

The Effect of Zooxanthellae Availability on the Rates of Skeletal Growth in the Red Sea Coral *Acropora Hemprichii*

Montaser Aly Mahmoud Al-Hammady¹ and Abd-Allah Alian²

¹ National Institute of Oceanography and Fisheries – Red Sea Branch – Hurghada – Egypt.

² Department of Zoology, Faculty of Science, Al-Azhar University.

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Abstract: Zooxanthellae density affects growth rate of *Acropora hemprichii* at reef flat and 10 m depth, where the correlations were significantly moderate at reef flat ($r = 0.461$ & $P < 0.01$) and significantly high at 10 m depth ($r = 0.636$ & $P = 0.424$). Non interactive effects were obtained at 20 and 25 m depths, where the correlations were insignificant ($r = 0.346$ & $P < 0.19$ and $r = 0.103$ & $P < 0.706$, respectively). Either zooxanthellae density, hosted by *Acropora hemprichii*, or growth rate was decreased with depth increase. Zooxanthellae density at reef flat ($1.55 \pm 0.303 \times 10^6$ cells/cm²) was twice higher than at 25 m depth ($0.706 \pm 0.253 \times 10^6$ cells/cm²). However, growth rate at reef flat was approximately three times higher than at 25 m depth (0.013 ± 0.0024 mm/day). The maximum growth rate (0.0335 mm/day) and zooxanthellae density (1.32×10^6 cells/cm²) were recorded during summer season, whereas the minimum growth rate (0.01769 mm/day) and zooxanthellae density (0.9311×10^6 cells/cm²) were recorded during autumn.

Keywords: zooxanthellae, growth rates, depths, *Acropora hemprichii* and Red Sea.

Introduction

The success of coral reefs which are considered as one of the most biodiverse ecosystems in the world is due in large part to obligate mutualistic symbioses involving invertebrates and photosynthetic dinoflagellate symbionts (Dustan, 1999; Stone *et al.*, 1999; Obura, 2009 and Al-Hammady, 2011). Scientists have been interested in the nutritional interrelationship between corals and their zooxanthellae (Muscatine & Porter, 1977; Barnes & Crossland, 1980; Furla *et al.*, 2000; Al-Horani *et al.*, 2005; Winters *et al.*, 2009, Fitt *et al.*, 2009 and Ammar *et al.*, 2012). Corals receive photosynthetic products (sugar and amino acids) in return for supplying zooxanthellae with crucial plant nutrients (ammonia and phosphate) from their waste metabolism (Trench, 1979 and Furla *et al.*, 2000). Muscatine (1990) found that, zooxanthellae provide energy and nutrients for coral host by translocating up to 95% of their photosynthetic production. Swanson and Hoegh-Guldberg (1998) mentioned that, zooxanthellae selectively leak amino acids, sugar, complex carbohydrates and small peptides across the host-symbiont barrier. Moreover, Papina *et al.* (2003) postulated that zooxanthellae provide the coral host not only with saturated fatty acids, but also with diverse polyunsaturated fatty acids. For the scleractinian corals, whose skeletons comprise the physical structure of reefs, calcification rate is also influenced by the presence of *Symbiodinium* (Pearse & Muscatine, 1971). One of the biggest threats to the health of coral reefs today is the increasing frequency of bleaching of hermatypic corals (whitening of corals due to loss of either symbiotic algae or their pigments, or both). In severe cases corals do not recover and subsequently die (Brown, 1997; Hoegh-Guldberg, 1999). The severity of the bleaching response differs greatly between species of corals (Marshall & Baird, 2000; Loya *et al.*, 2001) and even across individual colonies (Ralph *et al.*, 2002). It also varies spatially on local and regional scales (Glynn, 2001). Zooxanthellae inhabiting the tissue of corals normally show low rates of migration or expulsion to water column (Hoegh-Guldberg and Smith, 1989 and Winters *et al.*, 2009). Despite these low rates, population densities have been reported to undergo seasonal change (Fagoonee *et al.*, 1999). Population densities of zooxanthellae in reef building corals range between 0.5×10^6 and 5×10^6 cell cm⁻².

² (Drew, 1972; Porter *et al.*, 1984 and Hoegh-Guldberg & Smith, 1989b). The purpose of the present work is to study the effect of zooxanthellae density on the growth rate of the scleractinian coral *Acropora hemprichii* from the Red Sea, at different sea depths and seasons of the year.

Material and Methods

Growth rate measurements

Growth rates as linear extension of *Acropora hemprichii* were measured at the fringing reef of Al-Fanader site, which is located 11 km south Al-Qusier City (Fig. 1). Four colonies of the studied species were chosen and marked at four different depths (Reef Flat, 10, 20, and 25 m depth). Branches from each colony were tagged by plastic string about 1.5 - 2.0 cm apart from the tip of the branch. The linear extension was measured seasonally using vernier caliper to measure the length of the tagged branch from the plastic string to the tip of the branch.

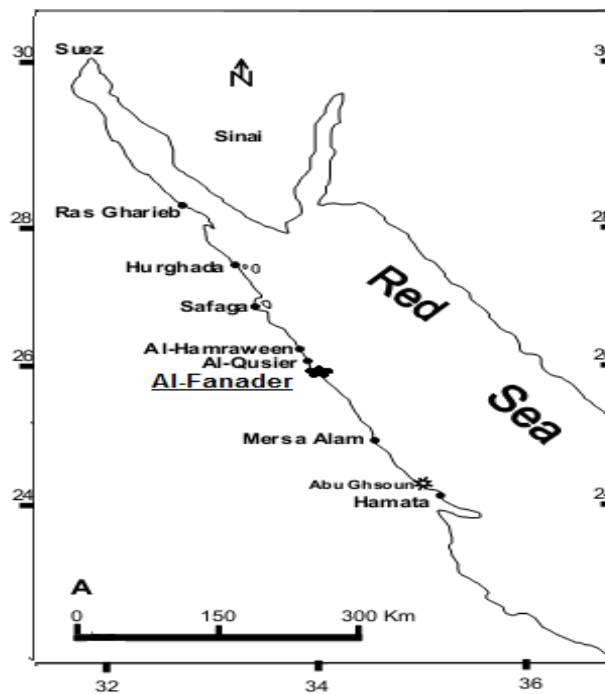


Fig. (1). Location map of the study site

Biomass Measurements

Skirt fragments (<5 cm fragment) from three separate colonies of *Acropora hemprichii* were seasonally collected at the same depths of the measured growth rates (reef flat, 10, 20 and 25 m depth). Only one terminal portion of the branch was sampled per coral colony, using a long nosed bone cutter. Samples were kept in dark by wrapping them in aluminium foil and placed in whirl-package under water. On the deck, water was removed from the bags and immediately transferred to foam box filled with ice waiting for transportation to NIOF laboratories for analysis zooxanthellae densities. In the laboratory, a tip of approximately equal size (1-2 cm) from each replicate was taken to measure the population densities of zooxanthellae. Tissues were striped from the skeletons with a jet of recirculated 0.45 µm membrane

filtered sea water using a water pikTM (Johannes and Wiebe, 1970). The slurry produced from the tissue-stripping process was homogenated in a blender for 30 second. and the volume of homogenate was recorded. The number of zooxanthellae in 10 ml aliquotes of homogenate was measured in triplicate by light microscope (X 400) using Count Rafter Cell. The total number of zooxanthellae per coral was measured after correcting the volume of homogenate. Zooxanthellae density was calculated as a number per unit surface area.

Zooxanthellae number / cm² = counted cells / cell surface area x cell depth x dilution

Surface area of the bare skeletons remaining after removal of tissue was measured independently using the paraffin wax technique (Stambler *et al.*, 1991), by immersing the skeleton bar in hot wax, the mass of wax added to the skeleton bare was determined by weighing the skeleton bare before and after immersion. A relationship between change in mass and surface area was obtained by immersing a known surface area cubes in the wax.

Results

Growth rates and zooxanthellae densities of *Acropora hemprechii* differed according to varying depths and seasons (Tab. 1). For growth rate the differences between depths and seasons were highly significant (ANOVA, $p < 0.01$) (Tab. 2). Turkey's Studentized Rang Statistical Analysis (HSD) (Tab.3) indicated that, growth rate at reef flat was significantly differed from those at 10 m, 10 m and 25 m depth. Recorded data indicated that the mean growth rate decreased with depth increasing, being 0.0412 ± 0.034 mm/day for *Acropora hemprechii* grow faster at reef flat than that at 10 m depth (0.0172 ± 0.003 mm/day). Moreover, at 10 m depth growth rate was faster than at 20 m depth being 0.0159 ± 0.0023 mm/day. While growth rate at reef flat was approximately three times higher than at 25 m depth being 0.013 ± 0.0024 mm/day. HSD also indicated that the mean value of growth rate in autumn was significantly differed from those in summer, winter and spring, meaning that growth rate in autumn (0.01769 mm/day) was lower than that in summer (0.0335 mm/day), winter (0.01831 mm/day) and spring (0.01835 mm/day) (Tab. 4). However, the highest growth rate was recorded during summer season.

A two – way Analyses of Variances (ANOVA) showed a highly significantly differences in the means of zooxanthellae densities between depths and seasons ($p < 0.01$) (Table 2). To detect the distinct variability between means of zooxanthellae density at the four depths and four seasons HSD (Zar, 1984) was applied (Tab. 5). It was shown that zooxanthellae density at reef flat was significantly differed from those at 10, 20 and 25 m depth. Zooxanthellae density at reef flat being $1.55 \pm 0.303 \times 10^6$ cells/cm² which is twice higher than at 25 m depth ($0.706 \pm 0.253 \times 10^6$ cells/cm²). At 10 m depth was $1.311 \pm 0.22 \times 10^6$ cells/cm² which is still higher than at 20 m depth ($0.88 \pm 0.036 \times 10^6$ cells/cm²) (Tab. 1). Furthermore, the lowest value of zooxanthellae was recorded at 25 m depth. In addition, HSD detected that zooxanthellae density recorded during summer was significantly different from those recorded during autumn or spring being 1.32×10^6 cells/cm² during summer, 0.931×10^6 cells/cm² during autumn and 1.09×10^6 cells/cm² during spring. Similarly zooxanthellae density recorded during spring still higher than those recorded during either winter or autumn being 1.106×10^6 cells/cm² during winter and 0.931×10^6 cells/cm² during autumn. (Table 6).

Correlations between zooxanthellae density and growth rate of *Acropora hemprichii* were pooled and the Pearson correlation analysis was applied. The correlation was significantly moderate at reef flat ($r = 0.461$ & $P < 0.01$) (Fig. 2), significantly high at 10 m depth ($r = 0.636$ & $P = 0.424$). Whereas at 20 and 25 m depth the correlations were insignificant ($r = 0.346$ & $P < 0.19$ and $r = 0.103$ & $P < 0.706$, respectively).

Finally, in the present study, the maximum growth rate of *Acropora hemprichii* was recorded at reef flat and the minimum rate was recorded at 25 m depth, meaning that growth rate decreased while depth increased. At the same manner, zooxanthellae density of *Acropora hemprichii* was highest at reef flat and the lowest was at 25 m depth. However, zooxanthellae density of *Acropora hemprichii* affects growth rate at reef flat and 10 m depth.

Discussion

Results of the present study demonstrated an interactive effect of zooxanthellae density on growth rate of *Acropora hemprichii* at reef flat and 10 m depth. This could be explained in view of Falkowski *et al.* (1984) that the total energy requirement in well-lit at reef flat and 10 m depth met by zooxanthellae photosynthetic production (Falkowski *et al.*, 1984). Papina *et al.* (2003) postulated that zooxanthellae provide the coral host not only with saturated fatty acid, but also with diverse polyunsaturated fatty acids. Moreover, Muscatine (1990) found that, zooxanthellae provide energy and nutrients for coral host by translocating up to 95% of their photosynthetic production. For the scleractinian corals, whose skeletons comprise the physical structure of reefs, calcification rate is influenced by the presence of *Symbiodinium* (Pearse & Muscatine, 1971). One of the biggest threats to the health of coral reefs today is the increasing frequency of bleaching of hermatypic corals (whitening of corals due to loss of either symbiotic algae or their pigments, or both) (Al-Hammady, 2011). The non interactive effects of zooxanthellae density on growth rate at 20 and 25 m depth could be caused by the obligate heterotrophy due to the lack of light at deeper depths. Similarly, Falkowski *et al.* (1984) reported that corals may obtain up to 60% of their energy at 20 and 25 m depth through feeding. This result agrees with McCloskey and Muscatine (1984) that the daily carbon fixed by zooxanthellae to animal respiration demands at 35 m was less than half that at 3 m, suggesting that deeper corals have an obligate requirement for heterotrophically obtained carbon. Anthony and Fabricius (2000) showed that heterotrophy increases both tissue and skeletal growth. In contrast, Wellington (1982) determined that heterotrophy has minimum effect on the skeletal growth of scleractinian corals. The importance of feeding as a supplemental source of nutrients depends on several environmental parameters, such as light availability (Falkowski *et al.*, 1984) or seawater turbidity (Anthony and Fabricius, 2000). Light enhancement of calcification is attributed to photosynthesis by the symbiont, though the exact mechanism of this enhancement is not well established (Allemand *et al.*, 1998 and Gattuso *et al.*, 1999). The decrease in number of symbiotic zooxanthellae, hosted by *Acropora hemprichii*, with depth increase is clearly explained as adaptations to limited photosynthetically active radiation at the deeper depth. McCloskey and Muscatine (1984), found that, *Stylophora pistillata* from 35 m showed a decrease in zooxanthellae density, and an increase in chlorophyll *a* per algal cell as compared to colonies from 3 m. Contrary, Falkowski and Dubinsky (1981) observed that the wide range of light intensities tolerated by the reef coral *Stylophora pistillata* is not necessarily due to zooxanthellae population of distinct ecotypes.

In contrast, Ammar (2004) found that zooxanthellae associated with *Favites persi* and *Porites solida*, increased especially in deeper areas, enables them to utilize the lowest amount of light, favouring this deeper area. Differences in the response of these species of coral to different depths may result from difference in tissue thickness that is associated with difference in the initial protein content (Warner *et al.*, 2002). Fitt *et al.* (2009) observed physiological and biochemical differences of both symbiont and host origin in response to high-temperature stress of *Porites cylindrica* and *Stylophora pistillata*. However, Al-Hammady (2011) reported that *Acropora humilis* had a higher decrease in its zooxanthellae densities than *Stylophora pistillata* at the same treatment. Ammar *et al.* (2011) detected a significant species variation in the susceptibility to bleaching stress. Celliers & Schleyer (2002) and Mc Field (1999) ascribed this phenomenon to difference in symbiont clade composition.

The higher growth rate of *Acropora hemprichii* at reef flat as compared to that at 25 m depth could be correlated to the increasing in density of zooxanthellae at reef flat than at 25m depth. Since the polyp receives a substantial part of its energy from the zooxanthellae (Muller-Parker and D'Elia, 1997), and any decrease in zooxanthellae densities will affect photosynthetic potential and coral growth (Szmant & Gassman, 1990 and Richmond, 1997). Previously it has been recorded that the rate of coral calcification is higher in light than in dark (Goreau, 1959; Pearse & Muscatine, 1971 and Chalker & Taylor, 1975). This light enhancement of calcification was attributed to photosynthesis by the symbiont, though the exact mechanism of this enhancement is not very well established (Allemand *et al.*, 1998 and Gattuso *et al.*, 1999). However, Houlbreque, *et al.* (2003) found that the dark calcification rates were four times lower than the rates of light calcification. Ammar (2004) observed that *Acropora hemprichii* prefer both extremes of illuminations at 10 m and 30 m depth zones, but it does not stand the strong waves of the reef flat zones. The present study indicated that *Acropora hemprichii* grow faster during the warm periods (summer and spring). This result coincides with the result of Vine (1986), Al-Hammady (2011) worked on different species of corals.

Results of the present study, contribute to the growing body of evidence showing that zooxanthellae plays a significant role in coral growth at shallow areas, and its effect must be taken into account in models explaining coral distributions. Mechanisms involved in the enhancement of the skeletal growth also require further investigation.

Conclusion

- The present study demonstrated that growth rate of *Acropora hemprichii* or zooxanthellae density decreased with depth increase. Growth rate at reef flat was approximately three times higher than at 25 m depth, and zooxanthellae density at reef flat was twice higher than at 25 m depth.
- Zooxanthellae density induced growth rate of *Acropora hemprichii* at reef flat and 10 m depth, while non interactive effects were obtained at 20 and 25 m depth.

Recommendations

- Eliminate factors that may enhance the effects of climate change and zooxanthella lost especially at shallow area.
- Further investigations dealing with the mechanisms involved in the enhancement of the skeletal growth in deeper area are required.

Acknowledgements

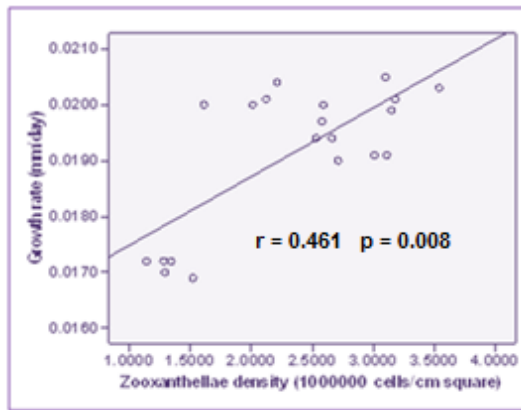
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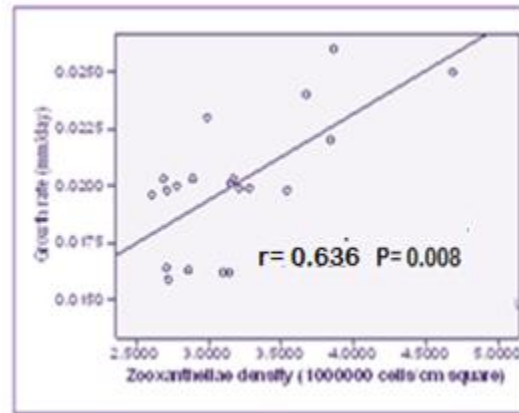
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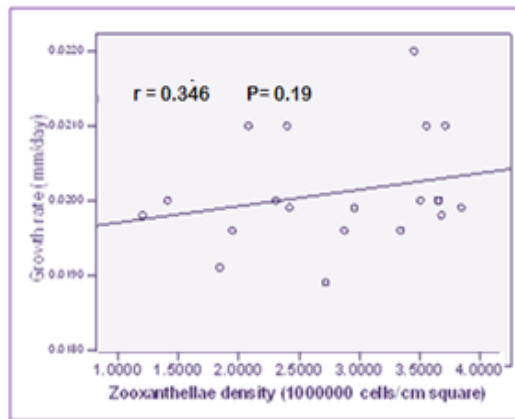
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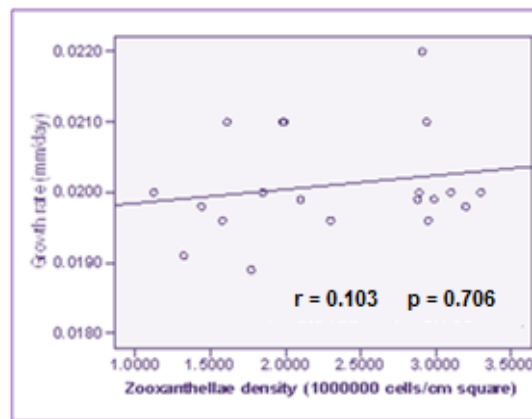
1.



(B)



(C)



(D)

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

Fig. (2). Pearson correlation between zooxanthellae densities (10^6 cells/cm²) and growth rate (mm/day) of *Acropora hemprichii*, (A): Reef flat, (B): 10 m depth, (C): 20 m depth and (D): 25 m depth.

Table (1). Seasonal mean of growth rate (mm/ day) and zooxanthellae densities (10^6 cells/cm²) of *Acropora hemprichii* at four different Depths (Reef Flat, 10, 20 and 25 m depth)s.

		Reef Flat	m depth 10	m depth 20	m depth 25
Autumn	G.r. X [±] S. D.	0.023±0.0013	0.0167±0.00017	0.0164±0.00037	0.0147±0.0012
	Zoox. X [±] S. D.	1.45±0.389	1.235±0.793	0.525± 0.005	0.51±0.028
Winter	G.r. X [±] S. D.	0.0203±0.0004	0.0188±0.00032	0.018±0.0008	0.0162±0.00079
	Zoox. X [±] S. D.	1.42±0.309	1.31±0.295	0.98±0.539	0.707±0.44
Spring	G.r. X [±] S. D.	0.0223± 0.00034	0.0202±0.00021	0.0172±0.00058	0.0135±0.001
	Zoox. X [±] S. D.	1.56±0.279	1.46±0.211	0.827±0.116	0.507±0.061
Summer	G.r. X [±] S. D.	0.0988±0.0027	0.0129±0.0046	0.0123±0.0012	0.0102±0.00013
	Zoox. X [±] S. D.	1.79±0.15	1.23±0.264	1.2±0.216	1.09±0.095
Annual mean	G.r. X [±] S. D.	0.0412±0.034	0.0172±0.003	0.0159±0.0023	0.013±0.0024
Annual mean	Zoox. X [±] S. D.	1.55±0.303	1.311±0.22	0.88±0.036	0.706±0.253

G. r.: growth rate (mm/ day) , Zoox. = zooxanthellae densities (10^6 cells/cm²), X': mean, S. D.: Standard deviation

Table (2). Two way analyses of variances (ANOVA) for the growth rate) and zooxanthellae densities of *Acropora hemprichii* at the studied Depths during the period of the study (four seasons).

Source	Growth rate					Zooxanthellae density				
	D.F.	ANOVAs SS	Mean Squares	F Value	Pr > F	D.F.	ANOVAs SS	Mean Squares	F Value	Pr > F
Depths	3	0.00618982	0.00216323	285.31	< .0001	3	76.86923	26.95341	314.7	< .0001
Seasons	3	0.00573948	0.00211314	214.27	< .0001	3	6.08196	2.15062	24.65	< .0001
Depths × Seasons	9	0.01362521	0.00123832	219.26	< .0001	9	7.187625	0.787847	24.23	< .0001
Total	15	0.0255545				15	90.13382			

Table (3). Turkey’s studentized range statistical analysis (HSD) for the differences between the measured growth rates of *Acropora hemprichii* by using the depths as dependent variables.

	Reef Flat (0.0412)	10 m depth (0.0172)	20 m depth (0.0159)	25 m depth (0.013)
Reef Flat (0.0412)				
10 m depth (0.0172)	0.024 **			
20 m depth (0.0159)	0.0253 **	0.0013 *		
25 m depth (0.013)	0.0282 **	0.0042 *	0.0029 *	

Number in parentheses= Growth rate (mm/day). Minimum significant difference 0.0007

** = Highly significant differences * = Significant differences

Table (4). Turkey’s studentized rang statistical analysis (HSD) for the differences between the measured growth rates of *Acropora hemprichii*, during the four seasons as dependent variable.

	Autumn (0.01769)	Winter (0.01831)	Spring (0.01835)	Summer (0.0335)
Autumn (0.01769)				
Winter (0.01831)	0.00062--			
Spring (0.01835)	0.00066--	0.00004 --		
Summer (0.0335)	0.0158**	0.015**	0.015**	

Number in parentheses= Growth rate (mm/day) Minimum significant difference 0.0007

** = Highly significant differences * = Significant differences

Table (5). Turkey's studentized rang statistical analysis (HSD), for the differences between the measured zooxanthellae density (10^6 cells/cm²), by using the depths as dependent variables.

	Reef Flat (1.55)	10 m depth (1.311)	20 m depth (0.88)	25 m depth (0.706)
Reef Flat (1.55)				
10 m depth (1.311)	0.239*			
20 m depth (0.88)	0.67**	0.431**		
25 m depth (0.706)	0.844**	0.605**	0.174--	

Number in parentheses= Zooxanthellae density (10^6 cells/cm²)

Minimum significant difference 0.2302 ** = Highly significant differences

* = Significant differences -- = Non-Significant differences

Table (6). Turkey's studentized rang statistical analysis (HSD), for the differences between the measured zooxanthellae density (10^6 cells/cm²) during the four seasons as dependent variable.

	Autumn (0.931)	Winter (1.106)	Spring (1.09)	Summer (1.32)
Autumn (0.931)				
Winter (1.106)	0.175--			
Spring (1.09)	0.159--	0.16--		
Summer (1.32)	0.389*	0.214--	0.23*	

Number in parentheses= Zooxanthellae density (10^6 cells/cm²)

Minimum significant difference 0.2302 -- = Non-Significant differences

* = Significant difference