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Physicochemical and bacteriological seawater quality and sustainability of Cabo Branco (Brazil) coral reef

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Abstract. The quality of water in many coastal regions is affected by human activities and may cause degradation of coral reefs and coral disease development. This study aimed to analyze the physicochemical and microbiological parameters of tide pools seawater of Cabo Branco coral reef, Paraiba state, Brazil, and quantify cultivable bacteria in the coral *Siderastrea stellata* tissue. During one year study the seawater temperature varied between 27.3 and 29.8 ºC, and the salinity was maintained between 32 and 35 ‰. The most values of seawater pH (>8.5), and 50% values of dissolved oxygen (<6 mg/L) were not in accordance with the Brazilian Regulation (CONAMA Resolutions: 274/2000 and 375/2005). The levels of fecal coliforms in seawater were within the limits allowed by the Brazilian legislation, however**,** the presence of *E. coli* and enterococci, observed in 42% and 17% of samples, respectively, indicates the potential risks of contamination. The density of total heterotrophic bacteria (10^2 - 10^4 CFU/mL) and *Vibrio* spp. (10^1 - 10^4 UFC/mL) in seawater samples were significantly higher during some months of dry season. The numbers of total heterotrophic bacteria (10^4 – 10^6 CFU/cm^2) and *Vibrio* spp. (10^1 - 10^4 CFU/cm^2) associated with tissue of healthy colonies of *S. stellata* varied significantly among the colonies analyzed. These results show the vulnerability of this coral reef located in the peri-urban area and point out the need of constant monitoring of seawater quality.

Key words: Coral reef, *Siderastrea stellata,* seawater quality, coliforms, entreroccci

Resumo. Qualidade físico-química e bacteriológica da agua do mar e a sustentabilidade do recife de coral do Cabo Branco, Brasil. A qualidade da água em muitas regiões costeiras é afetada pelas atividades humanas, as quais podem causar degradação dos recifes de coral e desenvolvimento da doença coral. Este estudo teve como objetivo analisar os parâmetros físico-químicos e microbiológicos das poças de maré do recife de coral do Cabo Branco, estado da Paraíba, Brasil, bem como quantificar bactérias cultiváveis associadas ao tecido do coral *Siderastrea stellata*. Durante o período de doze meses a temperatura da água do mar variou entre 27,3 e 29,8 ºC, e a salinidade foi mantida entre 32 e 35 ‰. A maioria dos valores de pH da água do mar (> 8,5), e 50% dos valores de oxigênio dissolvido (<6 mg / L) não estavam em conformidade com as Resoluções do CONAMA: 274/2000 e 375/2005. Os níveis de coliformes fecais na água do mar estavam dentro dos limites permitidos pela legislação brasileira, no entanto, a presença de *E. coli* e enterococos, observada em 42% e 17% das amostras, respectivamente, indica os riscos potenciais de contaminação. A densidade de bactérias heterotróficas totais (10^2-10^4 UFC/mL) e *Vibrio* spp. (10^1-10^4 UFC/mL) em amostras de água do mar foram significativamente maiores durante alguns meses de estação seca. Os números de bactérias heterotróficas totais (10^4-10^6 UFC/cm^2) e *Vibrio* spp. (10^1 - 10^4 UFC/cm^2) associadas ao tecido das colônias saudáveis de *S. stellata* variou significativamente entre as colônias analisadas. Estes resultados mostram a vulnerabilidade deste recife de coral localizado na zona urbana e apontam também a necessidade de monitoramento constante da qualidade da água do mar.

Palavras chave: Recife de corais, *Siderastrea stellata*, qualidade da água, coliformes, enterococos

Introduction

Coral reefs provide an important range of environmental, social and economic services. They offer protection against erosion caused by hydrodynamics, serve as potential source of pharmaceuticals (antiviral drugs, anti-cancer and anti-inflammatory agents), and are extremely important in ecotourism and fishery. Despite their ecological and economic importance, coral reefs have been threatened its vitality and diversity caused mainly by human activities such as urban development, runoff in the coastal zone, uncontrolled marine tourism, exploitation of natural resources, and industrial pollution (Schaffelke *et al.* 2005, Erftemeijer *et al.* 2012).

The abiotic and biotic factors, acting alone or in synergy, can cause the loss of coral tissue, leading this way to the deterioration of coral reefs observed around the world (Hughes *et al.* 2003, Harvell *et al.* 2007). A diverse range of stress factors (e.g. low salinity, high temperature, high sedimentation rate, aerial exposure, cyanide exposure) can initiate the bleaching response and may cause higher incidence and severity of diseases in corals, although the combination of high irradiance and increase in sea surface temperature, is the primary triggering condition for the occurrence of large-scale mass bleaching events (Schaffelke *et al.* 2005, Brodie & Waterhouse 2012, Erftemeijer *et al.* 2012).

Discharges of industrial and domestic effluents may release chemicals that lead to a change in the environmental conditions (Brodie & Waterhouse 2012) and affect localized reef areas, especially those located close to shore, causing high impact on the reefs functioning. Annual fluxes of terrestrial sediments, freshwater and nutrients to coastal and coral reef environments have increased with soil erosion, deforestation, coastal development, urbanization, agricultural practices, and mining (Kroon *et al.* 2014). Kelly *et al.* (2014) observed that local human populations influence the reef-associated microbial community indirectly by influencing the composition of benthic microorganisms.

The fecal pollution of coastal environments may involve health risks leading to human exposure to pathogenic organisms, such as protozoa, bacteria and virus (Arnone & Waling 2007, Touron *et al.* 2007, Stewart *et al.* 2008, González-Fernández *et al.* 2011). The health risk of infectious diseases transmitted by water can be measured by detection the universal microorganisms indicators of fecal contamination, the coliforms bacteria and fecal streptococci (APHA, 1998). These indicators provide information about fecal discharges that may affect the local biota and water use (Kamizoulis & Saliba 2004, Stewart *et al.* 2008). In addition to indicators of fecal contamination, the levels of total mesophilic aerobic bacteria and *Vibrio* may serve as support in the evaluation of the water microbiological quality. Different species of the *Vibrio* genus occur naturally in marine environments, however, some species, such as *V. parahaemolyticus*, *V. vulnificus*, *V. alginolyticus, V. harveyi* and *V. cholerae*, are potentially pathogenic to humans and various marine organisms, including fish, shellfish, crustaceans, corals and echinoids (Sussman *et al.* 2008, Chen *et al*. 2011, Chatterjee & Haldar 2012, Ruwandeepika *et al.* 2012, Tran *et al*. 2013, Garren *et al.* 2014, Clemente *et al.* 2014, Rubio-Portillo *et al.* 2014). Vibrios are considered as the most common and serious pathogens in fish and shellfish marine aquaculture (Chatterjee & Haldar 2012) and some *Vibrio* species such as *V. coralliilyticus* and *V. shiloi* play important role in the infection and bleaching of some coral species (Kushmaro *et al.* 2001, Ben-Heim *et al.* 2003, Garren *et al.* 2014).

Siderastrea stellata is a scleractinian coral widely distributed in coral reefs of Brazil occupying generally horizontal substrates up to 10 m deep (Segal & Castro 2000, Castro & Pires 2001). This coral species is considered to be relatively resistant to the action of waves, temperature variations, salinity and sedimentation, however, there are reports of *S. stellata* bleaching along the coastal Brazilian reefs (Costa & Amaral 2000, Costa *et al.* 2001, Costa *et al.* 2004, Leão *et al.* 2008, Poggio *et al.* 2009). The severe bleaching events of *S. stellata* in Cabo Branco reef (Paraiba state, Brazil) were observed in 2005, 2007 and 2008 (Sassi *et al.* 2015). In the face of seasonal coral bleaching, rising coastal development and increasing environmental impacts on the Cabo Branco reef the understanding of the pattern of physicochemical and bacteriological indicators of water quality may help to establish management strategy and sustainability of coral reef studied.

Materials and Methods

Study site: The reef of Cabo Branco Beach (7°08'50 "S, 34° 47'51" W) is situated in João Pessoa, Paraíba State, Northeast of Brazil (Fig. 1). This beach is outlined by a cliff and situated approximately 2 km from the discharge of Cabelo River. The Cabo Branco reef is formed by a large number of rocks which overlie a wide beach area and also extend for hundreds of meters into the sea. The rocks along with calcareous structures of living organisms form the base of the reef, and delimit the tide pools with diverse micro-habitats. The tide pools of Cabo Branco reefs are reach of microplankton, loricate protozoans, sponges, tunicates, reef fish, zoanthids and reef-building corals such as *Favia gravida*, *Porites astreoides* and *Siderastrea stellata*, the latter in higher quantity (Sassi & Melo 1989, Rosa *et al.* 1997).

The regional climate is classified as tropical humid with two distinct seasonal periods: a dry season that normally extends between September and February, and a rainy season from March to August. The monthly rainfall data of the study period were obtained from the website of AESA-PB (www.aesa.pb.gov.br).

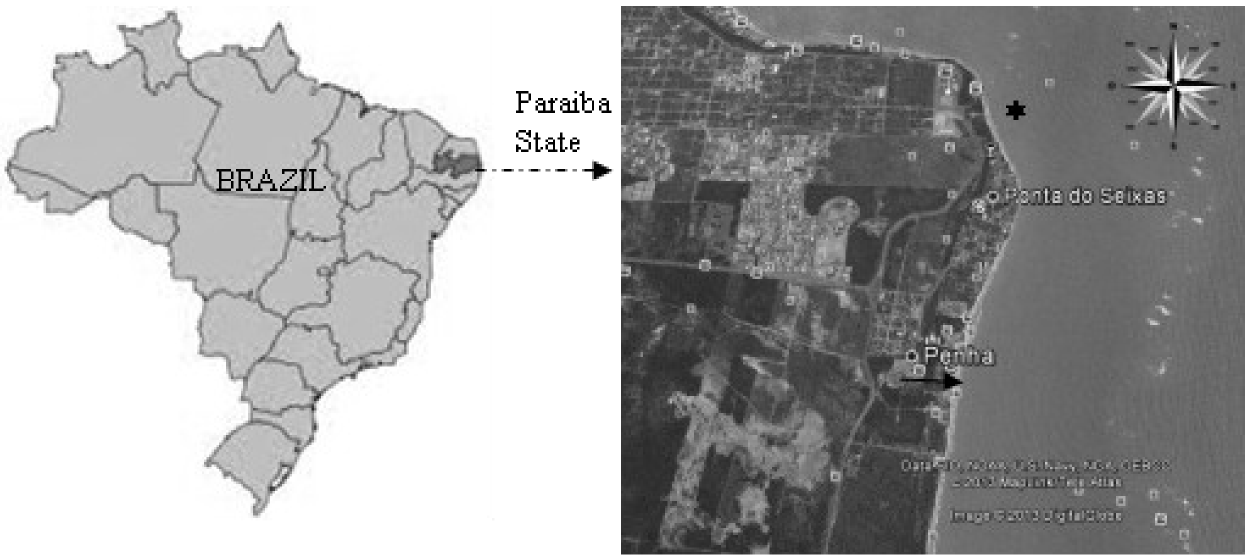

Figure 1. The Cabo Branco beach area at João Pessoa, Paraíba state, Brazil. The coral reef of Cabo Branco is marked with an asterisk. The Cabelo river discharge is shown by a solid arrow. Image source: GoogleEarth (accessed in 10 of April 2013).

Physicochemical monitoring of seawater: The physicochemical parameters (temperature, salinity, pH and dissolved oxygen) of seawater of selected tide pool at Cabo Branco reef were measured *in site* monthly, during the period from October 2011 to September 2012, using the multiparameter water quality meter (HANNA ® HI 9828). The measurements were done always at low tide in the morning (10:00 – 12:00 a.m.).

Sampling and bacteriological analysis of water and S. stellata colonies: Water samples form tide pool were collected monthly from October 2011 to September 2012, in 500 mL sterilized bottles, transported on ice to the laboratory and kept at 4 °C until the performance of microbiological analyses done within 24 hours after sampling.

Colonies of *S. stellata* with a diameter less than 12 cm were collected from the tide pool in November 2011, January, June, July and September

2012. The coral samples were placed in plastic bags containing seawater, sealed hermetically, and transported on ice to the laboratory, where the coral tissue was extracted using a high-pressure jet of sterile seawater (Waterpik®) according to protocol of Costa *et al.* (2008).

Decimal dilutions of seawater and *S. stellata* tissue samples were made with sterile filtered seawater. The counts of total aerobic bacteria were evaluated on Zobell Marine Agar (HiMedia) (triplicate) incubated at 30°C for 5 days. The counts of *Vibrio* spp. were obtained on TCBS Agar (HiMedia) (triplicate) incubated at 35°C for 24 hours. Bacterial density was expressed as colony-forming-units (CFU) per ml of water or per cm^2 of coral colony.

The fecal contamination of seawater was assessed by analysis of thermotolerant coliforms, *Escherichia coli* and enterococci. The enumeration of thermotolerant coliforms were realized by the Most Probable Number Technique (MPN) with 3 replicates according to APHA (1998) using Lauril Sodium Sulfate Broth (HiMedia) (presumptive test); Lactose Bile 2% Brilliant Green Broth (HiMedia) (confirmative test for the total coliforms) with incubation at 37ºC during 24 and 48 hours. The medium EC Broth (Himedia) was used for the presumptive test for E*scherichia coli* (thermotolerant coliforms) from positive Lactose Bile 2% Brilliant Green Broth tubes by MPN technique, with incubations at 44.5 ± 0.5 ºC for 24 hours. The presence of *E. coli* was confirmed with the Agar MUG EC (HiMedia). Colonies of *E. coli* exhibit on this medium blue fluorescence under the long-wave (366 nm) UV light.

The enterococci determination was carried out as described by Chigbu & Sobolev (2007). Serial dilutions of water samples were added to the test tubes (triplicate) containing Azide Dextrose Broth (HiMedia) and incubated at 35ºC for 24 – 48 hours. Confirmation of the presence of enterococci in the positive tubes was done on Pfizer Selective Enterococcus Broth (HiMedia) incubated at 35ºC for 24 hours. Brownish black colonies with brown halos indicated the presence of enterococci in the culture.

Data Analysis: Mean values and standard deviation were generated for each microbial data group and physical and chemical parameter. The one-way analysis of variance (ANOVA) and Tukey test ($P < 0.05$) were performed on the bacterial density data using Statistica (version 5) software. The Pearson correlation coefficient was used to assess the relationship between the physicochemical parameters and density of bacterial groups, considering a significance level of 5%. All statistical analyses followed Sokal & Rohlf (1983).

Results

The monitoring of physicochemical and bacteriological parameters of water of tide pool, where the colonies of coral *S. stellata* are found, at Cabo Branco reef were performed monthly over one year study, while the coral colonies were collected two and three times at rainy and dry season, respectively. The rainy season lasted three months (May, June and July 2012) with the highest rainfall in June (540.0 mm) (Table I). The lowest rainfall was observed in October (9.0 mm) and December (8.4 mm) 2011 (Table I).

The temperature of tide pool seawater varied between 26.3 and 29.8 ºC and salinity between 32 and 35 ‰ (Table 1). The pH values were over 8.5 during nearly all the study (92%), being above the limit of CONAMA resolution Nº 357/2005 for class I saline water: pH around 8.5 with no change greater than 0.2 units (Brasil 2005).

The dissolved oxygen varied from 3.1 mg/L in September 2012 to 12.5 mg/L in February 2012 (Table I). The 50% of dissolved oxygen values were below the permitted limit for seawater designed to protect aquatic communities (DO values not lower than 6 mg/L in any sample) (Brasil 2005).

Bacteriological analysis of seawater included evaluation of thermotolerant coliforms, *E. coli* and enterococci presence, counts of total aerobic mesophilic bacteria and *Vibrio* spp. The thermotolerant coliforms ranged from <3 to 43 MPN/100 mL of seawater, and their highest densities were observed in March and August (Table II). The presence of *E. coli* and enterococci was confirmed in 42% and 17% of the samples, respectively (Table II; Figure 1). The simultaneous presence of *E. coli* and *Enterococcus* was observed only in sample collected in February.

The levels of thermotolerant coliforms in seawater used for primary recreation and protection of aquatic communities according to CONAMA Resolutions 274/2000 and 357/2005 (Brasil 2005) should not exceed value of 10^3/100mL in 80% or more samples collected bimonthly during one year. Our results showed that levels of thermotolerant coliforms in Cabo Branco reef water were in accordance with Brazilian Regulation.

Table I. Monthly variations of rainfall and variables of seawater of the Cabo Branco reef, Paraiba state (October 2011 to September 2012).

Month	Rainfall (mm)	Temperature (°C)	pH	Salinity ‰	Dissolved oxygen (mg/L)
October [a]	9.0	29.8	7.9	35.0	3.3
November [a]	44.9	29.5	9.5	33.9	7.6
December [a]	8.4	28.6	8.8	35.0	9.7
January [a]	186.2	29.7	10.2	31.9	10.2
February [a]	141.9	28.4	10.0	32.0	12.5
March [a]	71.0	28.7	9.1	34.7	6.7
April [a]	46.6	29.5	9.0	34.9	5.6
May [b]	216.5	28.7	9.0	35.0	6.4
June [b]	540.7	26.3	9.2	32.0	5.4
July [b]	290.5	28.3	9.1	32.9	5.0
August[a]	81.1	27.5	9.1	33.5	3.4
September [a]	36.7	27.3	9.1	33.4	3.1
Mean	58.9	28.6	9.2	33.8	6.6
SD*	154.0	1.1	0.6	1.3	2.9

[a] – Dry season; [b] – Rainy season; *SD - Standard Deviation.

Table II. Microbiological parameters of seawater of Cabo Branco reef, Paraiba state, during the period from October 2011 to September 2012.

Month	Thermotolerant coliforms (MPN/100 mL)	*E. coli* presence	Enterococci presence	Total bacteria (CFU/mL)	*Vibrio* spp. (CFU/mL)
October [a]	<30.0	-	-	1.20×10^3	1.33×10^1
November [a]	<30.0	-	-	6.03×10^3	1.13×10^2
December [a]	<30.0	-	-	2.30×10^3	2.56×10^2
January [a]	<30.0	-	-	5.36×10^4	1.14×10^4
February [a]	15.0	+	+	2.86×10^4	1.35×10^3
March [a]	43.0	+	-	3.33×10^3	1.76×10^2
April [a]	<3.0	-	-	2.33×10^2	4.00×10^1
May [b]	9.2	+	-	3.66×10^3	1.50×10^2
June [b]	<3.0	-	+	1.53×10^3	3.66×10^1
July [b]	<3.0	-	-	1.36×10^3	2.80×10^2
August [a]	43.0	+	-	5.56×10^3	1.00×10^1
September [a]	9.2	+	-	1.60×10^4	N.D.
Mean				1.03×10^4	1.26×10^3
SD*				1.59×10^4	1.25×10^3

[a] – Dry season; [b] – Rainy season; + Positive samples; - Negative samples; *SD - Standard Deviation.

The density of total heterotrophic bacteria in seawater samples ranged from 2.33×10^2 to 5.36×10^4 CFU/mL, while *Vibrio* spp. counts showed stronger variation over the study period (1×10^1 – 1.14×10^4 UFC/mL) (Table II). The total bacteria and *Vibrio* spp. counts were significantly higher in January and February 2012 ($P<0.05$), and *Vibrio* spp. number also in December 2011. There was no significant difference between dry and rainy periods (Student's t-test, $P> 0.05$) for these bacterial groups.

The counts of total bacteria showed strong positive correlation with pH and dissolved oxygen ($r>0.7$, $P<0.05$). However, thermotolerant coliforms and *Vibrio* counts did not show statistically significant correlations with any physicochemical parameter analyzed.

The densities of total bacteria and *Vibrio* spp. in the tissue extracted from colonies of *S. stellata* are shown in Table III.

Table III. Density of total aerobic mesophilic bacteria and *Vibrio* spp. in the tissue of *S. stellata* collected in Cabo Branco reef, Paraiba state, during the period from November 2011 to September 2012.

Month	Total bacteria (CFU/cm²)	*Vibrio* spp. (CFU/cm²)
November [a]	3.01×10^6	2.70×10^4
January [a]	3.92×10^4	7.28×10^1
June [b]	2.13×10^5	7.83×10^2
July [b]	6.88×10^6	8.96×10^2
September [a]	3.12×10^5	5.00×10^3
Mean	2.09×10^6	6.67×10^3
SD*	0.27×10^6	6.75×10^3

[a] – Dry season; [b] – Rainy season; *SD - Standard Deviation.

The total bacterial densities in coral tissue ranged from 3.92×10^4 to 6.88×10^6 CFU/cm^2 and their counts were significantly higher for samples collected in November and July ($P<0.05$). The values of *Vibrio* spp. were between 7.28×10^1 CFU/cm^2 and 2.70×10^4 CFU/cm^2, and only coral sample collected in November showed *Vibrio* spp. density significantly higher than other samples ($P<0.05$).

There was no significant correlation between the bacterial counts in seawater and *S. stellata* tissue neither for total mesophilic bacteria ($r = -0.5$, $P<0.05$) nor for *Vibrio* spp. ($r = -0.2$, $P<0.05$).

Discussion

The contamination of coastal water can be due to point discharges of sewage from shoreline outfalls and no-point discharges such as run-off from naturally vegetated areas, urban, commercial and industrial lands (Stewart *et al.* 2008, Pandey *et al.* 2014). The Cabo Branco reef is situated near the beach, being exposed to the recreation activities, trampling, fishing and scientific research (Costa *et al.* 2007), as well as to runoff from natural vegetation and urban areas, including discharge of Cabelo river. All these factors may affect the near-shore reef environment and the water quality.

Among physical parameters of seawater the temperature is one of the most important coral stress inducers, since the rise of only one Celsius degree of the usual summer maxima temperature for some period may leads to coral bleaching, because of breakdown of the endosymbiosis between corals and the dinoflagellate *Symbiodinium* (Glynn 1993, Hoegh-Guldberg 1999, Donner *et al.* 2005). The bleaching of colonies of *S. stellata* in the reefs of Cabo Branco (Paraíba State), Gaibu and Tamandaré (Pernambuco State) were observed when the seawater temperature reached values between 29 and 30 °C, but the colonies had the normal brown pigmentation between 26 and 28 °C (Costa & Amaral 2000, Costa *et al.* 2004).

The ranges of temperature and salinity of water for tropical corals are 23 – 29°C and 32 –40‰, respectively (Lalli & Parsons 1995). The coral responses to the salinity changes depend on the coral species, magnitude of changes and exposure time (Berkelmans *et al.* 2012). During this study the tide pool seawater temperature varied between 27.3 and 29.8 ºC, and the salinity was maintained between 32 and 35 ‰. The highest temperature (29.8°C) of seawater was observed in January 2012, but the salinity value was also the highest (35.1 ‰). Stable level of salinity is important because, according with Coles & Jokiel (1978), the coral will resist short period of temperature rise if the salinity not declines.

The seawater pH values were over 9.0 during nearly all the study. Middelboe & Hansen (2007) has shown that pH is higher and more variable in marine shallow-water habitats than previously thought, and suggest that these habitats in general may experience high pH during a large part of the growth season. Some marine habitats, such as high intertidal rockpools, have pH variations of up to three units over a diel cycle as a result of photosynthesis and respiration (Poole & Raven 1997).

The levels of fecal coliforms in seawater of Cabo Branco reef were in accordance with Brazilian Regulation (Brasil 2000, 2005). However**,** the presence of *E. coli* and enterococci, observed in 42% and 17% of seawater samples, indicates the potential risks of contamination. The storm water run-off into coastal waters in tropical and sub-tropical environments can potentially influence water quality (Solo-Gabriele *et al.* 2000), and the coastal rivers can be one of the source of fecal pathogens released to seawater (Pandey *et al.* 2014). The Cabelo river empties into the ocean nearby Cabo Branco reef, which, according to Farias et al. (2007, 2008), suffers from pollution and contamination by industrial and domestic sewage, solid waste exploitation of mining and agriculture.

Shikuma & Hadfield (2010) showed that *E. coli* may persist associated with biofilms in shallow marine environments being a source of dissemination of these bacteria. Many bacteria, including indicator and pathogenic bacteria, belonging to such families as *Enterobacteriaceae* and *Vibrionaceae*, which exist in aquatic environments may enter into viable but non-culturable state (VBNC) in suboptimal conditions regarding the temperature, nutrients, salinity fluctuations, etc. (Colwell & Grimes, 2000). Zimmerman *et al.* (2009) showed that VBNC *E. coli* exist in marine water and suggest that this indicator bacteria may be present in higher numbers than observed by culture method. The enterococci are characterized by greater salt tolerance than fecal coliforms and *Escherichia coli,* and may be more adequate for seawater monitoring (Byappanahalli *et al.* 2012). However, *Enterococcus faecalis* also can enter a VBNC state (Signoretto *et al.* 2004) leading to underestimating of fecal pollution. Stewart *et al.* (2008) pointed out the need to improve the water quality standards and the detection methods of pathogens and water contamination level.

The seawater samples collected at summer months (December 2011, January and February 2012), that correspond to dry season, showed the highest density of total bacteria and *Vibrio* spp. The water collected in February was unique sample that showed simultaneously *E. coli* and enterococci. The summer period represents increased tourism (vacation period) and number of bathers on the beaches in the region, as well as the season with low rainfall and the highest temperature. Stewart *et al.* (2008) reported that swimmers may themselves be sources of microbes in water releasing significant numbers of microbes. All these factors may contribute to the significant increase in bacterial counts in seawater observed in the summer months. The reef environments of the study area have an increasing tourist-recreational flow that may affect the Cabo Branco reef environment.

In general, the marine environment is oligotrophic; however there are some niches rich in nutrients, such as the mucus layer found on the surface of corals, that support numerous and diverse bacterial populations (Shnit-Orland & Kushmaro 2009). Microorganisms found on coral surfaces and tissue may be saprophytic or pathogenic, and may provide important functions such as the host protection from pathogens through production of antimicrobial substances (Shnit-Orland & Kushmaro 2009, Rohwer *et al.* 2002) and/or supply nutrients that are not provided by the coral-symbiotic zooxanthellae (Kushmaro *et al.* 1996)

The density of bacteria associated with tissue colonies of *S. stellata*, that showed healthy brown color, with no signs of bleaching or disease, varied significantly among the colonies analyzed. The total heterotrophic bacteria reached the levels of $10^4 – 10^6$ CFU/cm^2, while densities of *Vibrio* spp. ranged from 10^1 to 10^4 CFU/cm^2. The microbial communities associated with corals are not merely a reflection of the bacterial communities present in the surrounding seawater (Knowlton 2001), and various kinds of not pathogenic bacteria are probably involved in stable associations very important for coral survival (Lesser 2004).

According to several authors, bacteria of the genus *Vibrio* when present in the coral tissue may cause or may be associated with the prevalence of various diseases, being capable of reducing the beneficial properties of zooxanthellae during bleaching, and having increased proliferation favored by temperature (Kushmaro *et al.* 2001, Rosenberg & Ben-Haim 2002; Cervino *et al.* 2004, Gil-Agudelo *et al.* 2006, Ritchie 2006, Sussman *et al.* 2008). Although several *Vibrio* species such as *V. alginolyticus, V. harveyi, V. campbellii* and *V. parahaemolyticus* being related with corals diseases, they are also important nitrogen-fixing species (N_2), that can have a positive effect on the coral reef (Chimetto *et al.* 2008).

Microorganisms detected in the microbiota of diseased corals suggest that sewage effluents, fish farm effluents, overland pollution and diseases derived from other marine organisms contribute significantly to the development of disease in corals (Frias-Lopez *et al.* 2004, Garren *et al.* 2009, Sutherland *et al.* 2011).

Monitoring of the health of *S. stellata* colonies in the reefs of Cabo Branco, simultaneous to this study in the period from September 2011 to May 2012, performed by Farias *et al*. (2012), showed that there was a prevalence of healthy colonies of *S.*

stellata, with low incidence of colonies with altered pigmentation. However, the phenomenon of seasonal changes in the pigmentation of colonies of *Siderastrea* spp. during bleaching events that affected high percentage of coral colonies in Cabo Branco reefs were observed in the summer of 2005, 2007 and 2008 (Sassi *et al.* 2015). Therefore, there is a need of constant registration of physicochemical and biological factors variations that affect the seawater quality of coral reef studied. The better understanding of contamination sources of coastal and marine environments and the relationships between reef ecosystem characteristics may help to prevent the breakdown of homeostasis and onset of conditions that favor the bleaching and coral diseases of Cabo Branco reef.

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References

AESA – **Agência Executiva de Gestão das Águas do Estado da Paraíba**. World Wide Web electronic publication accessible at http://aesa.pb.gov.br (Accessed 03/01/2013).

APHA - American Public Health Association. 1998. **Standard methods for the examination of water and wastewater**. Washington: American Public Health Association. 1155 p.

Arnone, R.D. & Walling, J.P. 2007. Waterborne pathogens in urban watersheds. **Journal of Water and Health** 5(1): 149–162.

Ben-Haim, Y., Thompson, F. L., Thompson, C. C., Cnockaert, M. C., Hoste, B., Swings, J. & Rosenberg, E. 2003. *Vibrio coralliilyticus* sp. Nov., a temperature-dependent pathogen of the coral *Pocillopora damicornis*. **International Journal of Systematic and Evolutionary Microbiology**, 53: 309–315.

Berkelmans, R., Jones, A.M. & Schaffelke, B. 2012.Salinity thresholds of *Acropora* spp. on the Great Barrier Reef. **Coral Reefs**, 31:1103–1110.

Brasil. **Resolução CONAMA nº 274, de 29 de novembro de 2000.** Diário Oficial da União, Brasília. Accessible at http://www.mma.gov.br/port/conama/legiabre.cfm?codlegi=272. (Accessed 27/08/2013).

Brasil. **Resolução CONAMA nº 357, de 17 de março de 2005.** Diário Oficial da União, Brasília. Accessible at http://www.mma.gov.br/port/conama/res/res05/res35705.pdf (Accessed 27/08/2013).

Brodie, J. & Waterhouse, J. 2012.A critical review of environmental management of the 'not so Great' Barrier Reef. **Estuarine, Coastal and Shelf Science,** 104-105 (1): 1-22.

Byappanahalli, M. N., Nevers, M. B., Korajkic, A., Staley, Z. R. & Harwood, V. J. 2012. Enterococci in the environment. **Microbiology and Molecular Biology Reviews**, 76: 685–706.

Castro, C. B. & Pires, D. O. 2001. Brazilian coral reefs: what we already know and what is still missing. **Bulletin of Marine Science,** 69(2): 357-371.

Cervino, J. M., Hayes, R. L., Polson, S. W., Polson, S. C., Goreau, T. J., Martinez, R. J. & Smith, G. W. 2004. Relationship of *Vibrio* species infection and elevated temperatures to yellow blotch/band disease in Caribbean corals. **Applied and Environmental Microbiology,** 70(11): 6855–6864.

Chatterjee, S. & Haldar, S. 2012. Vibrio related diseases in aquaculture and development of rapid and accurate identification methods. **Journal of Marine Science: Research & Development**, S:1http://dx.doi.org/10.4172/2155-9910.S1-002.

Chen, M.X., Li, H.Y., Li, G. & Zheng, T. L. 2011. Distribution of *Vibrio alginolyticus*-like species in Shenzhen coastal waters, China. **Brazilian Journal of Microbiology**, 42: 884-896.

Chigbu, P. & Sobolev, D. 2007. Bacteriological Analysis of Water. Pp. 97–134. *In*: Nollet L.M.L. (Ed.). **Handbook of Water Analysis**. CRC Press, Boca Raton, Florida, 769 p.

Chimetto, L. A., Brocchi, M., Thompson, C. C., Martins, R. C.R., Ramos, H. R. & Thompson, F. L. 2008. Vibrios dominate as culturable nitrogen-fixing bacteria of Brazilian coral *Mussismilia hispida*. **Systematic and Applied Microbiology**, 31(4): 312–319.

Clemente, S., Lorenzo-Morales, J., Mendoza, J. C., López, C., Sangil, C., Alves, F., Kaufmann, M. & Hernández, J. C. 2014. Sea urchin *Diadema africanum* mass mortality in the subtropical eastern Atlantic: role of waterborne bacteria in a warming ocean. **Marine Ecology Progress Series**, 506: 1-14.

Coles, S. L. & Jokiel, P. L. 1978. Synergistic effects of temperature, salinity and light on the hermatypic coral *Montipora verrucosa*. **Marine Biology,** 49(3): 187–195.

Colwell, R. R. & Grimes, D. J. 2000. "Semantics and strategies". Pp. 1–6. *In*: **Nonculturable Microorganisms in the Environment.** Colwell R. R. & Grimes D. J. (Eds.), ASM Press, Washington, DC, USA. 354 p..

Costa, C. F. & Amaral, F. D. 2000. Density and size differences of symbionts dinoflagellates from five reef-building coral species from Brazil. **9th International Coral Reef Symposium (ICRS) Proceedings.** Bali, Indonesia, 1: 23-27.

Costa, C. F., Sassi, R. & Amaral. F.D. 2001. Branqueamento em *Siderastrea stellata* (Cnidaria, Scleractinia) da Praia de Gaibu, Pernambuco, Brasil. **Revista Nordestina de Biologia**, 15(1): 15-22.

Costa, C. F., Coutinho, C. S., Sassi, R. & Lubambo de Brito, A. C. 2004. Microsymbionts of *Siderastrea stellata* (Cnidaria, Scleractinia) in coastal reefs of Cabo Branco, state of Paraíba, Northeastern Brazil. **Tropical Oceanography**, 32(2): 171-179.

Costa, C.F., Sassi, R., Costa, M.A. & Brito, A.C.L.. 2007. Recifes costeiros da Paraíba, Brasil: usos, impactos e necessidades de manejo no contexto da sustentabilidade. **Gaia Scientia**, 1: 37-45.

Costa, C. F., Sassi, R., & Gorlach-Lira, K. 2008. Uma abordagem metodológica para o estudo das zooxantelas de corais do Brasil. **Boletim do Laboratório de Hidrobiologia**, 21: 83-94.

Donner, S. D., Skirving, W. J., Little, C. M., Oppenheimer, M. & Hoegh-Guldberg, O. 2005. Global assessment of coral bleaching and required rates of adaptation under climate change. **Global Change Biology**, 11: 2251–2265.

Erftemeijer, P. L. A., Riegl, B., Hoeksema, B. W. & Todd, P. A. 2012. Environmental impacts of dredging and other sediment disturbances on corals: a review. **Marine Pollution Bulletin**, 64(9): 1737-1765.

Farias, M. S. S., Neto, J.D. & Lúcia, V. 2007. Riscos sociais e ambientais devido a presença de metais pesados nas águas superficiais no distrito industrial de Mangabeira. **QUALIT@S Revista Eletrônica.** ISSN 1677-4280, 6 (2).

Farias, M. S. S., Neto, J.D., Lira, V. M. & Franco, E.S. 2008. Impactos ambientais em uma bacia urbana e as consequências na qualidade da água superficial. **Enciclopédia Biosfera**, 5. 9 p.

Farias, G. M., Sassi, R. & Costa, C. F. 2012. Susceptibilidade de *Siderastrea stellata* Verril, 1880, ao branqueamento e ao aparecimento de doenças em poças de maré dos recifes costeiros do Cabo Branco, Paraíba. **Congresso Brasileiro de Oceanografia** (AOCEANO), Rio de Janeiro, Brasil: 593.

Frias-Lopez, J., Klaus, J.S., Bonheyo, G.T. & Fouke, B.W. 2004. Bacterial community associated with black band disease in corals. **Applied and Environmental Microbiology**, 70: 5955–5962.

Garren, M., Raymundo, L., Guest, J., Harvell, C. D. & Azam, F. 2009. Resilience of coral-associated bacterial communities exposed to fish farm effluent. **PLoS One**, 4 (10): e7319.

Garren, M., Son, K., Raina, J. B., Rusconi, R., Menolascina, F., Shapiro, O. H., Tout, J., Bourne, D.G., Seymour, J. & Stocker, R. 2014. A bacterial pathogen uses dimethylsulfoniopropionate as a cue to target heat-stressed corals. The **ISME Journal**, 8: 999–1007.

Gil-Agudelo, D. L., Smith G.W. & Weil, E. 2006. The white band disease type II pathogen in Puerto Rico. **Revista de Biología Tropical**, 54(3): 59–67.

Glynn, P. W. 1993. Coral reef bleaching: ecological perspectives. **Coral Reefs,** 12(1): 1-17.

González-Fernández, D., Garrido-Pérez, M.C., Nebot-Sanz, E. & Sales-Márquez, D. 2010. Fecal pollution in coastal marine sediments from a semi-enclosed deep embayment subjected to anthropogenic activities: an issue to be considered in environmental quality management frameworks development. **EcoHealth**, 7 (4): 473-478.

Harvell, D., Jordán-Dahlgren, E., Merkel, S., Rosenberg, E., Raymundo, L., Smith, G., Weil, E. & Willis, B. 2007. Coral disease, environmental drivers, and the balance between coral and microbial associates. **Oceanography**, 20 (1): 172-195.

Hoegh-Guldberg, O. 1999. Climate change, coral bleaching and the future of the world coral reefs. **Marine and Freshwater Research,** 50 (8): 839-866.

Hughes, T. P., Baird, A. H., Bellwood, D. R., Card, M., Connolly, S. R., Folke, C., Grosberg, R., Hoegh-Guldberg, O., Jackson, J.B., Kleypas, J., Lough, J.M., Marshall, P., Nyström, M., Palumbi, S.R., Pandolfi, J.M., Rosen, B. & Roughgarden, J. 2003. Climate change, human impacts and the resilience of coral reefs. **Science,** 301: 929.

Kamizoulis, G. & Saliba, L. 2004. Development of coastal recreational water quality standards in the Mediterranean. **Environment International**, 30 (6): 841-854.

Kelly, L.W., Williams, G.J., Barott, K.L., Carlson, C.A., Dinsdale, E.A., Edwards, R.A., Haas, A.F., Haynes, M., Lim, Y.W., McDole, T, Nelson, C.E., Sala, E., Sandin, S.A., Smith, J.E., Vermeij, M.J.A., Youle, M. & Rohwer, F. 2014. Local genomic adaptation of coral reef-associated microbiomes to gradients of natural variability and anthropogenic stressors. **Proceedings of the National Academy of Sciences, USA**, 111 (28): 10227-10232.

Knowlton, N., 2001. The future of coral reefs. **Proceedings of Academy of Natural Sciences of Philadelphia**, 98**:** 5419-5425.

Kroon, F.J., Schaffelke, B. & Bartley, R. 2014. Informing policy to protect coastal coral reefs: Insight from a global review of reducing agricultural pollution to coastal ecosystems. **Marine Pollution Bulletin** , 85 (1): 33–41.

Kushmaro, A., Loya, Y., Fine, M. & Rosenberg, E. 1996. Bacterial infection and coral bleaching. **Nature**, 380: 396.

Kushmaro, A., Banin, E., Loya, Y., Stackebrandt, E. & Rosenberg, E. 2001. *Vibrio shiloi* sp. nov., the causative agent of bleaching of the coral *Oculina patagonica*. **International Journal of Systematic Evolutionary Microbiology,** 51(4): 1383–1388.

Lalli, C.M. & Parsons, T.R. 1995. **Biological Oceanography: An Introduction**. Butterworth-Heinemann Ltd., Oxford, UK, 337 p.

Leão, Z. M. A. N., Kikuchi, R. K. P. & Oliveira, M. D. M. 2008. Coral bleaching in Bahia reefs and its relation with sea surface temperature anomalies. **Biota Neotropica** 8(3): 6-82.

Lesser, M.P., 2004. Experimental biology of coral reef ecosystems**. Journal of Experimental Marine Biology and Ecology**, 300: 217-252.

Middelboe, A.L. & Hansen, P.J. 2007. High pH in shallow water macroalgal habitats. **Marine Ecology Progress Series***,* 338: 107–17.

Pandey, P.K., Kass, P.H., Soupir, M.L., Biswas, S., Singh, V.P. 2014. Contamination of water resources by pathogenic bacteria. **AMB Express**, 4: 51.

Poggio, C. A., Leão, Z. M. A. N. & Mafalda-Junior, P. 2009. Registro de branqueamento sazonal em *Siderastrea* spp. em poças intermareais do recife de Guarajuba, Bahia, Brasil. **Interciência**, 34: 502-506.

Poole, L.J. & Raven, J.A. 1997. The biology of *Enteromorpha*. **Progress in Phycological Research**, 12: 1–148.

Ritchie, K. B. 2006. Regulation of microbial populations by coral surface mucus and mucus-associated bacteria. **Marine Ecology Progress Series**, 322: 15–27.

Rohwer, F. Seguritan, V., Azam, F. & Knowlton, N. 2002. Diversity and distribution of coral-associated bacteria. **Marine Ecology Progress Series**, 243: 1-10.

Rosa, S. R., Rosa, I. L. & Rocha, L. A. 1997. Diversidade da ictiofauna de poças de maré da praia do Cabo Branco, João Pessoa, Paraíba, Brasil. **Revista Brasileira de Zoologia**, 14 (1): 201-212.

Rosenberg, E. & Ben-Haim, Y. 2002. Microbial diseases of corals and global warming. **Environmental Microbiology**, 4(6): 318–326.

Rubio-Portillo, E., Yarza, P., Peñalver, C., Ramos-Esplá, A.A. & Antón, J. 2014. New insights into *Oculina patagonica* coral diseases and their associated *Vibrio* spp. communities. **The ISME Journal**, 8(9): 1794-1807.

Ruwandeepika, H.A.D., Jayaweera, T.S.P., Bhowmick, P.P., Karunasagar, I., Bossier, P. & Defoirdt, T. 2012. Pathogenesis, virulence factors and virulence regulation of vibrios belonging to the Harveyi clade. **Reviews in Aquaculture**, 4: 59-74.

Sassi, R. & Melo, G.N. 1989. Hyaline Tintinnina (Protozoa-Ciliophora-Oligotrichida) from northeast Brazilian coastal reefs. **Boletim do Instituto Oceanográfico,** 37(1): 59-74.

Sassi, R., Sassi, C. F. C., Gorlach-Lira, K. & Fitt, W.

K. 2015. Pigmentation changes in *Siderastrea* spp. during bleaching events in the coastal reefs of northeastern Brazil. **Latin American Journal of Aquatic Research**, 43 (1): 176-185.

Schaffelke B., Mellors J. & Duke N. C. 2005. Water quality in the Great Barrier Reef region: responses of mangrove, seagrass and macroalgal communities. **Marine Pollution Bulletin**, 51: 279-296.

Segal, B. & Castro, C. B. 2000. Slope preferences of reef corals (Cnidaria, Scleractinia) in the Abrolhos Archipelago, Brazil. **Boletim do Museu Nacional**, 418: 1-10.

Shikuma, N.J. & Hadfield, M.G. 2010. Marine biofilms on submerged surfaces are a reservoir for *Escherichia coli* and *Vibrio cholerae*. **Biofouling** 26: 39 – 46.

Shnit-Orland, M. & Kushmaro, A**.** 2009**.** Coral mucus-associated bacteria: a possible first line of defense. **FEMS Microbiology Ecology**, 6: 371–380.

Signoretto, C., Burlacchini, G., Lleó, M.M., Pruzzo, C., Zampini, M., Pane, L., Franzini, G. & Canepari, P. 2004. Adhesion of *Enterococcus faecalis* in the nonculturable state to plankton is the main mechanism for persistence of this bacterium in both lake and seawater. **Applied and Environmental Microbiology**, 70: 6892–6896.

Sokal, R. R. & Rohlf, F. J. 1983. **Biometry. The principles and practice of statistical in biological research**. W.H. Freeman, 880p.

Solo-Gabriele, H.M., Wolfert, M.A., Desmarais, T.R. & Palmer, C.J. 2000. Sources of *Escherichia coli* in a coastal subtropical environment. **Applied and Environmental Microbiology**, 66 (1): 230-237.

Stewart, J.R.,, Gast, R.J., Fujioka, R.S., Solo-Gabriele, H.M., Meschke, J.S., Amaral-Zettler, L.A., Castillo, E., Polz, M. F., Collier, T.K., Strom, M.S., Sinigalliano, C.D., Moeller, P.D.R. & Holland, A.F. 2008. The coastal environment and human health: microbial indicators, pathogens, sentinels and reservoirs. **Environmental Health**, 7 (Suppl 2), S3: 1-14.

Sussman, M., Willis, B. L., Victor, S. & Bourne, D. G. 2008. Coral pathogens identified for White Syndrome (WS) epizootics in the Indo-Pacific. **PLoS One**, 3(6): e2393.

Sutherland, K. p., Shaban, S., Joyner, J. L., Porter, J.W. & Lipp, E. K. 2011. Human pathogen shown to cause disease in the threatened eklhorn coral *Acropora palmate*. **Public Library of Science One**, 6 (8): e23468.

Touron, A., Berthe, T., Gargala, G., Fournier, M., Ratajczak, M., Servais, P. & Petit, F. 2007. Assessment of faecal contamination and the relationship between pathogens and faecal bacterial indicators in an estuarine environment (Seine, France). **Marine Pollution Bulletin**, 54 (9): 1441–1450.

Tran, L., Nunan, L., Redman, R.M., Mohney, L.L., Pantoja, C.R., Fitzsimmons, K. & Lightner, D.V. 2013. Determination of the infectious nature of the agent of acute hepatopancreatic necrosis syndrome affecting penaeid shrimp. **Diseases of Aquatic Organisms**, 105: 45–55.

Zimmerman, A. M., D. M. Rebarchik, A. R. Flowers, Williams, J. L. & Grimes, D. J. 2009. *Escherichia coli* detection using mTEC agar and fluorescent antibody direct viable counting on coastal recreational water samples. **Letters in Applied Microbiology**, 49: 478–483.

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