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# Trends in the Density of Zooxanthellae in *Acropora millepora* (Ehrenberg, 1834) at the Palm Island Group, Great Barrier Reef, Australia

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**Abstract**

It is important to understand the natural variation of coral zooxanthellae considering the implications of loss of these micro-algal symbionts for coral survival during bleaching. This study explores the seasonal trend of zooxanthellae densities in the common coral, *Acropora millepora* over a period of seven months at a relatively undisturbed environment, the inshore reefs of the Palm Island Group in the Central Section of the Great Barrier Reef. Zooxanthellae densities increased twofold at both sites with the onset of colder temperatures and lower solar flux. This strongly suggests that a seasonal component is responsible for the observed changes in algal densities in *A. millepora*. Zooxanthellae densities also varied between colonies ($F=4.858$, $p=0.000$) and branches ($F=1.565$, $p=0.001$). The results of this study stress the importance of accounting for seasonal variation and for variation within and among colonies when designing studies to determine zooxanthellae densities and assessing the intensity of bleaching during mass bleaching events.

Keywords: Coral, zooxanthella density, *Acropora millepora*, Great Barrier Reef

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## 1. Introduction

Scleractinian corals are highly dependent on their symbiotic algae to meet their nutritional requirements and for light enhanced calcification (Goreau, 1959; Chalker and Taylor, 1975). The algal symbiont can translocate up to 95% of its photosynthates to the coral host, hence providing up to 143% of the daily costs of the association (Muscatine et al., 1984; Davies, 1991). Early work on the symbiosis between the scleractinian coral host and its micro-algal symbiont suggested that the association is usually stable under normal environmental conditions, with relatively constant algal densities averaging $1–2.5\times10^6$ cells/$cm^2$ (Drew, 1972; Muscatine, 1980; Wilkinson et al., 1988; Muscatine et al., 1989; Falkowski et al., 1993; Titlyanov et al., 1996) with each host cell accommodating 1–2 algae (Muscatine and Pool, 1979). Oliver (1984) noted variation in zooxanthellae density between inner and outer branches in the branching coral *Acropora formosa*, based on qualitative assessment of branch colour (white vs brown-tipped branches), however, it is not known whether such patterns are consistent among coral species.

In contrast to early suggestions that zooxanthellae densities are relatively constant, more recent laboratory studies have shown that algal density within the coral host vary in relation to exogenous factors such as nitrogen enrichment (e.g. Muscatine et al., 1989; Muller-Parker et al., 1994; Marubini and Davies, 1996; Grover et al., 2002), copper enrichment (Jones, 1997a), iron enrichment (Harland and Brown, 1989; Ferrier-Pages et al., 2001), cyanide (Cervino et al., 2001), starvation and osmotic shock (Titlyanov et al., 2000), increases in seawater temperature (Coles and Jokiel, 1978; Hoegh-Guldberg and Smith, 1989; Glynn and D'Croz 1990; Fitt and Warner, 1995), lowered seawater temperature (Gates et al., 1992), ultraviolet light (Jokiel and York, 1982; Gleason and Wellington, 1993) and Photosynthetic Active Radiation (PAR) (Titlyanov et al., 2001a,b).

Furthermore, field studies have shown that there are seasonal cycles in zooxanthellae densities in response to variations in environmental factors (Stimson, 1997; Fagoonee et al., 1999; Brown et al., 1999; Fitt et al., 2000). Stimson (1997) in his study of *Pocillopora damicornis* in Hawaii, found a positive correlation between algal density and dissolved nitrate concentrations and a negative correlation with algal density and solar radiation. Similarly Fagoonee et al. (1999) found that the algal density of *Acropora formosa* correlated positively with fluctuations in nitrate concentrations and time of year in Mauritius. In both studies, no significant correlation was found between seasonal changes in seawater surface temperature (SST) and solar radiation and algal density. In contrast, Brown et al. (1999) found a significant negative correlation between SST and PAR and algal density in four species of massive corals at Phuket, Thailand. Similarly Fitt et al. (2000) found that variation in

light and temperature caused seasonal cycles in coral algal density in *Montastrea annularis, M. faveolata, M. franksi, Acropora palmata* and *A. cervicornis*. On the other hand, Jones and Yellowlees (1997) did not find any seasonal changes in algal density in *Acropora formosa* on the Great Barrier Reef and concluded that algal density is set to the maximum upper limit determined by space availability.

If corals and their symbionts are highly dependent on a variety of physical variables, which vary seasonally, it is to be expected that the hosts and their algal symbionts likewise undergo seasonal changes and it would seem reasonable that zooxanthellae numbers should vary in response to such fluctuations. Considering the implications of loss of zooxanthellae for coral survival during bleaching events and the fact that density of zooxanthellae is now being used as a standard for quantifying the effects of temperature stress, UV stress or eutrophication on standing stocks of algal symbionts (e.g. Stimson and Kinzie III, 1991; Muller-Parker et al., 1994; Hoegh-Guldberg and Salvat, 1995), it is important to understand the natural variability of zooxanthellae over time and within corals. Documenting natural variability in zooxanthellae densities may also provide insights into seasonal patterns in coral growth (e.g. Oliver, 1984) given the clear evidence for light-enhanced calcification in corals (reviewed in Barnes and Chalker, 1990).

In this study, we examined the seasonal trend in zooxanthellae density in the coral, *Acropora millepora* on a reef under minor anthropogenic impact. We also looked at the degree of variability in zooxanthellae densities within and between colonies of *A. millepora*. In addition, we investigated a number of environmental factors, which might be correlated with potential seasonal variability in algal densities.

## 2. Materials and Methods

### *Study site*

This study was undertaken from February to August 1999 at two reef sites adjacent to continental islands of the Palm Island group (18°46'S; 146°15'E), in the Central section of the Great Barrier Reef (Fig. 1). One of the sites was situated on South-East Reef of Pelorus Island (Site A) and the other site was on North-East Reef of Orpheus Island (Site B). Both sites were exposed to the prevailing southeasterlies.

### *Field sampling techniques*

To look at seasonal variation in zooxanthellae densities, sampling was undertaken over two months in summer (February, March), one month

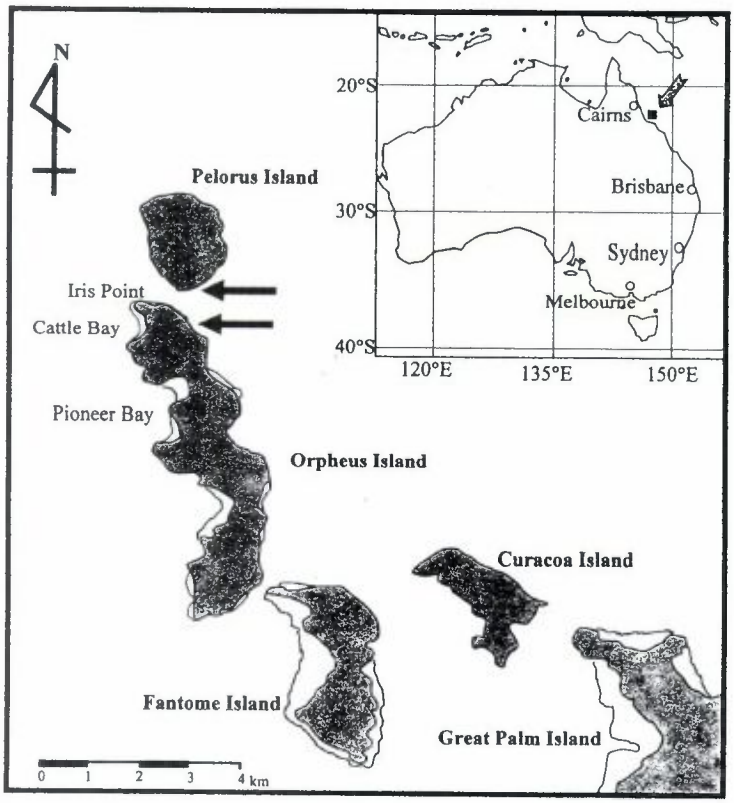


Figure 1. Location of sampling sites at South East reef of Pelorus Island and North East reef of Orpheus Island. Arrows indicate sampling sites.

representing the transition period between summer and winter (April) and two months in winter (May and August). Sampling at both sites was carried out on the reef slope at a depth of 4–6 m. Five colonies of *Acropora millepora* were randomly chosen at each site during each sampling period. Colonies were visually inspected before sampling to avoid sampling from the same colony so as to minimise stress, which might have caused corals to lose their symbiotic algae (e.g. Brown et al., 1995). Three branches approximately 4 cm in length were sampled only among the inner branches of each colony for consistency (see Oliver, 1979). The sampling at each site was modified in May to measure the variability of zooxanthellae density between inner and outer branches of each colony (within colony variation), inner branches being branches found in the innermost part of the corymbose coral and outer branches being those found on the peripheral part of the coral. Ten branches from each of five colonies were sampled, five from the inner and five from the peripheral part of the colony.

The branches were cut underwater with bonecutters and placed in plastic bags, taking care not to cause any abrasion of the tissues. Each branch was cut 4–5 cm below the tip of the branch to control the position at which algal densities were estimated. This protocol was adopted because we found algal density to vary along branches within a colony, the white portions (with reduced zooxanthellae densities) at the tips of branches varied from about 0.5 cm in length in inner branches to 2 cm in outer branches in *Acropora millepora*. Each branch was then wrapped in aluminium foil to occlude light, labeled, returned to the laboratory and kept frozen until processing.

*Determination of zooxanthellae density*

Tissue samples were removed from each branch with a jet of high-pressure air and seawater applied with an airbrush. This method generates a coral tissue/zooxanthellae slurry that is more concentrated than the more commonly used Water-Pik method (Johannes and Wiebe, 1970). We used 45 ml of 0.45 µm filtered seawater to produce the slurry for each branch. The slurry was then homogenised with an Ultra-Turrax T25 homogeniser at 20,500 rpm for 60 s. Samples for zooxanthellae counts were preserved by adding 5 ml of formalin. Thus tissue removed from each branch fragment was hence suspended in 50 ml of 10% formalin- seawater.

The number of zooxanthellae was counted from replicate (n=5) haemocytometer counts and standardised to tissue surface area by determining the live tissue area of each branch by a wax coating technique (Vytopil and Willis, 2001). To calculate the mean zooxanthellae density per $mm^2$ of tissue, the mean total number of zooxanthellae per branch sample (NBranch) was divided by the mean surface area per branch.

*Measurement of environmental factors*

Data on daily seawater temperature at Pelorus were obtained from loggers used as part of the long term temperature monitoring program conducted by the Great Barrier Reef Marine Authority. Data on the daily levels of Ultraviolet B (UVB) in Townsville from January 1997 to October 1999 were provided by A. Moise, James Cook University (JCU). Monthly mean solar irradiance and rainfall values from January 1997 to August 1999 for the area around Orpheus Island were obtained from the Bureau of Meteorology, Townsville. Data on salinity were not recorded during the study period, therefore we have used rainfall data as a proxy, as there is a strong negative correlation between amount of rainfall and salinity in an area (Walker, 1981).

To determine to what extent variation in zooxanthellae density could be

explained by simultaneous variations in several environmental parameters, scattergrams were fitted with a trendline. The critical value of the correlation coefficient, r was calculated (Zar, 1984) and its significance determined. The values of the environmental parameters are estimates of mean monthly values.

*Statistical analysis*

To examine temporal patterns in zooxanthellae densities, mean zooxanthellae densities were compared between months using a nested ANOVA. The factors 'time', 'site', 'colony' and 'branch' were taken as random. 'Colony' was nested within 'time' and 'site', 'branch' was nested within 'colony'. Due to the significant interaction detected between 'site' and 'time', we analysed the data for each site separately with a three-way ANOVA (Underwood, 1997; Coakes and Steed, 2001). For both these analyses, 'colony' was nested within 'time' and 'branch' was nested within 'colony'. When ANOVAs detected significant differences between means, *post hoc* tests (Student-Newman-Keuls (SNK)) were used to determine which months differed significantly (Underwood, 1997).

## 3. Results

*Seasonal patterns in zooxanthellae density*

Zooxanthellae density in *A. millepora* increased at both sites during the winter months (Fig. 2). At site A, there was a twofold increase in zooxanthellae density between February and August and an even greater increase was observed at site B. Density tended to be higher at site B except for the month of April, when higher mean densities were found at site A. This reversal in highest zooxanthellae densities between the two sites in April may explain the significant interaction between site and time (Table 1) and greater increase in August. When analysed separately, each site showed that zooxanthellae density differed significantly between sampling times with the highest densities in the colder months ($F=15.178$, $p=0.000$; $F=16.897$, $p=0.000$).

*Variation in zooxanthellae density between and within colonies*

Zooxanthellae densities varied significantly between colonies and branches (Table 1). The inner branches of *A. millepora* colonies had higher densities of zooxanthellae than the outer branches and this trend was observed at both sites (Fig. 3). At site A, mean zooxanthellae densities were $3.4\times10^6$ cells/cm$^2$ for inner branches of *A. millepora* and $3.0\times10^6$ cells/cm$^2$ for outer branches.

Table 1. Summary statistics for a 4-way nested ANOVA comparing zooxanthellae densities over time, between and within colonies. *Indicates significant differences for $\alpha$=0.05.

| Source of variation | df | MS | F | p |
|---|---|---|---|---|
| Time | 4 | $1.828\times10^{14}$ | 9.158 | 0.027* |
| Site | 1 | $1.686\times10^{13}$ | 0.845 | 0.410 |
| Colony (timexsite) | 40 | $6.204\times10^{12}$ | 4.858 | 0.000* |
| Branch (colony (timexsite)) | 100 | $1.277\times10^{12}$ | 1.565 | 0.001* |
| Timexsite | 4 | $1.996\times10^{13}$ | 3.217 | 0.022* |
| Error | 40 | $6.204\times10^{12}$ | | |

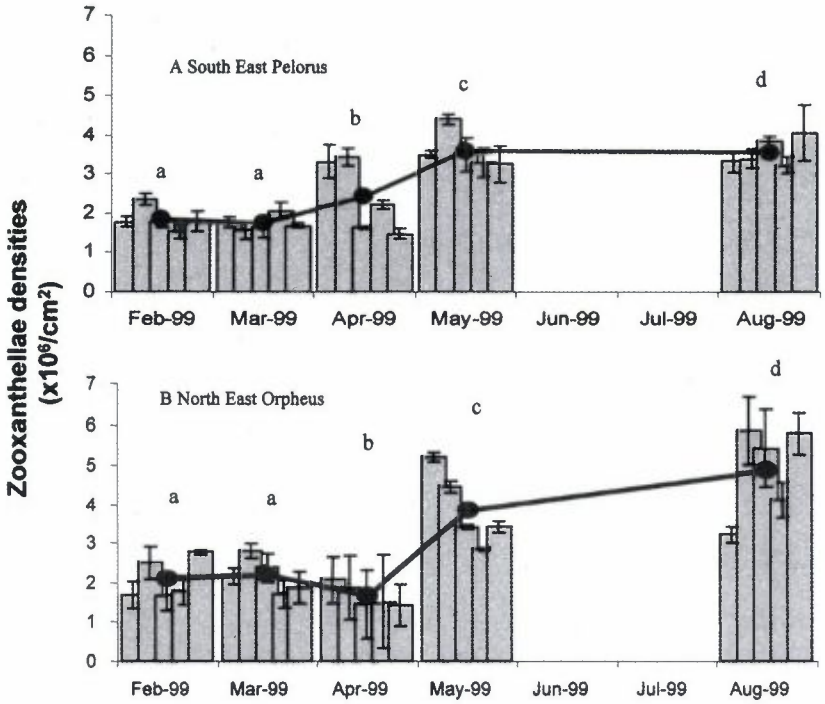


Figure 2. Mean algal densities (±S.E.) per colony (histogram) and mean algal density per sampling month (circles) for *A. millepora* over each sampling time at South East Pelorus and North East Orpheus. Lower case letters show membership to groups identified by Student-Newman-Keuls (SNK) means comparisons ($p<0.05$). (n=75 replicate algal counts/colony). The line joining the data points does not represent a continuous trend of monthly change as no samples were collected in the months of June and July.

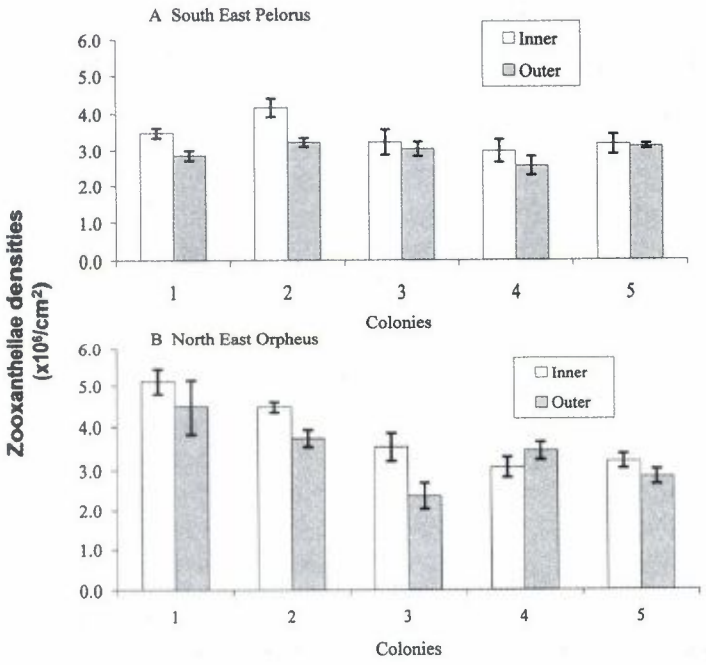


Figure 3. Mean zooxanthellae density (±S.E.) in inner and outer branches of *A. millepora* colonies in May 1999 at Pelorus and Orpheus. (n=75 algal counts/position (inner or outer)/colony).

At site B, mean densities were $4\times10^6$ cells/cm$^2$ for inner branches of *A. millepora* and $3.9\times10^6$ cells/cm$^2$ for outer branches. The pattern of higher zooxanthellae densities in inner branches was consistent for all colonies except one.

*Sea water measurement*

Seawater temperature obtained from data loggers over the past three years show a seasonal trend, with the highest temperatures being recorded in January–February during the summer and the lowest in July–August during the winter (Fig. 4).

*Ultraviolet radiation (UVB)*

Mean values of UVB radiation for the period January 1997 to October 1999 show that levels of UVB followed a seasonal trend in 1999, dropping to its lowest values in June and July (Fig. 5).

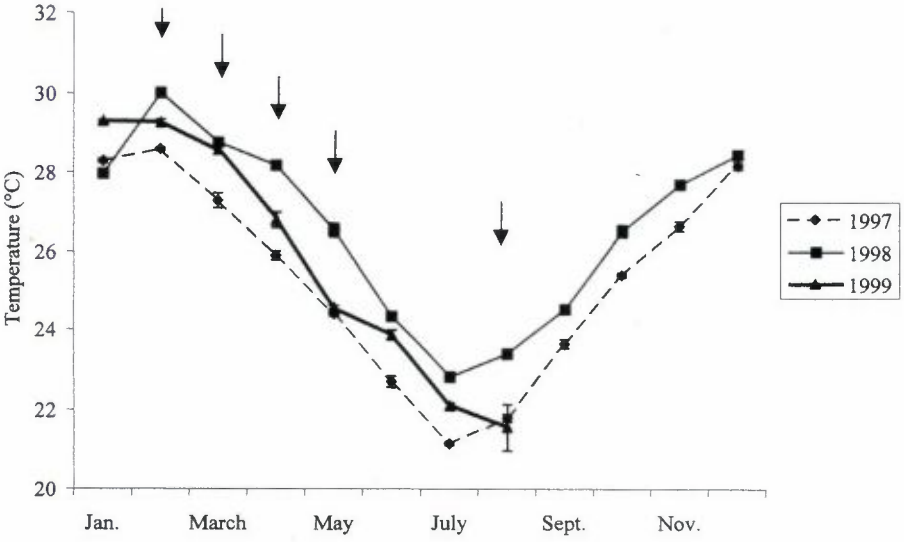


Figure 4. Monthly mean seawater temperature at Pelorus recorded by data loggers from 1997 – August 1999 (courtesy R. Berkelmans, GBRMPA). Arrows indicate months of sampling in the present study.

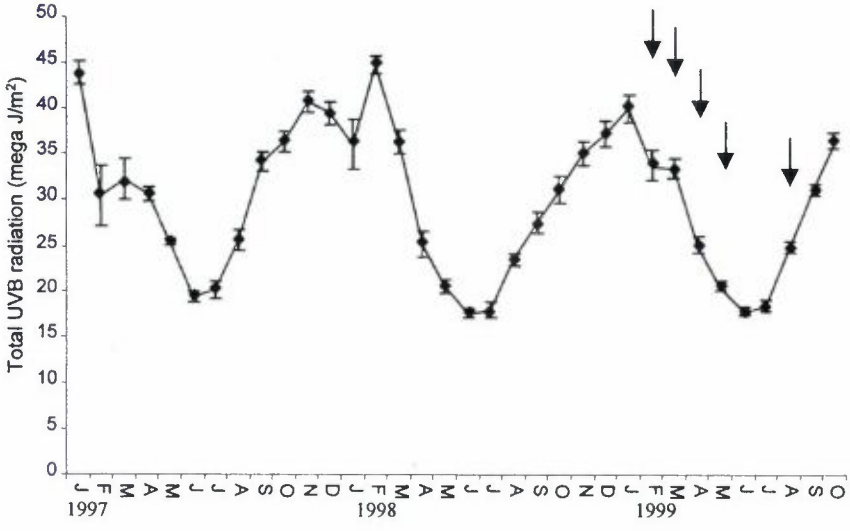


Figure 5. Mean UVB radiation levels during 1997, 1998 and until October 1999 in Townsville (courtesy of A. Moise, JCU). Arrows indicate month of sampling in the present study.

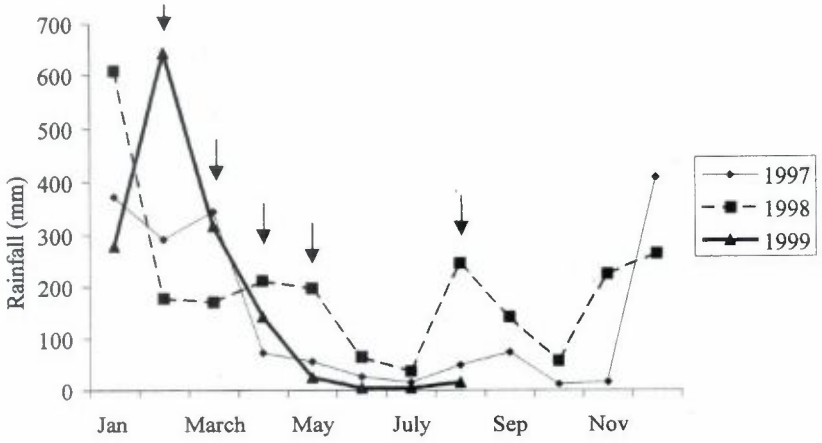


Figure 6. Monthly rainfall at Orpheus island from 1997 to August 1999. (Bureau of Meteorology, Townsville). Arrows indicate months of sampling in the present study.

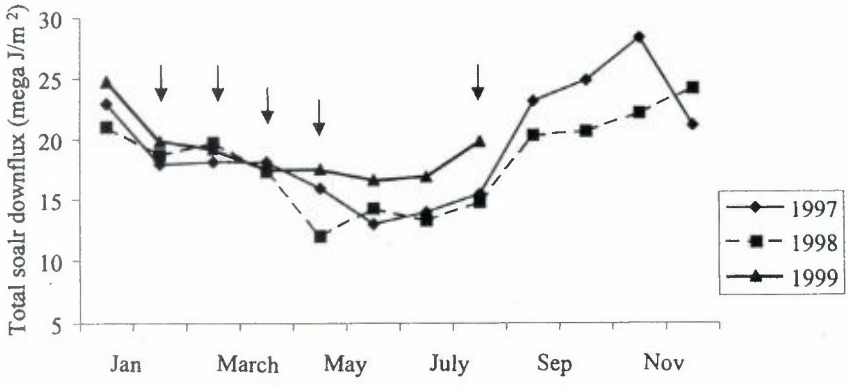


Figure 7. Mean total solar flux data during 1997, 1998 and up to August 1999. (Bureau of Meteorology, Townsville). Arrows indicate months of sampling in the present study.

## *Rainfall*

Mean rainfall values for the period January 1997 to August 1999 show that the dry winter season started in May 1999 and continued throughout the rest of the sampling period (Fig. 6). Average rainfall obtained over the period May to August 1999 was lower than that recorded for 1997 and 1998.

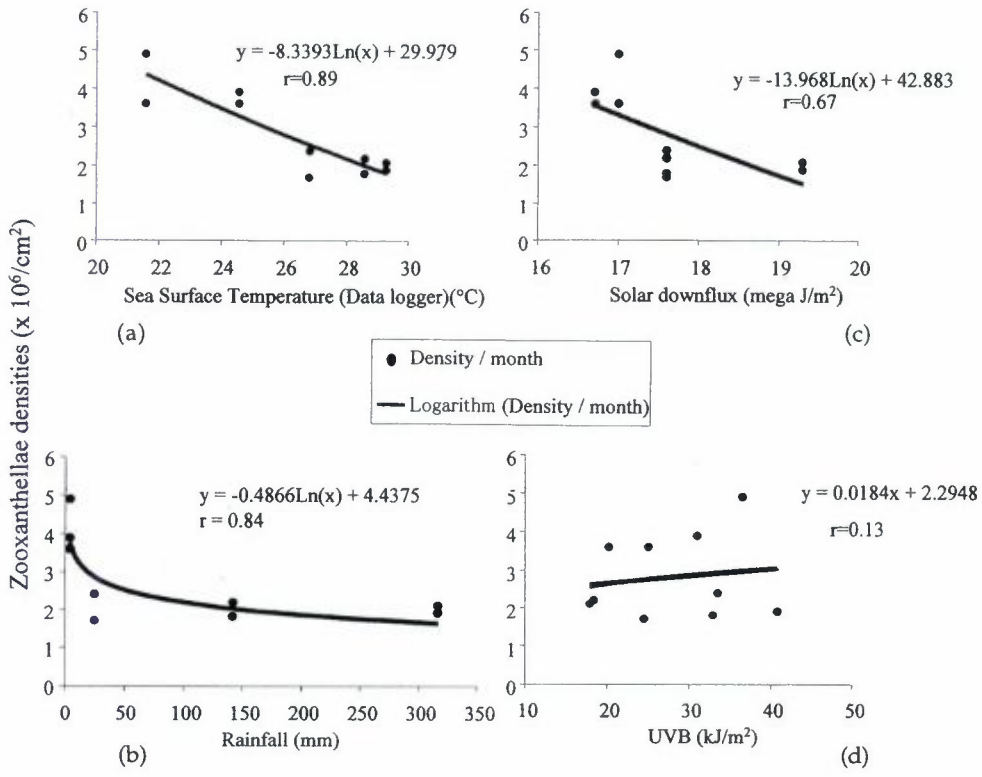


Figure 8. Relationship between zooxanthellae densities and environmental variables.

*Solar radiation*

Average monthly values for solar radiation from the Meteorological Services is presented in Fig. 7. Solar radiation showed a seasonal trend though the values of radiant heat were consistently higher from January to August in 1999, except for March and April. Minimum light values occurred from April to July.

*Association between environmental variables and zooxanthellae density*

Density of zooxanthellae was negatively correlated with sea surface temperature ($r=0.89$, $p<0.05$, Fig. 8a), rainfall (salinity) ($r=0.84$, $p<0.05$, Fig. 8b) and solar radiation ($r=0.67$, $p<0.05$, Fig. 8c). There was no indication of a relationship between density of zooxanthellae and ultraviolet radiation ($r=0.13$, $p<0.05$, Fig. 8d).

## 4. Discussion

*Seasonal patterns in algal densities*

Zooxanthellae densities in *Acropora millepora* increased significantly over the study period with the lowest algal density recorded in February–March (summer) at site A and from February to April at site B and the highest algal density in August (winter) at both sites. As mentioned earlier, other studies have reported similar seasonal changes in algal density in other species of corals at different geographic locations in response to various environmental factors (e.g Stimson, 1997; Fagoonee et al., 1999; Brown et al., 1999; Fitt et al., 2000).

In this study, algal density showed a negative correlation most strongly with temperature and also with solar radiation and rainfall (salinity). The observed seasonal trends in temperature and solar radiation at our study sites may have caused the algal densities to decrease during the warmer and high irradiance summer months and to peak during the cooler and low irradiance winter months. Our results confirm the findings of Brown et al. (1999) and Fitt et al. (2000). In fact these authors have suggested that seasonal changes in temperature and solar radiation are the most likely environmental factors responsible for the seasonal variation in zooxanthellae density.

Furthermore, several experimental studies have shown that algal density within a coral host can decrease in relation to factors such as an increase in temperature (Hoegh-Guldberg and Smith, 1989; Glynn and D'Croz, 1990), and the synergistic effects of high solar radiation and temperature (Jokiel and Coles, 1990; Smith and Buddemeier, 1992).

The lack of correlation between algal density and UVB levels suggests that seasonal patterns in UVB may not influence zooxanthellae densities. In an experimental study of the effect of temperature and light on zooxanthellae of four species of Caribbean corals, Fitt and Warner (1995) reported that the fluorescence ratio of the zooxanthellae were not affected by natural levels of UVB, most probably because most shallow-water corals have mycosporine-like amino acids (MAAs) to protect them from such dangerous wavelengths.

Two studies have reported that dissolved nitrates in seawater could be responsible for the observed seasonal pattern in zooxanthellae density (Stimson, 1997; Fagoonee et al., 1999) while Brown et al. (1999) and Fitt et al. (2000) have suggested that concentrations of nutrients might not be one of the factors responsible for the observed seasonal cycles of zooxanthellae density in corals. Levels of nutrients were not analysed in the present study and consequently we can only offer speculative evidence. Sources of elevated nutrients in the field are most likely to be from terrestrial run-off, stream or from storm-driven resuspension events (Muller-Parker and D'Elia, 1997) and

consequently are relatively low in GBR waters (Furnas, 1992). In comparison, ammonium and nitrate levels used in laboratory experiments that have demonstrated a link between nutrients and zooxanthellae densities have been relatively high, between 6 to 33 times levels recorded in typical field conditions (Stimson, 1997). The amplitude of variation in nutrient concentration in the present study is unlikely to have exceeded levels found to cause changes in algal densities and consequently may not be one of the factors responsible for the observed seasonal pattern in algal density in *A. millepora.*

There are several interpretations to explain natural changes in algal densities. This study shows lower zooxanthellae density in *A. millepora* during high summer temperatures and higher zooxanthellae density during low winter temperatures. The increase in algal density may be due to physiological seasonal adaptations by the coral to reduced light regime during the cooler months. In the presence of low irradiance, the corals may be increasing their light harvesting capacity by increasing the population density of the symbionts. Such a trend has been previously reported, as for example by Thinh (1991) who found a twofold increase in the algal density of *A. formosa* and *A. cuneata* when the irradiance levels were reduced experimentally from 50 to 4–5 $\mu mol\ m^{-2}\ s^{-1}$. Similarly, Titlyanov et al. (1999, 2001a,b) reported an increase in zooxanthellae population density when branches of *Stylophora pistillata* were experimentally exposed to low light intensity.

In contrast, other experimental studies have shown that corals adapt to low irradiance by a reduction in the algal population to reduce self shading (Vareschi and Fricke, 1986) while in others, low irradiance had no effect on the population density of the algal symbionts (e.g. Falkowski and Dubinski, 1981). It is likely that such contrasting results are indicative of different photoadaptive responses by different coral species. The increase in algal density noted in this study is highly indicative of endogenous control of zooxanthellae densities whereby algal densities increase under low light levels, may be along with a number of interacting biological mechanisms, for example by altering zooxanthellae size and photosynthetic pigment contents per cell (Vareschi and Fricke, 1986, and references therein). This hypothesis agrees with Titlyanov et al. (1980)'s natural experiment who found that corals sampled in the shade, at low irradiance, increase the size and number of their symbiotic algae together with an increase in pigment content and a change in photosynthetic pigment. As to whether the corals increase their zooxanthellae densities in response to only low irradiance or to combined low irradiance and temperatures remains to be determined. Some experimental studies have looked at the effects of high temperature and high solar radiation on zooxanthellae densities (e.g. Lesser et al., 1990; Jones et al., 1998) but there are no studies examining the conditions prevailing during the tropical winter season, when there are concurrent low irradiance levels and low temperatures. Experimental

studies incorporating such conditions would provide clues in relation to the response of zooxanthellae over the cooler season.

*Variation in zooxanthellae densities between and within colonies*

Both study sites showed a significant difference in zooxanthellae densities between colonies sampled at the same time. Similar observations were made by Jones (1997a,b) and Jones and Yellowlees (1997) in colonies of *Acropora formosa* from the Northern Great Barrier Reef. The authors suggest that such inter colony differences in algal densities are due to differences in algal sizes between colonies. In addition, Brown (1997) suggests that variability at the colony level may also be the result of fine scale fluctuations in illumination, hydrodynamics or sedimentation that may occur locally. For example the undersides of coral tissues, which do not receive an adequate light supply, often have no algal symbionts (Duerden, 1902, cited in Jones and Yellowlees, 1997).

Zooxanthellae densities differed between inner and outer branches of *A. millepora* and this trend was consistent at both sites. Intra specific differences in zooxanthellae densities has been reported by Titlyanov (1991), in the coral *Pocillopora damicornis* under natural light conditions. Zooxanthellae concentration was observed to decrease in older branches in shallow high light habitat due to self shading by the younger outer branches. In our study, we observed a higher concentration of zooxanthellae in inner branches of *A. millepora* whereby self shading by outer branches is limited due to the coral's corymbose morphology. Most probably, the observed difference in algal densities might be due to fine scale exogenous environmental parameters and /or endogenous control of zooxanthellae densities by the coral host or the algae.

This study has clearly shown that there is a seasonal pattern in zooxanthellae densities in *A. millepora* with an increase in densities in the colder months when solar flux is also reduced. A second finding of this study is the relatively high variability of zooxanthellae density even at the between and within-colony levels. These findings stress the importance of considering seasonal, intra-colony and inter-colony variation in zooxanthellae densities when designing field or experimental studies to measure zooxanthellae densities, for example as an indicator of temperature stress, UV stress, eutrophication or bleaching/impact.

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