

POPULATION DENSITY AND PHOTOSYNTHETIC PIGMENT CONTENT IN
SYMBIOTIC DINOFLAGELLATES IN THE BRAZILIAN
SCLERACTINIAN CORAL *MONTASTREA CAVERNOSA* (LINNAEUS, 1767)Cristiane F. Costa¹, Roberto Sassi² & Fernanda D. Amaral³

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A B S T R A C T

The seasonal dynamics of cell density and photosynthetic pigment contents of the zooxanthellae hosted by *Montastrea cavernosa* were investigated on coastal reefs off Picãozinho (06°42'05"/07°07'30"S and 34°48'37"/34°50'00" W), Northeast Brazil between September 1999 and 2000. A distinct pattern of these parameters was found: cell numbers were greater during the rainy season (autumn/winter) while photosynthetic pigments were greater during the dry season (summer). Both parameters showed drastic reductions during heavy rains (June and July 1999). We speculate that this pattern is largely influenced by the rain cycles which, owing to their magnitude and frequency, affect the water clarity and the seasonal physiological condition of the cells.

R E S U M O

A dinâmica sazonal na densidade de células e na concentração de pigmentos fotossintetizantes das zooxantelas de *Montastrea cavernosa* foram analisados no período setembro/1999 a setembro/2000 nos Recife do Picãozinho (06°42'05"/07°07'30" S e 34°48'37"/34°50'00" W), Nordeste do Brasil. Verificou-se que existe um padrão distinto entre estes parâmetros, com maior quantidade de células no período chuvoso e maior concentração de pigmentos fotossintetizantes na época de estiagem. Ambos os parâmetros apresentaram, no entanto, uma nítida redução em seus valores nos meses de maiores índices pluviométricos (junho e julho /1999). Especulamos que tal fato deve estar relacionado com o regime de chuvas que pode variar em magnitude e frequência, afetando a qualidade ótica da água e o estado fisiológico das células.

Descriptors: Brazil, *Montastrea cavernosa*; Symbiotic dinoflagellates; Zooxanthellae.
Descritores: Brasil, *Montastrea cavernosa*; Dinoflagelados simbióticos; Zooxantelas.

I N T R O D U C T I O N

Studies on the temporal variations of population density and photosynthetic pigment content of the zooxanthellae of hermatypic corals are scarce, particularly throughout a complete annual cycle. Most previous studies have been done over relatively short

temporal scales and do not provide sufficient data for reliable conclusions on the monthly and seasonal fluctuations of these characteristics of zooxanthellae (e.g. Fitt *et al.*, 1993; Fitt & Warner, 1995). Such aspects are nevertheless important for a detailed understanding of the functional dynamics of coral habitats, as well as the bleaching process.

Most studies done on symbiotic dinoflagellates in coral have only attempted to explain specific functional processes such as photosynthesis, photoacclimation, and the contribution of zooxanthellae to coral metabolic requirements (Porter *et al.*, 1984; Lesser, 2000). Further information is required on the population density and photosynthetic pigments of zooxanthellae throughout the year in the host tissues.

Using materials collected from reefs at Kaneohe Bay (Hawaii), Stimson (1997) completed one of the first investigations emphasizing variations in the zooxanthellae density of *Pocillopora damicornis* throughout a complete annual cycle. Although the data set comprised several disparate years, the existence of seasonal effects on cell densities was demonstrated, with lower values in spring and summer than in winter. Verde & McCloskey (1998) also provided data on the temporal variations in dinoflagellate densities, photosynthetic pigments, and mitotic index, in addition to information on production, respiration, and photophysiology of symbionts of the jellyfish *Cassiopea xamachana* in mangroves of Florida; but this work involved only two months: September, 1999 and January, 1993.

Significant reductions in the density and photosynthetic pigment content of zooxanthellae of hermatypic corals were reported by Brown *et al.* (1999) in the Indo-Pacific Ocean during the dry season. Fitt *et al.* (2000) also observed seasonal variations of zooxanthellae densities in five species of hermatypic corals, relating these to alterations in tissue biomass and coral bleaching. Seasonal fluctuations in the photosynthetic capacity of photosystem II were recently investigated by Warner *et al.* (2002) in three species of *Montastrea* in the Bahamas. This study also observed potential intra and interspecific differences at different depths.

In Brazil, the few existing studies on zooxanthellae were done in the northeast, emphasizing the use of dinoflagellates as a complement to the systematics of corals and hydrocorals (Amaral & Costa, 1998; Costa & Amaral, 2002), and also on some aspects of coral bleaching (Costa *et al.*, 2001). C. F. Costa *et al.* (unp. data) studied monthly fluctuations in the number of cells and photosynthetic pigment concentration of the zooxanthellae of endemic Brazilian corals (*Mussismilia harttii*, *M. hispida* and *Siderastrea stellata*).

The present study aims to evaluate possible variations in population density and photosynthetic pigment content of zooxanthellae hosted by the coral *Montastrea cavernosa*, a species occurring in other regions of the world. This is the only species of *Montastrea* found in all Brazilian reef areas, except in the Cape of São Roque, Northern Brazil (Castro & Pires, 2001). Although aspects of these symbionts

have been investigated in other regions (Baker & Rowan, 1997; Billingham *et al.*, 1997), no data exist from Brazilian reefs.

MATERIALS AND METHODS

Study site and collection of corals - Field studies were performed on the reefs of Picãozinho (06°42'05"/07°07'30" S and 034°48'37"/34°50'00" W), Northeast Brazil. During each month between September 1999 and September 2000, three 10-cm diameter fragments of the coral *Montastrea cavernosa* were collected using a rock pick and hammer. Samples were placed in labeled plastic bags filled with seawater and transported to the laboratory in an insulated cooler.

Monthly water temperature and salinity were determined at the surface using a thermometer ($\pm 0.1^\circ\text{C}$) and an American Optical refractometer (model 10419), respectively. Pluviometric data were obtained from the LMRS — Laboratório de Meteorologia, Recursos Hídricos e Sensoriamento Remoto da Paraíba.

Zooxanthellae - The tissue covering 1cm² of each coral sample was waterpiked from each colony using a WaterPik™ with filtered seawater, the volumes of which were mechanically homogenized and recorded (Dustan, 1979). To avoid contamination in pigment analysis, all specimens were carefully checked for macroalgae adhered to the coral. The density of zooxanthellae was determined in a Fuchs-Rosenthal chamber and four samples obtained from each homogenized preparation were examined. Pigments from subsamples were extracted in acetone (90%) for analyzing chlorophyll-*a* (Chl-*a*) and chlorophyll-*c* (Chl-*c*) and absorbencies read on a spectrophotometer. The contents were calculated using the method of Parsons & Strickland (1963).

Statistical analyses - Statistical analyses were performed using STATISTICA version 4.0 software, at 5% level of significance. Homoscedasticity of variances of all data sets was confirmed using Levene's test. The means of variables measured over the year were compared by ANOVA and tukey HSD post hoc test. The Student's *t*-Test was applied to compare the means differences among the dry and rainy seasons. Pearson's correlation test was used for the linear correlation between population density and photosynthetic pigment content of symbionts.

RESULTS

The highest pluviometric values (total monthly) were recorded between April and July 2000

(autumn/winter; rainy season), with a peak of 500 mm (Fig. 1). The temperature and salinity of superficial waters fluctuated throughout the year, with the highest values recorded during January and February (dry season). The lowest temperatures were recorded in September 1999 and June and July 2000, while the lowest salinity was during September 2000 (Fig. 1).

Cell density and photosynthetic pigment content varied throughout the study period but showed no correlation (Figs 2, 3; Pearson's correlation, $P > 0.05$). The number of cells increased clearly and simultaneously to the contents of Chl-*a* and Chl-*c* only during January 2000. These parameters were strongly reduced during the driest months (November and December 1999), at the beginning of the rainy season (February 2000) and during the highest rainfall (Figs 2, 3).

There were significant fluctuations in zooxanthellae density (ANOVA; $P < 0.05$), with an annual mean (\pm sd) of $2.6 \pm 1.2 \times 10^6 \text{ cm}^{-2}$. The difference between the maximum and minimum values observed was significant (tukey's Test; $P < 0.05$). The variation in the numbers of cells obtained during the dry and rainy seasons was significant (t -test; $P < 0.05$).

The photosynthetic pigment content showed significant fluctuations throughout the year (ANOVA; $P < 0.05$), but the difference obtained between the dry and rainy seasons was not significant (t -Test; $P > 0.05$). The means of zooxanthellae parameters obtained from the dry and rainy seasons showed a distinct pattern with an increase in the number of cells and a decrease in contents of Chl-*a* and Chl-*c* during the rainy season (Figs 3 and 4).

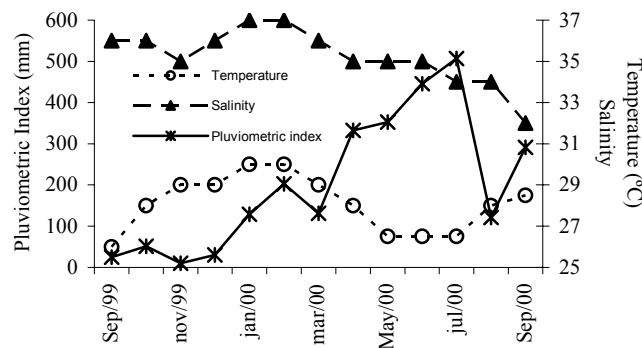


Fig. 1. Seasonal variations of pluviometric index (total monthly), water temperature and salinity of coral reefs at Picãozinho, Brazil, between September 1999 and 2000.

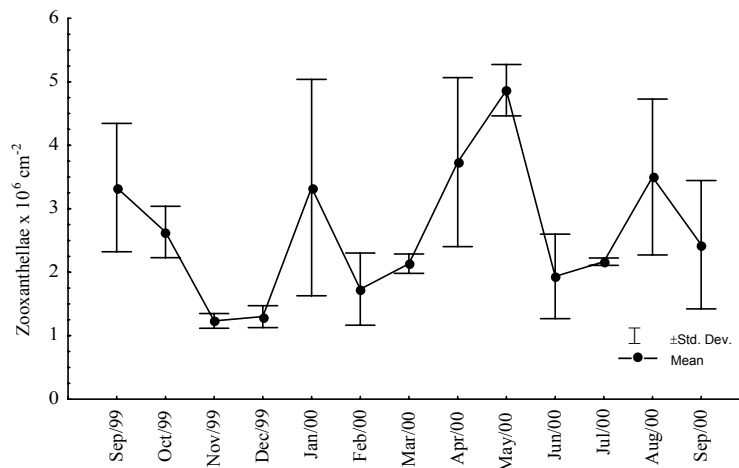


Fig. 2. Seasonal variations of zooxanthellae mean densities of *Montastrea cavernosa*, collected between September 1999 and 2000 in the reefs of Picãozinho, Brazil.

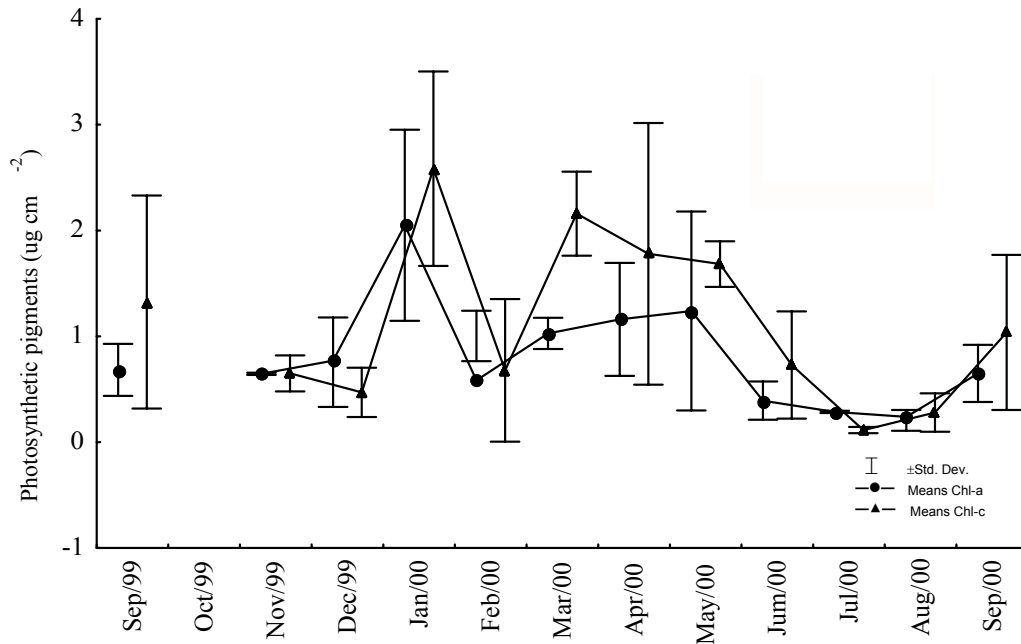


Fig. 3. Seasonal variations of chlorophyll-*a* (Chl-*a*) and chlorophyll-*c* (Chl-*c*) of *Montastrea cavernosa* zooxanthellae, collected between September 1999 and 2000 in the reefs of Picãozinho, Brazil.

DISCUSSION

Light and water temperature have been reported as the most important factors affecting the physiology of zooxanthellae (Lombardi *et al.*, 2000; Gorbunov *et al.*, 2001; Glynn *et al.*, 2001; Warner *et al.*, 2002). These parameters are certainly co-responsible for the temporal variations of the zooxanthellae in shallow and coastal waters in the studied area, although light seems to have the most influence.

The data we obtained showed fluctuations of symbiotic parameters of *Montastrea cavernosa* throughout the year that were related to rainfall in the littoral region. Specifically, cells density and photosynthetic pigments showed higher values during first rains, but decreased strongly during the months of heavy rains (June and July). This pattern may be attributed to the continental runoff in the area, which affects water clarity during high rainfall, increasing stress on the coral. C. F. Costa *et al.* (unp. data) has also suggested that coastal erosion from cliffs southwards of the area also could increase sedimentation, owing to suspended materials carried northwards by tidal currents.

In other regions, seasonal changes in zooxanthellae frequently have been associated with

water temperature, season and light conditions. But other factors also related to season may be important to control zooxanthellae densities hosted by corals, particularly in shallow water with large environmental fluctuations (Fagoonee *et al.*, 1999). A clearly significant and predictable seasonal variation in cell numbers (normally higher in cool season) and pigment content (higher at the beginning of cool season) was found by Brown *et al.* (1999) in near-equatorial Thailand and by Stimson (1997) and Fagoonee *et al.* (1999) in zooxanthellae from coral species in the Pacific Ocean. Correlations from the Caribbean correlates include those observed by Fitt *et al.* (2000), relating zooxanthellae density fluctuations to changes in host tissue biomass.

We don't discount the seasonal influence of thermal conditions on the parameters of zooxanthellae in the studied area, but here these variations exhibit small differences between values of minimum and maximum temperature throughout the year, probably improving small actions on the symbiotic cells of corals. Temperature patterns are very stable in the region with the mean monthly air temperature varying by only about 4.5°C over the past thirty years (INMET - Instituto Nacional de Meteorologia, Laboratório de Meteorologia, Recursos Hídricos e Sensoriamento Remoto da Paraíba, LMRS/PB, <http://www.lmrs-semarh.ufpb.br>).

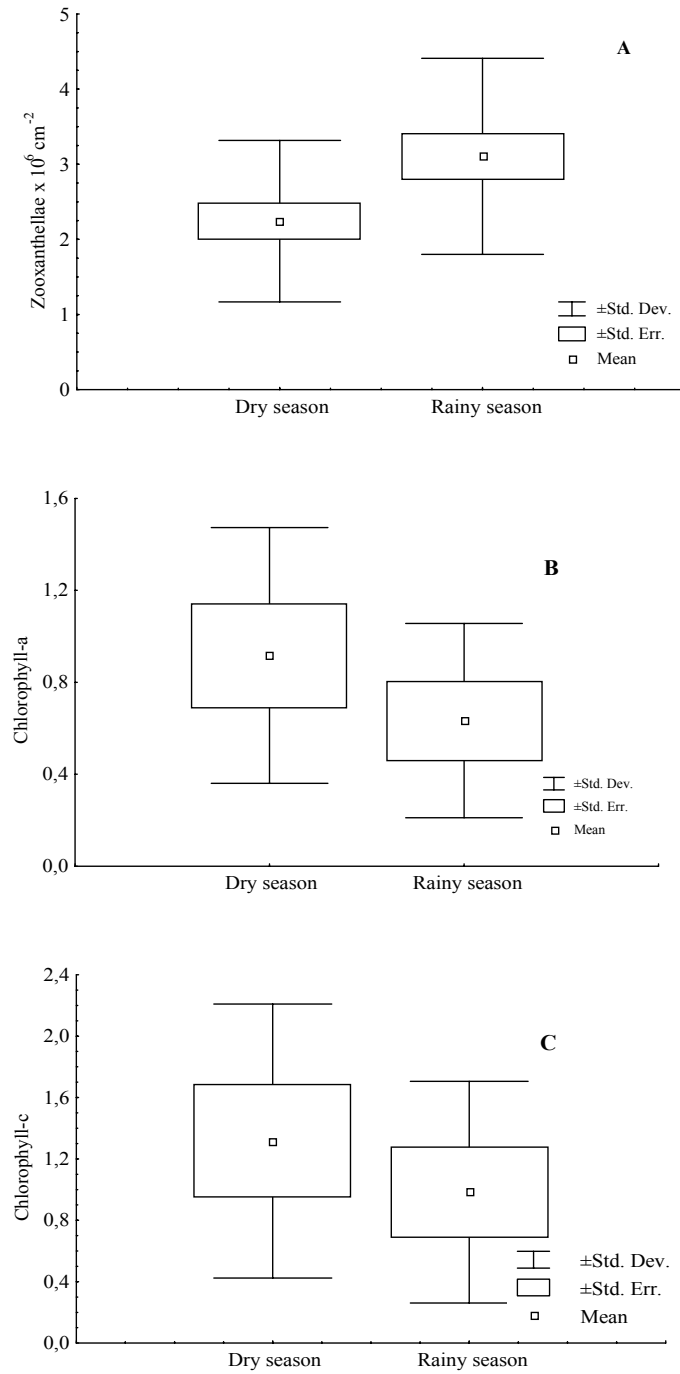


Fig. 4. Mean values of population density (A) and contents of chlorophyll-*a* (B) and chlorophyll-*c* (C) ($\mu\text{g cm}^{-2}$) from *Montastrea cavernosa* zooxanthellae, collected in the dry and rainy seasons in the reefs of Picãozinho, Brazil.

When the mean values from dry season (September, 1999 to March, 2000) were compared with mean values from rainy season (April to July, 2000), a clear relationship was observed between photosynthetic pigment contents and cell densities, which decreased and increased, respectively during the rainy season. Such asynchrony between pigment content and cell densities seems to be uncommon in zooxanthellae data.

Any attenuation mechanism of light (refraction, reflection, and scattering) due to an increase in suspended materials during the rainy months probably explains why zooxanthellae lose their pigments despite the increase in cell numbers: a likely compensation of zooxanthellae during increased photic stress. Some researchers have reported photoprotection and photoacclimation processes carried out by zooxanthellae in response to excess and reduction of light, respectively (Gorbunov *et al.*, 2001; Titlyanov *et al.*, 2001).

The asynchrony we observed between cells densities and photosynthetic pigment contents may be due to: (1) the seasonal differences on cell physiology (e.g. the existence of many cells with 'accessory pigments' in relation to Chl-*a*, therefore more mature and less active), and (2) the existence of distinct symbionts strains in *Montastrea cavernosa* (Baker *et al.*, 1997; Carlos *et al.*, 2000), which probably show distinct physiological rates in coral tissues.

The peculiarities of coastal reefs in the region frequently could change water conditions, particularly the light owing to cliff erosion southwards and the lateral inputs of fine sediments. These factors could increase stress on coral species and so cause their zooxanthellae to instigate physiological and biochemical repairs. If this is the case, strains of zooxanthellae of *M. cavernosa* across Picãozinho reefs could present distinct successional patterns related to these environmental stresses. These hypotheses are speculative, however, and require more detailed testing.

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