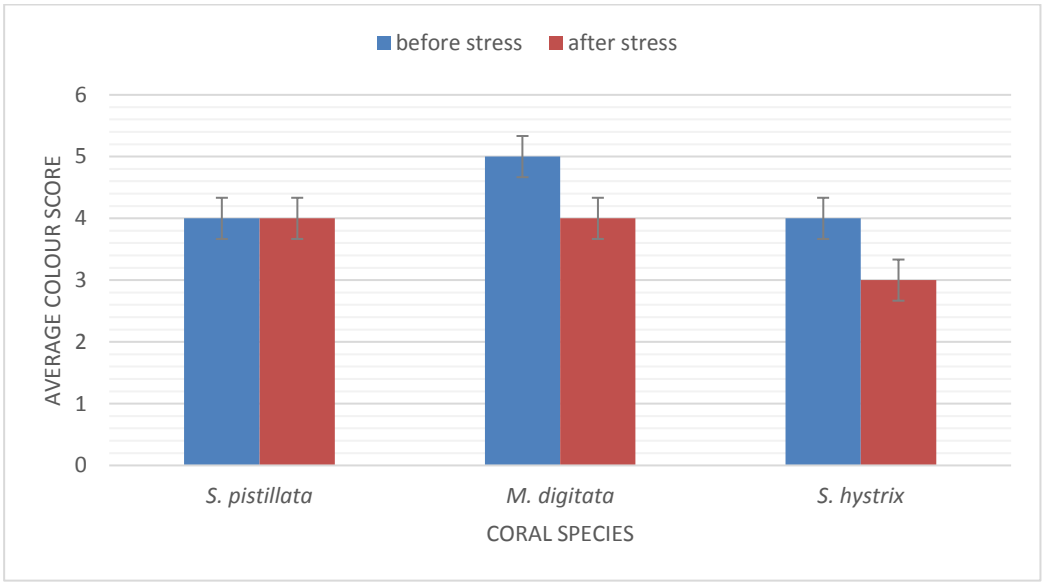

Figure 6. This bar graph shows the average colour score for coral species during normal state and after stress experiments (high temperature and high nitrate).

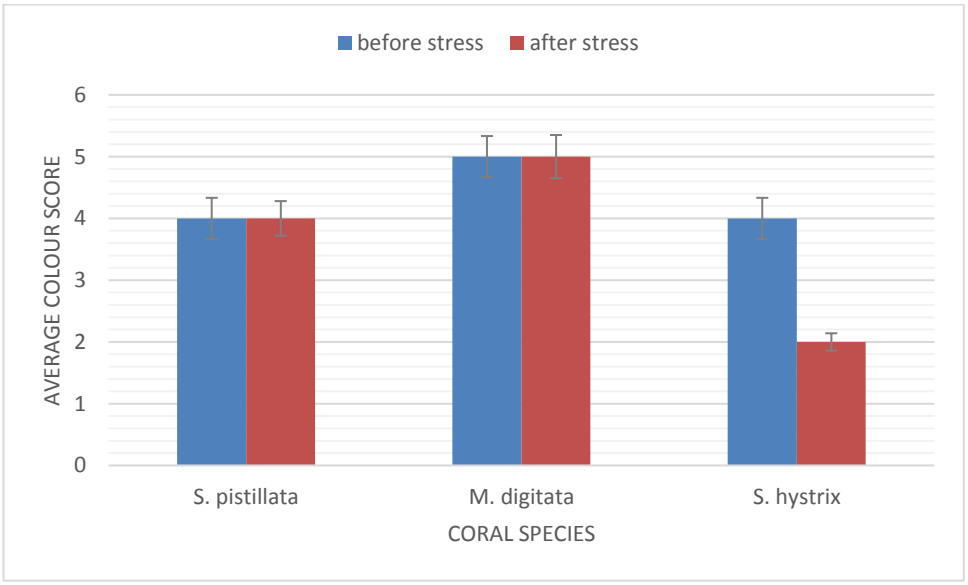

Figure 7. This bar graph shows the average colour score for coral species during normal state and after stress experiments (high temperature and ambient nitrate).

4. Discussion

4.1 Coral species response towards high temperature

The results indicate that high temperature is the dominant stressor to the coral species and the corals are more susceptible to high temperatures (30°C) than the high nitrate levels. Humanes et al. [31] stated temperature stress alone can cause impact to the coral *Acropora tenuis*, not in combination with high nitrate levels. Similarly, an elevated temperature of 32°C reduced gamete fertilization, larval survivorship and larval settlement of the coral [31]. Elevated temperature decreased rates of photosynthetic efficiency of PSII in *S. pistillata*.

4.2 Coral species respond towards nitrate stress

According to Faxneld et al. [32], high temperature (31°C) combined with nitrate enrichment and decreased salinity could lead synergistic effect. *Turbinaria mesenterina* was exposed to ambient temperature (25°C) with nitrate enrichment (+5 μM NO_3^-) plus low salinity (20ppm), the corals did not show any effects on metabolism and survival [32]. Nordermar et al. [25] suggested that corals on nutrient-exposed reefs may be more stressed when the combined effects of nitrate enrichment and elevated seawater temperature reduced the gross primary production rate of zooxanthellae. Higuchi et al. [33] also suggested that coral bleaching accelerated when *M. digitata* were exposed to high temperatures (32°C) and high nitrate concentrations (10 μM), rather than only a single stress condition. They reported that the nitrate stressor alone was not sufficient to induce stress, based on the F*v*/F*m* values during the combination of nitrate enrichment and ambient temperature or low light intensity condition. This finding is similar to this present study where the corals species had a none significant value of F*v*/F*m* during nitrate enrichment with ambient temperature stress treatment.

High nutrient levels have indirect effects on corals [33]. And also ammonium the major source for primary production of marine organisms [34] and normally, corals can tolerate low level inorganic nutrient waters, with nitrogen concentrations of 0.3 to 1 μM L^{-1} [35]. Nitrate is the major form of nitrogen that is present in tropical coastal waters [20Organisms which contain endosymbiotic zooxanthellae are effected by elevated levels of nutrients e.g. coral colony growth (i.e. coral calcification rate and linear extension) and coral larval settlement [36]. *Porites porites* and *Montastrea annularis* exhibited decreased calcification because of the nitrate enrichment of 5 μM and 20 μM [13]. According to Fabricius [37], increased dissolved inorganic nitrogen (as nitrate and ammonium) can affect zooxanthellae density, increasing the content of nitrogen and chlorophyll *a* per zooxanthella and photosynthetic rates. Nitrate-enriched condition (17 μM of NH_4^+) proved to have a greater algal standing stock and more than doubled algae densities, which means that the zooxanthellae are nutrient limited [38].

4.3 Paling colours on coral species after stress treatments

In this study, nitrate enrichment used was 15 μM, similar to Nordermar et al. [25] used 15 μM as high nitrate. Fabricius [37] had found that high nitrate alone can negatively affect the corals. This study showed that, high nitrate and high temperature; ambient nitrate and high temperature; and high nitrate and ambient temperature significantly affect the F*v*/F*m* measurement towards all coral species. Ammonium enrichment did not contribute significantly to PSI and PSII yield measurement in high temperature waters of *Turbinaria reniformis* corals [39]. Wiedenmann et al. [40] suggested that the photosynthetic efficiency of zooxanthellae can be decreased under a combination of increased temperature and light stress with imbalanced nutrient levels. They proved that increased levels of DIN (dissolved inorganic nitrogen) combined with temperature and light stress can increased a coral's susceptibility to bleaching. That can be seen in this study, after 5-h stress of combined stressors, *M. digitata* and *S. hystrix* experienced paling colours, an early sign of stressed corals. However, they recovered after 24-h in a controlled condition. For bleaching conditions, stressors can cause the breakdown of coral-algal symbiosis and zooxanthellae loss [40]. Paling coral shows a decline in density of the zooxanthellae and less pigments within the zooxanthellae [41] [42]. The pale appearance is because the cnidarian's calcareous skeleton is showing through the translucent tissues [43].

The changes in a coral colour after 5-hr stress can be related to the sensitivity of the corals used in the studies. *S. pistillata, M. digitata* and *S. hystrix* had significantly decreased F*v*/F*m* for combined high nitrate and high temperature. So, if the corals were exposed in a longer period (compared to only 5-hr stress) to either only high nitrate, or only high temperature, or by combination, they might experience bleaching. *Turbinaria mesenterina* was found to be bleached after being exposed to 24-hour stress of increased temperature (31°C), nitrate enrichment (5 μM), low salinity (20) and the combinations of the stressors [32]. Schlöder and D'Croz [44] recorded mortality of *P. damicornis* corals with treated with high nitrate and high temperatures and also pale colours for high temperatures stress treatment. The effect of nutrient enrichment on coral varies depending on the coral species [44] [45]. Lower maximum

quantum yield was found in nutrient-enriched samples of *S. pistillata* but not in *Acropora* spp. corals [45]. While in Schlöder and D'Croz [44], 90% of *P. lobata* had survived but only 30% of *P. damicornis* remained healthy after a nitrate enrichment stress treatment. According to Yuen et al. [45], slow growth corals (*S. pistillata*) can survive longer than the faster growing species (*Acropora* spp.). It is similar to the finding of Schlöder and D'Croz [44], where slow-growing species (*P. lobata*) can cope better with nitrate enrichment conditions than fast-growing species of *P. damicornis.* In this study, *S. pistillata* had shown normal colours throughout the stress experiments but not *M. digitata* and *S. hystrix*. It can be concluded that the survival level of corals species in this experiment is *S. pistillata* > *M. digitata* > *S. hystrix*, based on paling colour changes of corals after the treatments. It can be related to the previous studies, where slow growth corals (*S. pistillata*) are coping better with the environmental changes than the fast growth corals (*M. digitata* and *S. hystrix*).

This study suggests that coral health might not be affected by nutrient enrichment, but only high temperatures alone can give a significant decreased F*v*/F*m* values for all coral species. Maximum quantum yield of *S. pistillata*, *M. digitata* and *S. hystrix* were negatively affected its values by the nitrate enrichment, whether in ambient or high temperatures. There is a suggestion that both elevated temperature and nitrogen enrichment can cause a destabilized coral symbiosis [25], but this present study showed high temperature alone can significantly affect the photosynthetic yield. This study shows that the nitrate enrichment itself does not affect the F*v*/F*m* values, but if the nitrate concentrations increased to more than 10μM, in a high temperature conditions, it might increase the coral's susceptibility to bleaching [33]. Each coral species showed different responses to nutrient enrichment, in terms of their paling colours and survival. *S. pistillata* no response in terms of colouration before stress, after stress and after 24-hr recovery period all stress treatments. This study suggests that the effects of high temperature and ambient/high nitrate on corals varies according to coral species.

This study only examined the maximum quantum yield (F*v*/F*m*), and in a short period of time. Increased nutrient influxes in coral reefs surrounding have long-term consequences for the corals. Increased of nutrient levels can reduce the heat stress tolerance on corals which expose the corals to bleaching risk [46]. Nitrate effect onto coral can give significant changes of decreasing photosynthesis and calcification in symbiont in a short period of time of days to weeks [39] [36]. Nitrate concentration experiments might contribute as an initial response of the symbiont's physiologic, which lead to the understanding of corals behaviour to environmental change. The changes in the environmental conditions can affect nitrogen cycling in corals, which are the capacity of shifting within coral microbiome [47]. Besides as an initial response, a short-term nitrate stress onto corals can be resulted in changes of coral colour and physical appearances.

For future investigation, the synergistic impacts of elevated nitrate concentration with other environmental stressor (light, salinity, water motions etc.) should be further study on a larger dynamic of coral-symbiotic association (e.g. zooxanthellae density, chlorophyll *a* content) and in a longer stress-time.

References

[1] Stanley GD 2003 The evolution of modern corals and their early history *Earth-Science Reviews* **60** 195-225.

[2] Muscatine L, McCloskey LR, Marian RE 1981 Estimating the daily contribution of carbon from zooxanthellae to coral animal respiration *Limnol Oceanogr* **26** 601-610.

[3] Falkowski PG, Dubinsky Z, Muscatine L, McCloskey L 1993 Pupulation control symbiotic corals. Ammonium ions and organic materials maintain the density of zooxanthellae *BioScience* **43** 606-611.

[4] Sakka A, Legendre L, Gosselin M, Niquil N, Delesalle B 2002 Carbon budget of the planktonic food web in an atoll lagoon (Takapoto, French Polynesia) *J. Plankton Res* **24** 301-320.

[5] Praveena SM, Siraj SS, Aris AZ 2012 Coral reef studies and threats in Malaysia: a mini review *Rev Environmen Sci Biotechnol* **11** 27-39.

[6] Rodriguez-Román A, Iglesias-Prieto R 2005 Regulation of photochemical activity in cultured symbiotic dinoflagellates under nitrate limitation and deprivation *Marine Biology* **146** 1063-1073.

[7] Jakobsen F, Hartstein N, Frachisse J, Golingi T 2007 Sabah shoreline management plan (Borneo, Malaysia): Ecosystems and pollution *Ocean & Coastal Management* **50** 84-102.

[8] Knowlton N 2001 The future of coral reefs *Proceedings of the National Academy of Sciences* **98** 5419-5425.

[9] Nixon SW, Furnas BN, Lee V, Marshall N, Ong JE, Wong CH, et al. 1984 The role of mangroves in the carbon and nutrient dynamics of Malaysia estuaries *Proc. As. Symp. Mangr. Env.-Res. and Manag.*

[10] Szmant AM 2002 Nutrient enrichment on coral reefs: is it a major cause of coral reef decline? *Estuaries* **25** 743-766.

[11] Herbert RA 1999 Nitrogen cycling in coastal marine ecosystems *FEMS microbiology reviews* **23** 563-590.

[12] Suratman S, Latif MT 2014 Reassessment of nutrient status in Setiu Wetland, Terengganu, Malaysia *Asian Journal of Chemistry* **27** 239-242.

[13] Marubini F, Davies PS 1996 Nitrate Increases Zooxanthellae Population Density and Reduces Skeletogenesis in Corals *Marine Biology* **127** 319-328.

[14] Marubini F, Atkinson MJ 1999 Effects of lowered pH and elevated nitrate on coral calcification *Marine Ecology Progress Series* **188** 117-121.

[15] Wilkinson CR, Gomez LM, Ridzwan AR, Soekarno S, Sudra S. Status of coral reefs in Southeast Asia: Threats and responses. Global Aspects of Corals: Health, Hazard and History. ; 1993.

[16] Lundin CG, Lindén O 1993 Coastal Ecosystems: attempts to manage a threatened resource *Ambio* **22** 468-473.

[17] Malaysia RC 2016 Status of coral reefs in Malaysia, 2015 *RCM Annual Survey Report*.

[18] Somchit N, Somchit MN, Hadi SA, Zakaria MP 2009 Persistent organic chemicals in Malaysian waters: a review *Research Journal of Environmental Toxicology* **3** 101-112.

[19] C.W. B, Lee CW 2008 Nearshore and offshore comparison of marine water quality variables measured during SESMA 1 *Malaysian Journal of Science* **27** 25-31.

[20] Abdul-Adzis KA, Amri AY, Oliver J, Mohamed CAR 2009 Effective and maximum quantum yield of the lace coral Pocillopora damicornis (Anthozoa: Scleractinia: Pocilloporidae) in Pulau Tioman, Malaysia *Journal of Science and Technology in the Tropics* **5** 13-17.

[21] Mazlan AG, Zaidi CC, Wan-Lotfi WM, Othman BHR 2005 On the current status of coastal marine biodiversity in Malaysia *Indian Journal of Marine Sciences* **34** 76-87.

[22] Loh WK, Loi T, Carter D, Hoegh-Gulberg O 2001 Genetic variability of the symbiotic dinoflagellates from the wide ranging coral species Seriatopora hystrix and Acropora longicyathus in the Indo-West Pacific *Marine Ecology Progress Series* **222** 97-107.

[23] Warner ME, Fitt WK, Schmidt GW 1999 Damage to photosystem II in symbiotic dinoflagellates: a determinant of coral bleaching *National Academy of Sciences* **96(14)** p. 8007-8012.

[24] Hoegh-Guldberg O, Jones RJ 1999 Photoinhibition and photoprotection in symbiotic dinoflagellates from reef-building corals *Marine Ecology Progress Series* **183** 73-86.

[25] Nordemar I, Nyström M, Dizon R 2003 Effects of elevated seawater temperature and nitrate enrichment on the branching coral Porites cylindrical in the absence of particulate food *Marine Biology* **142** 669-677.

[26] Bhagooli R, Hidaka M 2002 Physiological responses of the coral Galaxea fascicularis and its algal symbiont to elevated temperatures *Journal of the Japanese Coral Reef Society* **2002** 33-42.

[27] Hennige SJ, McGinley MP,GAG, Warner ME 2011 Photoinhibition of Symbiodinium spp. within the reef corals Montastraea faveolata and Porites astreoides: implications for coral bleaching *Marine Biology* **158** 2515-2526.

[28] Jones RJ, Hoegh-Guldberg O 2001 Diurnal changes in the photochemical efficiency of the symbiotic dinoflagellates (Dinophyceae) of corals: photoprotection, photoinactivation and the relationship to coral bleaching *Plant, Cell and Environment* **24** 89-99.

[29] Anthony KR, Connoly SR, Hoegh-Guldberg O 2007 Bleaching, energetics, and coral mortality risk: Effects of temperature, light, and sediment regime *Limnology and oceanography* **52** 716-726.

[30] Ferrier-Pagès C, Richard C, Forcioli D, Allemand D, Pichon M, Shick JM 2007 Effects of temperature and UV radiation increases on the photosynthetic efficiency in four scleractinian coral species *The Biological Bulletin* **213** 76-87.

[31] Humanes A, Noonan SHC, Willis BL, Fabricius KE, Negri AP 2016 Cumulative Effects of Nutrient Enrichment and Elevated Temperature Compromise the Early Life History Stages of the Coral Acropora tenuis *PLoS ONE* **11** e0161616.

[32] Faxneld S, Jörgensen TL, Tedengren M 2010 Effects of elevated water temperature, reduced salinity and nutrient enrichment on the metabolism of the coral Turbinaria mesentria. Estuarine *Coastal and Shelf Science* **88** 482-487.

[33] Higuchi T, Yuyama I, Nakamura T 2015 The combined effects of nitrate with high temperature and high light intensity on coral bleaching and antioxidant enzyme activities *Regional studies in marine science* **2** 27-31.

[34] Codispoti LA 1989 Phosphorus vs. nitrogen limitation of new and export production *Productivity of the Ocean: Present and Past* 377-394.

[35] Grover R, Maguer JF, Allemand D, Ferrier-Pagès C 2003 Nitrate uptake in the scleractinian coral Stylophora pistillata *Limnology and Oceanography* **48** 2266-2274.

[36] Koop K, Booth D, Boadbent A, Brodie J, Bucher D, Capone D, et al. 2001 ENCORE. The Effect of Nutrient Enrichment on Coral Reefs. Synthesis of Results and Conclusions *Marine Pollution Bulletin* **42** 91-120.

[37] Fabricius KE 2005 Effects of terrestrial runoff on the ecology of corals and coral reefs: review and synthesis *Marine Pollution Bulletin* **50** 125-146.

[38] Stimson J, Kinzie RA 1991 The temporal pattern and rate of release of zooxanthellae from the reef coral Pocillopora damicornis (Linnaeus) under nitrogen-enrichment and control conditions *Journal of experimental marine Biology and Ecology* **153** 63-74.

[39] Béraud E, Gevaert F, Rottier C, Ferrier-Pagès C 2013 The response of the scleractinian coral Turbinaria reniformis to thermal stress depends on the nitrogen status of the coral holobiont *Journal of Experimental Biology* jeb-085183.

[40] Wiedenmann J, D'Angelo C, Smith EG, Hunt AN, Legiret FE, Postle AD,AEP 2012 Nutrient enrichment can increase susceptibility of reef corals to bleaching *Nature Climate Change*.

[41] Hoegh-Guldberg O, Smith GJ 1989 Influence of the Population-Density of Zooxanthellae and Supply of Ammonium on the Biomass and Metabolic Characteristics of the Reef Corals Seriatopora hystrix and Stylophora pistillata *Marine Ecology Progress Series* **57** 173-186.

[42] Kleppel GS, Dodge RE, Reese CJ 1989 Changes in pigmentation associated with the bleaching of stony corals *Limnology and Oceanography* **34** 1331-1335.

[43] Bucheim J 2005 Coral reef bleaching *Journal of Odyssey Expeditions, Tropical Marine Biology Voyages* **10** PMCid: PMC1739595.

[44] Schlöder C, D'Croz L 2004 Responses of massive and branching coral species to the combined effects of water temperature and nitrate enrichment *Journal of Experimental Marine Biology and Ecology* **313** 255-268.

[45] Yuen YS, Nakamura T, Yamazaki SS'YH 2008 Long-term effects of inorganic nitrogen enrichment on the reef-building corals Stylophora pistillata and Acropora spp. *11th International Coral Reef Symposium*.

[46] D'Angelo C, Wiedenmann J 2014 Impacts of nutrient enrichment on coral reefs: new perspectives and implications for coastal management and reef survival *Current Opinion in Environmental Sustainability* **7** 82-93.

[47] Rädecker N, Pogoreutz C, Voolstra CR, Wiedenmann J, Wild C 2015 Nitrogen cycling in corals: the key to understanding holobiont functioning? *Trends in Microbiology* **23** 490-497.

[48] Tan ASH, Ismail IA, Yasin Z 2009 Diversity of Family Fungiidae in Malaysian Waters *Publication of the Seto Marine Biological Laboratory - Special Publication Series* **9** 15-24.

[49] Hallock P 2005 Global change and modern coral reefs: New opportunities to understand shallow-water carbonate depositional processes *Sedimentary Geology* **175** 19-33.

[50] Tomascik T, Sander F 1985 Effects of eutrophication on reef-building corals *Marine Biology* **87** 143-155.

[51] Tomascik T 1990 Growth rates of two morphotypes of Montastrea annularis along a eutrophication gradient, Barbados, WI *Marine Pollution Bulletin* **21** 376-381.

[52] Hodgson G, Yah EPM 1997 Physical and biological controls of coral communities in Hong Kong *Proceedings of the Eight International Coral Reef Symposium* p. 459-461.

[53] Morton B 1994 Hong Kong's coral communities: Status, threats and management plans *Marine Pollution Bulletin* **29** 74-83.

[54] Loya Y 2004 The coral reefs of Eilat – past, present and future: three decades of coral community structure studies *Coral Reef Health and Disease* (Springer).

[55] Stimson J, Kinzie RA 1991 The temporal pattern and rate of release of zooxanthellae from the reef coral Pocillopora damicornis (Linnaeus) under nitrogen-enrichment and control conditions *Journal of experimental marine Biology and Ecology* **153** 63-74.

[56] van Woesik R, Tomascik T, Blake S 1999 Coral assemblages and physico-chemical characteristics of the Whitsunday Islands: evidence of recent community changes *Marine and Freshwater Research* **50** 427-440.

[57] Cortes J, Risk MJ 2008 A coral reef under siltation stress: Cahuita, Costa Rica *Bulletin of Marine Science* **36** 339-365.

[58] Godinot C, Houlbrèque F, Grover R, Ferrier-Pagès C 2011 Coral Uptake of Inorganic Phosphorus and Nitrogen Negatively Affected by Simultaneous Changes in Temperature and pH *PLoS ONE* **6** e25024.

[59] Li X, Huang H, Lian J, Huang L, Dong J 2009 Effects of the multiple stressors high temperature and reduced salinity on the photosynthesis of the hermatypic coral Galaxea fascicularis *Acta Ecologica Sinica* **29** 155-159.

[60] Kinsey DW, Davies PJ 1979 Effects of elevated nitrogen and phosphorus on coral reef growth *Limnology Oceanography* **24** 935-940.