



## Seasonal changes in zooxanthellae harbored by zoanthids (Cnidaria, Zoanthidea) from coastal reefs in northeastern Brazil

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**Abstract.** The zooxanthellae hosted by the zoanthidae *Palythoa caribaeorum*, *Protospalythoa variabilis*, and *Zoanthus sociatus* were examined to determine their cell densities, cell diameters, mitotic index, and genetic identity over one year study. The densities of zooxanthellae were significantly higher in *Z. sociatus* ( $44.2 \pm 22.7 \times 10^6 \cdot \text{cm}^{-3}$ ) than in *P. variabilis* ( $11.24 \pm 4.33 \times 10^6 \cdot \text{cm}^{-3}$ ) and *P. caribaeorum* ( $5.6 \pm 1.4 \times 10^6 \cdot \text{cm}^{-3}$ ). The significant differences in the density of zooxanthellae between dry and rainy seasons were observed only for *P. caribaeorum* with higher densities in rainy season. Zooxanthellae associated with *P. caribaeorum* showed higher mitotic index ( $7.8 \pm 1.7\%$ ) than symbionts of *P. variabilis* ( $5.9 \pm 10.7\%$ ) and *Z. sociatus* ( $5.2 \pm 0.8\%$ ), and higher levels of cell division occurred for this species during dry season. The values of zooxanthellae cell diameters of *P. caribaeorum* symbionts were significantly higher than for other zoanthids species. The significant difference between zooxanthellae diameters in dry and rainy season was observed only for *Z. sociatus*. Denaturing gradient gel electrophoresis (DGGE) fingerprinting of the ribosomal internal transcribed spacer 2 showed that *Palythoa caribaeorum* and *Protospalythoa variabilis* harbored subclad type C1 *Symbiodinium goreau*, while individuals of *Zoanthus sociatus* harbored predominantly subclad type A4 *Symbiodinium*.

**Key words:** coral reefs, *Symbiodinium*, dinoflagellates, environmental conditions

**Resumo. Mudanças sazonais nas zooxantelas hospedadas por zoantídeos (Cnidaria, Zoanthidea) dos recifes costeiros do nordeste do Brasil.** As zooxantelas hospedadas pelos zoantídeos *Palythoa caribaeorum*, *Protospalythoa variabilis* e *Zoanthus sociatus* foram analisadas durante o período de um ano, para determinar a densidade, diâmetro celular, índice mitótico e sua identidade genética. As densidades das zooxantelas foram significativamente maiores em *Z. sociatus* ( $44,2 \pm 22,7 \times 10^6 \cdot \text{cm}^{-3}$ ) do que em *P. variabilis* ( $11,24 \pm 4,33 \times 10^6 \cdot \text{cm}^{-3}$ ) e *P. caribaeorum* ( $5,6 \pm 1,4 \times 10^6 \cdot \text{cm}^{-3}$ ). Diferenças significativas nas densidades de zooxantelas estudadas entre as estações de seca e chuva foram observadas apenas para *P. caribaeorum* com densidades mais elevadas na estação chuvosa. *P. caribaeorum* teve maiores índices mitóticos de suas zooxantelas ( $7,8 \pm 1,7\%$ ) do que *P. variabilis* ( $5,93 \pm 10,7\%$ ) e *Z. sociatus* ( $5,2 \pm 0,8\%$ ), e níveis mais altos de divisão celular ocorreram para esta espécie durante a estação seca. Os diâmetros celulares das zooxantelas de *P. caribaeorum* foram significativamente maiores do que as outras espécies de zoantídeos estudadas. Diferenças significativas nos diâmetros das zooxantelas analisadas entre as estações de seca e chuva foram observadas apenas para *Z. sociatus*. A eletroforese em gel com gradiente desnaturante (DGGE) do espaçador ribossômico interno transcrito II mostrou que *Palythoa caribaeorum* e *Protospalythoa variabilis* abrigam *Symbiodinium goreau* pertencente ao subclado C1, enquanto que indivíduos de *Zoanthus sociatus* abrigam predominantemente *Symbiodinium* pertencente ao subclado A4.

**Palavras chaves:** recifes de corais, *Symbiodinium*, dinoflagelados, condições ambientais

## Introduction

Zoanths (Cnidaria: Anthozoa) are conspicuous representatives of inter-tidal regions and are commonly found on reefs, rocky coastal platforms as well as infralitoral areas in tropical regions (Sebens 1982, Ryland & Babcock 1991). These organisms are characterized by having two rows of tentacles, and are typically colonial, with a single ventral siphonoglyph and detritus encrustation in their mesoglea (Fujii & Reimer 2011). The order comprises diverse families and various genera, among them *Isaurus*, *Palythoa*, *Protopalythoa*, and *Zoanthus*, which can be found in agitated as well as calm waters as polyps typically colonial. The genera *Acrozoanthus*, *Parazoanthus* and *Epizoanthus* are usually found associated with sponges, hydroids, polychaetes and gorgonians (Mather & Bennett 1993).

Most of the zoanths host zooxanthellae, symbiotic dinoflagellates (*Symbiodinium*), in their gastrodermal tissues (Steen & Muscatine 1984, Davies 1992, Kemp *et al.* 2006). These symbionts provide the zoanths with nutrition through by translocation photosynthetic products (von Holt & von Holt 1968a, 1968b, Parker 1982), necessarily restricting these animals to shallow-water habitats. This relationship offers additional services including the removal of metabolic by-products such as carbon dioxide, ammonia, nitrates and phosphates that then serve as raw materials for their photosynthetic activities (Muscatine 1974). In exchange, the zooxanthellae obtain shelter and protection in the interior of their animal hosts.

The density of zooxanthellae found in tissues of scleractinians corals varies under different environmental conditions (Fitt *et al.* 2000, Costa *et al.* 2004a, 2005). Variations in these environmental parameters can influence the stability of the host/symbiont relationship and can lead to the loss of the zooxanthellae or of their photosynthetic pigments (Glynn 1993, Costa *et al.* 2001a, 2005), resulting in a reduction of density of symbionts (=bleaching) in hosts exposed to extreme environmental conditions (Glynn *et al.* 2001, Costa *et al.* 2001a). The bleaching has become increasingly prominent on reefs throughout the world, resulting in vast degradation of coral reefs in the tropics (Glynn 1993, Wilkinson 2008; Amorim *et al.* 2011) with serious implications for the trophic economy of these ecosystems and for overall marine productivity.

However, for the zoanths there are no data showing how zooxanthellae vary among different species and almost nothing is known about their

relationship with different environmental variables. Kemp *et al.* (2006) experimentally demonstrated that increasing water temperatures due to a 2005 El Niño in Florida resulted in a loss of zooxanthellae from *Palythoa caribaeorum* Duchassaing & Michelotti, 1860 and bleaching of colonies harboring a combination of *Symbiodinium trenchi* (D1a) and *Symbiodinium goreau* (C1).

Zoanths demonstrate extensive latitudinal distributions in Brazil and are encountered in different environments along the coast and on oceanic islands (Rohlf de Macedo & Belem 1994, Amaral *et al.* 2002). The genus *Zoanthus* Lamarck, 1801 and *Palythoa* Lamouroux, 1816 are among the most common representatives in shallow water communities in Brazil (Rohlf de Macedo & Belem 1994). Research focusing on this group of zoanths in Brazil has investigated aspects of their reproduction and taxonomy (Boscolo & Silveira 2005); diseases (Acosta 2001), ecology (Perez *et al.* 2005), pharmacological applications (Soares *et al.* 2006), and predation by sea turtles (Stampar *et al.* 2007).

The present work aimed to characterize the annual cycles of the zooxanthellae hosted by three species of zoanths (*Palythoa caribaeorum* Duchassaing & Michelotti, 1860; *Zoanthus sociatus* Ellis & Solander, 1786, and *Protopalythoa variabilis* Duerden, 1898) occurring on coastal reefs at Picãozinho, João Pessoa, Paraíba, in relation to environmental variables, and to identify the *Symbiodinium* types occurring in zoanths tissues. Similar studies have been undertaken with scleractinians corals investigating variations in zooxanthellae population densities, symbiont diversity, and extent of bleaching (Amaral & Costa 1998, Costa *et al.* 2001a, 2001b, Costa & Amaral 2002, Costa *et al.* 2004a, 2004b, Costa *et al.* 2005, Costa *et al.* 2008). We hypothesized that the zooxanthellae harbored by different species of zoanths belongs to distinct strains and that different patterns of seasonal variations would be present among them throughout an annual cycle.

## Materials and Methods

### Sampling and water analyses

Four fragments from each of the zoanths *P. caribaeorum*, *Z. sociatus* and *P. variabilis* were collected monthly, always in the morning (ca. 10AM), during the period between June/2007 and June/2008, in the intertidal zone along coastal reefs at Picãozinho, João Pessoa, Paraíba State, Brazil (06°42'05" - 07°07'30" S; 34°48'37" - 34°50'00" W). The harvesting of fragments of each

zoanthids species was made by removing a standard surface area of 4.52 cm<sup>2</sup> from each colony using a spatula. This area allowed removing approximately 10-20 polyps from *P. caribaeorum*, 20-30 polyps from *Z. sociatus*, and 6 polyps from *P. variabilis*.

To identify the *Symbiodinium* types occurring in zoanthids tissues, fragments (area of 4.52 cm<sup>2</sup>) of *P. caribaeorum* (n= 4), *Z. sociatus* (n= 7), and *P. variabilis* (n= 5) were also clipped of substrate in May/2008 and preserved in DMSO-salt buffer (20% dimethylsulfoxide, 0.25M EDTA in NaCl saturated water) and stored in 1.5ml microcentrifuge tubes at 4°C (Seutin *et al.* 1991) as described by Baker *et al.* (1997).

For each sampling date the following water parameters were determined: the sea surface temperature - SST (mercury thermometer ± 0.1 °C), the salinity (American Optical refractometer, model 10419), the quantity of solid material in suspension (gravimetric method), and the amount of dissolved oxygen (method of Strickland & Parsons 1960). Salinity values are based on the Practical Salinity Scale of 1978 (UNESCO 1981). The rainfall indices for the sampling period were obtained from the State Secretary of Water Resources, Paraíba State - SEMARH ([www.semarh.pb.gov](http://www.semarh.pb.gov)).

#### *Determination of densities, mitotic index, and cell diameter of zooxanthellae*

The volume of each sample collected was determined by the volume displacement technique, using a graduated beaker containing 60ml of filtered seawater. The fragments of *P. caribaeorum* and the polyps of *Z. sociatus* and *P. variabilis* were placed individually into the graduated beaker, the displaced volume was noted and considered as the volume of the polyps or fragments and the sum of volume over the initial volume of the beaker were considered as the total sample volume. Then, the zooxanthellae of each sample were subsequently disassociated from the zoanthids tissues by mechanical maceration and homogenizing.

The extracted material was fixed in Lugol 10% and examined using a Zeiss microscope and Fuchs Rosenthal counting chambers at a total of six subsamples of the homogenized material for each sample. For each sample of the zoanthids species the densities, mitotic index, and diameter of zooxanthellae were determined from four randomly-selected replicates by counting the homogenate in a Fuchs-Rosenthal chamber, using standard microscope. The cell densities were expressed per sample volume (cells.cm<sup>-3</sup>); the mitotic index (%) was determined by counting dividing cells; and the zooxanthellae diameters were measured using a

previously calibrated micrometric eyepiece to examine a total of 40 cells per sample each month.

#### *Molecular analyses of zooxanthellae*

The isolation of zooxanthellae from the coral tissue and all subsequent DNA extractions, PCR amplifications, and denaturing-gradient gel electrophoresis (DGGE) were done using the protocols of LaJeunesse *et al.* (2003). DNA was extracted using the Wizard DNA preparation protocol (Promega). Between 20 and 40mg of material was placed into 1.5ml micro centrifuge tubes with 250µg of 0.5mm glass beads and 600µl nuclei lysis buffer (Promega) and beaten for 140s at 2400rpm in a Biospec Mini-Beadbeater. The lysis was then incubated with 0.1mg ml<sup>-1</sup> proteinase K for 1h at 65°C. 250µl of protein precipitation buffer (Promega) was then added and the extract incubated on ice for 10–15min. After centrifugation for 5min at 15.000g, 550µl of supernatant was transferred to a second 1.5ml tube with 700µl 100% isopropanol and 25µl 3M sodium acetate, pH 5.6. After incubation on ice for 10min, the precipitated DNA was centrifuged and the pellet washed with 70% ethanol. The DNA was centrifuged again for 5min, dried, and re-suspended in 95µl H<sub>2</sub>O and 5µl of 10x Tris-EDTA (10mM Tris Base, 1mM EDTA Solution, 0.05% Tween 20, pH 9).

Denaturing-gradient gel electrophoresis (DGGE) was used to analyze the ITS2 region of nuclear ribosomal RNA genes (LaJeunesse 2001; LaJeunesse 2002). PCR-DGGE analyses of the ITS2 region were conducted using the forward primer, “ITSintfor2” (5'- GAATTGCAGA ACTCCGTG-3') (LaJeunesse & Trench 2000), which anneals to a “*Symbiodinium*-conserved” region in the middle of the 5.8S ribosomal gene, and the highly conserved reverse primer that anneals to the LSU “ITS2CLAMP” (5'-CGCCCCGCCGCCCGCC CGTCCCGCCG CCCCCGCC GGGATCCATA TGCTTAAGTT CAGCGGGT-3'), and ITS-reverse universal primer modified with a 39-bp GC clamp (italicized) (LaJeunesse & Trench 2000). A “touchdown” amplification protocol with annealing conditions 10°C above the final annealing temperature of 52°C was used to ensure PCR specificity. The annealing temperature was decreased by 0.5°C after each of 20 cycles. Once the annealing temperature reached 52°C, amplified samples were loaded onto an 8% polyacrylamide denaturing gradient gel (45-80% urea-formamide gradient; 100% consists of 7M urea and 40% deionized formamide) and separated by electrophoresis for 9.5h at 150V at a constant temperature of 60°C (LaJeunesse 2002). Samples

were run with previously published standards (LaJeunesse 2002).

#### Statistical analyses

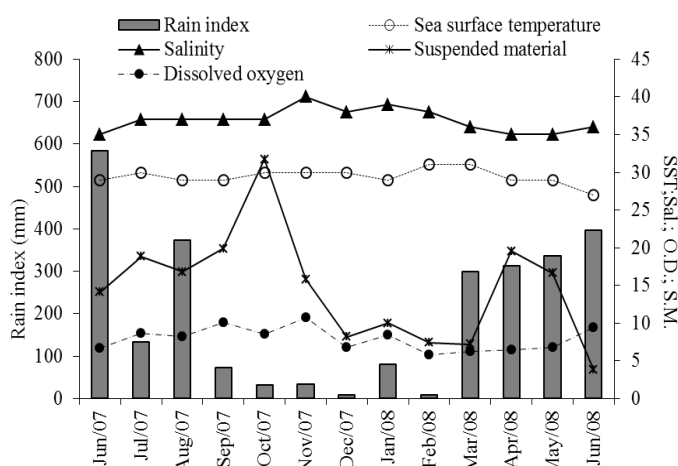
All statistical analyses were carried out using the Statistica 7.0 for Windows. The homoscedasticity of the variances of all zooxanthellae parameters analyzed were confirmed utilizing the Levene test. One-way ANOVA was used to analyze means (population densities, mitotic index, and cellular diameter of zooxanthellae), with least significant difference at the 5% level. When ANOVAs detected significant differences between monthly means at the  $p < 0.05$  level, Tukey's post hoc test was used to determine which months differed significantly. The Student t -Test was applied to compare the differences in the symbiont parameters means for the dry and rainy seasons. Pearson's Correlation test was used to determine,

whether there was significant correlation between the environmental data and zooxanthellae parameters. All statistical analyses followed Sokal & Rohlf (1983).

## Results

### Environmental parameters

The monthly rainfall during rainy seasons (June – August 2007; March – June 2008) varied from 134.0 to 590.0 mm, while during dry season (from September 2007 to February 2008) was between 8.0 and 73.0 mm (Figure 1). The sea surface temperature – SST (27-31°C), salinity (35-40) and dissolved oxygen (ca. 5.8-10.8 mg. L<sup>-1</sup>) values fluctuated slightly over all period of study (Fig. 1). The concentration of material in suspension was greatest just after the rainy season of 2007 and during the rainy season in 2008.



**Figure 1.** Average values of environmental variables at the Picãozinho reefs, João Pessoa, Paraíba State, Brazil, during the period from June/2007 to June/2008. SST= Sea surface temperature (°C); Sal. = Salinity; O.D.= Dissolved Oxygen (mg L<sup>-1</sup>); S.M. = Suspended material (mg L<sup>-1</sup>).

### Variations in zooxanthellae density associated with zoanthids species

The densities of zooxanthellae were significantly higher in *Z. sociatus* than in *P. caribaeorum* and *P. variabilis* (df= 147, F= 129.9, p= 0.000). *Z. sociatus* showed the zooxanthellae numbers ranged from 13.8 to 124.1 x 10<sup>6</sup>.cm<sup>-3</sup>, while in *P. variabilis* and *P. caribaeorum* were observed 5.4 to 31.3 x 10<sup>6</sup>.cm<sup>-3</sup> and 2.6 to 8.9 x 10<sup>6</sup>.cm<sup>-3</sup>, of zooxanthellae, respectively (Table I).

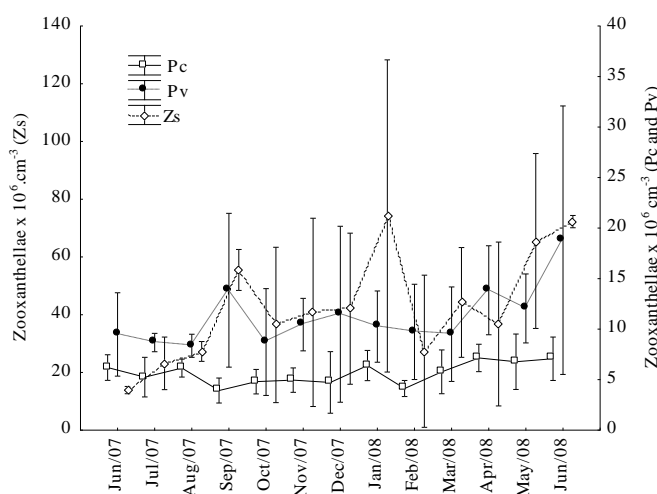
The significant differences of zooxanthellae

density between samples collected in different months were observed for all species studied. The densities of *P. caribaeorum* zooxanthellae were lower in September 2007 and February 2008 than in April, May and June 2008 (df= 37, F= 3.66, p= 0.001) (Fig. 2). The lower numbers of zooxanthellae in *P. variabilis* were observed at July, August and October 2007 than in June 2008 (df= 37, F= 2.42, p= 0.019). *Z. sociatus* showed lower counts of zooxanthellae in June, July and August 2007 than in January and June 2008 (df= 37, F= 4.84, p= 0.000).

**Table I.** Average estimates of densities, mitotic index, and cell diameter of zooxanthellae associated with *Palythoa caribaeorum* (Pc), *Protopythoa variabilis* (Pv), and *Zoanthus sociatus* (Zs) at Picãozinho reefs, João Pessoa, Paraíba State, Brazil, analyzed during the period from June 2007 to June 2008.

	Density (cells x 10 <sup>6</sup> cm <sup>-3</sup> )			Mitotic index (%)			Cell diameter (µm)		
	Pc	Pv	Zs	Pc	Pv	Zs	Pc	Pv	Zs
Mean	5.62 <sup>a</sup>	11.24 <sup>a</sup>	44.20 <sup>b</sup>	7.81 <sup>a</sup>	5.93 <sup>b</sup>	5.21 <sup>b</sup>	10.65 <sup>a</sup>	10.36 <sup>b</sup>	10.42 <sup>b</sup>
SD (±)	1.45	4.33	22.75	1.72	2.52	0.87	0.32	0.19	0.36
Minimum	2.60	5.40	13.80	0.00	1.00	3.20	9.94	9.94	9.42
Maximum	8.90	31.30	124.10	9.80	10.66	7.87	11.32	10.94	10.88
Valid N	50	50	50	50	50	50	40	40	40

Mean values marked with same letter do not differ significantly (p>0.05)



**Figure 2.** Average monthly density of the zooxanthellae associated with the zoanthids from the reefs at Picãozinho, João Pessoa, Paraíba State, Brazil, analyzed during the period from June 2007 to June 2008. (Zs = *Zoanthus sociatus*; Pc = *Palythoa caribaeorum*; Pv= *Protopythoa variabilis*).

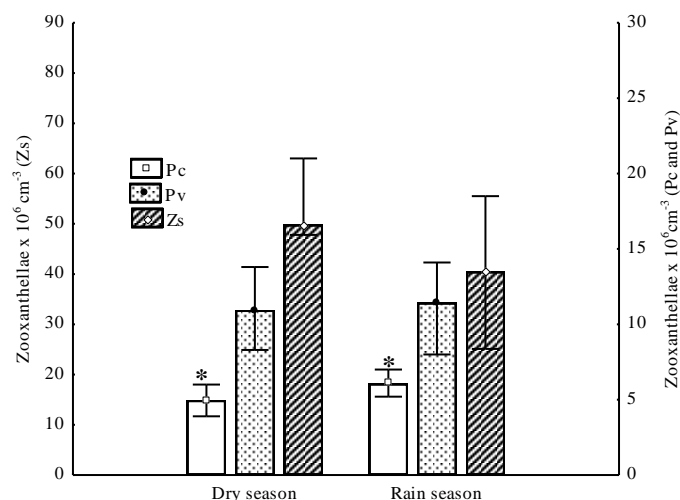
Among the species studied only *P. caribaeorum* showed significant differences in the density of zooxanthellae between the dry and rainy seasons ( $t = 2.779, p = 0.007$ ) (Fig. 3). This species showed higher zooxanthellae densities during the rainy season of 2008 than in dry season.

The density of all species studied demonstrated weak negative correlation with sea surface temperature and weak positive correlation with rainfall (Table 2). Zooxanthellae density of *Palythoa caribaeorum* showed also weak negative correlation with salinity.

*Variations of mitotic index and cell diameters of zooxanthellae*

Zooxanthellae associated with *Palythoa caribaeorum* showed significant higher mitotic index than symbionts of *Protopythoa variabilis* and *Zoanthus sociatus* ( $F = 26.958; p = 0.000$ ) (Table I). The mitotic index of zooxanthellae associated with

the three zoanthids species showed significant fluctuations throughout the year (Fig. 4). *P. caribaeorum* showed significant lower values of mitotic index in June 2007 (2.8%) than in other months (6.3 - 9.2%), except for July 2007 (5.4%) ( $df = 37, F = 11.277, p = 0.000$ ). *P. variabilis* showed significant lower mitotic index in November and December 2007 (3.3% and 1.5% respectively) than in August 2007 (8.8%) ( $df = 37, F = 3.534, p = 0.000$ ), and *Z. sociatus* showed significant higher values of this parameter in September 2007 (6.9%) than in all other months (Fig. 4). The mitotic index of zooxanthellae associated with *P. caribaeorum* and *Z. sociatus* showed weak positive correlation with sea surface temperature and dissolved oxygen. The mitotic index of zooxanthellae hosted by *P. variabilis* showed weak negative correlation with salinity and positive correlation with dissolved oxygen (Table II).



**Figure 3.** Average cell densities ( $\pm$ Standard error) of the zooxanthellae associated with zoanthids from the reefs at Picãozinho, João Pessoa Paraíba State, Brazil, analyzed during the dry and rainy seasons of the period from June 2007 to June 2008. (Zs= *Zoanthus sociatus*; Pc= *Palythoa caribaeorum*; Pv= *Protospalythoa variabilis*; \*= significant differences).

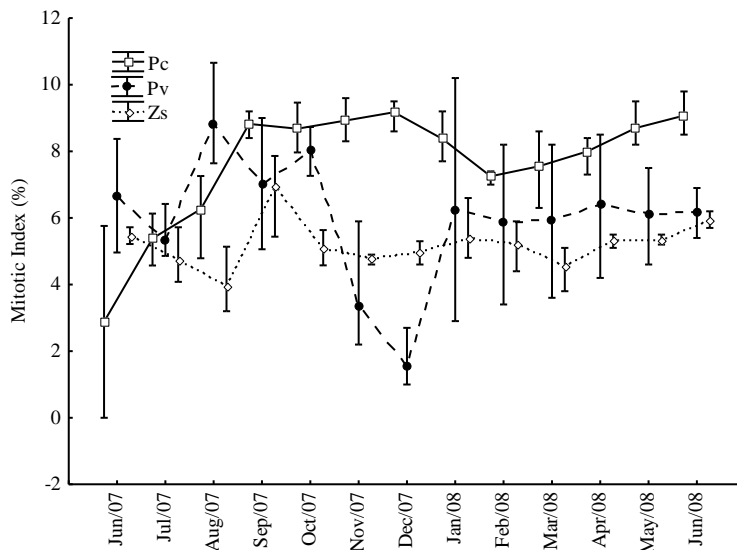
**Table II.** Correlation matrix between environmental parameters and variables of zooxanthellae associated with *Palythoa caribaeorum* (Pc), *Protospalythoa variabilis* (Pv), and *Zoanthus sociatus* (Zs) at Picãozinho reefs, João Pessoa, Paraíba State, Brazil, analyzed during the period from June 2007 to June 2008.

Parameter	Correlation coefficient ( $p < 0.05$ , $n = 40$ ) for:								
	Cell density			Mitotic index			Cell diameter		
	Pc	Pv	Zs	Pc	Pv	Zs	Pc	Pv	Zs
Temperature	-0.45	-0.54	-0.51	0.46		0.48			
Salinity	-0.40				-0.32				0.32
Monthly rainfall	0.63	0.38	0.32						
Dissolved oxygen				0.51		0.35			0.35
Suspended material					0.32				

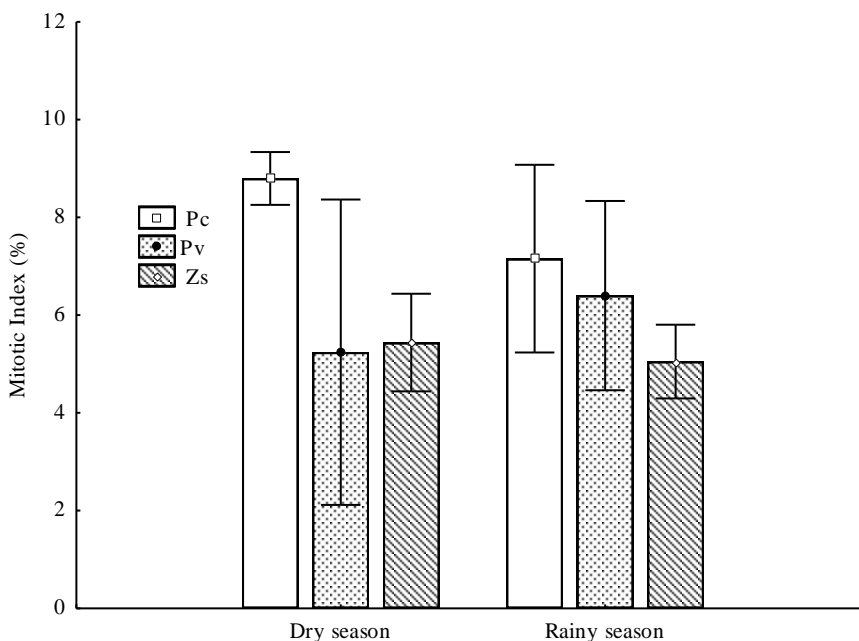
The significant differences in the mitotic index of zooxanthellae between dry and rainy seasons were observed only for *P. caribaeorum* ( $df = 48$ ,  $t = -3.7121$ ,  $p = 0.0005$ ), that showed higher levels of cell division during dry season (Fig. 5).

The values of cell diameters of *Palythoa caribaeorum* symbionts ranged from  $9.94\mu\text{m}$  to  $11.32\mu\text{m}$ , and were higher than for other zoanthids species studied ( $df = 117$ ,  $F = 13.946$ ,  $p = 0.000$ ; Table

D). The significant difference between zooxanthellae diameters in dry and rainy season was observed only for *Z. sociatus* ( $df = 38$ ,  $t = 3.2322$ ,  $p = 0.002$ , Fig. 6), but no variations of this parameter were observed between monthly samples for the all species studied ( $p > 0.05$ ). The zooxanthellae cell diameter of *Z. sociatus* was found to be weakly positive correlated with salinity and dissolved oxygen (Table II).



**Figure 4.** Average ( $\pm$ Standard error) of the mitotic index of the zooxanthellae associated with three zoanths species from the reefs at Picãozinho, João Pessoa, Paraíba State, Brazil, analyzed during the period from June 2007 to June 2008. (Zs= *Zoanthus sociatus*; Pc = *Palythoa caribaeorum*; Pv= *Protopalycha variabilis*).

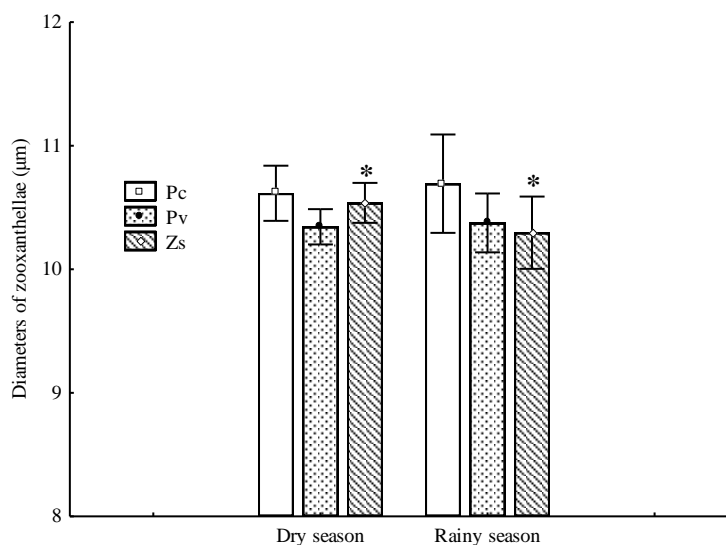


**Figure 5.** Average monthly ( $\pm$  Standard error) of the mitotic index of the zooxanthellae associated with three zoanths species from the reefs at Picãozinho, João Pessoa, Paraíba State, Brazil, analyzed during the dry and rainy seasons of the period from June 2007 to June 2008. (Zs= *Zoanthus sociatus*; Pc= *Palythoa caribaeorum*; Pv= *Protopalycha variabilis*; \*= significant differences).

*Zooxanthellae clades associated with zoanths species*

Two divergent groups of zooxanthellae corresponding to Clades A and C were identified in zoanths species. ITS2-DGGE fingerprinting of *Symbiodinium* populations harbored by *P.*

*caribaeorum* and *P. variabilis* identified *Symbiodinium goreau* C1 (sensu LaJeunesse 2001). Most of the *Z. sociatus* specimens analyzed harbored primarily subcladal type A4 *Symbiodinium* (n= 5), however one individual contained a mixed population of *S. goreau* C1 and subcladal type A3.



**Figure 6.** Average of the diameters ( $\pm$  Standard error) of the zooxanthellae associated with three zoanthids species from the reefs at Picãozinho, João Pessoa Paraíba State, Brazil, analyzed during the dry and rainy seasons of the period from June 2007 to June/2008. (Zs= *Zoanthus sociatus*; Pc= *Palythoa caribaeorum*; Pv= *Protopalythoa variabilis*; \*= significant differences).

## Discussion

Zooxanthellae densities have often been observed to be inversely related to sea surface temperatures, and this observation has been widely used to explain bleaching episodes affecting zooxanthellate corals, hydrocorals, and zoanthids (Fitt *et al.* 1993, Glynn *et al.* 2001, Saxbay *et al.* 2003, Costa *et al.* 2005, Kemp *et al.* 2006). There is good evidence, however, that synergistic stress factors such as temperature, sedimentation processes (Philipp & Fabricius 2003), coastal nutrification (Philipp & Fabricius 2003, Philipp & Fabricius 2003), rainfall indices (Costa *et al.* 2004a, 2005; Downs *et al.* 2009; Amorim *et al.* 2011), fluctuations in the optical quality of the water, and other environment factors related to anthropogenic activities (Fagoonee *et al.* 1999) acting jointly can influence bleaching among cnidarians.

In the present study, temperature effects were only apparent in *Palythoa caribaeorum*, which demonstrated significant reductions in zooxanthellae densities during the summer. The densities of the zooxanthellae in *Zoanthus sociatus* were greater during this same period of elevated temperatures, however, suggesting the influence of other factors in regulating the concentrations of these symbionts in host tissues.

All of the species studied here colonize substrates that lay exposed for long periods of time during certain low tides, and are thus subjected to high temperatures, strong solar radiation levels that favor desiccation, and increased sedimentation that

can cause smothering and death of coral polyps, shading, and tissue necrosis death (Erftemeijer *et al.* 2012). As this intermittent stress favors desiccation, the cnidarians are obliged to produce more photo-protective mucus, which suggests that they must maintain high metabolic levels under extreme environmental conditions. Zoanthids are also subject to predation and sedimentation under these conditions (Mendonça *et al.* 2008; Francini-Filho & Moura 2010), so that multiple stress factors can act upon them simultaneously.

Our results indicated that zooxanthellae sizes and densities are inversely related variables. *Palythoa caribaeorum*, the most conspicuous species in the study area, has very large zooxanthellae, although these symbionts are present at lower densities than those found in *Zoanthus sociatus* and *Protopalythoa variabilis*. The same situation has been observed with the coral *Siderastrea stellata*, the most abundant species on the coastal reefs of Paraíba State, which also has large zooxanthellae but at lower densities than other corals such as *Mussismilia harttii* and *Mussismilia hispida*, both endemic to Brazil (Costa *et al.* 2005).

Changes in the numbers of zooxanthellae reflect changes in cell division rates, but specific responses to environmental factors will vary from host to host. The observed differences in the mitotic indices among the species studied here indicated different responses to external factors affecting the homeostasis of the symbiotic relationships between these organisms (Cervino *et al.* 2003, Hoegh-



Guldberg 1994) – so that increasing or decreasing cell division rates act as adjustment mechanisms to environmental factors, maintaining dinoflagellate densities within ranges most beneficial to the host (Wilkerson *et al.* 1988).

Stress situations can result in the expulsion or death of zooxanthellae (Kemp *et al.* 2006), which will then increase the cell division rates among the remnant populations—demonstrating a form of resilience in the relationship between endosymbionts and their hosts. As symbiosis is essential to guarantee the quality of life of zooxanthellate cnidarians, it can be inferred that the costs of maintaining this relationship is quite high and that the physiological and reproductive equilibrium of zooxanthellae must occur in consonance with the interests of the host.

These homeostatic mechanisms also have a genetic component. Certain host coral species are dominated by particular types of *Symbiodinium*, whose prevalence appear to be associated with growth rates, nutrient capture, photosynthetic efficiency, and the capacity for photo-acclimation – attributes that can affect species distributions and competition (LaJeunesse 2002).

The subcladal types of *Symbiodinium* found in *Palythoa caribaeorum* and *Protospalythoa variabilis* are similar to those found in Caribbean species of *Palythoa* and *Protospalythoa* (the host-generalist subcladal type **C1**, *Symbiodinium goreau*; LaJeunesse 2002); the identification of subcladal type **A4** as the primary symbiont of *Z. sociatus* was unusual, however, since this subclade occurs jointly in this host with the subcladal types **A3**, **B1**, and **C1** (Lajeunesse 2002).

Other reports have demonstrated that clade **A4** shows no strict host –specificity, as it has also been reported in *Zoanthus sansibaricus* (Reimer *et al.* 2007) and the reef-building corals *Montastraea franksi* (Garren *et al.* 2006) and *Acropora* spp. (Lajeunesse 2002). Reimer *et al.* (2011) reported that most specimens of several species of zoanthids (*Isaurus tuberculatus* Gray, 1828; *Zoanthus zansibaricus* Carlgren, 1900; *Zoanthus kuroshio* Reimer, Ono, Iwama, Takishita, Tsukahara & Maruyama, 2006; *Palythoa mutuki* Haddon & Shackleton 1891) from Ogasawara Island maintained symbiotic relationships with *Symbiodinium* clade **C**, while only one specimen of *Z. kuroshio* was observed to host *Symbiodinium* clade **A1**.

Hill *et al.* (2011) found clade **A** zooxanthellae in *Zoanthus sociatus* from Jamaica, in various

species of sponges living on Caribbean reefs, and in one sponge species from Pacific reefs, and *Symbiodinium* clade **A** was the most common type found in *Cliona aprica*, *C. caribbaea*, *C. tenuis*, and *C. laticavicola* in the Caribbean (Granados *et al.* 2008). Symbiotic relationships with *Symbiodinium* clade **A** were also observed in ten species of scleractinian coral, sea anemones, and scyphomedusae in the Mediterranean Sea, while only one species of sea anemone (*Bunodeopsis strumosa*) harbored clade **B**, indicating the common occurrence of clade **A** in the Mediterranean region (Visram *et al.* 2006).

The prevalence of clade **C** in three zoanthid species reinforces its generalist character and abundance at tropical latitudes (Baker 2003). The additional presence of subclades **A3** and **A4** in *Zoanthus sociatus* indicates that this species has the flexibility to select its zooxanthellae, making it (in the view of Rowan *et al.* 1997 and Glynn *et al.* 2001) one of the zoanthids most resistant to bleaching on the reefs at Picãozinho (with clade **A>B>C>D**).

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