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Vírus-Bacteria Dynamics In The Coral Siderastrea Stellata

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ABSTRACT

Coral reef systems constitute reservoirs of extensive genomic diversity sustaining ecosystems characterized by high levels of biodiversity and primary productivity. Nevertheless, anthropogenic activities have markedly compromised coral reef health. Alterations in the physicochemical properties of seawater have direct repercussions on marine microsymbiotic associations, including diverse marine taxa. Among the most critical microsymbiosis in reef environments are the associations between cnidarians and dinoflagellates (zooxanthellae), in conjunction with diverse bacterial communities. In addition, viruses have been documented in association with prokaryotic - eukaryotic and coral organisms, contributing to modulation of coral health and exerting control over specific bacterial populations. Our study aimed to quantify bacterial and viral abundances present in the coral *Siderastrea stellata* at two locations - Porcos Island and Forno Beach, both situated in Arraial do Cabo, Rio de Janeiro, Brazil. Concurrently, analyses seawater physicochemical parameters at depths (5m and 15m) to determine their potential influence on virus-bacteria dynamics. Our findings revealed that temperature and nutrient concentrations exhibited variation with depth. Total bacterial abundance exceeded that of metabolically active bacteria and was positively correlated with dissolved organic carbon concentrations. The abundance of metabolically active bacteria appeared to be associated with ammonium levels and was comparatively higher at Forno Beach. Although marine viruses were detected in coral samples, the absence of viral genome sequencing precluded definitive taxonomic classification. These results underscore the necessity for further investigations incorporating additional environmental parameters and metagenomic approaches to elucidate the complex interactions among viruses, bacteria, and coral hosts within reef ecosystems.

Keywords: Siderastrea stellata; Coral Microbiome; Marine Viruses; Host-Microbe Interactions; South Atlantic Reefs

INTRODUCTION

The marine viruses are represented by bacteriophages, or phage in most of the aquatic systems [1,2]. Despite being pathogenic entities, they are also responsible for several functions in the environment as nutrient cycling, abundance and diversity control of prokaryotes (bacteria and archaea) and eukaryotes (phytoplankton) and gene lateral or vertical transfer [3]. The reef environment is considered one of the oldest, stable, biodiverse and complex of the planet [4]. However, in the last years, factors as biological and/or chemical, physical stresses, overexploitation, pollution, exotic species introduction, climate change and urban development have contributed to the coral reef's degradation [5]. Microsymbiosis in marine invertebrates has been recognized throughout the past century. Among the main symbioses are cnidaria with dinoflagellates as well as the associated of zooxanthellae and bacteria in corals [6,7]. Now, there are few studies showed the relationship between virus and holobionts corals. A previous study that isolated viral particles from cnidarians was unable to perform genetic sequencing due to the low purity of the samples, although its visualization has been completed by epifluorescence microscopy [1,8]. On the other hand, the literature has shown the association of Virus Like Particles (VLPs) with anemones zooxanthellae in the reef environment with consequent corals bleaching [9]. Also, VLPs were found in Pavona danai tissue, these Scleractinia have high temperature and associated with coral-stress and comorbidity [10]. Given the known abundance and diversity of microorganisms associated to corals, most of which are bacteria, is it possible quantify the number of marine viruses associated with corals in relation to the bacterial abundance? According to this hypothesis, we conducted a comparative study about the presence of marine bacteria and virus in the reef microenvironment, and we analyse the parameters (pH, temperature, salinity, turbidity and concentration of ammonia, nitrite, nitrate, phosphate and chlorophyll a) that could influence the virus-bacteria relationship.

MATERIAL AND METHODS

Sample collection of S. stellata

The samples of S. stellata were collected from different colonies in the two places mentioned above. Samples were taken at the same depths, surface (5m) and depth (15m) and stored in bottles identified with the specific sites of collection. To remove the coral reef fragments used a chisel to take off the fragments to surface of water and stored in plastic bag. At the laboratory, the coral samples were one more time washed, and mucus extracted. All collects were performed through Scuba Diving.

Water collection

The sea water samples were collected in two beaches of Arraial do Cabo, Rio de Janeiro (Porcos Island and Forno Beach) with aid of cylinder-Van Dorn between the April and May months. Depths of 5 m and 15 m were chosen and 2L collected in each depth for analysis of physicochemical parameters (salinity, pH, turbidity, temperature, nitrite, nitrate, phosphorus and chlorophyll-a) and total and active metabolically bacteria concentration.

Analysis of physicochemical parameters

The temperature was mensurated by reverse thermometer attached to a bottle Nansen. The inorganic nutrients, chlorophyll-a, total phosphorus and nitrogen were determined according with methodology described by Grasshoff & Almgreen (1983) [11]. The pH was determined by pH benchmark InoLab. For determination of salinity was used a hydrometer Self-salt Guildeline brand, model 8400A and the turbidity determined by Secchi disk.

Determination of total bacteria number and active metabolically

The determination of the number of active metabolically bacteria and total were used the methodology described by [12, 13]. Orange acridine and fluorescein diacetate were used as chromophores. Quantification was performed by epifluorescence microscope with a magnification of 1000 times with fluorescence microscope Axiops 50, Zeiss, triple filter Texas Red, fluorescein-Iisotiocianato DAPI. Briefly, 0.5 mL of samples were removed for analysis and counting of pretreated bacteria with acridine orange 1mg/mL [13]. The total volume (0.5mL) was serialized diluted 320 times and added 0.75 µl of acridine orange. The dilution third content was filtered by a polycarbonate membrane (Isopore Membrane filters ™) GTBP pore size 0.2 m, diameter 25 mm, followed by membrane preparation in slides to be analysed in fluorescence microscope. Ten integer fields of all samples were counted in triplicate. To analysis active metabolically bacteria and samples aliquot from coral were diluted twenty times in monobasic sodium phosphate (NaH2PO4) and stained with chromophores (100 µl to 20 mL final solution). For the total bacteria quantification, samples aliquot pretreated with solution of 20 mg fluorescein diacetate were diluted in 1 mL of acetone PA and incubated in a rotary shaker for 75 minutes. After, 2 mL of solution was removed and filtered by a polycarbonate membrane (Isopore Membrane filters ™) GTBP.

Viral DNA extraction and Podovirus detection

Viral DNA was extracted from Siderastrea stellata coral samples using the High Pure PCR Template Preparation Kit (Roche), following the manufacturer's instructions with some adaptations from Maciel (2002). The presence of marine viruses was investigated using three primer pairs derived from degenerate primers Podo-F and Podo-R, targeting ~1200 bp fragment of the conserved DNA polymerase gene of the Podoviridae family (Labonté et al., 2009). This gene region was selected based on its high representation among marine bacteriophages (Suttle 2007). Primer sequences, host organisms, and GenBank accession numbers are provided in Table 1.

Table 1: Bacteriophage primers for *Prochlorococcus* sp. (P60) and *Escherichia coli* (T7 and T3), all targeting the DNA polymerase gene with corresponding GenBank accession numbers.

Bacteriophage	Primers (5' → 3')	GenBank Accession No.
P60	**F:** GACACACTCATATCTGTCTGACGTG **R:** CTGAAGGCCCTAGACGTAGG	AF338467
T7	**F:** GACACACTCATATCTGTCTGACGTG **R:** ATTAAAGTTGGTGGTGGTCT	V01146
T3	**F:** GACACCCTTGTCGTCTCACGCTTG **R:** ATTAAAGGTCTGGATGGTCGT	AJ318471

PCR reactions (25 µL) were prepared with 16.6 µL of DNA template, Taq DNA polymerase, 1× PCR buffer, 2 mM $MgCl_2$, 0.2 mM of each dNTP, and 0.5 µM of each primer. Thermocycling conditions included initial denaturation at 94 °C for 90 s, followed by 39 cycles of denaturation at 94 °C for 45 s, annealing at 56 °C for 45 s, and extension at 72 °C for 60 s, with a final extension at 72 °C for 5 min. Negative controls lacking DNA

template were included in all reactions. PCR products were analysed by electrophoresis on a 2.5% agarose gel stained with ethidium bromide. Amplified bands were visualized under UV light, and product size was compared to a 1000 bp DNA ladder.

RESULTS

Physicochemical parameters of seawater

The analyse of physicochemical parameters of seawater at the two sampling sites (Porcos Island and Forno Beach) is shown in Table 2. Turbidity and pH remained relatively constant across both sites and depths. Temperature exhibited an inversely relationship with depth, with surface waters recording 24.7°C (Porcos Island) and 24.5°C (Forno Beach), while deeper water deeper waters consistently showed 21.1°C. Salinity displayed slight variations, ranging from 33.3 and 33.6 at Porcos Island and 33.6 to 34.6 at Forno Beach. Concentrations of nitrite (NO_2^-), nitrate (NO_3^-), phosphate (PO_4^{3-}), and chlorophyll α varied with depth at both sites, except for chlorophyll α at Forno Beach, which remained constant. Generally, higher concentrations of NO_2^-, NO_3^-, and PO_4^{3-} were observed at greater depths.

Table 2: Physicochemical parameters of seawater at the two sampling sites

Sample	Sampling Station		
Water	Porcos Island	pH	7
		T (°C)	24.7°C / 21.1°C
		Salinity	33.3 / 33.6
	Forno Beach	pH	7
		T (°C)	24.5°C / 21.1°C
		Salinity	33.6 / 34.6

Determination of total bacteria number and active metabolically

Due the capacity of marine viruses to associate with bacteria in reef ecosystems, the total and metabolically active bacteria abundances within the mucus of Siderastrea stellata corals were quantified from samples collected at Porcos Island and Forno Beach. At both sampling sites, the total bacterial abundance consistently exceeded the number of metabolically active bacteria. Notably, a higher abundance of metabolically active bacteria was observed at Forno Beach compared to Porcos Island (Table 3). The bacterial organic carbon was also analysed, being directly proportional to the bacteria number.

Table 3: Total bacteria number (AO), metabolically active bacteria number (DF) and bacterial organic carbon. AO = acridine orange and DF = fluorescein diacetate.

Sample	Sampling Station	Bacteria number/ cm^3	Bacterial Organic Carbon
Water	Porcos Island - AO	2.85×10^8	3.425926
	Porcos Island - FD	2.82×10^2	0.065355
	Forno Beach - AO	2.03×10^8	2.440741
	Forno Beach - FD	1.23×10^7	0.147222

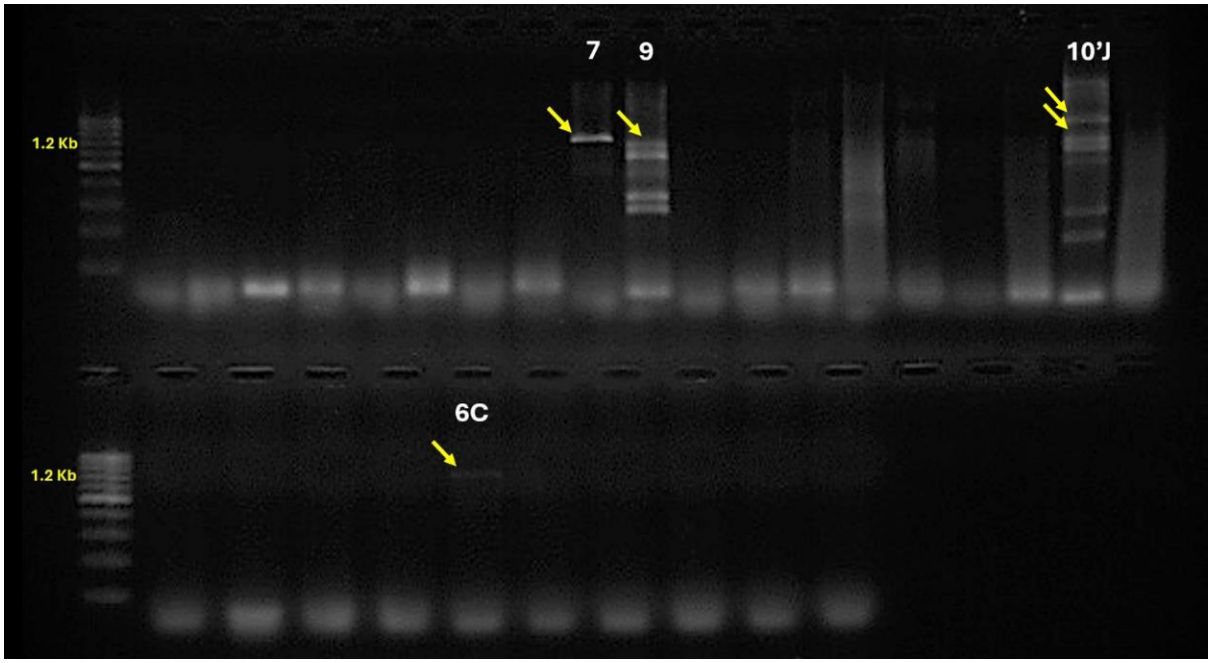

Figure 1: Detection of the host in S. stellata samples by agarose gel electrophoresis of PCR amplification products using the P60, T3 and T7 primer. Note: The bands amplified in the 1.2 kb region of the Molecular Weight Standard (scale on the left); yellow arrows indicate the amplified target regions: 7 = 2SIP - culture with 8 days of Porcos Island; 9 = 2SPF – culture with 12 days of Forno Beach; 10'J = Mangrove bacteria; 6C = 2SIP – culture 6/13 with days of Porcos Island.

DISCUSSION

Marine environments cover approximately 70% of the Earth's surface and are subject to frequent fluctuations in temperature, pressure, and salinity. These ecosystems harbour a wide diversity of microorganisms including Archaea, Bacteria, and Eukarya - that contribute to about 98% of marine primary production [14,15]. Arraial do Cabo, a Brazilian municipality located within a sustainable-use conservation unit, is characterized by clear waters, rich biodiversity, and complex ecological interactions, including endosymbiotic bacteria [16]. The region is also influenced by upwelling events that bring cold, nutrient-rich waters to the surface, enhancing biomass production and stimulating the metabolic activity of aerobic anoxygenic phototrophic bacteria [17]. However, environmental constraints such as particle association, temperature, light attenuation, nutrient limitation, and predation pressure can limit bacterial abundance [18,19]. Several studies have demonstrated that viral abundance in marine environments is closely linked to bacterial populations [20,21,22,23]. Bacteriophages commonly known as phage represent the dominant group of marine viruses and may exist freely or associated with organisms as cyanobacteria [24], bacterioplankton [25], and other bacterial taxa [26]. This association is also well documented in coral reef ecosystems [27,28]. Coral reefs represent microenvironments that host approximately 30% of marine biodiversity [29]. These systems are characterized by intrinsic symbiotic relationships among corals, microbes, and viruses, which together drive organic matter cycling and nutrient turnover, thereby sustaining complex trophic webs [30]. Research on coral-microbe-virus interactions has increased significantly over the past 15 years [31,32], with estimates indicating 10^6–10^8 viral particles per mL of reef seawater higher than counts observed for other microorganisms [33]. Viral predation of bacteria plays a

central role in modulate bacterial regulatory networks within coral reefs primarily through viral lysis, which contributes to the high abundance of viruses associated to the corals [34]. Moreover, viral infections may promote the emergence of bacterial pathogens and, upon lysis, significantly contribute to the dissolved organic carbon (DOC) pool [35]. Some viruses also enhance microbial host fitness by encoding auxiliary metabolic genes involved in photosynthesis, carbon metabolism, and nucleotide synthesis [36,37]. Despite potential benefits, viruses can also induce coral diseases, either through primary infection, reactivation of latent infections, immune suppression, or immune senescence, often manifesting as bleaching events [31]. Environmental stressors as elevated temperatures, UV radiation, low pH, and excess nutrients are also implicated in increased viral propagation and coral bleaching [2]. In this context, we analysed the physicochemical parameters of seawater and the presence of bacteriophages in the mucus of Siderastrea stellata corals collected at two sites Porcos Island and Forno Beach, at Arraial do Cabo, Brazil. Our data sowed that turbidity and ph remained constant independent of depth. Temperature and salinity varied inversely and directly with depth. Ammonia (NH_3) levels varied independently of depth, being highest at 5 m in Porcos Island and at 15 m in Forno Beach. These nutrients particularly nitrate and phosphate are essential macronutrients used by phytoplankton and autotrophic bacteria and commonly used as indicators of water quality and organic matter processing [19]. The elevated nutrient levels observed at greater depths may be attributed to increased activity of nitrifying and denitrifying bacteria [38]. Additionally, excessive nutrient concentrations may indicate anthropogenic contamination from industrial, domestic, or agricultural sources. Despite these variations, the measured parameters were within the limits set by Brazilian environmental standards, indicating good water quality [39]. Interestingly, ammonia concentrations did not correlate with temperature in this study, in contrast with previous findings that showed ammonia-oxidizing archaea and bacteria closely linked to temperature, salinity, oxygen, pH, and light concentration, even though these controls can interact and vary by habitat and substrate supply [40-42]. Thus, the role of temperature in regulating NH_3 dynamics in this setting remains inconclusive. Lower chlorophyll α levels were observed in this study compared to previous data collected in summer, due to seasonal variation. Our samples, collected in autumn, coincided with reduced solar radiation, limiting photosynthetic activity and explaining the lower chlorophyll α concentration [43-45]. Bacterial abundance was assessed comparing total and metabolically active bacteria. As expected, total bacterial counts were bigger than metabolically active bacteria in all samples. This difference is explained because acridine orange, which stains both live and dead cells, is opposite to fluorescein diacetate, which only stains viable cells through esterase activity [13,46]. Across sites, metabolically active bacteria were higher at Forno Beach and were positively associated with higher ammonia, a pattern consistent with enhanced primary production and microbial activity, as also reported by Coelho-Souza et al. (2013) [17]. Total bacterial abundance increased with dissolved organic carbon [47], with the highest dissolved organic carbon measured at the Porcos Island sampling site. Numerous studies have demonstrated virus-bacteria-coral interactions [34,48,49]. Coral microbiomes harbour a wide diversity of viruses that modulate host health [43,50]. These viruses can regulate bacterial populations via selective infection and lysis, forming a "lytic barrier" that prevents pathogen colonization [24, 25]. Viral abundance is especially high in the coral surface mucus layer, where they can facilitate genetic exchange and potentially enhance coral survival by modulating holobiont functions [36]. Under stable conditions, these interactions are beneficial to coral health. However, stressors such as ocean warming, eutrophication, and hypoxia can disrupt this balance, promote viral infections and compromise reef resilience

[34,51]. Our electrophoresis results showed the presence of marine viruses in coral mucus, corroborating with known association of viruses and coral microbial communities [34]. However, due viral genomes are highly diverse and evolve rapidly, detection typically targets conserved loci with optimized (often degenerate) primer sets. In marine systems, ubiquitous short-tailed dsDNA phages include T7/T3-like coliphages (family Autographiviridae; historically placed within Podoviridae) and Prochlorococcus podoviruses such as cyanophage P60 [32,52,53]. Although marine viruses were detected in coral samples, the absence of viral genome sequencing precluded definitive taxonomic classification, particularly with respect to the Podoviridae family being needed further investigations, involving primers targeting other viral families, genetic sequencing, environmental parameters and metagenomic approaches that make it easier to clarify the complex interactions among viruses, bacteria, and coral hosts within reef ecosystems.

CONCLUSION

In this study, we evaluated the influence of physicochemical parameters seawater on the bacterial and viral abundance associated to the coral Siderastrea stellata collected at Porcos Island and Forno Beach, two prominent coastal sites in Arraial do Cabo, Rio de Janeiro, Brazil. Our findings indicate that, regardless of depth (5m and 15m), the sea water quality at both sampling sites is in accordance withpf the criteria established by the CONAMA resolution, suggesting a stable marine environment. Ammonia and organic carbon concentrations were respectively linked to the abundance of metabolically active and total bacteria, suggesting that nutrient availability plays a direct role in modulating the microbial communities present on S. stellata. Marine viruses were also consistently found in areas with higher bacterial concentration. Although viral presence was confirmed, sequencing was not performed, preventing the identification of specific viral families as Podoviridae. These findings provide relevant baseline data for this coastal region.

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