



Pigmentation patterns of *Siderastrea stellata* Verrill, 1868 (Cnidaria, Scleractinia) from coastal reefs in Northeastern Brazil and its relation with zooxanthellae and other microsymbionts

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ABSTRACT: Changes in the pigmentation patterns of 70 randomly-selected colonies of *Siderastrea stellata* growing on coastal reefs at Cabo Branco, Paraíba State, Brazil, were examined using photographic records and visual inspections following Coral Health Chart criteria, between September/2008 and May/2009. Fragments of healthy colonies with different pigmentation patterns were collected monthly to analyze the zooxanthellae and other associated microsymbionts, and to identify the *Symbiodinium* clades. The highest percentages of colonies demonstrating color pattern alterations were observed in the summer and at the beginning of the rainy season. Colonies showing color alterations demonstrated expressive losses of zooxanthellae (often up to 100%) and significant increases in zooxanthellae mitotic index. Molecular studies indicated that *Siderastrea stellata* contains clade C zooxanthellae. Healthy (tonalities C or D, and scores 5-6), moderate bleached (tonalities C or D, and scores 3-4), bleached (tonalities C or D, and scores 1-2), and pink colonies (tonality C, and scores 3-2) demonstrated wide diversity of associated symbionts, including foraminiferans, diatoms, cyanobacteria, worms (like nematodes), and microcrustaceans.

Key Words: *Symbiodinium*, Coral reefs, coral diseases, scleractinian corals.

RESUMO: Padrões de pigmentação de *Siderastrea stellata* Verrill, 1868 (Cnidaria, Scleractinia) de recifes costeiros no Nordeste do Brasil e sua relação com zooxantelas e outras microssimbiontes. Alterações dos padrões de pigmentação de 70 colônias aleatoriamente selecionados de *Siderastrea stellata* dos recifes costeiros de Cabo Branco, Paraíba, Brasil, foram examinadas utilizando registros fotográficos e inspeções visuais, seguindo os critérios do *Coral Health Chart*, sendo o estudo desenvolvido entre setembro/2008 e maio/2009. Fragmentos de colônias saudáveis e com diferentes padrões de pigmentação foram coletados mensalmente para analisar as zooxantelas e os outros microssimbiontes associados, e para identificar os clados de *Symbiodinium*. Os maiores percentuais de colônias que demonstram alterações no padrão de coloração foram observados no verão e no início da estação chuvosa. Colônias com alterações na coloração demonstraram perdas expressivas de zooxantelas (chegando até 100% de perdas) e aumentos significativos no índice mitótico das zooxantelas. Estudos moleculares indicaram que *Siderastrea stellata* abriga zooxantelas do clado C. Colônias saudáveis (tonalidade C ou D, e score 5-6), moderadamente branqueadas (tonalidade C ou D e score 4-3), branqueadas (tonalidades C ou D e score 2-1) e colônias rosa (tonalidade C, e scores 3-2) demonstraram grande diversidade de simbiontes associados, incluindo diatomáceas, cianobactérias, vermes (ex. nematóides) e micro crustáceos.

Palavras chave: *Symbiodinium*, recifes de corais, doenças em corais, corais escleractínios.

Introduction

Significant declines of coral reefs in the last three decades (Hughes *et al.* 2003, Pandolfi *et al.* 2003, Bellwoode *et al.* 2004, De'ath *et al.* 2012) have stimulated a number of studies focusing on these degradation processes. A great deal of attention has been directed towards understanding bleaching and diseases that affect scleractinian corals and other marine invertebrates associated with the zooxanthellae (Rosenberg & Loya 2004, Harvell *et al.* 2007, Work & Aeby 2011, Mydlarz *et al.* 2013) as well as the stress factors caused by anthropogenic activities that appear to be intensifying these episodes (Jackson *et al.* 2001, Harvell *et al.* 2007, Palmer *et al.* 2012, Mydlarz *et al.* 2013).

Pigmentation changes associated with environmental stress make coral vulnerable to potentially lethal infections caused by parasites and other opportunist organisms (Fitt *et al.* 1993, Glynn 1993, Glynn *et al.* 2001, Willis *et al.* 2004, Bourne *et al.* 2008, Porter *et al.* 2011, Palmer *et al.* 2012).

Ever since the discovery of Black Band Disease (BBD), the first reported coral disease (Garrett & Ducklow 1975, Antonius 1981, 1988, Richardson 1998, 2004), more than 35 different types of diseases have been found to affect coral reefs around the world, and the numbers of published papers dealing with this subject have dramatically increased in the last 10 years (Ward & Lafferty 2004, Harvell *et al.* 2007). These diseases lead to instability of the symbiotic relationships maintained with their zooxanthellae (Antonius 1988, Weil *et al.* 2006, Palmer *et al.* 2012), resulting in a number of negative effects on the coral reefs.

The appearance of diverse types of syndromes in scleractinian corals, such as Dark Spot Syndrome (Borger 2005), White Syndrome (Roff *et al.* 2008, Luna *et al.* 2010), Brown Band Syndrome (Ulstrup *et al.* 2007), Pink Line Syndrome (Ravindran & Raghukumar 2006), and Pink Blue Spot Syndrome (PPBS) (Bongiorni & Rinkevich 2005) indicate specific responses of different coral species to environmental stress and manifestations of distinct bleaching patterns.

These events have been affecting coral reefs throughout the world, and have been associated with changes in water temperatures (Fitt *et al.* 1993, 2000, Glynn 1993, Glynn *et al.* 2001, Costa *et al.* 2001a, Weil *et al.* 2006, Harvell *et al.* 2007;), salinity, illumination, and pollution (Goreau & Macfarlane 1990, Brown 1997, Saxby *et al.* 2003), among others, with resulting alterations of the symbiotic stability that these hosts normally

maintain with their zooxanthellae (Kühlmann 1988, Birkeland 1997, Ateweberhan *et al.* 2013). Corals have been observed to lose between 60 and 90% of their zooxanthellae under severe stress conditions, and the zooxanthellae that remain within the host tissues demonstrate 50 to 80% reductions in the concentrations of their photosynthetic pigments (Glynn 1996, Fitt *et al.* 1993, 2000).

A number of bleaching events have been reported in Brazil (Migotto 1997, Castro & Pires 1999, Costa *et al.* 2001a, Leão *et al.* 2003, Dutra *et al.* 2004, Amaral *et al.* 2006, Costa, 2006, Leão *et al.* 2008, Amorim *et al.* 2011), as well as a highly aggressive disease that affected the zoanthid *Palythoa caribbaeorum* (Acosta 2001). Various cases of diseases affecting corals have been reported by Francini-Filho *et al.* (2008) in recent years on the Abrolhos reefs of the coast of southern Bahia State, but detailed data on these bleaching events are still very scarce.

The present work examined changes in the color patterns in colonies of *Siderastrea stellata* on coastal reefs at Cabo Branco in northeastern Brazil, analyzed the seasonal dynamics of their zooxanthellae and other components of their microbiota associated with these changes, and characterized the genotypes of the zooxanthellae present in healthy colonies.

Methods

Monitoring colonies and samples collection

We performed this study on the coral reefs at Cabo Branco, João Pessoa, PB, Brazil (07°09'16"S and 34°48'17"W) during the period between September/2008 and May/2009 to accompany changes in the color patterns of the coral *Siderastrea stellata* by monthly monitoring of 70 randomly chosen colonies on the reef plateau during low tides. Photographs were taken and visual inspections were made (following the criteria of the Coral Health Chart and the recommendations of Siebeck *et al.* 2006) to determine the frequencies of: healthy colonies (tonalities C or D, and scores 6-5); moderate bleaching colonies or pale colonies (tonalities C or D, and scores 4-3); bleached colonies (tonalities C or D, and scores 2-1); and pink colonies (tonality C, and scores 3-2) and dead colonies. The colonies and their respective colors (tonalities and scores) were recorded on PVC boards in the field. Four fragments ($\approx$ 5 cm) from healthy colonies, as well as those demonstrating color alterations (moderate bleaching, bleached and pink) were collected every month. The coral samples were

placed in plastic bags containing seawater, sealed hermetically, and transported to the Laboratory of Reef Environments and Microalgae Biotechnology of the Federal University of Paraíba. Samples of healthy colonies (n=38) were also collected and analyzed in the Laboratory of Molecular Biology and Ecology, Federal University of Paraíba, for genotypic characterization of their zooxanthellae.

Sea surface temperatures – SST (± 0.1 °C, using a Watanabe Keiki reversion thermometer), salinity (portable refractometer), material in suspension (gravimetric determinations), and dissolved oxygen (using the Winkler technique, following Parsons & Strickland 1963) measurements were taken each month during the field samples. Rainfall data for the study area was obtained from the Agência Executiva de Gestão das Águas do Estado da Paraíba (AESa-PB): <http://www.aesa.pb.gov.br/>.

Laboratory analyses: Zooxanthellae and other microsymbionts

Coral tissues were extracted from each sample using a high-pressure jet of water (waterpik®), the extracted volume was registered, and the materials were subsequently homogenized mechanically and fixed in 10% Lugol solution. Zooxanthellae densities were estimated using a Fuchs Rosenthal chamber and four samples obtained from each homogenized preparation were examined in a Leica light microscope, and the values expressed as cells/cm² (Costa *et al.* 2008a).

The mitotic index and the cell diameters (n=60 measurements per sample) of the zooxanthellae were determined in each sample using a microscope fitted with an ocular micrometer. The other principal associated microbiota components from each sample were quantified using a Sedgwick-Rafter chamber and photographed using a Pixera digital camera coupled to the microscope. Diatoms were identified by consulting Cupp (1943), Hendey (1964), Ricard (1987), and Silva-Cunha & Eskinazi-Leça (1990), among others.

Genotypic characterization of the zooxanthellae

The DNAs of the zooxanthellae from healthy samples of *Siderastrea stellata* were extracted following Lajeunesse *et al.* (2003), with some modifications. The amplifications of the small subunit of ribosomal DNA (SSUrDNA) from the zooxanthellae were performed using the primers: ss5 (*forward*) (5'GCA GTT ATA ATT TAT TTG ATG GTC ACT GCT AC-3') and ss3Z (*reverse*) (5'-AGC ACT GCG TCA GTC CGA ATA ATT CAC CGG-3') (Rowan *et al.* 1997, Costa *et al.* 2008b). The

following amplification conditions were used: initial denaturation at 94°C for 5 minutes, 30 cycles of denaturation at 94°C for 45 seconds, annealing at 56°C for 45 seconds and extension at 72°C for 2 minutes; final extension at 72°C for 8 minutes (Rowan & Knowton 1995, Costa *et al.* 2008b).

To analyze Restriction Fragment Length Polymorphisms (RFLP) of SSUrDNA the amplicons were digested separately with the enzymes *Taq* I (Invitrogen) and *Hae* III (Qbiogene). The restriction fragments were separated by electrophoresis in 2% agarose gel, using a 100 bp standard (Invitrogen). The restriction patterns were photographed and analyzed using Image Master VDS version 2.0 software (Pharmacia Biotech).

Statistical analyses

All statistical analyses were carried out using Statistica 7.0 for Windows software, at 5% level of significance. The homoscedasticity and normality of the variances of all zooxanthellae parameters analyzed were confirmed utilizing respectively the Levene's test and the Shapiro's test. The means of the population densities, mitotic index, and cellular diameters of the zooxanthellae measured over the year were compared by One-way ANOVA and Tukey HSD post hoc test. The Student's *t*-Test was applied to compare the means differences of the symbiont parameters among the dry and rainy seasons. Pearson's Correlation tests were used to determine whether there were significant correlations between the environmental data and zooxanthellae parameters. All statistical analyses followed Zar (2010).

Results

Siderastrea stellata colony monitoring and zooxanthellae analyses

A total of 840 colonies of *Siderastrea stellata* were monitored, recording the presence of healthy, moderate bleached, bleached, and pink colonies (Fig. 1). Monthly variations in the numbers of healthy colonies were related to the local climate, with fewer healthy colonies at the end of the summer (n= 27 in Jan/09) and at the beginning of the rainy season (n= 21 in Mar/09) (Fig. 2). Hydrological and climatic data from the study area demonstrated defined seasonal variations, with less rainfall from September/2008 to January/2009 and the highest rainfall during April and May/2009; water temperatures and salinity were highest during the dry season (summer) and lower during the rainy season (winter) (Fig. 2).

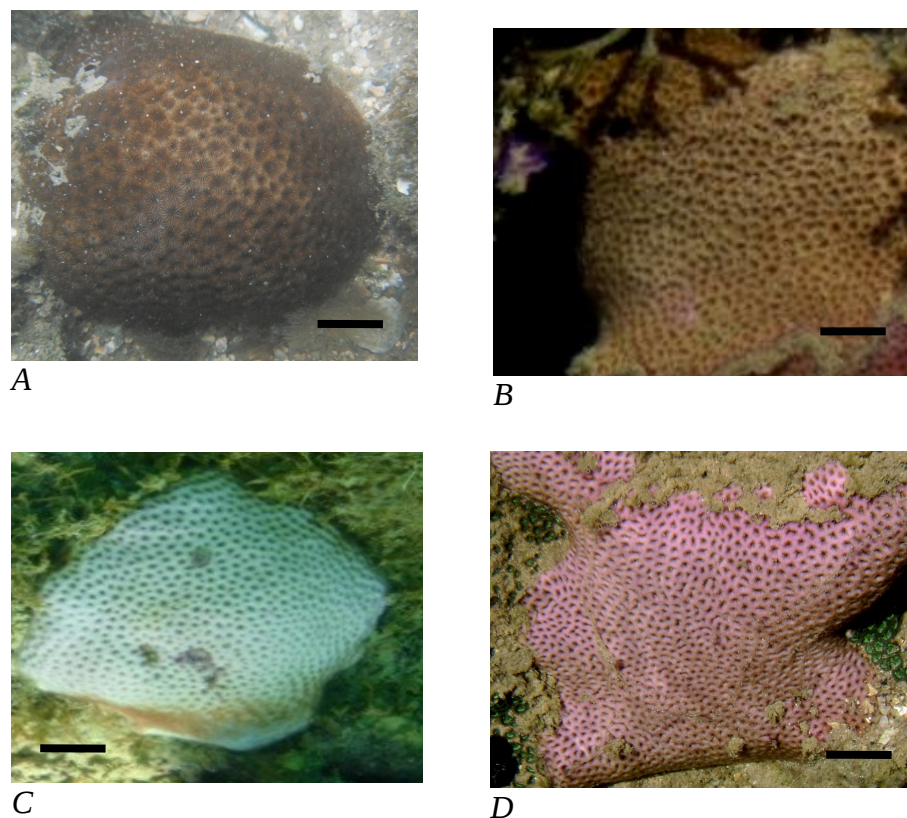


Figure 1. Pigmentation pattern observed in the colonies of *Siderastrea stellata* on the Cabo Branco reef, João Pessoa, PB, Brazil, during the period between September/2008 and May/2009. (A = healthy colonies, B= moderate bleached colonies; C= bleached colonies; D= pink colonies, following the criteria of the Coral Health Chart; Scale bar = 1.4cm).

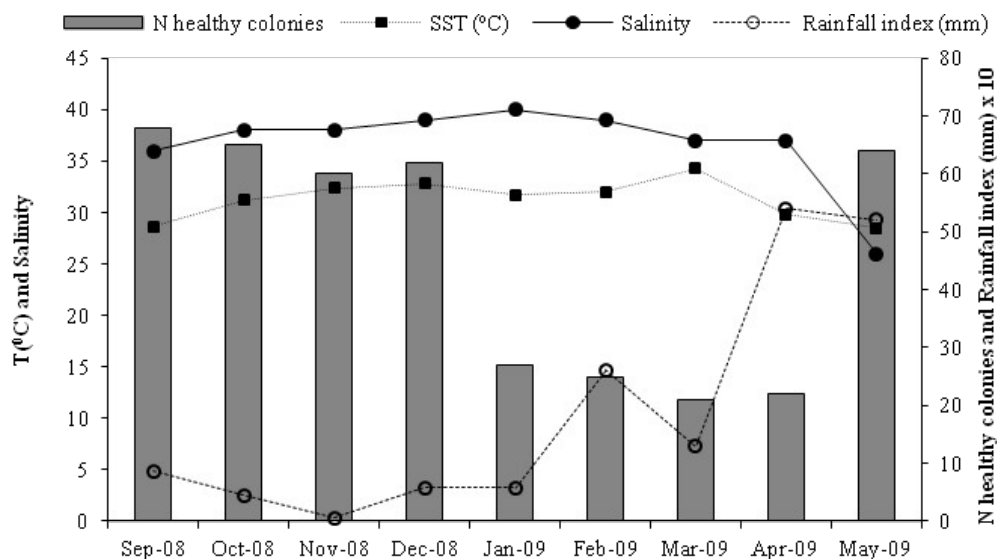


Figure 2. Monthly variations in the numbers of healthy colonies of *Siderastrea stellata* and of the environmental variables on Cabo Branco reef, João Pessoa, PB, Brazil, during the period between September/2008 and May/2009. (N= Number of healthy colonies; SST= Sea Surface Temperature).

Evident decreases in the stability of the symbiotic associations of the corals with zooxanthellae were observed, and the higher numbers of moderate bleached (paled), pink, and dead colonies were registered at the end of the summer and beginning of the rainy season (Fig. 3).

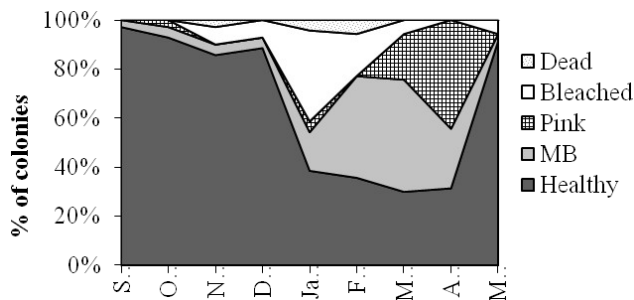


Figure 3. The conditions of *Siderastrea stellata* colonies monitored on the Cabo Branco reef, João Pessoa, PB, Brazil, during the period from September/2008 to May/2009. (MB= Moderate bleached colonies).

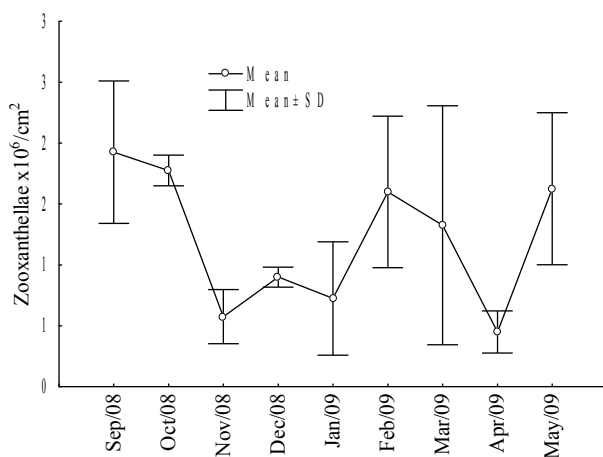


Figure 4. Densities of the zooxanthellae associated with healthy colonies of *Siderastrea stellata* collected on the Cabo Branco reef, João Pessoa, PB, Brazil, during the period between September/2008 and May/2009.

Moderate bleached colonies (paled) were observed throughout the year, but in higher percentages between January and April/2009; higher numbers of pink colonies occurred in March and April/2009; bleached colonies were observed starting in November/2008, but with the highest percentages in January and February/2009; dead colonies were only registered in November/2008 and in January and February/2009, although always in low numbers (Fig. 3).

Healthy colonies of *Siderastrea stellata* demonstrated significantly higher zooxanthellae densities during September and October/2008 and significantly lower densities in November/2008 and April/2009 ($df= 8$; $F= 4.653$; $p< 0.01$; Fig. 4). The population densities of the zooxanthellae were greater during the dry season and lower during the rainy season, although these values were not statistically significant ($df= 34$; $t= 0.466$; $p= 0.643$; Fig. 5).

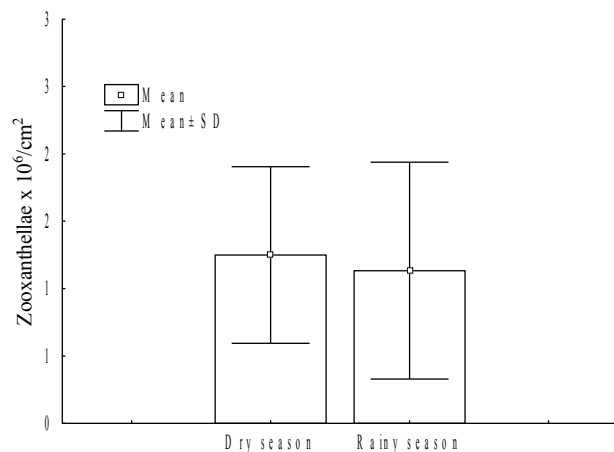


Figure 5. Densities of the zooxanthellae associated with healthy colonies of *Siderastrea stellata* collected on the Cabo Branco reef, João Pessoa, PB, Brazil, during the dry and rainy seasons for the period between September/2008 and May/2009.

Table I. Correlation matrix between environmental variables and parameters of zooxanthellae associated with the coral *Siderastrea stellata* collected at the Cabo Branco reef, João Pessoa, PB, Brazil between September/2008 and May/2009.

Variables	Correlation coefficient for:		
	Cell density	Mitotic index	Cell diameter
Sea surface temperature ($^{\circ}\text{C}$)	-0.24	0.45*	0.27
Salinity	-0.27	0.38*	0.64*
Suspended material (mg/L)	-0.35*	-0.02	0.44*
O ₂ (mg/L)	-0.37*	0.01	0.55*
Rainfall index (mm)	-0.03	-0.19	-0.64*

*Significant correlation at $p< 0.05$)

Among the environmental variables analyzed, only the levels of material in suspension and dissolved oxygen contents demonstrated negative significant correlations with zooxanthellae population densities (Pearson's correlation coefficient, $p < 0.05$; Table I).

The coral colonies that showed alterations of pigmentation (moderate bleached, bleached, and pink colonies) had consistently lower zooxanthellae densities in their tissues than healthy colonies (Fig. 6). Zooxanthellae reductions during these months reached up to 100% in relation to healthy colonies (Table II). Colonies with color alterations, usually also demonstrated high percentages of mitotic index of zooxanthellae. The highest mitotic index values were recorded in bleached colonies in March/2009 and May/2009, and in moderate bleached colonies in April/2009 (Table II).

The percentages of zooxanthellae undergoing cell division in healthy colonies varied throughout the study period, with the largest values observed in

January, February, and March/2009 and the lowest percentages in September/2008, although these differences were not statistically significant between months ($df = 27$; $F = 2.21$; $p = 0.06$; Fig. 7) or between dry and rainy season ($df = 34$, $t = 0.58$; $p = 0.6$). Among the environmental variables analyzed, only the sea surface temperature and salinity demonstrated positive significant correlations with mitotic index of zooxanthellae (Pearson's correlation coefficient, $p < 0.05$; Table I).

The average cell diameters of the zooxanthellae varied between $7.7 \mu\text{m}$ and $13.5 \mu\text{m}$ (Table III) and demonstrated significant variations during the study period, with higher values in December/2008 ($12.7 \mu\text{m}$) and lower ones in May/2009 ($10.4 \mu\text{m}$) ($df = 8$; $F = 13.880$; $p < 0.01$; Fig. 8). The zooxanthellae cell diameters were higher during the dry season ($12.3 \mu\text{m}$) and lower during the rainy season ($11.4 \mu\text{m}$) ($df = 34$; $t = 3.373$, $p < 0.01$; Fig. 9).

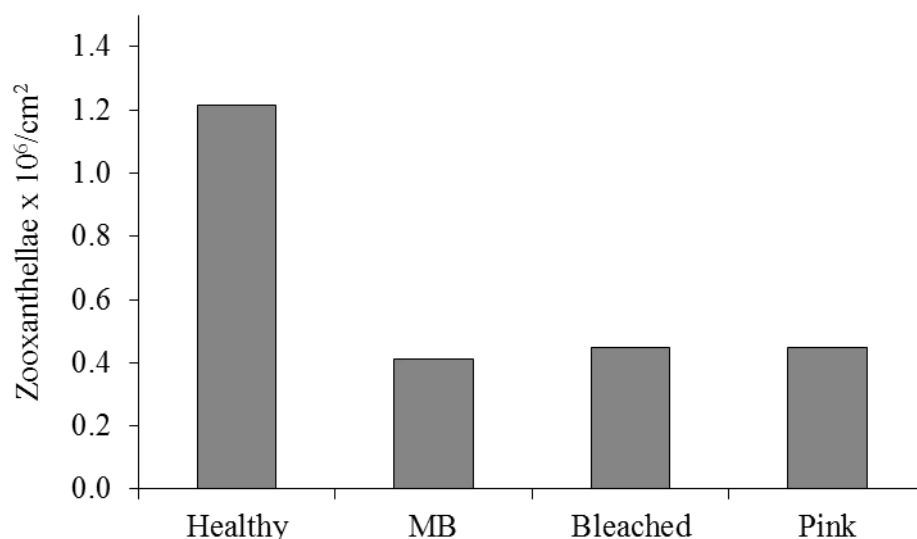


Figure 6. Mean densities of zooxanthellae of colonies *Siderastrea stellata* monitored on the Cabo Branco reef, João Pessoa, PB, Brazil, during the period from September/2008 to May/2009. (MB= Moderate bleached colonies).

Table II.- Percentages of zooxanthellae losses and variations in the mitotic index of healthy, moderate bleached (MB), bleached, and pink colonies of *Siderastrea stellata* collected on the Cabo Branco reef, João Pessoa, PB, Brazil, during the period between September/2008 and May/2009.

Months	% of zooxanthellae losses	Mitotic index (%)			
		Healthy	MB	Bleached	Pink
Jan/2009	33.3	5.6	-	0.3	-
Mar/2009	14.0 - 66.9	4.3	-	4.2	-
Apr/2009	72.9 - 93.7	2.8	4.2	0.9	1.3
May/2009	65.6	1.85	-	6.2	2.4
Other months	>10	1.5 - 5.5	<0.2	<0.2	-

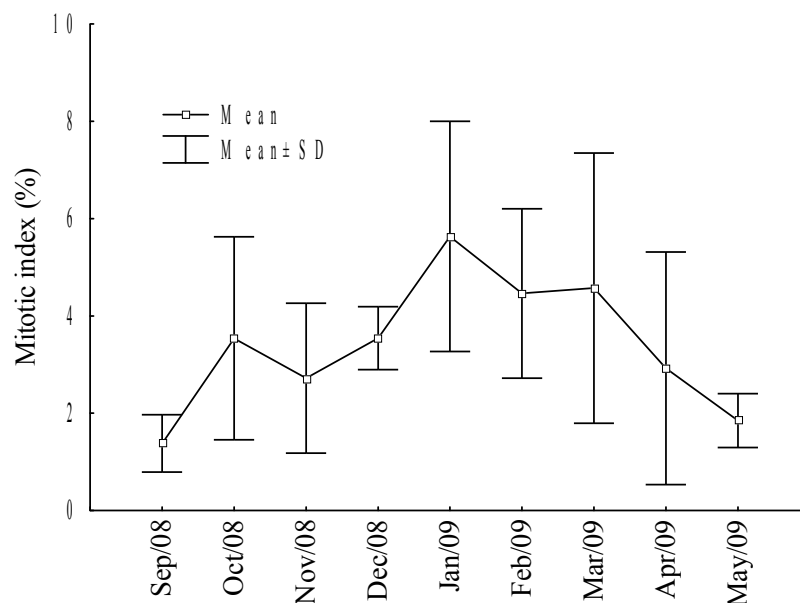


Figure 7. Mitotic index of the zooxanthellae associated with healthy colonies of *Siderastrea stellata* collected on Cabo Branco reef, João Pessoa, PB, Brazil between September/2008 and May/2009.

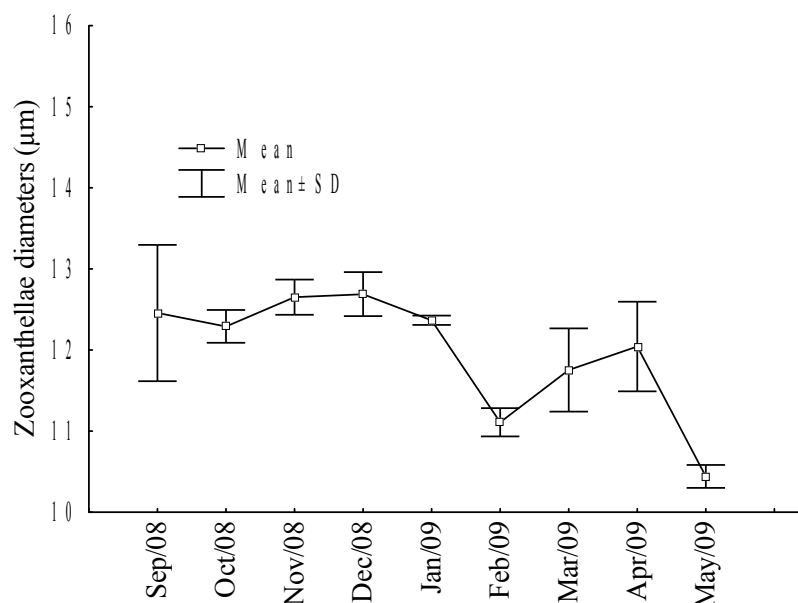


Figure 8. Mean diameters of zooxanthellae associated with healthy colonies of *Siderastrea stellata* collected on Cabo Branco reef, João Pessoa, PB, Brazil between September/2008 and May/2009.

Analyses of other microsymbionts

In addition to the zooxanthellae, other diverse microorganisms were observed in the tissues of healthy colonies of *Siderastrea stellata*, including foraminiferans, cyanobacteria, worms (like nematodes), and diatoms (the latter being the most highly represented organisms in all of the samples analyzed). These microorganisms demonstrated

fluctuations in their population densities throughout the study period, with greater values in October/2008 and lower ones in February/2009 ($df=8$; $F=11.425$; $p<0.01$; Fig. 10). The densities of these microsymbionts were higher during the dry period, and lower during the rainy season ($df=34$; $t=4.756$; $p<0.01$; Fig. 11).

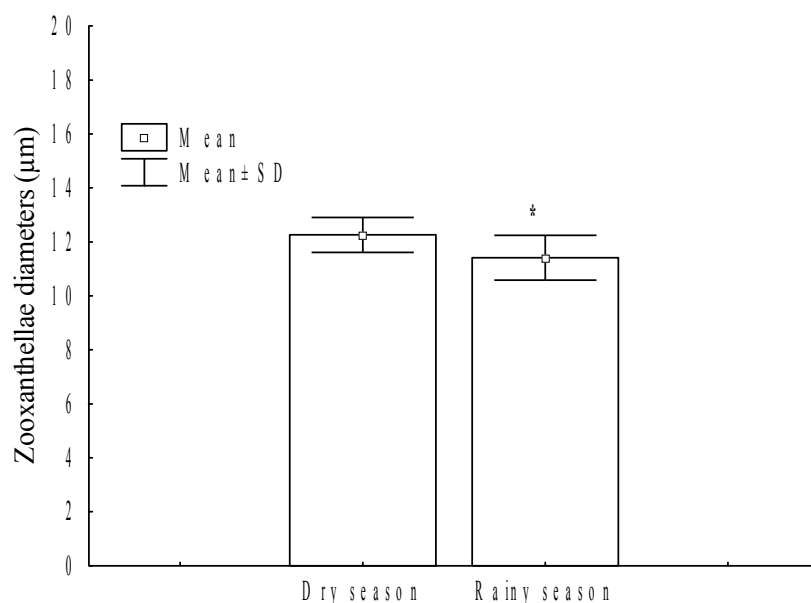


Figure 9. Mean diameters of zooxanthellae associated with healthy colonies of *Siderastrea stellata* collected on Cabo Branco reef, João Pessoa, PB, Brazil, during the dry and rainy seasons between September/2008 and May/2009 (*significant difference at $p \leq 0.05$).

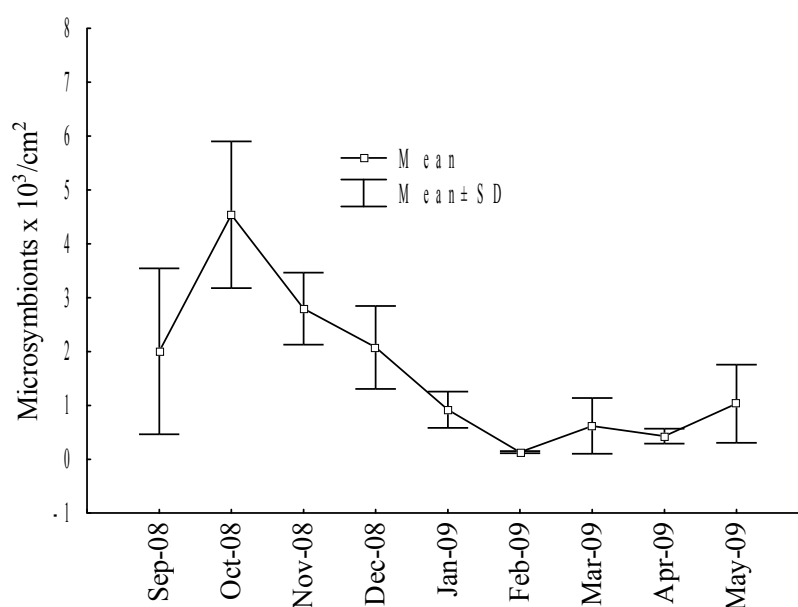


Figure 10. Mean densities of other microsymbionts associated with healthy colonies of *Siderastrea stellata* collected on the Cabo Branco reef, João Pessoa, PB, Brazil between September/2008 and May/2009.

Our data indicated that alterations in the pigmentation patterns of the colonies were associated with the increment in the densities of such microsymbionts like foraminiferans, cyanobacteria, and diatoms. The pink, moderate bleached and bleached colonies demonstrating higher densities of these microsymbionts than healthy colonies (Fig. 12).

Genotypic characterization of the zooxanthellae

Among the 38 samples of genomic DNA extracted from zooxanthellae obtained from healthy colonies of *Siderastrea stellata*, only six demonstrated amplifications of the SSU rDNA fragment (with approximately 1700 bp). All samples demonstrated the same restriction fragmentation patterns obtained using the enzymes *Taq I* and *Hae*

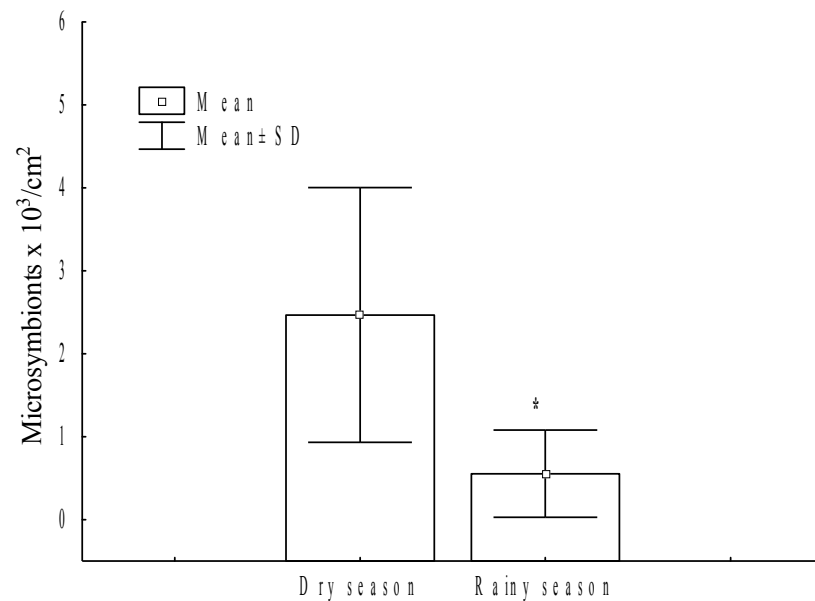


Figure 11. Mean densities of other microsymbionts associated with healthy colonies of *Siderastrea stellata* collected on the Cabo Branco reef, João Pessoa, PB, Brazil, during the dry and rainy seasons between September/2008 and May/2009. (*significant difference at $p \leq 0.05$).

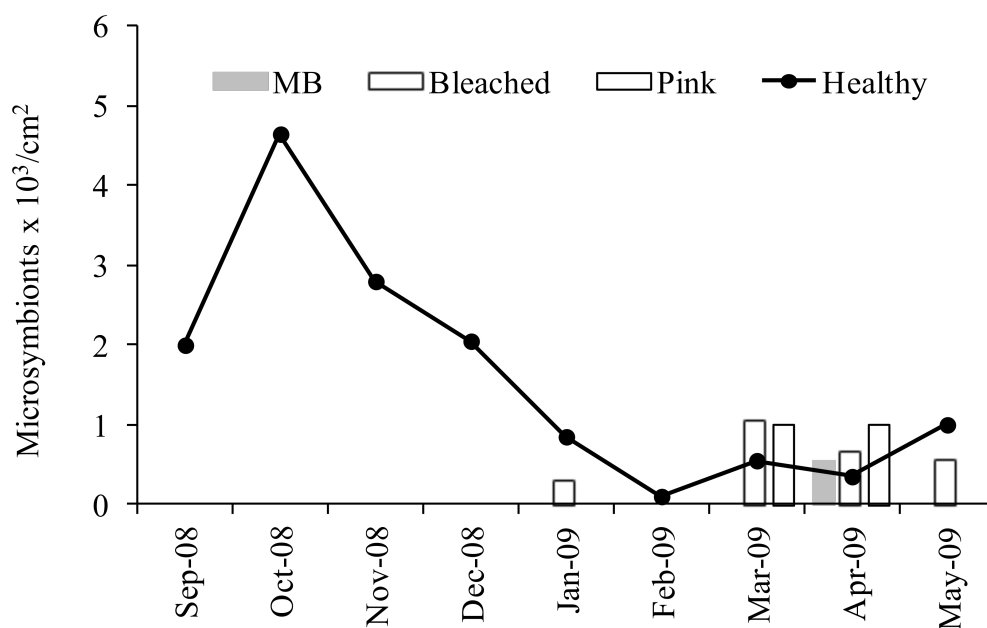


Figure 12. Mean densities of other microsymbionts associated with bleached, healthy, moderate bleached (MB), and pink colonies of *Siderastrea stellata* collected on the Cabo Branco reef, João Pessoa, PB, Brazil between September/2008 and May/2009.

III, which produced two (approximately 700 and 1000 bp) and three restriction fragments (approximately 280, 420 and 900 bp) respectively (Fig. 13).

The RFLP analyses of the SSU rDNA, utilizing the enzymes *Taq* I and *Hae* III, showed that

the zooxanthellae samples isolated from *Siderastrea stellata* had restriction patterns associated with clade C, according to data published by Rowan *et al.* (1997), Toller *et al.* (2001 a, b), and Costa *et al.* (2008b).

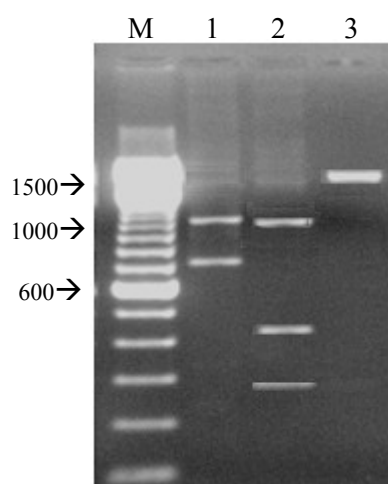


Figure 13. Restriction patterns of SSU rDNA of the zooxanthellae associated with healthy colonies of *Siderastrea stellata* as generated by the enzymes *Taq* I (line 1) and *Hae* III (line 2). M - molecular weight marker 100 bp; Line 3 – amplified SSU rDNA (1700 pb).

Discussion

The correlations between the environmental variables and zooxanthellae densities observed in the present work corroborated with the reports of Costa *et al.* (2004a), Costa *et al.* (2005) and Costa *et al.* (2013a) for the same coral species and other Brazilian scleractinian species in the same study area.

It appears that alterations in the pigmentation patterns of healthy coral colonies lead to losses of zooxanthellae, as was shown also by Glynn (1996), Costa *et al.* (2001a), Putnam & Edmunds (2010), and Palmer *et al.* (2012) during bleaching events. The exact percentages of zooxanthellae losses during these episodes depend on the coral species involved and the bleaching intensity (Fitt *et al.* 2000, Putnam & Edmunds 2010, Palmer *et al.* 2012).

The increases in zooxanthellae cell division rates in colonies demonstrating color alterations can be interpreted as a process of population recomposition in the coral that is manifested in a much accentuated form under stress conditions in attempts to maintain zooxanthellae population densities within ranges compatible with host necessities (Wilkerson *et al.* 1988). The diameters of the zooxanthellae determined here are in agreement with those reported by Amaral & Costa (1998) and Costa & Amaral (2002) for different Brazilian scleractinian corals.

The presence of wide varieties of other microsymbionts in addition to the zooxanthellae (including diatoms, foraminiferans, worms (like nematodes), cyanobacteria, and bacteria) has also been reported in the zoanthid *Palythoa*

caribbaeorum (Costa *et al.* 2013b), the calcareous hydroid *Millepora alcicornis* (Amorim *et al.* 2011), various corals (Costa *et al.* 2001b, Costa *et al.* 2004b, Costa *et al.* 2013a) on reefs in northeastern Brazil, in the coral *Mussismilia braziliensis* of the Itacolomis Reef, Corumbau – BA (Reis *et al.* 2009), and in corals found in the Gulf of Thailand (Piyakarnchana *et al.* 1986).

We observed that environmental conditions influenced the density of zooxanthellae and other microsymbionts. But the distinct behaviors of zooxanthellae and the other microsymbionts appeared to indicate that the health conditions of the scleractinian host determine the densities of other associated microbiota components (foraminiferans, cyanobacteria and diatoms). On the other hand, the highest quantities of zooxanthellae and other microsymbionts were observed during the dry period, demonstrating that rainfall destabilizes both types of symbionts inside the colonies (with alterations in coral pigmentation patterns at the beginning of the rainy season).

Several researchers have reported the presence of many different organisms in the planktonic populations of coral reef environments (Paul *et al.* 1986, Wild *et al.* 2004, Reis *et al.* 2009) and in the interstitial spaces of their calcareous walls (Ferrier-Pagès *et al.* 1998). The advantages and disadvantages of these associations are still unknown (Costa *et al.* 2004b, Costa *et al.* 2013a,b). It is assumed that even though they are not directly involved in mutualistic intracellular symbiotic relationships, alterations in the amounts of these organisms may reflect ruptures in the relationships

that zooxanthellae would otherwise maintain with their hosts.

The reduction of microsymbiotes (foraminiferans, cyanobacteria, worms, and diatoms) population densities in bleached colonies suggests that these organisms are important for host health and could be useful as bio-indicators of environmental stress, similarly to zooxanthellae. Alterations of pigmentation of *Siderastrea stellata* colonies might be useful to indicate stress conditions that reduce their ability to maintain high densities of zooxanthellae within their tissues, and consequently, reduce supplies of nutrients for other components of the epibiont microbiota.

The monitoring of *Siderastrea stellata* in the study area demonstrated the existence of healthy, pale (moderate bleached), bleached, and pink colonies, with the greatest percentages of altered colonies being observed at the end of summer and beginning of rainy season. Costa (2006) also observed that coral bleaching on the Cabo Branco reef at João Pessoa is not as closely related to increase in water temperatures as has often been suggested (Fitt *et al.* 2000, Glynn *et al.* 2000), although local stress conditions can affect certain species and destabilize their relationships with zooxanthellae. The appearance of bleached colonies on the Cabo Branco reef may occur due to local hydrodynamic conditions that lead to sediment cover of the colonies (Costa 2006).

The pink colonies of *Siderastrea stellata* observed at certain periods of the year demonstrate similarities to pigmentation responses commonly seen in colonies of the genus *Porites* that appeared to be associated with the cyanobacteria *Phormidium valderianum* (Raymundo *et al.* 2005). This syndrome is characterized by irregular pinkish regions of dead colony sites bordered by strong purple pigmentation (Ravindran & Raghukumar 2006). This phenomenon is recurrent in *Siderastrea stellata* on Brazilian reefs (Costa & Sassi 2008) but not lethal.

The pink pigmentation of colonies of *Siderastrea stellata* observed in the present study represents a type of bleaching never before been documented in Brazilian coastal waters, and only previously having been referred to as a characteristic of shallow water specimens (Laborel 1969). Significant losses of zooxanthellae and reductions in the densities of other microsymbiotes during bleaching events in *Siderastrea stellata* on the Cabo Branco reef makes this species vulnerable to various types of diseases and should be considered a

warning sign for more intensive monitoring of reefs in Paraíba State.

The results of the RLFP analyses of the SSU rDNA of isolated zooxanthellae revealed the occurrence of clade C in the *Siderastrea stellata* samples analyzed. Our knowledge of the diversity of zooxanthellae clades in Brazilian corals is still incipient, with only a single reports by Costa *et al.* (2008b) and by Monteiro *et al.* (2013) documenting the presence of clade C in samples of zooxanthellae associated with *Siderastrea stellata* on the Cabo Branco and Picãozinho reefs, João Pessoa, PB during the years from 2003 – 2005 and 2008 respectively. This finding suggests the stability of this clade in its association with *Siderastrea stellata* on these reefs. Similarly, Thornhill *et al.* (2006) detected clade C of *Symbiodinium* in *Siderastrea siderea* in the Bahamas, and Lajeunesse (2002) identified clade B5a in *Siderastrea radians* from Caribbean coral reefs.

The distribution of *Symbiodinium* among scleractinian corals varies considerably between the tropical western Atlantic (Caribbean) and Indo-Pacific regions (Baker & Rowan 1997, Baker 2003, Lajeunesse 2002, Lajeunesse *et al.* 2003). Clades A-D of *Symbiodinium* are common in the Caribbean at shallow depths (<7 m), and a given species of coral is typically associated only with a single zooxanthellae clade (although they can occasionally be associated with up to three clades) (Van Oppen *et al.* 2009). Scleractinian corals in the Indo-Pacific Ocean at similar depths are dominated by clade C and D symbionts (Van Oppen *et al.* 2009).

Clade C of *Symbiodinium* is considered a generalist and can be encountered in both the Atlantic and Pacific oceans, suggesting that it arose before the closing of the Panama Isthmus (Lajeunesse 2005). The high adversities of sub-clades within clade C observed in both oceans suggest independent evolutionary paths during their adaptation to different radiation levels (Lajeunesse 2005).

Loh *et al.* (1998) analyzed nineteen species of scleractinian corals from the Australian Pacific utilizing RFLP analysis of SSU rDNA and observed that clade C was predominant among corals on the One Tree Island reefs, being encountered in eighteen coral species. Bongaerts *et al.* (2011) detected various sub-clades of clade C in zooxanthellae associated with corals belonging to ten different genera on the Great Barrier Reef of Australia. Zhou & Huang (2011) reported that most of the

scleractinian coral species (88.6%) off Hainan Island, China had only clade C zooxanthellae, generally sub-clades C1 and C3.

Lien *et al.* (2012) detected three clades (C, D and F) of *Symbiodinium* in scleractinian corals at Tanabe Bay, Japan, although clade C was hosted by most species. These authors reported that symbiotic relationships between the majority of the coral species in the study area (temperate climate) and clade C was stable over many years, suggesting that clade C has significant physiological flexibility (or that there are genetic differences among its various sub-clades that generate physiological differences). These findings may help explain the high observed ecological plasticity of the *Siderastrea stellata* and its ability to colonize many different habitats – making it the most conspicuous and widely distributed coral on Brazilian reefs.

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