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Research Article

**Pigmentation changes in *Siderastrea* spp. during bleaching events
in the costal reefs of northeastern Brazil**

Roberto Sassi¹, Cristiane Francisca Costa Sassi¹, Krystyna Gorlach-Lira² & William K. Fitt³

¹Laboratório de Ambientes Recifais e Biotecnologia com Microalgas, Departamento de Sistemática e Ecologia, Universidade Federal da Paraíba (UFPB/DSE/LARBIM)
Cidade Universitária, João Pessoa, 58059-900, Paraíba, Brasil

²Laboratório de Biologia Molecular e Ecologia, Departamento de Biologia Molecular
Universidade Federal da Paraíba (UFPB/DBM/LABIME)
Cidade Universitária, João Pessoa, 58059-900, Paraíba, Brasil

³Institute of Ecology, Bioscience 711, University of Georgia, Athens, Georgia 30602, USA
Corresponding author: Roberto Sassi (sassi_rs@yahoo.com.br)

ABSTRACT. We report here the occurrence of seasonal changes in the pigmentation of colonies of *Siderastrea* spp. during bleaching events on coastal reefs in northeastern Brazil. Bleached affected almost 50% of coral colonies analyzed in Cabo Branco reefs (Paraíba state) in the summer of 2005. The same phenomenon was detected along various coastal reefs in northeastern Brazil during the summer of 2007 and 2008. These events were seasonal, and began with the emergence of pale colonies that became pale-pink and then purple during the rainy months. The patterns and intensity of colonies pigmentation changes varied with the studied sites. The decrease in zooxanthellae density and chlorophyll-*a* content was quantified in the colonies with their pigmentation pattern altered (bleaching). Microbiological analyses revealed higher densities of bacteria in pink colonies (bleached) as compared to brown colonies (normal). Environmental disturbances may lead to the pigmentation changes in *Siderastrea* spp., but the immediate causes of this phenomenon are not clear and require further investigations.

Keywords: zooxanthellae, bacteria, marine ecology, pink colonies, Brazilian reefs.

**Cambios en la pigmentación de colonias de *Siderastrea* spp. durante los
eventos de blanqueamiento en arrecifes costeros del noreste de Brasil**

RESUMEN. Se relata aquí la aparición de cambios estacionales en la pigmentación de colonias de *Siderastrea* spp. durante los eventos de blanqueamiento en arrecifes costeros del noreste de Brasil. En verano de 2005, existían casi 50% de colonias blanqueadas en los corales analizados en Cabo Branco, Estado de Paraíba. El mismo fenómeno se detectó a lo largo de varios arrecifes costeros en el noreste del país durante el verano de 2007 y 2008. Estos eventos son asociados a la variación estacional, que comenzaron con la aparición de colonias pálidas que luego se convirtieron en rosa pálido y después a morado, durante los meses de lluvias. Los patrones e intensidad de los cambios en la pigmentación de las colonias fueron diferentes en los sitios estudiados. La disminución de la densidad de zooxantelas y del contenido de clorofila-*a* fue cuantificada en las colonias y comparados con el patrón de pigmentación alterada (blanqueo). Los análisis microbiológicos revelaron densidades más altas de bacterias en colonias rosas (blanqueada) en comparación con las colonias marrones (considerados normales). Las alteraciones ambientales pueden conducir a estos cambios de pigmentación en *Siderastrea* spp., pero las causas inmediatas de este fenómeno no están claras y requieren más investigación.

Palabras clave: zooxantelas, bacterias, ecología marina, colonias rosas, arrecifes brasileños.

INTRODUCTION

The substantial increase in occurrence of coral bleaching around the world has led to the degradation

of extensive areas of coral reefs (Glynn, 1993; Fitt *et al.*, 2000), and has disturbed reef trophic relationships and marine productivity (Bryant *et al.*, 1998). Bleaching is associated with stress conditions that decrease the di-

sease resistance of coral colonies (Rosenberg & Ben-Haim, 2002; Borger, 2005; Santavy *et al.*, 2005; Weil *et al.*, 2006; Palmer *et al.*, 2008, 2010), and brakes down the symbiotic relationship with zooxanthellae (Kaczmarzsky, 2006; Weil *et al.*, 2006).

During the bleaching process corals experience loss of symbionts and/or photosynthetic pigments (Fitt *et al.*, 1993; Glynn, 1993; Costa *et al.*, 2001), and a reduction of the host biomass, growth colonies rate, and uptake of carbohydrates and lipids (Fitt *et al.*, 1993). Usually, the coral tends to be more stressed, with reduced natural immunity against diseases, and, in extreme cases (persistence of high temperatures), the phenomenon can lead to coral death.

Bleaching events have been observed in coral reefs around the world, and most of them have been associated with changes in water temperature (Fitt *et al.*, 1993, 2000; Glynn, 1993; Costa *et al.*, 2001; Glynn *et al.*, 2001; Weil *et al.*, 2006). Other factors include extreme salinity, photic conditions, pollution (Goreau & MacFarlane, 1990; Saxby *et al.*, 2003; Weil *et al.*, 2006) and UV radiation (Brown *et al.*, 2000). In severe situations, corals can lose between 60 and 90% of their zooxanthellae, and those that remain within the host tissues show photosynthetic pigment concentrations reduced by 50 to 80% (Fitt *et al.*, 1993, 2000; Glynn, 1993).

Bleaching events were registered in Brazil during El Niño Southern Oscillation of 1997-1998 and 2005 (Costa *et al.*, 2001; Leão *et al.*, 2003). Migotto (1997), Castro & Pires (1999), Costa *et al.* (2001) and Leão *et al.* (2003) also reported the occurrence of the bleaching in many endemic and non-endemic coral species along the Brazilian coast.

In addition to bleaching events, disease in the zoanthid *Palythoa caribaeorum* was observed along the São Paulo coast, São Sebastião Channel, Brazil (Acosta, 2001). Various diseases have been described in Brazilian scleractinian corals, on the Abrolhos reefs, Bahia State, Brazil (Francini-Filho *et al.*, 2008). Different types of anthropogenic factors that affect Brazilian coastal environments were discussed by Costa *et al.* (2007).

We report here the occurrence of seasonal bleaching in colonies of *Siderastrea* spp. during the years 2005 and 2007-2008 on the coastal reefs of northeastern Brazil. All colonies analyzed in this study were reported as *Siderastrea* spp. in view of the difficulty in distinguishing two species of *Siderastrea* that occur in the Brazilian reefs (Neves *et al.*, 2008): *S. radians* (Pallas, 1766) and *S. stellata* Verrill, 1868. We hypothesized that bleaching is a function of environmental variables, and coral pigmentation shows

various stages and intensity, that can be identified and monitored.

MATERIALS AND METHODS

Monitoring of *Siderastrea* spp. pigmentation patterns

The present research was conducted on coastal reefs in the states of Rio Grande do Norte, Paraíba, and Pernambuco, Brazil. Pigmentation alterations in colonies of *Siderastrea* spp. were monitored during field surveys recording the diameters and colors of colonies following the criteria of the Coral Health Chart (<http://www.coralwatch.org>) and Siebeck *et al.* (2006).

Colonies showing brown color and bleached areas; brown color and white and pink areas; or pink and pale areas were considered to have a pigmentation pattern altered (PPA) colonies. The colonies were photographed using a Canon G9® digital camera. The percentages of colonies of each pigmentation pattern at each site studied were calculated.

The monitoring of pigmentation patterns was conducted only in shallow waters on the reef top, during three different periods, in order to relate the bleaching and changes in the pattern of colors in *Siderastrea* spp. with the local climatological changes:

From January to March 2005 (first rainy period): in the Cabo Branco reef, Paraíba State (7°08'44"S, 34°47'43"W) where 530 colonies of *Siderastrea* spp. were analyzed at three sites at different depths: 1 m (A1-A3); <1 m (B1-B4); tidal pools (C1-C3), to check the habitat/depth effects on the spreading of PPA in a same reef.

From September 2007 to June 2008: in the Cabo Branco (7°08'44"S, 34°47'43"W) and Picãozinho reefs (7°07'30"S, 34°48'28"W), Paraíba State, where 60-70 colonies at depths of near 1 m were analyzed monthly, to check the seasonal effects the climatologic changes on the PPA appearance in a same reef.

From March to April 2008: when 70 colonies of *Siderastrea* spp. were analyzed in each of the following coral reefs: Carapibus, Paraíba State (7°18'03"S, 34°47'52"W), Buzios, Rio Grande do Norte State (6°00'14'S, 35°06'19"W), and Porto de Galinhas, Pernambuco State (8°30'15"S, 34°59'54"W), viewing to assess the latitudinal extension of bleaching in the northeastern coastal reefs of northern Brazil.

In all sites the water temperatures (mercury thermometer, $\pm 0.5^\circ\text{C}$) and salinities (portable refractometer, American Optical) were measured at the Cabo Branco and Picãozinho reefs during the fieldwork and the monthly rainfall at João Pessoa City was obtained from AESA-PB (2009).

Analyses of zooxanthellae and bacteria in brown and pink colonies of *Siderastrea* spp. at Cabo Branco reef

Fragments of 10 unbleached (brown) colonies and 10 pink (bleached colonies) haphazardly selected, were collected during January and February 2005 in shallow waters of the infralittoral zone at Cabo Branco reef and transported in plastic bags containing sea water to the laboratory for zooxanthellae analysis (densities and chlorophyll-*a*) and culturable and total bacteria counts. To prevent deterioration of tissues and/or proliferation of bacteria, the samples were kept in sterile flasks inside a styrofoam box during their transportation to the laboratory.

The coral tissues were extracted using high-pressure sterile filtered seawater (Water Pik®), and the zooxanthellae densities were determined using a Fuchs-Rosenthal counting chamber at 400x. The densities of viable bacteria in the colonies of *Siderastrea* were evaluated using marine agar (pH 7.6) composed of 5 g of peptone (Difco), 0.1 g of yeast extract (Difco), 15 g of agar, and filtered seawater collected at the study site. Tenfold serial dilution of extracted coral tissues were made using sterile seawater, plated on marine agar, and incubated at 30°C for 7 days. The numbers of bacteria were expressed as CFU (colony forming units) per cm² of coral surface. Direct counts of bacteria in samples of extracted coral tissues fixed with Lugol's solution were made using a Neubauer chamber and a phase-contrast microscope (Leica).

Chlorophyll-*a* concentrations were analyzed using a Turner-Design fluorometer model 10005R, with correction for phaeopigments, as described by Strickland & Parsons (1972) and Arar & Collins (1992).

Statistical analyses

Statistical analyses were performed using Statistica version 7.0 software, at a 5% level of significance. The homoscedasticity of the variances of all data sets was confirmed using Levene's test. The Student's *t*-test was applied to compare the mean differences of zooxanthellae densities and chlorophyll-*a* data among the healthy brown and pink colonies. All statistical analyses followed Sokal & Rohlf (1983).

RESULTS

Siderastrea spp. colonies pigmentation patterns

Siderastrea spp. in the reefs of northeastern Brazil had brown colonies (Coral Health Chart: D5, D6, normal pigmentation for *Siderastrea* spp.) (Fig. 1a) as well as various multi-signs of bleaching, with different types of pigmentation pattern alterations (PPA) such as brown

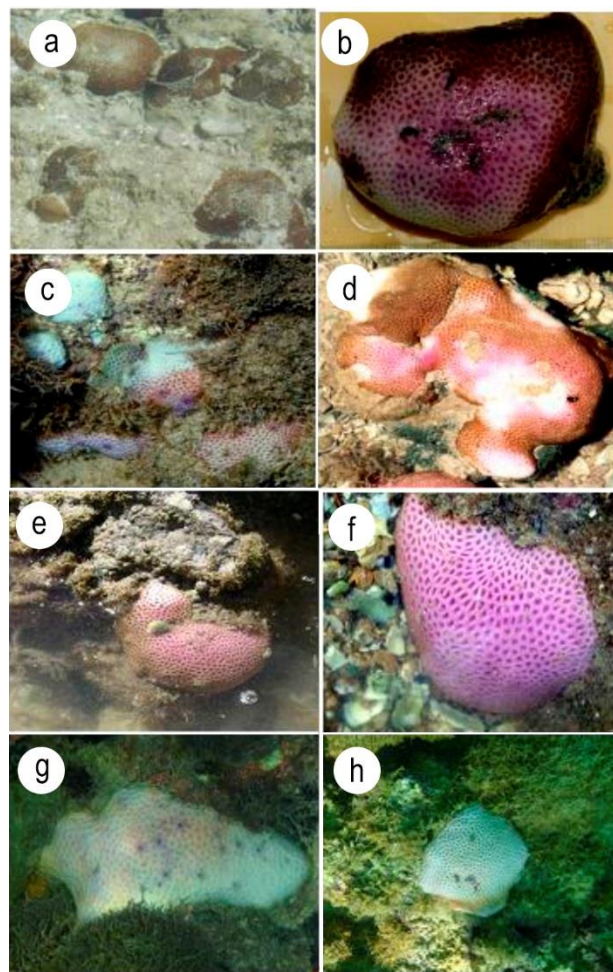


Figure 1. Variations in the pigmentation pattern observed in *Siderastrea* spp. in the reefs studied. a) brown colonies, b-e) pigmentation patterns altered (PPA) colonies, b,c,d) brown colonies with pink and bleached spots, e) pink colonies with bleached spots, f) pink colonies, g-h) bleached colonies.

colonies with pink and white spots (Fig. 1d), pink colonies with white spots (Figs. 1b-1e), pink colonies (Fig. 1f), and bleached colonies (Figs. 1g-1h).

Among 530 colonies analyzed in 2005 on the coastal reefs of Cabo Branco only 9.2% were heavily bleached (Coral Health Chart: D2, D1), 45.7% were brown healthy (Coral Health Chart: D5, D6), and 45.1% showed pigmentation pattern alterations (PPA). The highest numbers of brown colonies (D5, D6) were recorded among submerged corals at 1 m depths (sites A1-A3), while the PPA colonies were the most frequently observed in the intertidal pools (sites C1-C3). Bleached colonies were observed mostly at sites A1 (n = 24 colonies), B1 (n = 5 colonies), and C2 (n = 5 colonies) (Fig. 2).

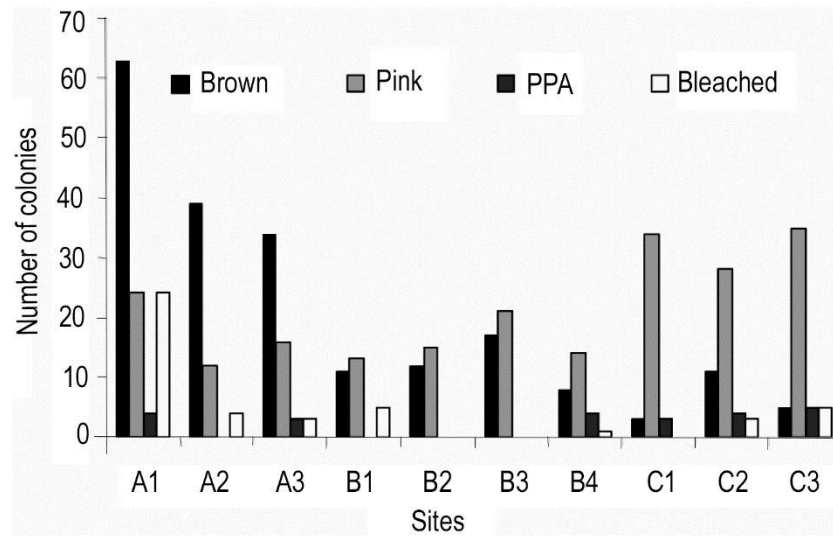


Figure 2. Variations of pigmentation of coral *Siderastrea* spp. at different depths of Cabo Branco reefs, Paraíba State, registered during February and March 2005. A1-A3 = 1 m depth, B1-B4 = <1 m depth, C1-C3 = tide pool (PPA: pigmentation patterns altered).

More detailed investigations along the Cabo Branco and Picãozinho reefs undertaken in 2007 and 2008 demonstrated that pink pigmentation was common in *Siderastrea* colonies, with high numbers of pink colonies in March, April, and May (Fig. 3a). The monthly monitoring of the Cabo Branco and Picãozinho reefs revealed that occurrence of pink colonies varied with habitat at each site. During the same period of study, the Picãozinho reefs showed less brown colonies (<20%), while moderately bleached colonies (paled colonies) were dominant (Fig. 3b). Pink colonies were observed in the Picãozinho reefs almost all over monitoring period, with higher frequencies from December 2007 to June 2008 (25-40%).

Seasonality of PPA was strongly observed during the period corresponding to the rainy season, which typically starts in March (Fig. 4). Paled colonies (moderately bleached, Coral Health Chart: D3, D4) first appeared at this period, then developed into pale-pink colonies, and finally into pink colonies, but later returned to brown pigmentation (Coral Health Chart: D5, D6) (Fig. 3a). The *Siderastrea* of the reef of Cabo Branco were the most affected at March 2008, when up to 92% of the shallow water colonies showed alterations of pigmentation pattern. Brown colonies were dominant from September to December 2007; in June 2008 no PPA colonies were observed.

High percentages of PPA colonies were observed on the reefs at Porto de Galinhas, Carapibus, and Buzios in 2008 (March-April) (Fig. 5). Only 2% of the colonies analyzed at Porto de Galinhas and Buzios during this

period had brown color (Coral Health Chart: D5, D6); more colonies were brown at Carapibus reefs (25%). The percentages of pink colonies varied from 45 to 70% in the studied reefs (Fig. 5). The PPA colonies varied in size, from young recruits to colonies up to 27 cm in diameter.

Density of zooxanthellae and bacteria in brown and pink colonies of *Siderastrea* spp.

Microscopic analyses of the tissue extracted from *Siderastrea* spp. collected at Cabo Branco reef (Paraíba State) revealed significantly reduced numbers of zooxanthellae ($df = 30$; $t = 7.498$; $P = 0.000$; Fig. 6), and significantly lower concentrations of Chl-*a* in PPA colonies ($df = 8$; $t = 5.314$; $P = 0.000$; Fig. 6).

Microbiological analyses of tissue extracted from *Siderastrea* revealed significantly higher densities of viable bacteria in the tissue of pink colonies than in the brown colonies (t -test; $P < 0.05$). Average counts of cultivable heterotrophic bacteria of brown colonies were 2.9×10^4 CFU·cm⁻², while higher numbers (6.8×10^5 CFU·cm⁻²) were observed in pink colonies. Direct counts of bacteria were higher than those found by the culture method, with average numbers of 7.0×10^7 cells·cm⁻² and 6.9×10^8 cells·cm⁻² in brown and pink colonies of *Siderastrea* spp., respectively.

DISCUSSION

The bleaching phenomenon that affected colonies of *Siderastrea* on coastal reefs in northeastern Brazil during

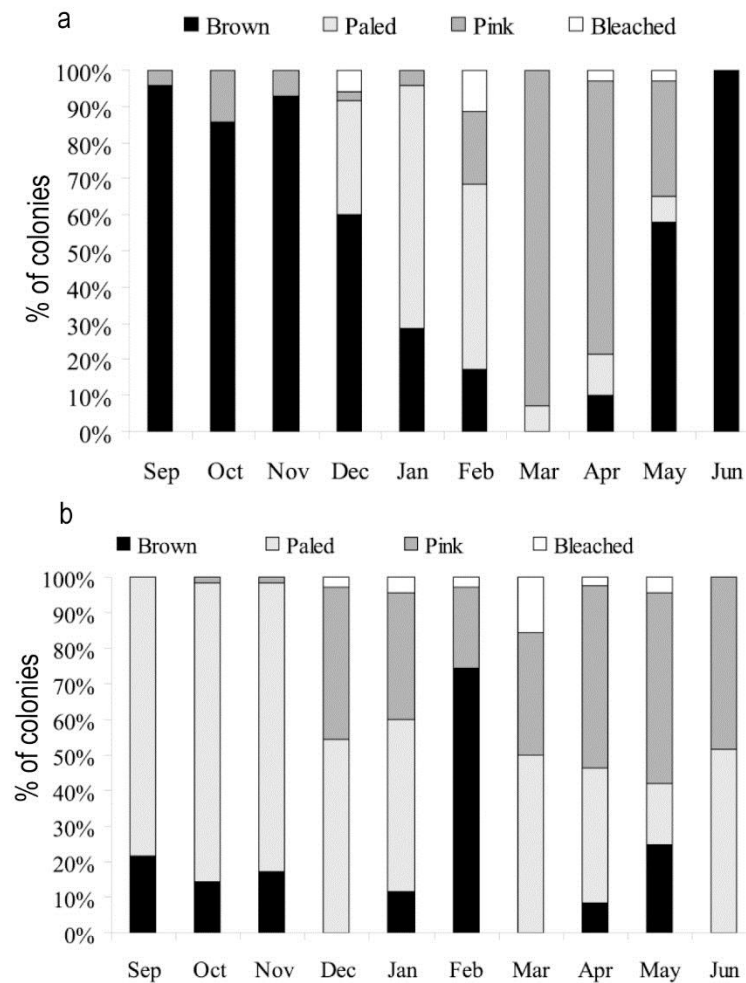


Figure 3. Monthly evolution of the pigmentation changes in colonies of *Siderastrea* spp. in the reefs of a) Cabo Branco and b) Picãozinho Paraíba State, in the period from September 2007 to June 2008.

the summer of 2005 and in the years 2007-2008 suggests the existence of stressful conditions in the area studied. The episode is seasonal and show maximum intensities during rainy season. Evidence of seasonal bleaching has previously been documented for a number of species (Antonius, 1981; Kaczmarzsky, 2006; Weil *et al.*, 2006).

Multi-signs of bleaching were observed covering the scales D and C, and all tonality (1-4) of Coral Health Chart and Siebeck *et al.* (2006). We observed brown colonies; brown colonies with white spots; brown colonies with pink and white spots; moderate bleached (paled) colonies; moderate bleached colonies with pink spots; moderate bleached colonies with pink and white spots; and pink colonies. Colony recovery after the bleaching was observed in all bleaching types, and occasional cases of mortality were observed only among pink colonies.

The persistence of pink colonies for long periods could increase host and/or zooxanthellae mortality, as can be judged by the fact that some colonies became fully bleached. However, the re-establishment of normal pigmentation status (Coral Health Chart: C5, C6, D5, and D6), observed concurrently when rainfall decrease and temperature increase, strongly indicates that this phenomenon is natural and cyclical, and may be based on genetic contribution, with a protective function for the species (Palmer *et al.*, 2008).

According to Bongiorno & Rinkevich (2005) this phenomenon is a 'pigmentation response' of the host to a variety of stressors, possibly an immune response, and the purple coloration occurs due to the presence of pocilloporin fluorescent pigments (molecular weights of 28.7 kD and a maximum peak of absorption at 580 nm) in the underlying tissue after the loss of zooxanthellae. Once there are less zooxanthellae and

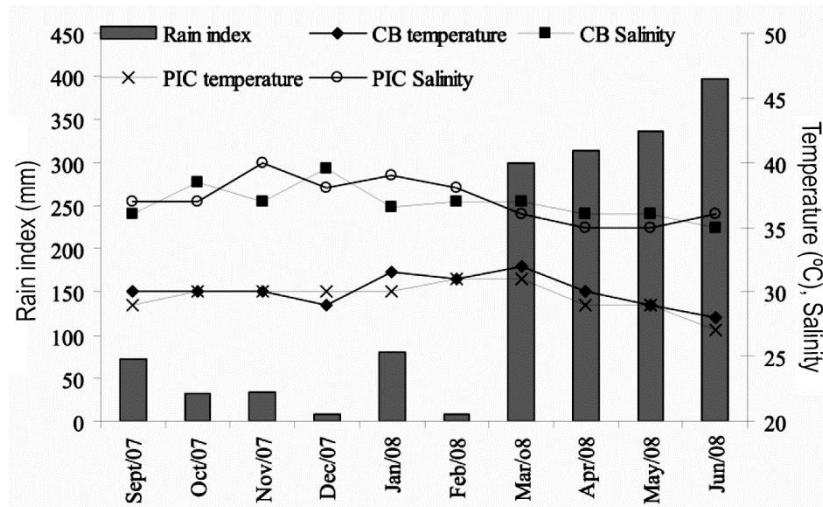


Figure 4. Monthly seasonal variation of rain index, water temperature and salinity, in Picãozinho (PIC) and Cabo Branco (CB) reefs, Paraíba State, between September 2007 and June 2008.

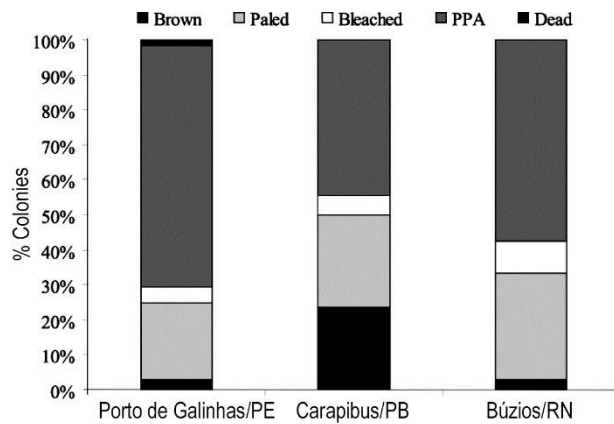


Figure 5. The prevalence of pigmentation changes in colonies of *Siderastrea* spp. in the reefs of Carapibus (Paraíba State), Porto de Galinhas (Pernambuco State) and Búzios (Rio Grande do Norte State) during March and April 2008. PPA: Pigmentation pattern altered.

their pigments, the pocilloporins may become visible (pink-blue pigmentation of colonies). If zooxanthellae numbers are reduced, the mortality of the entire coral colonies or parts of them may occur ("bleached" or white colonies; colonies with several white spots). Once stressful conditions go down, the pink colonies return to brown color because of the increase of the zooxanthellae pigment concentrations, and therefore the pocilloporins are not distinguishable.

Seawater surface temperatures (Antonius, 1981, 1988; Alker *et al.*, 2001; Martínez & Baquero, 2002; Cervino *et al.*, 2004; Borger, 2005) and anthropogenic impacts (Lesser, 2004) have been associated with the emergence of bleaching and new diseases in marine

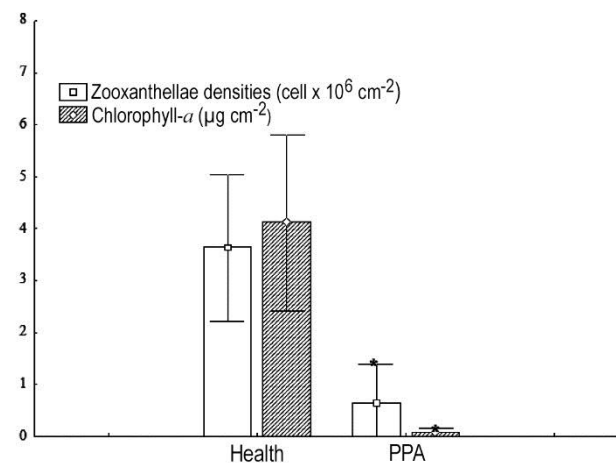


Figure 6. Mean values of population density and contents of zooxanthellae and Chl-*a* from healthy (brown) colonies and colonies with pigmentation pattern altered (PPA) of *Siderastrea* spp. of the Cabo Branco reefs, Paraíba State, Brazil, collected during January and February 2005. *Significantly different.

environments (Hayes & Goreau, 1998; Harvell *et al.*, 1999; Green & Bruckner, 2000; Shinn *et al.*, 2000). However, based on the environmental data obtained here we cannot generalize that the coral bleaching in all reef environments encompassed in this study has the same causes, although the habitat had a strong influence on pigmentation changes of *Siderastrea* colonies.

The studied reef areas show some specific characteristics as follows: i) The northeast coast of Brazil is usually outside of the zone of thermal anomalies; however, temperature anomalies of about

0.75 to 1°C were observed in the region from mid-March 2005, and also in March-April 2008 along the most northern part of Brazilian Northeast, including the coast of the Rio Grande do Norte state (see <http://www.osdpd.noaa.gov/ocean/cb/hotspot/html>).

No thermal anomaly was observed in the period from September to December 2007 and in June 2008. The local differences in seasonal pattern of the PPA colonies as observed in Cabo Branco and Picãozinho reefs (Paraíba State) during periods without occurrence of thermal anomalies indicate other conditions for bleaching, ii) all studied sites are quite ecologically similar regarding the substrate, depth, solar radiation exposure, seasonal rains and temperature, indicating that such variables may not have strong influence on coral bleaching. Temperatures were almost constant throughout the annual cycle and the salinity was always greater than 35 even at the rainy season. However, more intense bleaching always occurred at the beginning of the rains and during the rainy season, when the colonies showed pink pigmentation that might be associated with increasing cloudiness in this period. However, in the tidal-pool habitats, the temperature can be high during solar exposure and the salinity is quite variable, depending on the contribution of continental groundwater. Sassi *et al.* (1988) observed salinity of only 6 and temperature of 42°C inside tidal pools in the reef of Cabo Branco, and iii) the Cabo Branco reef is near to an active cliff and is subjected to a quite strong wave action which promotes a high sedimentation. Its proximity to the beach (near shore) results in an intensively pressure due to recreation, trampling, fishing, educational activities and scientific research, among others (Costa *et al.*, 2007). Picãozinho (Paraíba State) and Porto de Galinhas (Pernambuco State) reefs are also intensively visited by tourists, have similar structure and are 500 to 1000 m distant from the beach (off shore) (Costa *et al.*, 2007). Buzios (Rio Grande do Norte State) and Carapibus (Paraíba State) reefs are much closer to the beach but have most accidentally rocks, and so are less stepped. Such pigmentation changes could be associated with local conditions of stress generated by biological and/or environmental factors, including anthropogenic land-use impacts. The pigmentation changes include low and highly fluorescent forms of pigments that seem to provide a photobiological protective system for regulating the light environment of the host coral tissues (Dove *et al.*, 1995, 2001; Salih *et al.*, 2000; Roth *et al.*, 2010). Light impinging on corals can reach very high intensities, depending on tissue thickness and zooxanthellae densities (Enríquez *et al.*, 2005).

The bleaching phenomenon affecting *Siderastrea* spp. in northeastern coastal reefs of Brazil favored the

proliferation of opportunistic microorganisms, particularly bacteria that showed the higher numbers in pink colonies than in colonies with brown pigmentation. The microbial communities associated with corals are not merely a reflection of the bacterial communities present in the surrounding sea water (Knowlton, 2001), and various kinds of bacteria are probably involved in stable, not pathogenic, associations. Recent reports have noted that healthy coral colonies showed distinct associated bacterial communities from those observed in diseased corals (Rohwer *et al.*, 2001), although their exact composition depends on the coral species. The composition of coral-associated bacterial communities is shaped by various environmental and host factors, including specific nutrients (such as dimethylsulfoniopropionate – DMSP, produced by dinoflagellates symbionts) (Raina *et al.*, 2009), and the antimicrobial activity of coral mucus (Ritchie, 2006). The functions of most bacteria remain obscure, but many of them seem to be important for coral survival (Lesser *et al.*, 2004).

To date *S. stellata* was considered as an endemic species for Brazilian waters (Laborel, 1970; Leão, 1986; Castro & Pires, 2001; Neves & Silveira, 2003; Santos *et al.*, 2004), and several authors described this species as being red-brown with pale walls, or pink with bluish tonalities (Laborel, 1970; Leão, 1986; Santos *et al.*, 2004), but these authors never associated these different color patterns with seasonal bleaching events. The pink colonies of *Siderastrea* of Brazilian reefs may result from the synergetic effects of several recurrent and natural factors in Brazilian waters. Our results are sufficiently robust to support this argument, as pink colonies of *Siderastrea* spp. were observed on the coastal reefs of Cabo Branco in the summer of 2005, and then again in 2007-2008.

Some colonies identified in the field as *S. stellata* could be in fact *S. radians* since, according to Neves *et al.* (2008), both species may exhibit similar patterns of pigmentation changes during bleaching events in Brazilian reefs. We suggest that episodes of pigmentation changes in the *Siderastrea* of Brazilian reefs have been overlooked for the last 40 years (since Laborel 1970), and need to be examined more deeply. The fact that no mortality was ever documented leads us to speculate that these corals are extremely temperature tolerant and have evolved great resistance to the "bleaching events" that have increased in frequency over the past three or four decades (Eakin *et al.*, 2010).

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