Malaysian Applied Biology (2023) 52(2): 21-31
https://doi.org/10.55230/mabjournal.v52i2.2510

Research Article

Symbiodiniaceae Density Pattern in Relation To Colony Morphology of Scleractinian Corals in Pulau Tioman and Pulau Bidong, Malaysia

Muhammad Arif Samshuri[1], Che Din Mohd Safuan[2]*, Nur Atiqah Maznan[1], Siti Nurtahirah Jaafar[1,2] and Zainudin Bachok[2]

1. Faculty of Science and Marine Environment, Universiti Malaysia Terengganu, 21030 Kuala Nerus, Terengganu, Malaysia
2. Institute of Oceanography and Environment, Universiti Malaysia Terengganu, 21030 Kuala Nerus, Terengganu, Malaysia
Corresponding author: chedinmohdsafuan@umt.edu.my

ABSTRACT

A healthy coral reef is linked to the mutualistic relationship between scleractinian coral and the symbiotic Symbiodiniacea (SD). However, there is limited research on SD in Malaysia, despite its important role in reef-building coral. In this study, the SD density of scleractinian corals from the family Acroporidae was evaluated to (i) examine the pattern of SD density and (ii) comprehend the regulation of the SD density by the host. The mean SD density ranged between 0.46 ± 0.01 × 10^6 cell cm^{-2} and 2.98 ± 1.17 × 10^6 cell cm^{-2}. It is hypothetically proven that the SD density differed significantly between genera and morphological factors such as colony surface area (CSA) and dry weight coral tissue per unit colony surface area (DWCT/CSA) were significantly correlated with the SD density. The results show that the significant variation in SD density among coral genera can be influenced by coral growth forms and tissue biomass. There was a significant relationship between SD density and CSA as well as DWCT/CSA. Coral genera with a wider CSA and lower DWCT/CSA such as *Anacropora* and *Acropora* with branching, digitate and tabulate growth forms contained lower SD density than massive, laminar, and encrusting such as *Montipora* and *Astreopora* which hold more DWCT/CSA at lower CSA, resulting in higher SD density. The findings provide valuable information on SD density in different types of corals from the southern part of the South China Sea and reveal the coral host's SD regulation.

Key words: Symbioniaceae, scleractinian coral, symbiotic algae, growth form, South China Sea

Article History
Accepted: 11 May 2023
First version online: 30 June 2023

Cite This Article:
Samshuri, M.A., Safuan, C.D.M., Maznan, N.A., Jaafar, S.N. & Bachok, Z. 2023. Symbiodiniaceae density pattern in relation to colony morphology of scleractinian corals in Pulau Tioman and Pulau Bidong, Malaysia. Malaysian Applied Biology, 52(2): 21-31. https://doi.org/10.55230/mabjournal.v52i2.2510

INTRODUCTION

Symbiodiniaceae (SD) is a type of dinoflagellate that lives in a mutual relationship with scleractinian corals and helps to keep the reef healthy by providing them with energy daily (Papina *et al*., 2003; Teece *et al*., 2011; Muller-Parker *et al*., 2015). The SD plays an important role in reef structure formation (Fransolet *et al*., 2012) by enhancing the calcification rate of scleractinian coral (Ladrière *et al*., 2008). The SD transfers over 90% of the energy excess (in terms of the carbon they produce through photosynthesis) to their coral host (Davies, 1984). The carbon was used by coral for growth and respiration from its SD and under low light conditions, SD could not provide enough carbon for coral energy metabolism (Treignier *et al*., 2008). These SD also provide the coral with essential nutrients such as lipids and fatty acids, which help to meet its daily energy needs (Papina *et al*., 2003; Teece *et al*., 2011).

Healthy corals possessed a high density of SD, ranging from 0.88 × 10^6 cm^{-2} to more than 8 × 10^6 cells cm^{-2} of coral surface (Muller-Parker *et al*., 2015). Several studies have shown that the SD density varied among the species, even within the same genus (Fitt *et al*., 2000; Li *et al*., 2008). For instance, in the Caribbean region, Fitt *et al*. (2000) found that the SD density in *Acropora palmata* is significantly higher compared to *Ac. cervicornis*. In another study from the tropical island located in southernmost China, *Ac. pulchra* contained higher SD density compared to *Ac. hyacinthus* (Li *et al*., 2008).

Several factors are known to influence the SD density in scleractinian corals. On the environmental aspect, depth was found to affect the SD density in scleractinian corals. For example, Li *et al*. (2008) found that corals living at 4 m depth have a higher SD density than those living at the bottom (~7 m depth). Similarly, higher SD density in *Stylophora pistillata* was found at 3 m depth (4.5 × 10^6 cells cm^{-2}) than at 35 m depth (3 × 10^6 cells cm^{-2}) (McCloskey & Muscatine, 1984), while Al-Hammady (2013) found that SD density in *Ac. hemprichii* at depths less than 10 m (1.55 ± 0.303 × 10^6 cells cm^{-2}) was twice as high as at 25 m depth (0.71 ± 0.25 × 10^6 cells cm^{-2}). Besides that, SD density was

also affected by seasonal cycles in response to the change in environmental conditions (Fagonee *et al*., 1999; Pillay *et al*., 2005; Mwaura *et al*., 2009). Time-series data shows that SD density was higher during the colder season as compared to the warmer season (Fagoonee *et al*., 1999; Fitt *et al*., 2000). It is indicated that light and temperature might influence the SD density in *Montastrea annularis, M. faveolata, M. franksi, Ac. palmata* and *Ac. cervicornis* from the Caribbean region (Fitt *et al*., 2000). Meanwhile, in the Great Barrier Reef, a similar trend was also observed in *Ac. millepora* where higher SD density was found in August when seawater temperatures are lower than in February (Pillay *et al*., 2005). However, in another study, Mwaura *et al*. (2009) reported that some coral species display higher SD density during the northeast monsoon when the seawater temperature and radiation levels are the highest. The inconsistency between the studies specifies that other environmental parameters influence the population density of SD in scleractinian corals (Leletkin, 2000; Mwaura *et al*., 2009).

In another aspect, the host itself can regulate the density of SD in their tissue. Using the example of scleractinian coral *Plerogyra* sp., Drew (1972) implied that the SD density is regulated by the coral host. An experimental investigation demonstrated a substantial decline in the SD density in *M. annularis* as depth increased due to the reduction in the volume of its living tissue, which subsequently reduced the coral's metabolic demand on the SD (Dustan, 1979). Likewise, a decrease in coral tissue as depth decreased was found in *Fungia* spp. collected in tropical Micronesia, and a corresponding decrease in SD density was observed (Masuda *et al*., 1993). Apart from that, different morphologies of scleractinian corals have also influenced the SD density. For example, branching corals contained a lower SD density compared to massive corals (Drew, 1972; Li *et al*., 2008). Even within the species, the SD density in *Ac. millepora* varied between colonies and branches (Pillay *et al*., 2005).

Over the past two decades, coral reefs worldwide are continuously degraded and bleaching is one of the phenomena that contribute to coral mortality (De'ath *et al*., 2012; Guest *et al*., 2012; Phongsuwan *et al*., 2013). Therefore, there is an urgent need to understand the mechanism of coral bleaching to apply mitigation measures to coral reefs. The increasing incidence of coral bleaching and concern for the future of coral reef ecosystems have heightened interest in the study of SD density in the face of climate change. Assessing the variation in SD density is crucial for predicting the vulnerability of scleractinian coral to environmental changes, as SD plays a significant role in coral nutrition and their response to environmental perturbation (Muller-Parker *et al*., 2015; Xu *et al*., 2017). In Malaysia, there is limited research on SD, and most of the studies focused on the aspects of coral reproduction (Chelliah *et al*., 2015; Tan *et al*., 2020a), biodiversity (Mazlan *et al*., 2005), coral community structure survey (Ibrahim *et al*., 2006; Toda *et al*., 2007; Safuan *et al*., 2016; 2018; 2020), and SD genotyping (Tan *et al*., 2020b; Lee *et al*., 2022). Following the importance of SD density data in scleractinian corals, a baseline assessment of SD density was conducted in Pulau Bidong and Pulau Tioman, both located on the east coast of Peninsular Malaysia. This study aims to investigate the pattern of SD density in different growth forms of scleractinian corals and their relationship with tissue biomass. The resilience of certain coral reef ecosystems is associated with healthy corals; thus, it would contribute crucial information vital for developing coral reef ecosystem management.

MATERIALS AND METHODS

Study area

Examination of SD density in scleractinian corals was determined from the coral species collected from the fringing reefs in Pulau Bidong and Pulau Tioman in July and August 2017. Samples were collected via SCUBA diving at three sites in Pulau Bidong (5°37'7.79"N, 103° 3'49.01"E) and two sites in Pulau Tioman (2°48'33.75"N, 104° 8'58.38" E) as shown in Figure 1. Samples were collected at depths ranging from 3 m to 15 m. 12 healthy species of coral from the family Acroporidae (*Acropora, Montipora, Astreopora* & *Anacropora*) were collected at different sites (Table 1).

Sample collection

In this study, samples collected consist of *Acropora, Montipora, Astreopora* and *Anacropora*. (Figure 2). Samples were collected using a hammer and chisel by breaking them into smaller fragments. Samples collected for branching (*An. forbesi*, *Ac. muricata*), digitating (*Ac. digitifera*), and tabulating (*Ac. specifera*, *Ac. hyacinthus*, *Ac. florida*) coral species were taken by breaking approximately 5 cm to 7 cm length of coral fragments. Meanwhile, around 5 cm × 5 cm of the fragment were taken from each encrusting (*M. aequituberculata*, *M. informis*), laminar (*M. hispida*), and massive (*As. ocellata*, *As. sugessta*, *As. myriopthalma*) coral species. In this study, only healthy corals were selected based on intact tissues, and no sign of predation or diseases. From the intensity of colour, the selection of healthy coral species was done using Coral Color Reference Card developed by Siebeck *et al*. (2006) and all samples was taken from non-shaded areas to prevent inadequate light exposure from affecting the SD density. In total, three fragments per colony were taken for each coral species (colony replicated, n=3). Depending on the species, samples were collected at a depth ranging from 3 m to 20 m. In the field, all coral fragments were placed separately in transparent plastic bags and kept in a container filled with ice before reaching land. Back on land, samples were directly kept in a freezer at -20°C before further analysis. Identification of hard coral samples was done in the laboratory based on external morphology (underwater photograph and skeleton) following Veron (2000).

Sample preparation

Each sample of coral tissue was removed from the coral skeleton using a jet of high-pressure air from an artist airbrush containing chilled distilled water (80 psi, approximately 1 cm distance from the tip of the airbrush to the coral) and placed in a thick, transparent polyethene bag inside a container with ice (Johannes & Wiebe, 1970). To maximize the tissue removal from the skeleton, the fragment was sprayed for 5 min and visibly confirmed by the exposed white surface coral skeleton. The tissue slurry was then poured into a 50 mL tube and the plastic bag was rinsed using the airbrush to remove the remaining tissue from the bag. The total volume of the tissue slurry was then recorded.

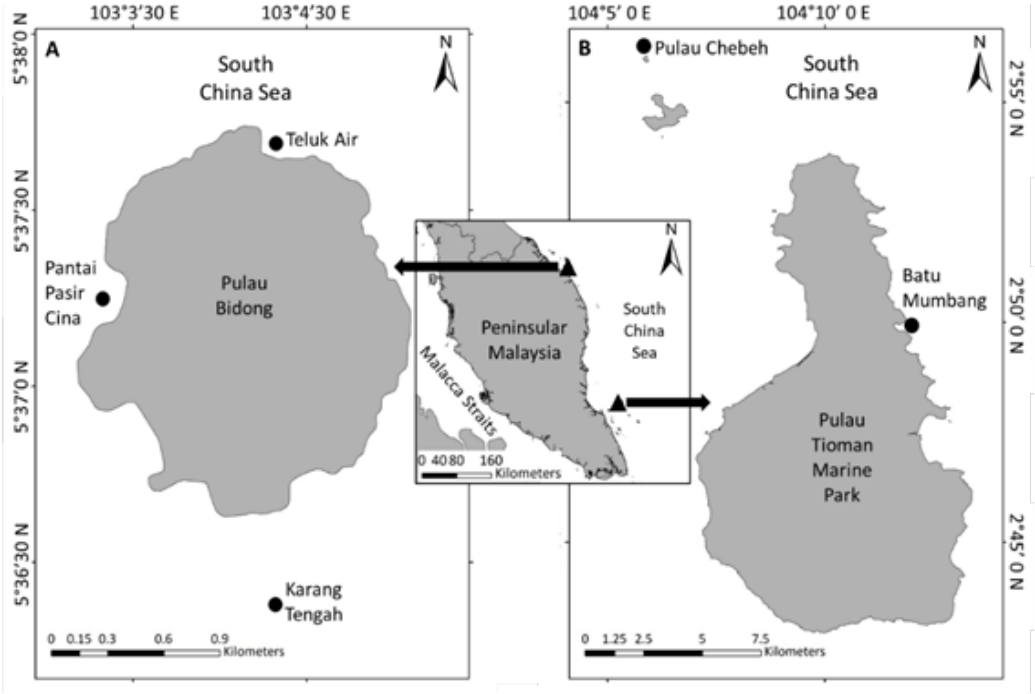

Fig. 1. Map (middle) shows the location of Pulau Bidong (PB) and Pulau Tioman Marine Park (PTMP) on the east coast of Peninsular Malaysia. Samples were collected in three sites at Pulau Bidong (A) and two sites at Pulau Tioman (B).

Table 1. List of 12 species of hard corals collected from sites in Pulau Bidong, PB (Karang Tengah, Pantai Pasir Cina & Teluk Air) and Pulau Tioman Marine Park, PTMP (Batu Mumbang & Pulau Chebeh)

Coral Genus	Coral Species	Code	Site	Depth (m)
Acropora	*Ac. spicifera*	ACP1	Pulau Chebeh (PTMP)	3
		ACP1	Pulau Chebeh (PTMP)	15
	Ac. digitifera	ACP2	Pantai Pasir Cina (PB)	3
		ACP2	Batu Mumbang (PTMP)	10
	Ac. hyacinthus	ACP3	Pantai Pasir Cina (PB)	3
		ACP3	Karang Tengah (PB)	10
		ACP3	Pulau Chebeh (PTMP)	15
	Ac. muricata	ACP4	Pantai Pasir Cina (PB)	3
		ACP4	Karang Tengah (PB)	15
	Ac. florida	ACP5	Pantai Pasir Cina (PB)	3
		ACP5	Karang Tengah (PB)	15
Montipora	*M. aequituberculata*	MON1	Karang Tengah (PB)	15
		MON1	Batu Mumbang (PTMP)	5
	M. hispida	MON2	Karang Tengah (PB)	15
		MON2	Pulau Chebeh (PTMP)	10
	M. informis	MON3	Karang Tengah (PB)	15
		MON3	Teluk Air (PB)	5
Astreopora	*As. ocellata*	AST1	Pulau Chebeh (PTMP)	15
		AST1	Pulau Chebeh (PTMP)	20
	As. sugessta	AST2	Teluk Air (PB)	15
		AST2	Teluk Air (PB)	10
	As. myriophthalma	AST3	Teluk Air (PB)	15
		AST3	Teluk Air (PB)	10
Anacropora	*An. forbesi*	ANA1	Batu Mumbang (PTMP)	10

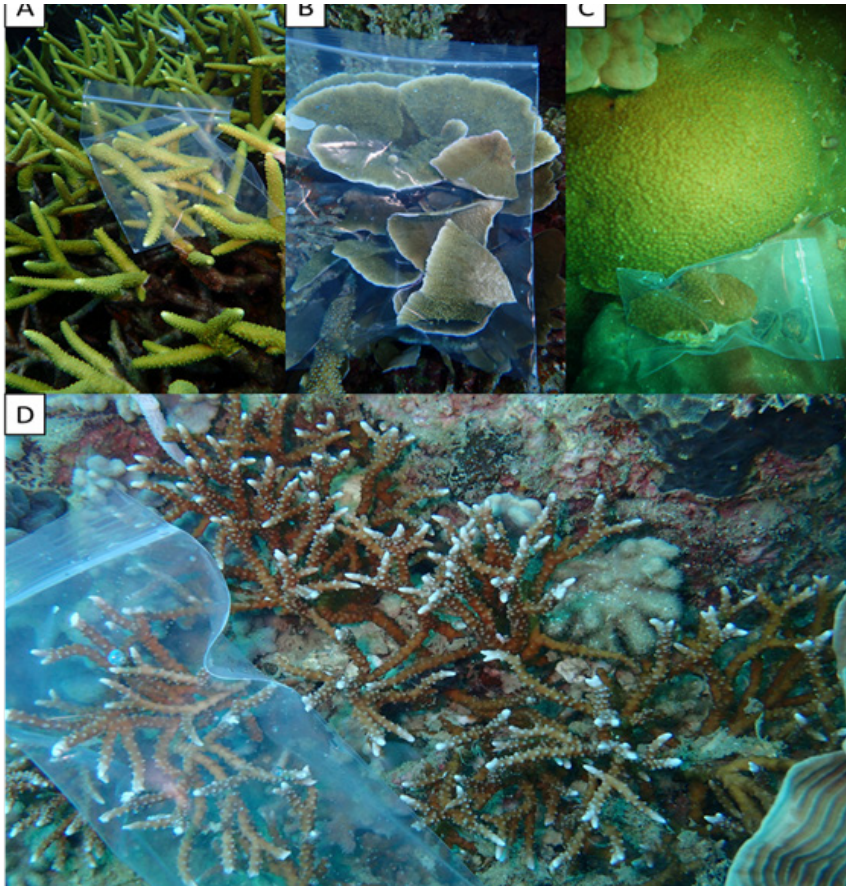

Fig. 2. Coral fragments collected in PTMP and PB. Coral from genus *Acropora* (A), *Montipora* (B), *Astreopora* (C) and *Anacropora* (D) were collected.

Tissue biomass

The remaining tissue slurry was filtered using a known weight of blank filter paper (Sartorius, Glass-Microfibre Discs, MGC, Ø 47 mm, & Retention Rate 1.2 µm) to trap the coral tissue and their SD. The sample was then freeze-dried (Labconco FreeZone 4.5 Liter Benchtop Freeze Dry System) for 12 hr at -40°C to remove the excess water from the sample. The tissue biomass of each sample, expressed as the dry-weight coral tissue (DWCT), was then determined by subtracting the dried sample's weight on filter paper from a previously known weight of blank filter paper.

Colony surface area

The denuded coral skeleton was used for determining the coral surface area (CSA). The CSA was determined using the 'aluminium foil' technique (Marsh, 1970). The fragment of the denuded coral skeleton was carefully wrapped, covering the entire surface with aluminium foil. The foil was then weighted, and its surface area was determined using a calibration curve plot ($y = 0.0044x + 0.0001$, r^2=0.9996, n=15) of the weights of aluminium foil (y) with known surface area (x). The surface area of the foil was used to represent the surface area of the denuded skeleton. The DWCT for each sample was standardized by dividing the weight of coral tissue with the CSA (DWCT/CSA).

Symbiodiniaceae cells count

The SD density was determined using a haemocytometer (Neubauer Improved Tiefe Fepth Profoundeur, 0.100 mm). The tissue slurry was shaken vigorously, and the sub-sample was placed onto the haemocytometer using a glass pipette and covered with a glass coverslip. The sample was viewed under 40 × magnification using a Leica DME compound microscope. The number of SD cells was calculated from the replicate haemocytometer count (n=8). The SD density (× 10^6 cells cm^{-2}) was calculated by multiplying the number of cells by 10^4 (to get 1 mL of sample in 0.0001 mL of haemocytometer chamber) by the total volume of the tissue slurry (mL) and dividing by the CSA.

Statistical analysis

The analysis of PERMANOVA was used to test the SD density between coral genera and growth forms based on the Bray-Curtis similarity index (Bray & Curtis, 1957) using normalized data with log (x + 1) transform function. Any significant data was further tested with PERMANOVA pairwise analysis. Similarly, PERMANOVA was also used to find any differences in pooled data of SD density, CSA, and DWCT/CSA between coral genera and colony growth forms based on Euclidean distance on standardizing data. PERMANOVA pairwise comparison was

also used for any significant data to find out the possible pair comparison that causes the differences. To obtain multivariate plots that follow the null hypotheses of 'no difference factors' (genus and colony form), a canonical analysis of principal coordinates (CAP) was used to illustrate the data's distribution plots. Vectors were then projected to the CAP diagram to determine the variables that contribute to the pooled data's separation by using the genus as a factor group. To further understand the relationship of SD density with CSA and DWCT/CSA, Spearman rank correlation was used. PERMANOVA and CAP analyses were done using PRIMER-E version 6 with PERMANOVA add-on while the correlation was performed by SPSS IBM statistical software.

RESULTS

Density of Symbiodiniaceae

The mean SD density of scleractinian corals collected at 3-5 m depth ranges from 0.46 ± 0.01 × 10^6 cells cm^{-2} to 2.98 ± 1.17 × 10^6 cell cm^{-2} (Table 2). There were clear differences in SD density among different genera and colony growth forms, as revealed by PERMANOVA analysis (Table 3). The pairwise test indicated that all genera were statistically different from each other. No significant difference was found between BC vs. TB, BC vs. DG, TB vs. DG, EN vs. LM, EN vs. TB, and LM vs. TB as indicated by a pairwise test (Table 3).

Colony surface (CSA) area and tissue biomass (DWCT/CSA)

In this study, two variables (CSA and DWCT/CSA) were used to investigate their influence on the SD density in scleractinian corals (Table 4). PERMANOVA analysis showed that both CSA and DWCT/CSA differed significantly among genera and colony growth forms (Table 5). A pairwise test among genera showed that all genera were significantly different from each other. Corals with BC form do not differ significantly from TB and DG corals. A similar condition was found in EN vs. LM and LM vs. MS (Table 5).

Interestingly, there was a similarity among coral genera in terms of SD density, CSA and DWCT/CSA based on PERMANOVA analysis, as shown in Tables 3 and 5. Additionally, there was a significant correlation between the SD density with CSA and DWCT/CSA (Table 6). The CSA was negatively correlated with SD density. In contrast, the SD density increase with the increased DWCT/CSA. The data presented here shows that host characteristics, such as morphological appearance or colony form as well as tissue biomass, partly regulate the SD density in the corals. This is further proven based on CAP plots, as shown in Figure 3. The dispersion of the pooled data meets the assumption made by the PERMANOVA and its pairwise test analysis, which shows a clear separation among coral genera (Table 5). Based on the analysis, *Acropora* was separated from other genera via CSA, while DWCT/CSA was responsible for both *Montipora* and *Astreopora*. Among the genera, *Anacropora* was well separated from other genera because the taxa contained the lowest SD density, CSA and DWCT/CSA as compared to others.

Overall, it can be summarized that the genus *Acropora*, which possesses BC, TB, and DG growth forms, had less SD density than *Astreopora* and *Montipora*, which possess EN, KM, and MS growth forms (1.56 ± 0.05 vs 2.25 ± 0.12 × 10^6 cells cm^{-2}, respectively; $t(48) = -5.11$, $p=0.00$), due to the differences in CSA and DWCT/CSA. On the other hand, the BC growth form of *Anacropora* had the lowest SD density due to its smaller skeletal structure and tissue biomass as compared to the other (CSA = 15.78 ± 0.9 cm^2, DWCT/CSA = 1.34 ± 0.11 mg cm^{-2}). Furthermore, *Astreopora* with EN and LM growth forms had a higher SD density compared to *Montipora* with MS growth forms, which differentiated the two taxa.

DISCUSSION

The pattern of Symbiodiniaceae density in scleractinian corals

The SD density in 12 scleractinian coral species varied from 0.46 ± 0.01 × 10^6 cells cm^{-2} to 4.05 ± 0.02× 10^6 cell cm^{-2}. However, it is a common occurrence that the SD density was varied even among the species of the same genus (Fitt *et al*., 2000; Li *et al*., 2008). The present data revealed that certain *Acropora* species recovered from 3 to 15 m (*Ac. specifera* and *Ac. muricata*) exhibited noticeable variation in SD density, whereas others (*Ac. digitifera*, *Ac. hyacinthus*, & *Ac. florida*) did not. In comparison with other work from tropical islands located in southernmost China at 2 m depth, SD density within the genus *Acropora* can range from 1.49 to 2.23 × 10^6 cell cm^{-2}, which is a wider range than that found in this study (Li *et al*., 2008). However, it is not clear that genotype could influence the SD population or that another limiting factor might involve such as differences in spatial (depth, geographical area) and temporal (seasons).

Apart from that, the variation of SD density was found to vary among coral with different colony morphologies (Li *et al*., 2008), suggesting a possible relationship between the SD density with the coral growth forms. Our results show a significant correlation between SD density, coral surface area (CSA), and tissue biomass (DWCT/CSA), which several factors can explain. Firstly, the colony growth form differences provide the SD with a variety of internal habitats (Yost *et al*., 2013). For instance, in branching type species, the SD density can be related to their comparable coral growth forms, which maximize the incident of light absorbed by a unit of coral surface area. For instance, the shadowing effect in branched colonies decreased the amount of light that was absorbed per area and the SD, thus lowering the SD density in some parts of the branching coral (Stambler & Dubinsky, 2005). The SD will accumulate at specific surface areas to gain optimal light absorption for their photosynthesis process. Moreover, branching coral has a thin tissue that does not necessarily penetrate the surface and mostly, the SD concentrate in the polyps (Yost *et al*., 2013) and the illuminated parts (Santos *et al*., 2009). Meanwhile, high SD density in massive coral can be related to their spherical or 'bumpy' surface, increasing surface exposure to sunlight (Dubinsky & Stambler, 2011). The exposed surface area of massive coral allowed them to obtain maximum sunlight exposure for the SD, allowing the SD to fully occupy the surface area of massive coral.

Table 2. The mean density of Symbiodiniaceae in 12 species of hard corals from the family Acroporidae

Genus	Species	Colony growth forms	Depth (m)	Density ($\times 10^6$ cell cm^{-2})
Acropora	*Ac. specifera*	Tabulate, TB	3 – 15	1.85 ± 0.02
	Ac. digitifera	Digitate, DG	3 – 10	1.46 ± 0.05
	Ac. hyacinthus	Tabulate, TB	3 – 15	1.49 ± 0.4
	Ac. muricata	Branching, BC	3 – 15	1.63 ± 0.47
	Ac. florida	Tabulate, TB	10 – 15	1.46 ± 0.22
Montipora	*M. aequituberculata*	Encrusting, EN	5 – 15	1.75 ± 0.03
	M. hispida	Laminar, LM	10 – 15	1.87 ± 0.16
	M. informis	Encrusting, EN	5 – 15	1.84 ± 0.12
Astreopora	*As. ocellata*	Massive, MS	15 – 20	2.47 ± 0.32
	As. sugessta	Massive, MS	10 – 15	2.59 ± 0.85
	As. myriopthalma	Massive, MS	10 – 15	2.98 ± 1.17
Anacropora	*An. forbesi*	Branching, BC	10	0.46 ± 0.01

Note: Values of SD density are mean ± SE

Table 3. Result of PERMANOVA including the pairwise test of the SD density among genera and colony form

Source	df	SS	MS	Pseudo-F	P(perm)	perms
Genus	3	2.6971	0.89903	44.004	0.001**	999
Res	70	1.4301	2.04E-02			
Total	73	4.1272				
Colony Form	5	2.0025	0.40051	12.818	0.001**	998
Res	68	2.1247	3.12E-02			
Total	73	4.1272				
Genus				t	P(perm)	perms
ANA, MON				26.944	0.001**	444
ANA, AST				6.8886	0.002**	652
ANA, ACP				7.3008	0.001**	901
MON, AST				4.6049	0.001**	996
MON, ACP				3.2159	0.003**	997
AST, ACP				7.1782	0.001**	998
Colony Form				t	P(perm)	perms
BC, EN				2.7335	0.011**	999
BC, LM				2.1536	0.048*	989
BC, MS				5.4969	0.001**	998
BC, TB				1.8473	0.067	996
BC, DG				0.69721	0.501	986
EN, LM				1.3512	0.168	845
EN, MS				3.9191	0.001**	997
EN, TB				1.6123	0.133	994
EN, DG				8.2219	0.001**	929
LM, MS				2.4437	0.021*	992
LM, TB				1.5582	0.124	970
LM, DG				5.9476	0.001**	665
MS, TB				4.9517	0.001**	995
MS, DG				4.5779	0.001**	997
TB, DG				1.2167	0.244	992

Note: Significant level denoted as * (p<0.05), ** (p<0.01) and *** (p<0.001)

Table 4. Mean values of CSA and DWCT/CSA in hard corals

Species	Colony	CSA, cm^2	DWCT/CSA, mg cm^{-2}
An. forbesi	Branching	15.78 ± 0.9	1.34 ± 0.11
M. aequituberculata	Encrusting	25.21 ± 1.04	4.25 ± 0.37
M. hispida	Laminar	27.04 ± 1.05	3.33 ± 0.11
M. informis	Encrusting	28.96 ± 1.59	3.75 ± 0.19
As. ocellata	Massive	17.59 ± 0.89	3.08 ± 0.22

Note: Values of SD density are mean ± SE.

Table 5. Result of PERMANOVA including the pairwise test of the CSA and DWCT/CSA among different genus and colony forms. Significant level denoted as * (p<0.05), ** (p<0.01) and *** (p<0.001)

Source	df	SS	MS	Pseudo-F	P(perm)	perms
Genus	3	44.767	14.922	45.096	0.001**	999
Res	70	23.163	0.3309			
Total	73	67.93				
Colony Form	5	44.308	8.8616	25.51	0.001**	999
Res	68	23.662	0.3484			
Total	73	67.93				
Genus				t	P(perm)	perms
ANA, MON				5.906	0.001**	650
ANA, AST				2.947	0.011*	701
ANA, ACP				3.200	0.001**	933
MON, AST				2.785	0.004*	997
MON, ACP				8.878	0.001**	999
AST, ACP				8.615	0.001**	998
Colony Form				t	P(perm)	perms
BC, EN				6.2807	0.001**	999
BC, LM				3.8281	0.001**	982
BC, MS				6.1112	0.001**	999
BC, TB				1.7126	0.085	999
BC, DG				1.1254	0.29	994
EN, LM				1.9338	0.066	965
EN, MS				2.6428	0.009**	999
EN, TB				7.1181	0.001**	999
EN, DG				8.8416	0.001**	981
LM, MS				1.665	0.088	994
LM, TB				4.6613	0.001**	993
LM, DG				10.027	0.002**	757
MS, TB				7.4666	0.001**	999
MS, DG				5.6265	0.001**	998
TB, DG				2.8793	0.003**	994

Table 6. Significant correlation between SD density with CSA and DWCT/CSA

	rho	*P*
SD Density		
CSA	-0.470**	< 0.001
DWCT/CSA	0.437**	< 0.001

** Correlation is significant at the 0.01 level (2-tailed)
* Correlation is significant at the 0.05 level (2-tailed)

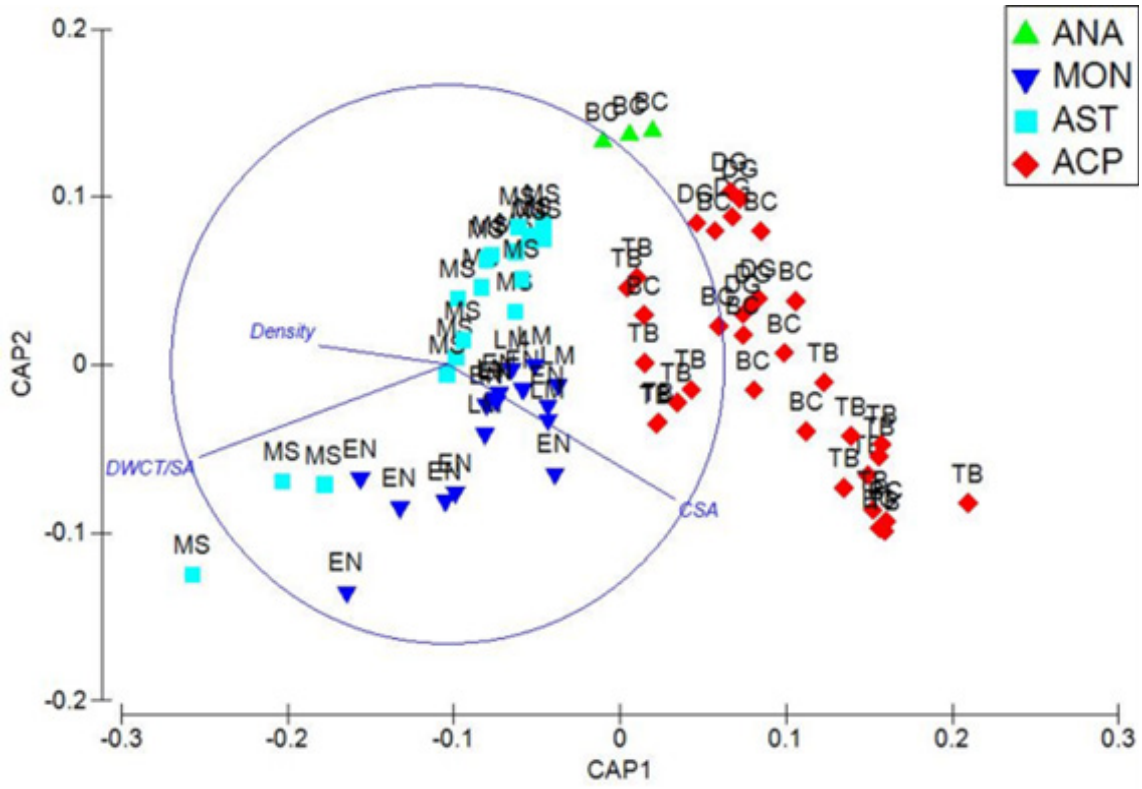

Fig. 3. Separation based on constrained (CAP) ordination of SD density, CSA and DWCT/CSA pooled data from coral species in the family Acroporidae. Vectors were used based on Spearman correlation to indicate variables that were responsible for the separation among the plots if r≥0.2. Labelled represent coral genera (ANA = *Anacropora*, MON = *Montipora*, AST = *Astreopora* and ACP = *Acropora*) and colony form (BC = branching, DG = digitate, TB = tabulate, EN = encrusting, MS =massive and LM = laminar).

Secondly, the proportion of tissue biomass per unit area positively influenced the SD density in corals. Higher SD density found in massive coral growth forms (i.e., *Astreopora*) compared to other coral growth forms were best described by different weights of coral tissue held by the host. Based on Fitt *et al*. (2000), SD density and coral tissue biomass were higher during the cold season and both parameters declined during summer. This might suggest that coral increases their tissue to accommodate the high density of SD by giving more space availability to hold more SD. Whether or not, these factors may acclimate through the host and SD cellular interactions requires future investigation. Otherwise, coral required high tissue biomass to hold the symbiont by the multilayered SD arrangement in coral tissue as described by Stambler and Dubinsky (2005). Massive corals such as *Astreopora* with higher tissue biomass can hold more symbionts than branching *Acropora* or *Anacropora*, which possessed lower tissue biomass. However, despite the growth form, tissue biomass is an influential limiting factor in SD density. For instance, in branching Red Sea coral, *Seriatopora hystrix*, the tissue biomass increases with depth, thus harbouring a higher SD density (Nir *et al*., 2011). In addition, the size of the SD may play a role in determining SD density in coral via space limitation in the coral tissue (Jones & Yellowlees, 1997). The size (diameter) of the SD is normally between 8 and 10 µm, and the higher tissue biomass would give the SD more space. However, an investigation by Stimson *et al*. (2002) of 26 coral species found no correlation between the SD's diameters with the density due to the potential overestimation of the coral tissue biomass and SD density. Further research that incorporates this variable may help to confirm the relationship between SD diameter and coral tissue biomass.

Role of Symbiodiniaceae density as a biological indicator

Scientists used quantitative estimation of SD density for assessing stress in scleractinian corals (Fujise *et al*., 2014; Xu *et al*., 2017). Thermal stress, which becomes more severe with global warming effects, has impacted a large scale of coral reefs worldwide. A detailed analysis of previous major bleaching scenarios has shown that branching corals were highly impacted by thermal stress compared to massive coral (Marshall and Baird, 2000; Edwards *et al*., 2001). Branching-type corals such as *Acropora* and *Pocillopora* are the most vulnerable species to bleaching due to their lower SD density (Stimson *et al*., 2002; Li *et al*., 2008; Xu *et al*., 2017). In contrast, Stimson *et al*. (2002) indicate that corals with a low mortality rate, such as *Porites*, have massive coral growth forms, harboured higher SD density, and density and contained more coral tissue per square centimetre of coral surface. This explains that coral with high tissue biomass, such as massive coral, could be less susceptible to bleaching than branching coral. Szmant and Gassman (1990) found that bleached coral loses 30% to 50% of their tissue showing that coral might rely more on their energy to survive the prolonged thermal stress period.

Conversely, in the bleaching event recorded in 2010, Guest *et al*. (2012) found that *Acropora* and *Pocillopora* were unaffected by abnormal seawater temperatures, whereas massive species bleached severely. Based on this case, we assumed that having low SD density has become a possible adaptation for the coral

Acropora and *Pocillopora* to reduce the source of stress which emerge from the symbiont. Cellular interaction between coral and its symbiont is critical for healthy symbiosis, especially during environmental perturbation. For example, long-term exposure towards thermal stress can help coral counter the radicals from the photosynthetic product of SD, reducing the mortality rate when the stress occurs. However, the strategies to maintain the redox balance vary among species in responding to oxidative stress (Dias *et al*., 2019). Experimental studies have found that a higher level of antioxidant activity was found in a coral with a high density of SD and the production of harmful substances by the symbiont causing damage to coral cells (Nesa and Hidaka, 2009; Yakovleva *et al*., 2009). Several studies indicate that stress will cause impairment of photosynthetic machinery (photosystem II, PSII) of the SD, resulting in excess production of reactive oxygen species (ROS), which is harmful to the host (Downs *et al*., 2002; Smith *et al*., 2005; Wooldridge, 2013; Safuan *et al*., 2021).

Additionally, Cunning and Baker (2013) found that coral with high densities of SD is more prone to bleaching regardless of SD type. As Baird *et al*. (2009) outlined, the differences in coral species' susceptibility do not directly rely on different types of symbionts hosted by the corals. During the coral bleaching event in Singapore and Malaysia, branching *Acropora* and *Pocillopora* were unaffected by abnormal seawater temperatures (Guest *et al*. 2012). This contradicts previous findings that branching corals have higher possibilities for bleaching (Stimson *et al*., 2002; Li *et al*., 2008). Since both massive growth and coral branching now have the potential to experience coral bleaching, regulation of SD density can be a mechanism to maintain healthy coral biology under normal or stressful conditions (Safuan *et al*., 2021). Notwithstanding the limitation of the knowledge about coral susceptibility towards environmental insults, there is a high potential that SD density may be a contributing factor. Hence, integrating antioxidant activities to evaluate stress response and SD density data at differential stages during stress scenarios can provide a piece of meaningful information.

CONCLUSION

This study highlighted the SD density in 12 scleractinian coral species collected in Pulau Bidong and Pulau Tioman. The SD density varied among coral species and genera. The interaction of SD density with the morphological difference among coral species was represented by the measures used in this study. The variation in SD density among coral genera can be influenced by factors such as colony growth form and tissue biomass. Coral with wider CSA and low DWCT/CSA such as branching, digitating and tabulate coral contained lower SD density than massive, laminar, and encrusting which hold more DWCT/CSA at lower CSA, resulting in higher SD density. As SD density is commonly used as an indicator of healthy corals, the data presented here is crucial for future analysis to determine the vulnerability of hard corals towards environmental perturbation. Therefore, it is important to provide a shred of evidence through field or experimental studies to understand further the role of SD towards the survivorship of scleractinian corals.

ACKNOWLEDGEMENT

This study was funded under a research grant of the Higher Institution Centre of Excellence (HICoE) in Marine Science by the Ministry of Higher Education Malaysia (MoHE) with grant number 66928 and the Department of Fisheries Malaysia.

ETHICAL STATEMENT

The study involved sample collection and was approved by the Department of Fisheries Malaysia under a research permit -ref. no JTLM 630-7 Jld. 8 (34).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Al-Hammady, M.A.M. 2013. The effect of zooxanthellae availability on the rates of skeletal growth in the Red Sea coral *Acropora hemprichii.* Egyptian Journal of Aquatic Research, 39(3): 177–183. https://doi.org/10.1016/j.ejar.2013.10.005

Baird, A.H., Bhagooli, R., Ralph, P.J. & Takahashi, S. 2009. Coral bleaching: the role of the host. Trends in Ecology & Evolution, 24(1): 16-20. https://doi.org/10.1016/j.tree.2008.09.005

Bray, J.R. & Curtis J.T. 1957. An ordination of the upland forest communities of Southern Wisconsin. Ecological Monographs, 27: 325–349. https://doi.org/10.2307/1942268

Chelliah, A., Amar, H. Bin, Hyde, J., Yewdall, K., Steinberg, P.D. & Guest, J.R. 2015. First record of multi-species synchronous coral spawning from Malaysia. PeerJ, 3: e777. https://doi.org/10.7717/peerj.777

Cunning, R. & Baker, A.C. 2013. Excess algal symbionts increase the susceptibility of reef corals to bleaching. Nature Climate Change, 3(3): 259-262. https://doi.org/10.1038/NCLIMATE1711

Davies, P.S. 1984. The role of zooxanthellae in the nutritional energy requirements of *Pocillopora eydouxi*. Coral Reefs, 2(4): 181-186. https://doi.org/10.1007/BF00263571

De'ath, G., Fabricius, K.E., Sweatman, H. & Puotinen, M. 2012. The 27–year decline of coral cover on the Great Barrier Reef and its causes. Proceedings of the National Academy of Sciences, 109(44):17995-17999. https://doi.org/10.1073/pnas.1208909109

Dias, M., Ferreira, A., Gouveia, R., Madeira, C., Jogee, N., Cabral, H., Diniz, M. & Vinagre, C. 2019. Long-term exposure to increasing temperatures on scleractinian coral fragments reveals oxidative stress. Marine Environmental Research, 150(May): 104758. https://doi.org/10.1016/j.marenvres.2019.104758

Downs, C.A., Fauth, J.E., Halas, J.C., Dustan, P., Bemiss, J. & Woodley, C.M. 2002. Oxidative stress and seasonal coral bleaching. Free Radical Biology and Medicine, 33(4): 533-543. https://doi.org/10.1016/S0891-5849(02)00907-3

Drew, E.A. 1972. The biology and physiology of alga-invertebrates symbioses. II. The density of symbiotic algal cells in a number of hermatypic hard corals and Alcyonarians from various depths. Journal of Experimental Marine Biology and Ecology, 9(1): 71–75. https://doi.org/10.1016/0022-0981(72)90008-1

Dubinsky, Z. & Stambler, N. 2011. Coral reefs: An ecosystem in transition. Springer, Dordrecht. 552 pp. https://doi.org/10.1007/978-94-007-0114-4

Dustan, P. 1979. Distribution of zooxanthellae and photosynthetic chloroplast pigments of the reef-building coral *Montastrea annularis* Ellis and Solander in relation to depth on a West Indian coral reef. Bulletin of Marine Science, 29(1): 79-95.

Edwards, A. J., Clark, S., Zahir, H., Rajasuriya, A., Naseer, A. & Rubens, J. 2001. Coral bleaching and mortality on artificial and natural reefs in Maldives in 1998, sea surface Temperature Anomalies and Initial Recovery. Marine Pollution Bulletin, 42(1): 7–15. https://doi.org/10.1016/S0025-326X(00)00200-9

Fagonee, I., Wilson, H.B., Hassell, M.P. & Turner, J.R. 1999. The dynamics of zooxanthellae populations: a long-term study in the field. Science, 283(February): 843–845. https://doi.org/10.1126/science.283.5403.843

Fitt, W.K., McFarland, F.K., Warner, M.E. & Chilcoat, G.C. 2000. Seasonal patterns of tissue biomass and densities of symbiotic dinoflagellates in reef corals and relation to coral bleaching. Limnology and Oceanography, 45(3): 677–685. https://doi.org/10.4319/lo.2000.45.3.0677

Fransolet, D., Roberty, S. & Plumier, J. C. 2012. Establishment of endosymbiosis: the case of cnidarians and *Symbiodinium*. Journal of Experimental Marine Biology and Ecology, 420: 1-7. https://doi.org/10.1016/j.jembe.2012.03.015

Fujise, L., Yamashita, H., Suzuki, G., Sasaki, K., Liao, L.M. & Koike, K. 2014. Moderate thermal stress causes active and immediate expulsion of photosynthetically damaged zooxanthellae (*Symbiodinium*) from corals. PLoS ONE, 9(12): e114321. https://doi.org/10.1371/journal.pone.0114321

Guest, J.R., Baird, A.H., Maynard, J.A., Muttaqin, E., Edwards, A.J., Campbell, S.J., Yewdall, K., Affendi, Y.A. & Chou, L. M. 2012. Contrasting patterns of coral bleaching susceptibility in 2010 suggest an adaptive response to thermal stress. PLoS ONE, 7(3): 1–8. https://doi.org/10.1371/journal.pone.0033353

Ibrahim, S., Mohd, W., Wan, R., Kassim, Z., Joni, Z.M., Zakaria, M.Z. & Hajisamae, S. 2006. Seasonal Abundance of Benthic Communities in Coral Areas of Karah. Turkish Journal of Fisheries and Aquatic Sciences, 136: 129–136.

Johannes, R.E. & Wiebe, W.J. 1970. Method for determination of coral tissue biomass and composition. Limnology and Oceanography, 15(5): 822-824. https://doi.org/10.4319/lo.1970.15.5.0822

Jones, R.J. & Yellowlees, D. 1997. Regulation and control of intracellular algae (= zooxanthellae) in hard corals. Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences, 352(1352): 457-468. https://doi.org/10.1098/rstb.1997.0033

Ladrière, O., Compere, P., Decloux, N., Vandewalle, P. & Poulicek, M. 2008. Morphological alterations of zooxanthellae in bleached cnidarian hosts. Cahiers de Biologie Marine, 49(3): 215-227. https://doi.org/10.1098/rstb.1997.0033

Leletkin, V.A. 2000. Trophic status and population density of zooxanthellae in hermatypic corals. Russian Journal of Marine Biology, 26(4): 231–240. https://doi.org/10.1007/BF02759502

Li, S., Yu, K., Shi, Q., Chen, T., Zhao, M. & Zhao, J. 2008. Interspecies and spatial diversity in the symbiotic zooxanthellae density in corals from northern South China Sea and its relationship to coral reef bleaching. Chinese Science Bulletin, 53(2): 295-303. https://doi.org/10.1007/s11434-007-0514-4

Marsh, J.J.A. 1970. Primary productivity of reef□building calcareous red algae. Ecology, 51(2): 255-263. https://doi.org/10.2307/1933661

Marshall, P.A. & Baird, A. H. 2000. Bleaching of corals on the Great Barrier Reef: Differential susceptibilities among taxa. Coral Reefs, 19(2): 155–163. https://doi.org/10.1007/s003380000086

Masuda, K., Goto, M., Maruyama, T. & Miyachi, S. 1993. Adaptation of solitary corals and their zooxanthellae to low light and UV radiation. Marine Biology, 117(4): 685–691. https://doi.org/10.1007/BF00349781

McCloskey, L.R. & Muscatine, L. 1984. Production and respiration in the Red Sea coral *Stylophora pistillata* as a function of depth. Proceedings of the Royal Society of London. Series B 222(1227): 215-230. https://doi.org/10.1098/rspb.1984.0060

Muller-Parker, G., D'elia, C.F. & Cook, C.B. 2015. Coral reefs in the Anthropocene. Springer, Dordrecht. 200 pp.

Mwaura, J., Grimsditch, G., Kilonzo, J., Amiyo, N. & Obura, D. 2009. Zooxanthellae densities are highest in summer months in equatorial corals in Kenya. Western Indian Ocean Journal of Marine Science, 8(2): 193-202. https://doi.org/10.4314/wiojms.v8i2.56980

Nesa, B. & Hidaka, M. 2009. High zooxanthella density shortens the survival time of coral cell aggregates under thermal stress. Journal of Experimental Marine Biology and Ecology, 368(1): 81-87. https://doi.org/10.1016/j.jembe.2008.10.018

Nir, O., Gruber, D.F., Einbinder, S., Kark, S. & Tchernov, D. 2011. Changes in scleractinian coral *Seriatopora hystrix* morphology and its endocellular *Symbiodinium* characteristics along a bathymetric gradient from shallow to mesophotic reef. Coral Reefs, 30(4): 1089–1100. https://doi.org/10.1007/s00338-011-0801-z

Papina, M., Meziane, T. & Van Woesik, R. 2003. Symbiotic zooxanthellae provide the host-coral *Montipora digitata* with polyunsaturated fatty acids. Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology, 135(3): 533-537. https://doi.org/10.1016/S1096-4959(03)00118-0

Phongsuwan, N., Chankong, A., Yamarunpatthana, C., Chansang, H., Boonprakob, R., Petchkumnerd, P., Thongtham, N., Paokantha, S., Chanmethakul, T., Panchaiyapoom, P. & Bundit, O.A. 2013. Status and changing patterns on coral reefs in Thailand during the last two decades. Deep Sea Research Part II: Topical Studies in Oceanography, 96: 19-24. https://doi.org/10.1016/j.dsr2.2013.02.015

Pillay, R.M., Willis, B. & Terashima, H. 2005. Trends in the density of zooxanthellae in *Acropora millepora* (Ehrenberg, 1834) at the Palm Island group, Great Barrier Reef, Australia. Symbiosis, 38: 209-226

Safuan, C.D.M., Ismail, K., Khalil, I., Ali, A., Chong, W.S., Chan, A.A., Ismail, M.N., Repin, I.M. & Bachok, Z. 2018. Quantification of coral reef benthos for coral health assessment in Labuan Marine Park, Malaysia. Journal of Sustainability Science and Management, 13(5): 1–26.

Safuan, C.D.M., Roseli, N.H., Bachok, Z., Akhir, M.F., Xia, C. & Qiao, F. 2020. First record of tropical storm (Pabuk - January 2019) damage on shallow water reef in Pulau Bidong, south of South China Sea. Regional Studies in Marine Science, 35(January 2019): 101216. https://doi.org/10.1016/j.rsma.2020.101216

Safuan, C.D.M., Samshuri, M.A., Jaafar, S.N.T., Tan, H.C. & Bachok, Z. 2021. Physiological response of shallow-water hard coral *Acropora digitifera* to heat stress via fatty acid composition. Frontiers in Marine Science, 8: 715167. https://doi.org/10.3389/fmars.2021.715167

Safuan, C.D.M., Wee, H.B., Ibrahim, Y.S., Idris, I. & Bachok, Z. 2016. Current status on community structure of coral reefs around west coast of peninsular malaysia using coral video transect technique. Journal of Sustainability Science and Management, 11(SpecialIssue1): 107–117.

Santos, S.R., Toyoshima, J. & Kinzie III,R.A. 2009. Spatial and temporal dynamics of symbiotic dinoflagellates (Symbiodinium: Dinophyta) in the perforate coral *Montipora capitata.* Galaxea, Journal of Coral Reef Studies, 11: 139–147. https://doi.org/10.3755/galaxea.11.139

Siebeck, U.E., Marshall, N.J., Klüter, A. & Hoegh-Guldberg, O. 2006. Monitoring coral bleaching using a colour reference card. Coral Reefs, 25(3): 453-460. https://doi.org/10.1007/s00338-006-0123-8

Smith, D.J., Suggett, D.J. & Baker, N.R. 2005. Is photoinhibition of zooxanthellae photosynthesis the primary cause of thermal bleaching in corals? Global Change Biology, 11(1): 1-11. https://doi.org/10.1111/j.1529-8817.2003.00895.x

Stambler, N. & Dubinsky, Z. 2005. Corals as light collectors: An integrating sphere approach. Coral Reefs, 24(1): 1–9. https://doi.org/10.1007/s00338-004-0452-4

Stimson, J., Sakai, K. & Sembali, H. 2002. Interspecific comparison of the symbiotic relationship in corals with high and low rates of bleaching-induced mortality. Coral Reefs, 21(4): 409–421. https://doi.org/10.1007/s00338-002-0264-3

Szmant, A.M. & Gassman, N.J. 1990. The effects of prolonged “bleaching” on the tissue biomass and reproduction of the reef coral *Montastrea annularis.* Coral Reefs, 8(4): 217–224. https://doi.org/10.1007/BF00265014

Tan, C.H., Safuan, C.D.M., Idris, I., Noor, M.R.H.M., Samshuri, M.A., Kamarrudin, N., Zaidi, P. N., Jaafar, S.N. & Baird, A.H. 2020a. Multispecific synchronous coral spawning on Pulau Bidong, Malaysia, South China Sea. Bulletin of Marine Science, 96(January): 10–12. https://doi.org/10.5343/bms.2019.0067

Teece, M.A., Estes, B., Gelsleichter, E. & Lirman, D. 2011. Heterotrophic and autotrophic assimilation of fatty acids by two scleractinian corals, *Montastraea faveolata* and *Porites astreoides.* Limnology and Oceanography, 56(4): 1285-1296. https://doi.org/10.4319/lo.2011.56.4.1285

Toda, T., Okashita, T., Maekawa, T., Alfian, B.A.A.K., Rajuddin, M.K.M., Nakajima, R., Chen, W., Takahashi, K.T., Othman, B.H.R., & Terazaki, M. 2007. Community structures of coral reefs around peninsular Malaysia. Journal of Oceanography, 63(1): 113–123. https://doi.org/10.1007/s10872-007-0009-6

Veron, J.E.N. 2000. Corals of the World. Volumes 1-3. Australian Inst Mar Sci. 1,382 pp.

Wooldridge, S.A. 2013. Breakdown of the coral-algae symbiosis: towards formalising a linkage between warm-water bleaching thresholds and the growth rate of the intracellular zooxanthellae. Biogeosciences, 10(3): 1647–1658. https://doi.org/10.5194/bg-10-1647-2013

Xu, L., Yu, K., Li, S., Liu, G., Tao, S., Shi, Q., Chen T, & Zhang, H. 2017. Interseasonal and interspecies diversities of Symbiodinium density and effective photochemical efficiency in five dominant reef coral species from Luhuitou fringing reef, northern South China Sea. Coral Reefs, 36(2): 477-487. https://doi.org/10.1007/s00338-016-1532-y

Yakovleva, I.M., Baird, A.H., Yamamoto, H.H., Bhagooli, R., Nonaka, M. & Hidaka, M. 2009. Algal symbionts increase oxidative damage and death in coral larvae at high temperatures. Marine Ecology Progress Series, 378: 105-112. https://doi.org/10.3354/meps07857

Yost, D.M., Wang, L.H., Fan, T.Y., Chen, C.S., Lee, R.W., Sogin, E. & Gates, R.D. 2013. Diversity in skeletal architecture influences biological heterogeneity and *Symbiodinium* habitat in corals. *Zoology*, 116(5): 262–269. https://doi.org/10.1016/j.zool.2013.06.001