Spatial variation of symbiotic Dinoflagellates on coral reefs of the northern Persian Gulf

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Abstract

Density, mitotic index, Chlorophyll-a content and cell size of symbiotic dinoflagellates of dominant reef-building corals were measured at two different depths in Kish Island and from one depth of Larak Island in the Persian Gulf. The higher densities of symbionts were found in shallow waters of Kish Island. However, ANOVA analyses of the mitotic index yielded mixed results. The cell sizes of symbionts did not significantly differ among depths, except for *Porites* corals at one site in Kish Island which displayed the larger cells in deeper water. The comparison of symbiont attributes between islands exhibited intraspecific variability. Density of zooxanthellae was only different in *Porites* and *Favia* corals between islands. Chlorophyll-a density (cm^{-2}) was significantly different between islands in all species, with higher values for Kish corals. Chlorophyll a $cell^{-1}$ was only significantly different in *Porites* and *Platygyra* corals, with higher values for Larak Island. The symbiont cell sizes usually exhibited no variability, except for Porites corals with larger sizes in Kish Island.

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Introduction

Located in the north-west of the Indian Ocean, the Persian Gulf is a semi-enclosed marginal sea with generally harsh environmental circumstances with regard to salinity, temperature, occasional extreme low tides and poor flushing rates (Sheppard, 1993). These circumstances have imposed adverse effects on coral reefs leading to low diversity comparing to corals of other parts of the Indian Ocean (Price, 1993). The incidence of bleaching in coral reefs due to loss of their symbiont dinoflagellates (zooxanthellae) or pigmented algae has mainly linked to heat stress (Hoegh-Guldberg, 1999; Goreau et al., 2000). Zooxanthellae are found to be the main item in the animal-algal endosymbioses relationship that indicate any changes in the environmental condition beyond the normal limits for corals survival (Pilcher et al., 2000). Zooxanthellae density per unit of some variables including surface area and protein content is regarded as an indication of coral health, especially with regard to bleaching. Changes in zooxanthellae density and its chlorophyll content are associated with different environmental conditions including light and nutrient levels (Putron et al., 2004). This indicates the importance of symbiont dinoflagellate in understanding the extent to which corals start to bleach and dye off. In recent years, coral bleaching has occurred in the Persian Gulf resulting in mass mortality of corals mainly due to the elevated temperature (Pilcher et al., 2000). Two mass mortality events of corals occurred in the northern part of the Persian Gulf around Kish Island during 1996, 1998 and 2006 (Shokri et al., 2000). However, coral bleaching in the

Persian Gulf has also linked to decline in water temperature (Coles and Fadlallah, 1991). Density of zooxanthellae and Chlorophyll-a content with regards to species, depth, site, and year has been studied. For example, zooxanthellae density and pigment content of five coral species at three different depths from the Bahamian sites were monitored. It was concluded that corals living in shallow water often had higher levels of all parameters measured compared to deeper corals, except for chlorophyll-a content which usually displayed the opposite trend (Fitt et al., 2000). Also, laboratory experiments have documented decreased densities of symbiotic dinoflagellate and pigment content in corals correlated with acute increases in temperature (Coles and Jokiel, 1978; Hoegh-Guldberg and Smith, 1989; Fitt and Warner, 1995). Brown et al., (1999) found a correlation between rising sea surface temperature and relatively lower symbiont density and pigments during the dry season for four species of shallow-water corals in Thailand. Limited attention has so far been paid to division rates, or mitotic indices of zooxanthellae. Knowledge of these aspects of population dynamics is important for understanding the flux of nutrients and energy through zooxanthellae, as well as the mechanisms which regulate symbiont population size. Environmental factors influencing mitotic index of zooxanthellae include light/dark photoperiod (Sweeney and Hastings, 1958), temperature (Heath, 1982), nutrients (Chisholm and Costello, 1980) and salinity (Steen and Muscatine, 1987; Hoegh-Guldberg and Smith, 1989). Studies have already demonstrated phased

cell division in natural populations of free-living dinoflagellates, in which mitotic indices had peaked in the early morning and ranging from 10 to 60% (Swift and Duflbin, 1972; Elbrachter, 1973; Dolye and Poore, 1974; Weiler and Chisholm, 1976).

Despite zooxanthellae importance to the health of coral reefs, few studies have focused on the association between symbiotic dinoflagellates and corals in the northern part of the Persian Gulf. In a recent effort, the molecules diversity of dinoflagellates on dominant corals was studied in the northern Persian Gulf (Mostafavi et al., 2007). There are still many questions about characteristics of symbiotic dinoflagellates to be answered in the Persian Gulf. Data were collected from Kish and Larak Islands in the northern Persian Gulf. The coral reefs of Kish Island are located in the inner Persian Gulf area and therefore tolerate the more saline and less nutrient-rich conditions, whereas corals of Larak Island are greatly exposed to flux of nutrient-rich and less saline oceanic water through Strait of Hormuz (Shokri et al., 2005). By collecting baseline data on main attributes of symbiotic dinoflagellates (i.e. density, mitotic index, chlorophyll-a content and cell size) on dominant coral species, response of symbiont attributes to environmental variations were explored in the present study. This issue was tested by looking at the intra and inter-specific variations of symbiont attributes within two different depths and two locations with predominant differences in their environmental conditions.

Materials and methods

The dominant reef-building coral specimens including *Acropora* sp., *Favia pallida, Platygyra daedellae* and *Porites compressa* were collected from two different depth intervals (i.e. 3-6 m and 8-12 m) in Kish Island and from one depth interval (i.e. 3-6 m) in Larak Island in the northern part of the Persian Gulf (Figure 1). Symbiotic dinoflagellates were separated from host coral tissues using Water Pik followed by hi-speed centrifuging (2500 rpm, 10 min). Concentration of zooxanthellae was determined using a hemacytometer by counting 9 separate cells (Putron et al., 2004). Surface area of bare coral skeleton was determined using aluminium foil method (Marsh, 1970). Mitotic index was determined by dividing the number of dividing cells by the total number of zooxanthellae (Suharsono et al., 1992). Cell size of zooxanthellae was measured using an eye micrometer (Stimson et al., 2002). Chlorophyll-a content was measured using spectrophotometer (Putron et al., 2004) and calculated following equations suggested by Jeffery and Humphery, 1975. The symbol E_x denotes the extinction at wave length x.

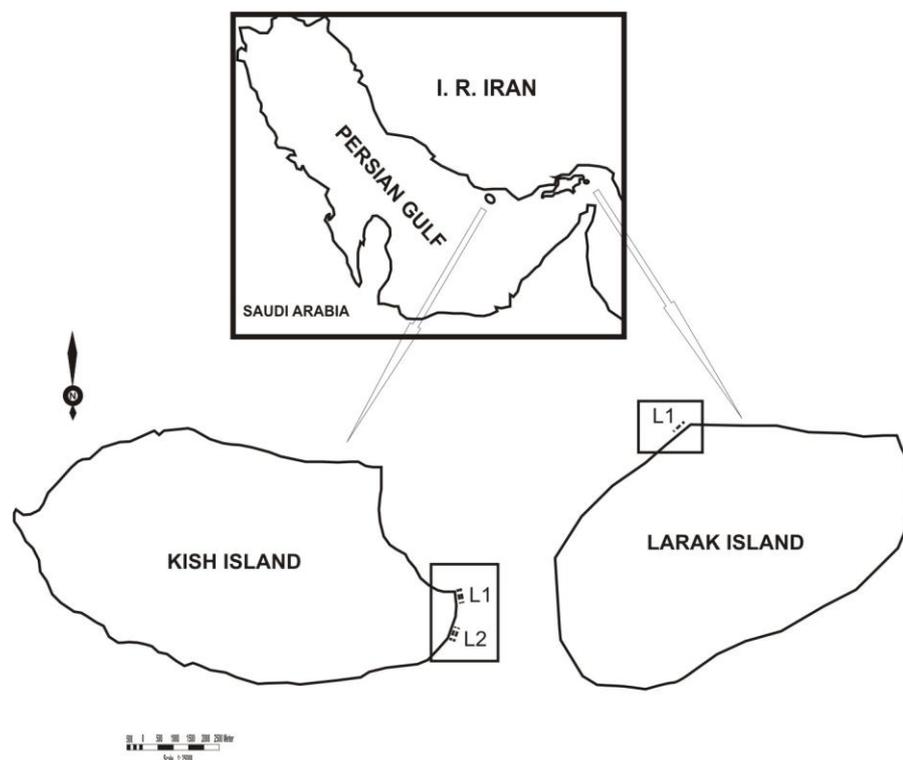Chl-a concentration = $(11.43 * E_{664}) - (0.64 E_{630})$.

Figure 1: Locations of the studied areas in Kish and Larak island

Differences of symbiont attributes (i.e. density, mitotic index, chlorophyll-a content cell^{-1} or cm^{-2} and cell size) between hosting coral species were tested by a 3-factor analysis of variance (ANOVA) in Kish Island where corals were collected from two different depths (3-6 m and 8-12 m). The three factors were: (1) Coral species: fixed, orthogonal, 4 levels; (2) Location: fixed, orthogonal, two levels; (3) Depth: fixed, orthogonal, two levels. The effect of depth on symbiont attributes in different coral species was indicated by a significant Coral×Species×Depth interaction. Significant interactions and differences between host coral species were explored by Student-Newman-Keuls procedure (Sokal and Rohlf, 1995). Since the corals at Larak Island were only found at one depth interval (3-6 m), intra and inter-specific differences of symbiont attributes were tested by one-factor analysis of variance (ANOVA) with one factor: Coral species: fixed, orthogonal, 4 levels. Cochran's test was applied on data to test the homogeneity of variances prior to ANOVA test and data sets with heterogeneous variances were transformed. A square-root transformation was applied to data on density, and heterogeneity was removed from data on chlorophyll content by a log-transformation. No transformation was applied to data of mitotic index and cell size, because of homogeneity of their variances. ANOVA analyses were undertaken using the GMAV5 Software (Underwood and Chapman, 1984).

Differences in symbiont attributes between two islands were tested by multivariate analysis using PRIMER 5 (Plymouth Marine Laboratories, Clarke, 1993; Clarke and Warwick, 1994). A non-

metric multidimensional scaling ordinations based on pooled data of all hosting coral species at three locations of two islands was used to explore differences in symbiont attributes between the two islands. Non-metric multidimensional scaling (nMDS) ordinations, based on Bray-Curtis dissimilarity matrices of the square-root transformed density, log-transformed chlorophyll content, and none-transformed mitotic index and cell size data, were used to visualize differences in symbiont attributes between the two islands. Patterns observed in the nMDS ordination were confirmed with cluster analysis.

Results

Density of symbiotic dinoflagellates among the 300 coral specimens from 4 different species at two locations averaged 4.27×10^6 (standard error = 1.54×10^5) cells/cm^2. Dinoflagellates density on 4 coral species ranged from 2.75×10^6 to 5.33×10^6 cells/cm^2 (Table 1). Pair wise comparisons showed that dinoflagellates density was significantly greater in massive *Favia pallida* and *Platygyra daedellae* and submassive *Porites compressa* than in branching *Acropora* sp. (F=13.73, 3 d.f., *p*=0.001 for Kish Island) (Table 2); F=36.44, 3 d.f., *p*=0.001 for Larak Island (Table 3). Within site comparisons, between depths at Kish Island yielded significantly greater zooxanthellae density (F=13.73, 3 d.f., *p*=0.001) on *Acropora* sp., *P. compressa* and *F. pallida* in the shallow depth (Table 2). Concentrations of Chl-a per zooxanthellae and Chl-a per cm^{-2} ranged from 0.10 to 3.16 μg and 1.67 to 5.73 μg, respectively (Table 1). Pair wise comparisons showed that Chl-a μg cm^{-2}

was greater in massive *F. pallida* and *P. daedellae* than in sub massive *P. compressa and* branching *Acropora* sp. at Kish Island (Table 2), whereas this was not the case in Larak Island (Table 3). Significant differences were found within coral species in Chl-a per zooxanthellae in Larak Island with greater values for massive and submassive corals (Table 3). Within site comparisons, between depths at Kish Island yielded significantly greater Chl-a μg cell^{-1} (F=8.95, 3 d.f., *p*=0.001) on *P. compressa* and *F. pallida*, and Chl-a μg cm^{-2} (F=550.95, 3 d.f., *p*=0.001) on *Acropora* sp., *P. compressa* and *F. pallida* in 8-12 m (Table 2). ANOVA analyses of mitotic index in Kish Island exhibited mixed significant differences among coral species between depths (F=22.27, 3 d.f., *p*=0.001) (Table 2). Within site comparisons, between depths yielded significantly greater mitotic index (F=8.95, 3 d.f., *p*=0.001) for *P. daedellae* (F=22.17, 1 d.f., *p*=0.001) at 8-12 m (Table2). Mitotic index was not compared in corals from Larak Island because of marginally zero values only in one coral species. Comparisons of dinoflagellates cell sizes among coral species yielded mixed results within depths. Significant differences were only found between *Acropora* sp. with *P. compressa* (*p*=0.01) and *F. pallida* (*p*=0.01) at the shallow depth of location 1 in Kish Island (Table 2) and between *P. compressa* and *F. pallida* in Larak Island (*p*=0.05) (Table 3). Cell sizes of symbionts were not significantly different between depths, except for *P. compressa* at location 1 in Kish Island that displayed larger cells at 8-12 m (F=4.57, 1 d.f., *p*=0.05) (Table 2). Spatial patterns in dinoflagellate attributes in Kish and Larak

Islands were compared using non-metric multidimensional scaling ordinations based on pooled data of all hosting coral species at each site. Spatial patterns of dinoflagellate attributes including dinoflagellate densities, cell sizes of dinoflagellates and concentrations of

chlorophyll content per zooxanthellae and per square centimetre at Larak Island were largely similar to those of Kish Island (Figure 2) whereas mitotic index in Larak Island was largely dissimilar to that of Kish Island.
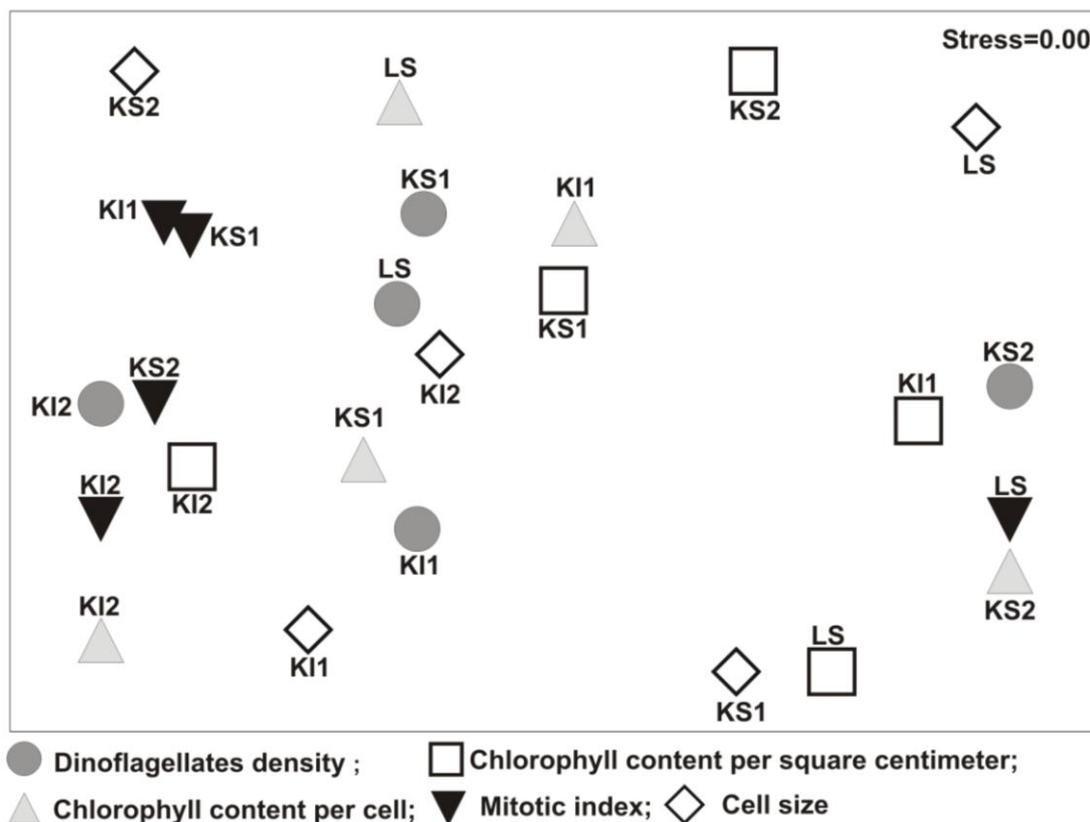

Figure 2: Non-metric multidimensional scaling ordinations based on pooled data of all hosting coral species at each site comparing the dinoflagellate attributes between Kish and Larak Islands. KS1=Kish shallow depth at location 1; KI1 = Kish intermediate depth at location 1; KS2 = Kish shallow depth at location 2; KI2 = Kish intermediate depth at location 2; LS = Larak shallow depth; LI = Larak intermediate depth.

Discussion

The estimation of symbiotic dinoflagellate attributes (density, chlorophyll-a cell $^{-1}$ or cm^{-2} of coral surface area, mitotic index and cell size) have been conducted on several species (e.g. Brown et al., 1999; Fitt et al., 2000; Saxby, 2001). In the

present study, conducted on 4 coral species, different dinoflagellate attributes have been found, which correspond with earlier works. The differences in dinoflagellate density and chlorophyll-a among species may be due to the types of

Table 1: Symbiont attributes on different coral species in Kish (N=6) and Larak (N=3) Islands. Mean and standard error (SE) are provided.

			Acropora sp.		*Porites compressa*		*Favia pallida*		*Platygyra daedellae*	
			Shallow (3-6 m)	**Intermediate (8-12 m)**	**Shallow (3-6 m)**	**Intermediate (8-12 m)**	**Shallow (3-6 m)**	**Intermediate (8-12 m)**	**Shallow (3-6 m)**	**Intermediate (8-12 m)**
Density cm^{-2}	Kish Island	Mean	4503333	3748333	5336667	3071667	5336667	3488333	4641667	4393333
		SE	362267.5	97139.65	406912.49	371056.30	460055.55	818141.86	392321.44	271780.14
	Larak Island	Mean	2753333		4720000		4453333		4446667	
		SE	133333.33		98657.66		26666.67		268473.05	
Mitotic index	Kish Island	Mean	0.035	0	0.05	0.075	0.1467	0.1767	0	0.23
		SE	1.565×10^{-2}	0	0.4472×10^{-2}	1.118×10^{-2}	1.801×10^{-2}	5.667×10^{-2}	0	3.958×10^{-2}
	Larak Island	Mean	0		0.15		0		0	
		SE	0		0		0		0	
Chl a µg cell^{-1}	Kish Island	Mean	90.17×10^{-8}	96×10^{-8}	49×10^{-8}	132.33×10^{-8}	77.50×10^{-8}	181.67×10^{-8}	101.5×10^{-8}	107.83×10^{-8}
		SE	10.36×10^{-8}	10×10^{-8}	9.40×10^{-8}	9.29×10^{-8}	14.30×10^{-8}	59.19×10^{-8}	9.25×10^{-8}	14.26×10^{-8}
	Larak Island	Mean	169×10^{-8}		37×10^{-8}		61×10^{-8}		108×10^{-8}	
		SE	0		0		0.58×10^{-8}		1.73×10^{-8}	
Chl a µg cm^{-2}	Kish Island	Mean	3.9017	3.645	2.5017	3.9117	4.8533	3.9583	4.57	4.6267
		SE	0.1577	0.435	0.3649	0.1853	0.1504	0.5930	0.07707×10^{-2}	0.4446
	Larak Island	Mean	4.67		1.67		2.73		4.81	
		SE	0		0		0.03464		0.06928	
Cell size µm	Kish Island	Mean	1.22×10^{-2}	1.20×10^{-2}	1.03×10^{-2}	1.13×10^{-2}	1.05×10^{-2}	1.15×10^{-2}	1.13×10^{-2}	1.17×10^{-2}
		SE	3.07×10^{-4}	2.58×10^{-4}	4.22×10^{-4}	4.22×10^{-4}	4.28×10^{-4}	2.24×10^{-4}	3.33×10^{-4}	4.22×10^{-4}
	Larak Island	Mean	1.13×10^{-2}		0.967×10^{-2}		1.23×10^{-2}		1.10×10^{-2}	
		SE	3.33×10^{-4}		3.33×10^{-4}		8.82×10^{-4}		5.77×10^{-4}	

Table 2: Summary of a three-factor analysis of variance results comparing symbionts attributes in Kish Island between depths and different host coral species. (***P < 0.001, **P < 0.01, *P < 0.05, ns P > 0.05)

Source of variation	df	Density cm$^{-2\,a}$		Mitotic index ^{b}		Chl a µg cell$^{-1\,c}$		Chl a µg cm$^{-2\,d}$		Cell size µm ^{e}	
		MS	F	MS	F	MS	F	MS	F	MS	F
Coral species	3	24678.51	1.84 ns	0.0471	22.27***	0.1149	1.79 ns	0.3826	654.34***	3.7986	4.93**
Location	1	70240.61	5.23*	0.0019	0.89 ns	0.7008	10.93**	0.9127	1560.83***	0.5208	0.68 ns
Depth	1	1269733	94.46***	0.0469	22.17***	1.982	30.91***	0.0117	20.05***	3.5208	4.57*
Coral sp. × Location	3	552929.20	41.13***	0.0107	5.05**	0.2763	4.31**	0.0439	75.04 ***	0.1875	0.24 ns
Coral sp. × Depth	3	184591.20	13.73***	0.04	18.93***	0.5741	8.95***	0.3222	550.95***	0.9653	1.25 ns
Location × Depth	1	101480.10	7.55**	0.004	1.91 ns	1.5147	23.62***	0.4639	793.31***	0.5208	0.68 ns
Coral sp. × Location × Depth	3	293451.70	21.83***	0.0197	9.32***	1.2351	19.26***	0.261	446.29***	1.6319	2.12 ns
Residual	32	13442.60		0.0021		0.0641		0.0006	654.34***	0.7708	
Total	47										

^{a} Sqrt(x+1) transformed, variances homogenous.
^{b} Untransformed, variances homogenous.
^{c} Ln(x+0.1) transformed, variances heterogeneous.
^{d} Ln(x) transformed, variances homogenous.
^{e} Untransformed, variances homogenous.

Table 3: Summary of one-factor analysis of variance results comparing symbionts attributes in Larak Island between different host coral species. (***P < 0.001, **P < 0.01, *P < 0.05, ns P > 0.05)

Source of variation	df	Density cm$^{-2\,a}$		Mitotic index ^{b}		Chl a µg cell$^{-1\,c}$		Chl a µg cm$^{-2\,d}$		Cell size µm ^{e}	
		MS	F	MS	F	MS	F	MS	F	MS	F
Coral species	3	169400.8	36.44***	-	-	1.0231	4842.45***	0.7569	2737.6***	3.6389	3.64 ns
Residual	8	4649.164		-		0.0002		0.0003		1	
Total	11										

^{a} Sqrt(x+1) transformed, variances homogenous.
^{b} Untransformed, variances homogenous.
^{c} Ln(x+0.1) transformed, variances homogenous.
^{d} Ln(x) transformed, variances homogenous.
^{e} Untransformed, variances homogenous.

zooxanthellae found in different species of corals. Coral species have been found to possess more than one strain of zooxanthellae (Rowan and Powers, 1991; Baker et al., 1997). Zooxanthellae strains have been found to have different physiological characteristics (Iglesias-Prieto and Trench, 1994; Warner et al., 1996; Banaszac et al., 2000). These differences among zooxanthellae strains, along with morphological and physiological differences in tissues of different host coral species, evidently result in a variety of symbiotic characteristics as represented in this study. Depth gradient of densities of symbiotic dinoflagellates and chlorophyll-a as seen here, have been known for years (e.g. McCloskey and Muscatine, 1984; Batty and Porter, 1988). Some authors have proposed that decline in symbiont density with depth is a response to decreased photon flux density (PFD); even as symbiotic algae acclimate through increased algal pigment (Fitt et al., 2000). The low density of zooxanthellae in corals at 8-12 m depth was probably due to decreased PFD. According to Wilkerson et al., (1983), environmental controlling factors for mitotic index of zooxanthellae include light/dark photoperiod (Sweeney and Hastings, 1958), temperature (Heath, 1982), nutrients (Chisholm and Costello, 1980) and salinity (Steen and Muscatine, 1987; Hoegh-Guldberg and Smith, 1989). Suharsono and Brown (1992) asserted that thermal increase is one of the main factors in increasing mitotic index during which zooxanthellae emerge from the coral tissue. As a result, to compensate decline algal density, mitotic index will increase. According to the present study, within site comparisons between different depths,

greater mitotic index was found for *Platygyra daedellae* at 8-12 m but the interpretation is not obvious.

Acknowledgements

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