ORIGINAL ARTICLE

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Response of the zooxanthellae of *Palythoa caribaeorum* (Cnidaria: Zoanthidea) to different environmental conditions in coastal and oceanic ecosystems of the Tropical Atlantic

Gleice S. Santos[1], Fernanda D. Amaral[2], Cristiane F. C. Sassi[3] and Ralf Schwamborn[1*]

Abstract

The present study tested the hypothesis that endosymbionts of the zoanthid *Palythoa caribaeorum* show specific responses in cell density, size, and mitotic index (as proxy for cell population growth) under different environmental conditions (water depth, position within the reef and within the colony). Colony fragments were sampled at the nearshore coastal reefs of Porto de Galinhas and at the oceanic St. Peter and St. Paul's archipelago (SPSP), Brazil. At the coastal reefs, zooxanthellae showed higher density on the reef flat (mean: 2.99×10^6 cm^{-2}) than at the back reef (mean: 2.07×10^6 cm^{-2}). Zooxanthellae were larger at the center of the colonies (mean: 10.61 μm) than at the edges (mean: 10.11 μm), where younger polyps were found. At SPSP, zooxanthellae showed smaller cell diameters at 3 m depth, than at 5–15 m. Cell size increased with depth, thus enhancing the photosynthetic efficiency of colonies in deeper environments. Strategies and adaptations of these endosymbionts to environmental stressors are discussed. Symbiont distribution patterns shown in this study have fundamental implications for the monitoring of the health of tropical reef ecosystems.

Keywords: Reef ecosystems, Zoanthids, Symbiosis, *Symbiodinium*, Benthos ecology, Brazil

Background

Most corals and zoanthids contain symbiotic dinoflagellates of the genus *Symbiodinium* (zooxanthellae) in their tissues. These zooxanthellae form symbiotic associations with invertebrates such as foraminiferans, sponges, cnidarians, and mollusks, which use them to obtain photosynthetic energy in the form of carbohydrates. This relationship is vital to many species of cnidarians, considering that the loss of zooxanthellae from host tissues causes the phenomenon known as bleaching, which can cause the host's death [16, 19, 20, 25, 49]. A plethora of studies on this symbiosis has been conducted with reef-building scleractinian corals [5, 18]. Furthermore, some

studies have been conducted on the zooxanthellae of zoanthids [22, 37, 39, 45], which are cnidarians that do not secrete any calcareous exoskeleton, but are often the main structuring organisms of benthic communities in intertidal reef flat and on rocky shores in tropical and temperate regions [23, 31, 38, 41].

Zoanthids are the animals with the highest biomass on many reefs along the Brazilian coast [9, 37, 40, 42], where they play an important role in reef health and stability, protecting reef surfaces from destructive grazers, such as sea urchins [31, 32, 38]. Also, they provide shelter for many animals [33].

Tropical coastal reef ecosystems of Brazil generally present high abundances of zoanthids, even under near-pristine conditions [40], as opposed to Caribbean and Indo-Pacific reefs, where corals often dominate and the dominance of zoanthids and macroalgae is regarded as an indicator of severe human impacts.

*Correspondence: rs@ufpe.br
[1] Departamento de Oceanografia, Universidade Federal de Pernambuco, Av. Professor Moraes Rêgo, 1235, Cidade Universitária, Recife, PE 50670-901, Brazil
Full list of author information is available at the end of the article

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The zoanthid *Palythoa caribaeorum* (Duchassaing & Michelotti, 1860) is distributed in coastal and oceanic environments of Brazil [1, 31]. It occurs at intertidal and shallow subtidal reef areas. When exposed at low tide on intertidal reef flats, this species secretes a characteristic mucus to prevent desiccation and predation. This species has been the focus of several ecological studies and the target of biochemical research that revealed several active agents and the applicability for toxins of the mucus secreted in huge amounts by these animals, especially with regard to compounds with bactericidal action [17, 24]. There is still little information on *Palythoa caribaeorum* symbionts. Most studies focus on temperature effects and bleaching [26, 28], seasonality [13], and symbiont diversity [36, 39]. At the oceanic St. Peter and St. Paul's Archipelago, the characteristic vertical zonation of the rocky shore ecosystems includes a *P. caribaeorum* zone, where colonies of this species of up to 15 m diameter (210 m^2) occur [1]. In spite of their dominance on many reef flats, the factors that affect the association between zoanthids and their zooxanthellae are still poorly understood.

To date, the major focus of symbiont research has been on scleractinians corals. Studies on scleractinian corals have shown that key characteristics of symbiont populations, such as cell density, mitotic index, cell diameter, and concentration of photosynthetic pigments, are directly influenced by seasonality, temperature, light, and other factors [4, 10, 11, 29]. A single coral colony can exhibit considerable variability in its symbionts, when different areas are observed, as occurs in some coral species [35, 47]. Changes in population parameters of zooxanthellae with depth are well described for scleractinian corals [29, 44, 47]. However, to date, it is still unknown if and how symbiont populations of zoanthids change with depth.

Despite the enormous ecological importance of *Palythoa caribaeorum* in coastal and oceanic reef environments, there is still little information on the zooxanthellae of this zoanthid species in coastal environments of Brazil and there is no information on zooxanthellae of this species on oceanic islands of the Tropical Atlantic Ocean.

The aim of the present study was to analyze the cell density, size, and mitotic index (as a proxy for cell population growth) of *Palythoa caribaeorum* zooxanthellae, testing the hypothesis that these key population parameters vary between reef zones (reef flat vs. back reef), within colonies (center vs. edge of the colonies), between sampling areas (coastal vs. oceanic sites), and at different depths.

Methods

This study was conducted at the nearshore reefs of Porto de Galinhas (PG—8°59′00″–8°33′33″S; 35°00′27″–34°59′00″W), northeastern Brazil, and at the rocky shores of the oceanic St. Peter and St. Paul's Archipelago (SPSP—00°56′N; 29°22′W), Brazil. The reefs of PG are adjacent to sandy beaches, being accessible on foot from the shore, and subject to seasonal oscillations in turbidity due to wind-driven turbulence and nutrients from continental runoff. The SPSP archipelago is inhabited only by scientists and navy personnel, being located approximately 1100 km from the coast, with year-round oligotrophic conditions and extremely transparent waters (Fig. 1).

While the oceanic SPSP archipelago points out of the Middle Atlantic Ridge like a sharp needle and is characterized by steep rocky shores, the PG reefs are located at a flat coastal location, with little if any slope, and extensive intertidal flats, that are bordered by back reef zones and reef fringes, a horizontal zonation that is typical for coastal sandstone reefs in the Tropical Atlantic. The sampling strategy reflects these different geomorphologies: Sampling at the steep rocky shores of SPSP was conducted at different depth strata, while at PG, sampling was performed in different reef zones (intertidal reef flat vs. subtidal back reef walls). Sampling in both areas was conducted in 2008 and 2009, during the dry season (September–February), under conditions of minimum wind and rainfall, and thus maximum transparency in this region.

At the coastal PG reefs, samples were taken from the extensive flat intertidal reef top (reef flat) and from the steep to vertical subtidal back reef walls (back reef), during the period from November 2008 to January 2009. In each zone, 24 fragments were collected from the center and 24 from the edge of *P. caribaeorum* colonies. Permanently submerged colonies were considered from the back reef walls.
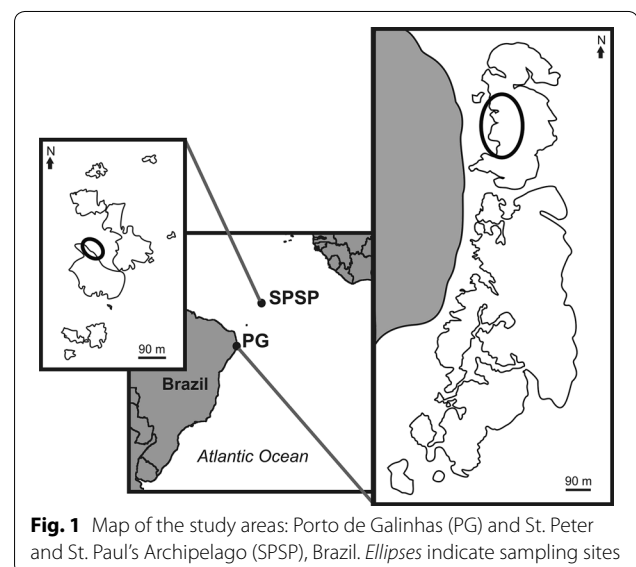
Fig. 1 Map of the study areas: Porto de Galinhas (PG) and St. Peter and St. Paul's Archipelago (SPSP), Brazil. *Ellipses* indicate sampling sites

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At the oceanic SPSP archipelago, sampling was performed on steep rocky shores in October 2008 and October 2009, with generally five fragments from the center of the colonies at 3, 5, 7, 10, and 15 m depth in both years.

Samples were removed from the substrate with the aid of a cylindrical sampler of 3.14 cm^2 through snorkeling or scuba diving. Forty-two fragments of the colonies were sampled at PG and 43 colonies at SPSP (4, 10, 9, 10, and 10 samples at 3, 5, 7, 10, and 15 m depth, respectively). After sampling, zoanthid fragments were properly labeled and placed in individual plastic jars containing filtered seawater (using Whatman GFC filters) and taken to the laboratory. Subsequently, the samples were ground with the aid of glass rod and a scalpel and homogenized in 25 ml filtered seawater. The homogenate was fixed with 10 drops of 10 % Lugol. All samples were analyzed in Fuchs–Rosenthal counting chambers. Population density, mitotic index, and cell diameter (ten cells for each colony sample) of the zooxanthellae were determined under a binocular microscope equipped with a micrometer ocular.

All variables were tested for normality using the Kolmogorov–Smirnov test. Since the data were not normally distributed, analyses were performed with Kruskal–Wallis ANOVA and Mann–Whitney U tests [50], to compare population parameters (cell density, cell size, and mitotic index), water depths (3–15 m), reef zones (back reef vs. reef flat), and positions within the colony (edge vs. center). Only samples taken from the centers of the colonies were considered for the comparisons of population parameters between study areas (PG vs. SPSP). All analyses were performed at $\alpha = 0.05$.

Results

Population density, cell size, and mitotic index of *P. caribaeorum* zooxanthellae showed clear responses to different environmental conditions and marked differences between study areas and reef zones.

When comparing different reef zones of the Porto de Galinhas reef, density of zooxanthellae on the reef flat (mean: 2.99×10^6 cm^{-2}, minimum: 1.16×10^6 cm^{-2}, maximum: 6.21×10^6 cm^{-2}) was significantly higher than that at the back reef (mean: 2.07×10^6 cm^{-2}, minimum: 0.82×10^6 cm^{-2}, maximum: 4.42×10^6 cm^{-2}) (Mann–Whitney U test, $p < 0.03$, $n = 42$; Fig. 2). Cell diameter and mitotic index did not differ between these reef zones.

When center and edge areas of individual colonies were compared, population density and mitotic index did not show any differences. Symbiont cell size was significantly larger at the center of the colonies (mean diameter: 10.61 µm, minimum: 9.94 µm, maximum: 11.55 µm; Table 1) than that at the edge of the colonies (mean: 10.11 µm, minimum: 9.17 µm, maximum: 11.25 µm, Mann–Whitney U test, $p < 0.001$, $n = 42$, Fig. 3).
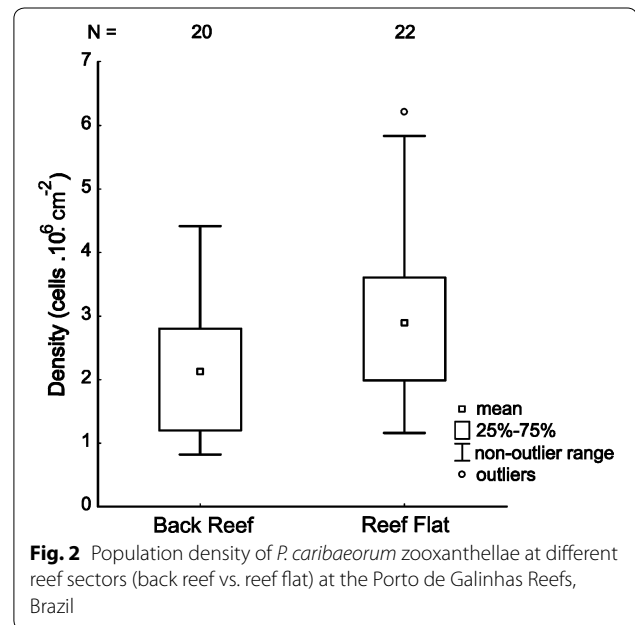
Fig. 2 Population density of *P. caribaeorum* zooxanthellae at different reef sectors (back reef vs. reef flat) at the Porto de Galinhas Reefs, Brazil

At the oceanic SPSP archipelago, population density and mitotic index did not show any differences between depth levels. Cell size showed significant differences between depths, with the formation of similar groups at some depths (Kruskal–Wallis ANOVA, $p < 0.006$, $n = 43$, Fig. 3). Smaller cell diameters were found at 3 m depth (11.3 µm) than at 5–15 m (diameters generally >12 µm).

When coastal (Porto de Galinhas) and oceanic (SPSP) reefs were compared (centers of the colonies only), population density of zooxanthellae showed significant differences, being higher at the coastal reef (Kruskal–Wallis ANOVA, $p < 0.03$; Fig. 4, $n = 63$; Table 1). Moreover, the mitotic index was higher and cell diameters were larger at oceanic reefs (Kruskal–Wallis ANOVA, $p < 0.001$; $n = 63$, $p < 0.001$; $n = 63$; Fig. 4; Table 1).
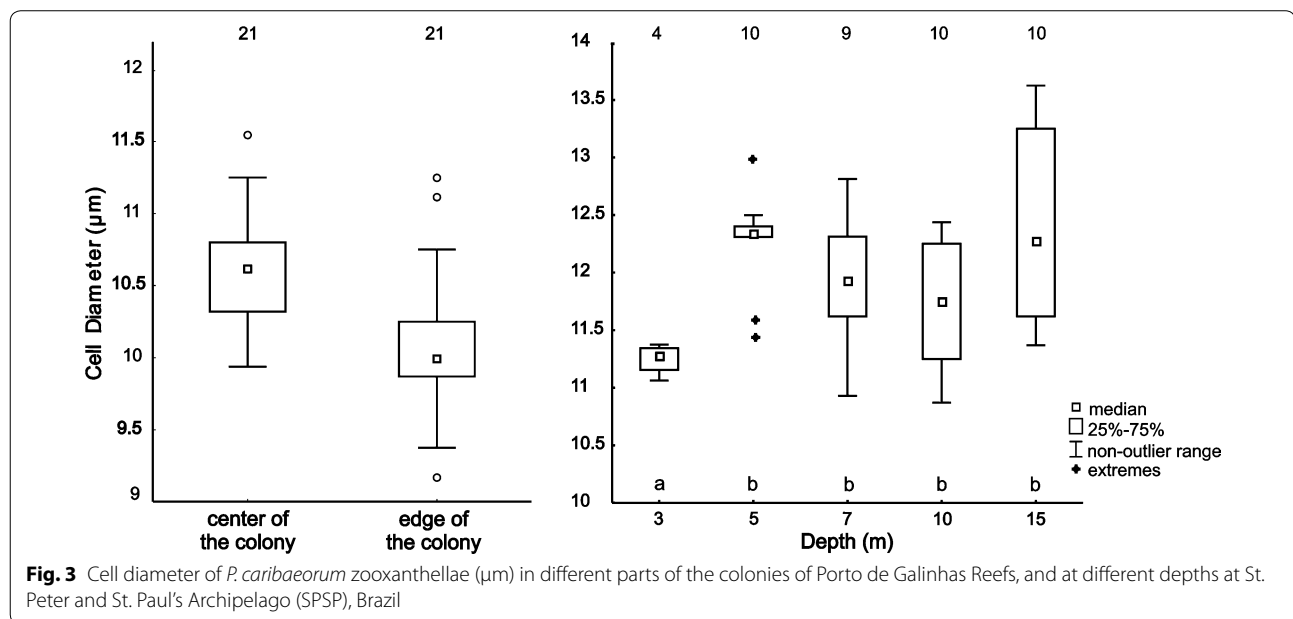
Discussion

This study contributes to the understanding of the variability in cell population density, size, and growth (assessed the mitotic index) of *P. caribaeorum* zooxanthellae in different reef areas. Furthermore, it contributes to elucidate the depth stratification of symbiont populations at a oceanic rocky shore environment that had not been studied so far.

The higher density of zooxanthellae observed in *P. caribaeorum* colonies located on the intertidal reef flats of Porto de Galinhas, when compared to the lower laying back reef, was unexpected, considering that reef flats have the most hostile environmental conditions, i.e., total exposure of colonies during low tides. These fragile animals are thus submitted to thermal, osmotic, and photic

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Table 1 Mean, minimum (min), maximum (max) values of density, mitotic index, and cell diameter of zooxanthellae of *P. caribaeorum* in Porto de Galinhas (PG) and at St. Peter and St. Paul's Archipelago (SPSP), Brazil

	Density (cells × 10^6 cm^{-2})			Mitotic index (%)			Cell diameter (µm)		
	Mean	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.
PG									
Reef flat	2.99	1.16	6.21	3.04	0.91	6.36	10.42	9.17	11.55
Back reef	2.07	0.82	4.42	2.96	1.36	6.1	10.29	9.5	10.75
CC	2.42	0.86	6.21	2.65	0.91	4.5	10.61	9.94	11.55
EC	2.68	0.82	5.84	3.33	1.45	6.63	10.11	9.17	11.25
SPSP (m)									
3	16.85	5.97	27.79	3.62	1.33	6.73	11.25	11.06	11.38
5	13.39	3.7	56.49	7.28	4.3	13.05	12.31	11.69	13.06
7	18.4	5.81	32.36	6.43	4.79	10.34	11.98	10.93	12.81
10	10.36	2.25	24.64	8.12	3.45	11.47	11.72	10.87	12.44
15	15.97	5.16	35.59	6.16	3.33	12.11	12.44	11.37	13.63

CC center of the colony, *EC* edge of the colony

Fig. 3 Cell diameter of *P. caribaeorum* zooxanthellae (µm) in different parts of the colonies of Porto de Galinhas Reefs, and at different depths at St. Peter and St. Paul's Archipelago (SPSP), Brazil
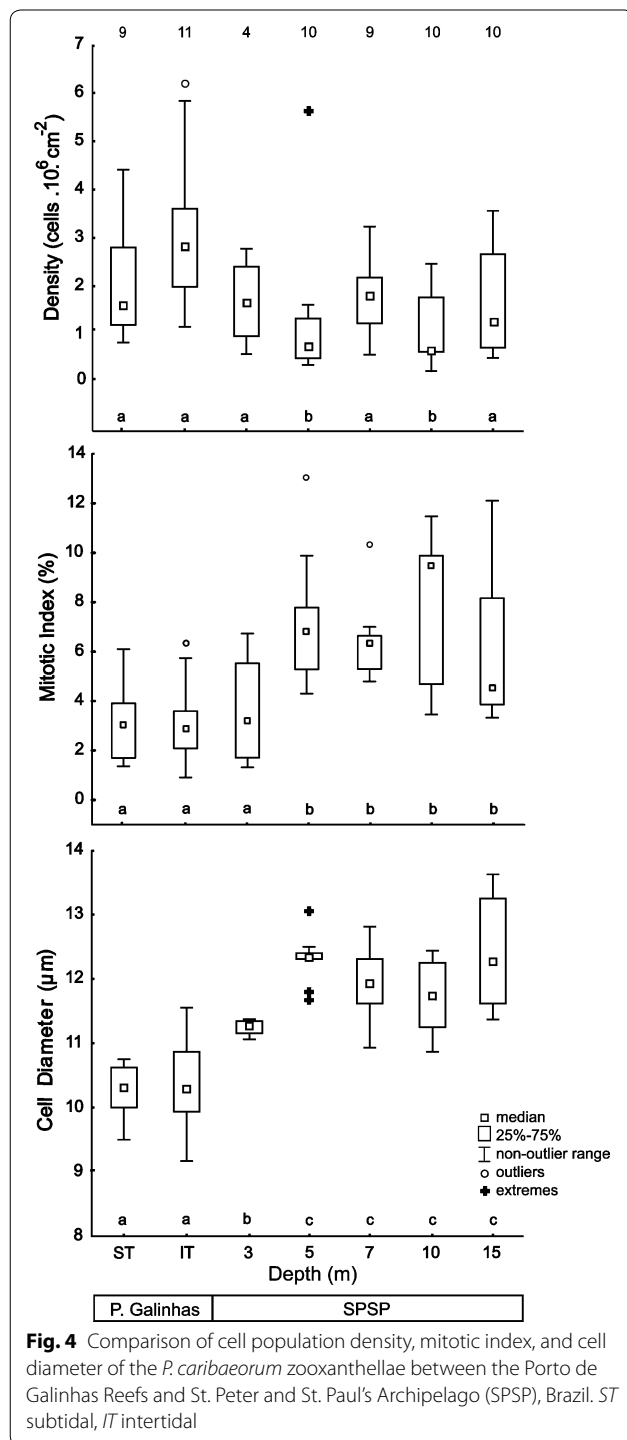
stress factors that do negatively influence symbiosis and could have lead to a lower density on the reef flat [16, 26]. Higher densities of symbionts on reef flats may be necessary to compensate for the energy needed to cope with multiple stressors in these hostile intertidal areas.

Under hostile environmental conditions, zooxanthellae can be degraded within the tissue of their hosts. This phenomenon can be observed in corals from shallow environments, since colonies that are in shady locations tend to be less degraded and have higher population densities of zooxanthellae as compared to colonies in light-exposed environments [43].

This zoanthid showed a strategy that is similar to many scleractinians, such as *Siderastrea stellata,* an endemic Brazilian coral species, which features the ability to successfully colonize shallow waters of coastal reefs due to symbiotic zooxanthellae and their adaptation to extreme and variable environments, such as reef flats that are fully exposed during low tides [12].

The maintenance of physiological stability of cnidarians that perform symbiosis with zooxanthellae is directly linked to the population density of symbionts in the host tissue, considering that the latter control the dynamics of zooxanthellae in their tissues [43]. Our

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Fig. 4 Comparison of cell population density, mitotic index, and cell diameter of the *P. caribaeorum* zooxanthellae between the Porto de Galinhas Reefs and St. Peter and St. Paul's Archipelago (SPSP), Brazil. *ST* subtidal, *IT* intertidal

results suggest that colonies on the reef flat require more energy in the form of carbohydrates to withstand extreme changes in the environment caused by the total exposure to the air during low tides and would have to compensate this by increasing the amount of zooxanthellae in their tissues.

Increased mucus production is also present in colonies that are in direct contact with sunlight (remaining completely exposed during low tides) as a means to avoid desiccation [41]. Piggot et al. [34] reported a similar strategy for the coral *Montastraea annularis*, where mucus production was directly influenced by light conditions. Increasing light intensity is thus a factor that significantly influences mucus production and higher density of symbionts in the tissues of scleractinians, similarly to our findings.

There is no information available on the energy required for mucus production in zoanthids. Yet, many studies have shown that the coral species that produce mucus invest large amounts of energy in this process. They may exude up to half of the carbon assimilated by their zooxanthellae as mucus [14, 15, 21, 46]. The mucus patches produced by *P. caribaeorum* cover considerable parts of the intertidal reef flats in Brazil. This mucus is one of the most conspicuous characteristics of this species, that is popularly known as "baba-de-boi" ("ox slobber") in Brazil [6, 7, 33, 42]. Obviously, this requires a considerable amount of energy. The energy needed for mucus production could explain the higher density of symbionts found in intertidal reef flats as compared to subtidal colonies.

Furthermore, the density of *P. caribaeorum* zooxanthellae showed no differences between the edge and center of the colonies, showing that zoanthid symbionts are distributed equally throughout the host tissue. These results validate the sampling methodology used in quantitative studies on the density of zooxanthellae, where any portion of the colonies is being sampled. This may be related to the massive, compact shape of *P. caribaeorum* colonies, which resemble massive corals. In branching corals, as in *Acropora millepora*, the population density of symbionts is different in different regions of colonies [35]. The results of this study indicate that the massive shape of zoanthid colonies can be an important factor in the uniform within-colony distribution of symbiont density, as observed in massive corals.

However, cell diameter significantly differed between different regions of *P. caribaeorum* colonies. This could be related to colony growth, as it occurs from the center to the edge [3, 23]. Probably, in these marginal areas with younger polyps, a considerable part of symbionts are cells originated from mitotic divisions of mature cells from the center of colonies, given that the cytoplasmic region of daughter cells has a smaller area compared with original cell [27]. The same pattern is observed for branching corals, where larger cells on the top and smaller ones on the base of colonies are observed [47].

In the oceanic SPSP archipelago, depth did not show any influence on the population density of symbionts.

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Conversely, Nir et al. [30] investigated changes in the physiology of *Seriatopora hystrix* at depths ranging from 10 to 60 m and observed that symbionts exhibited higher population density and greater amount of photosynthetic pigments at 50 m. The authors concluded that this behavior was due to reduced light intensity with increasing depth, since the increase in the number of symbionts would facilitate the capture of light, making photosynthesis more efficient so as to meet the physiological needs of the host.

Similarly to the present study, Carricart-Ganivet and Beltrán-Torres [8] investigated the population density and chlorophyll *a* concentration of zooxanthellae in the coral *Montastraea cavernosa* and observed that these variables did not significantly change between depths ranging from 3 to 18 m. Population density seems not to be influenced by small variations in depth in shallow areas. Only at extreme depths, such as in deep, mesophotic areas (depth > 30 m), cell density of coral zooxanthellae seems to increase to compensate for the extreme light deficiency, as shown in Nir et al. [30]. Since the present study was conducted well within the euphotic zone (maximum depth: 15 m), no changes in population density were observed.

On the other hand, cell diameter of *P. caribaeorum* symbionts showed a significant increase with depth. Our results corroborate the work of Wilkerson et al. [47], who studied the cell diameter of nine species of scleractinian corals in Jamaica at four different depths and observed that the cell diameter of zooxanthellae also increased with depth. Similarly, Winters et al. [48] studied *Stylophora pistillata* coral (Esper 1797) at 2 and 30 m deep and also found that this variable is directly influenced by local depth.

Therefore, symbionts probably compensate for decreasing light intensity by increasing their cell surface (i.e., cell diameter), as to obtain space for a greater amount of chloroplasts in their cytoplasm, thereby providing better light capture and making more efficient photosynthesis. Due to increased cell diameter in deeper regions, colonies in these environments probably do not have the need to increase the cell density of symbionts in their tissues.

Baseline data on zooxanthellae of cnidarians are essential for the understanding of current and future variations of tropical coastal and oceanic ecosystems, including extreme events, such as bleaching [2, 16, 26, 28]. The symbiont distribution patterns shown in this study have fundamental implications for future continuous monitoring programs of the health of tropical reef ecosystems.

Authors' contributions
GSS did the fieldwork, collected data, analysed data and wrote the manuscript. FDA designed the study and wrote the manuscript. CFC analysed data and wrote the manuscript. RS analysed data and wrote the manuscript. All authors read and approved the final manuscript.

Author details
[1] Departamento de Oceanografia, Universidade Federal de Pernambuco, Av. Professor Moraes Rêgo, 1235, Cidade Universitária, Recife, PE 50670-901, Brazil. [2] Departamento de Biologia, Universidade Federal Rural de Pernambuco, Rua Dom Manoel de Medeiros, s/n, Dois Irmãos, Recife, PE 52171-900, Brazil. [3] Departamento de Sistemática e Ecologia, Universidade Federal da Paraíba, Campus I - Cidade Universitária, s/n, João Pessoa, PB 58059-900, Brazil.

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