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Salinity Drives the Virioplankton Abundance but Not Production in Tropical Coastal Lagoons

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Abstract Viruses are the most abundant components of microbial food webs and play important ecological and biogeochemical roles in aquatic ecosystems. Virioplankton is regulated by several environmental factors, such as salinity, turbidity, and humic substances. However, most of the studies aimed to investigate virioplankton regulation were conducted in temperate systems combining a limited range of environmental variables. In this study, virus abundance and production were determined and their relation to bacterial and limnological variables was assessed in 20 neighboring shallow tropical coastal lagoons that present wide environmental gradients of turbidity (2.32–571 NTU), water color (1.82–92.49 m^{-1}), dissolved organic carbon (0.71–16.7 mM), salinity (0.13–332.1‰), and chlorophyll-a (0.28 to 134.5 μg L^{-1}). Virus abundance varied from 0.37×10^8 to 117×10^8 virus-like-particle (VLP) mL^{-1}, with the highest values observed in highly salty aquatic systems. Salinity and heterotrophic bacterial abundance were the main variables positively driving viral abundances in these lagoons. We suggest that, with increased salinity, there is a decrease in the protozoan control on bacterial populations and lower bacterial diversity (higher encounter rates with virus specific hosts), both factors positively affecting virus abundance. Virus production varied from 0.68×10^7 to 56.5×10^7 VLP mL^{-1} h^{-1} and was regulated by bacterial production and total phosphorus, but it was not directly affected by salinity. The uncoupling between virus abundance and virus production supports that the hypothesis that the lack of grazing pressure on viral and bacterial populations is an important mechanism causing virus abundance to escalate with increasing salt concentrations.

Keywords Virus-bacteria dynamics · Virus shunt · Dilution technique · Flow cytometry · Carbon cycling · Shallow lakes

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Introduction

Viruses have been widely recognized as the most abundant components of the biosphere [1]. Virioplankton reach very high abundances in surface waters of all sorts of aquatic ecosystems but have been found more abundant in inland and coastal waters than oceans [2]. They play important roles in aquatic ecosystems regulating microbial community composition [3] and altering ecosystem functioning and biogeochemical cycles by killing phytoplankton and bacterioplankton [4].

Viruses abundance can be directly affected by environmental factors. High-molecular-weight dissolved organic matter, such as humic substances, has been demonstrated to decrease

the abundance of viruses by either binding or destroying virus particles [5]. Organic matter can also decrease virus infectivity and replication rates in freshwater ecosystems [5]. Unsurprisingly, humic lakes have lower virus abundance [6]. High temperatures [7] and exposition to UV radiation [8] have also been suggested to negatively affect virus distribution in aquatic systems.

Most viruses are bacteriophages and their abundance is strongly correlated with bacterial abundance in aquatic systems [9], although the ratio of this relationship is widely variable across ecosystems [2, 10]. Thus, environmental factors that significantly impact bacterial productivity and density also have a strong and indirect effect on virus production and distribution [9]. Salinity has been described as an important indirect virus regulator as high salt concentrations drastically decrease both the prokaryotic community diversity and the eukaryotic grazing pressure on bacteria [11], which increase bacterial abundance and, in turn, virus infection [12]. Viruses are also affected by the trophic status of the ecosystem; there is evidence that eutrophic systems have higher virus abundance than oligotrophic ones as a response to higher bacterial and phytoplankton abundance [13]. Therefore, virus abundance correlates with chlorophyll-*a* in several aquatic environments such as lakes, estuaries, and marine systems [13–15] and high turbidity can negatively affect virus abundance by reducing the phytoplankton abundance [16].

In spite of these investigations about virus responses to different environmental factors in inland aquatic systems, none of them have been performed in aquatic systems covering large environmental gradients. Moreover, most studies were conducted in temperate zones. This bias may underestimate the importance of indirect effects on viruses mediated by bacteria because tropical systems are warmer and sunnier and present higher bacterial metabolic rates [17]. Different patterns of virioplankton abundance and activity might comparatively emerge from these systems.

We investigate virus abundance and production and their major bacterial and environmental drivers in tropical coastal lagoons. Virus-mediated mortality and the amount of carbon released from bacteria lyses by the viruses were also estimated in some of these systems. The tropical coastal lagoons sampled here present broad ranges of trophic state (from oligotrophic to hypereutrophic conditions), salinity (from freshwater to hypersaline systems), turbidity (from clear to highly turbid waters), water color, and dissolved organic carbon concentration (from clear water to extremely humic systems). Besides combining such widely ranging environmental characteristics, these coastal lagoons are relatively close to each other and are subject to similar broad-scale regional factors such as climate and proximity to the sea [18]. In summary, we found that bacterial abundance and salinity are the main drivers of viral abundance among the studied lagoons. However, virus and bacterial production were not directly affected by salinity,

bringing about an apparent paradox that is explored throughout this study.

Methods

Study Site

The study took place in 20 tropical coastal lagoons situated in the state of Rio de Janeiro, southeastern Brazil (Fig. 1). Most of them (16 out of 20 lagoons) are located in the Restinga de Jurubatiba National Park (between 22–22° 30′ S and 41° 15′– 42° W), which is characterized by patches of shrubs and small trees in sandy dunes. Inter-dune areas are dominated by both seasonally flooded forests and perennial or temporary coastal shallow lagoons. The studied lagoons are located within a maximum distance of 73 km from each other, present nearly the same distance to the sea, and are subject to similar climate factors [18]. Nevertheless, they present a wide range of limnological features such as dissolved organic carbon, chlorophyll-*a* and nutrient concentrations, salinity, and water color [18]. Water and nutrient inputs to these lagoons are mainly from the water table, the ocean, and small streams; however, the relative importance of these sources greatly depends on each lagoon genesis, position in relation to the ocean, and the watershed and may also vary over a seasonal scale [18]. Some lagoons (lagoons 5, 6, 9, 11, 12, and 19 in Fig. 1) are dystrophic systems with extremely high concentrations of dissolved organic carbon derived from the humic-rich water table [19]. Lagoons 1 and 2 are also humic ecosystems but receive nutrient inputs from both diffusive (agriculture activity) and punctual (sewage runoff from a nearby city) sources [19]. Lagoon 4 is fully inserted in an urban area and receives high nutrient loads from direct sewage discharges. Lagoons 3 and 10 are brackish lagoons [18], while lagoons 16, 17, 20, 13, and 14 are hypersaline (from 43 up to 332‰) because of their reduced water volume in response to the persistent drought periods lately observed in the studied region (Fig. 2). The regional weather is warm, with an average air temperature of 22.6 °C. The historical annual precipitation in the region is 1038 ± 336 mm (Instituto Brasileiro de Meteorologia, INMET). The precipitation accumulated during the year (2014) prior to our sampling was notably low (total of 676 mm, Fig. 2). Additional details about the local climate as well as the limnological and morphological traits of these lagoons are found in Caliman et al. (2010).

Sampling

Sub-surface water samplings and in situ measurements were carried out in a near-shore sampling station of each lagoon in January 2015. Water temperature and dissolved oxygen were in situ evaluated using specific probes (YSI-30 and YSI-61,
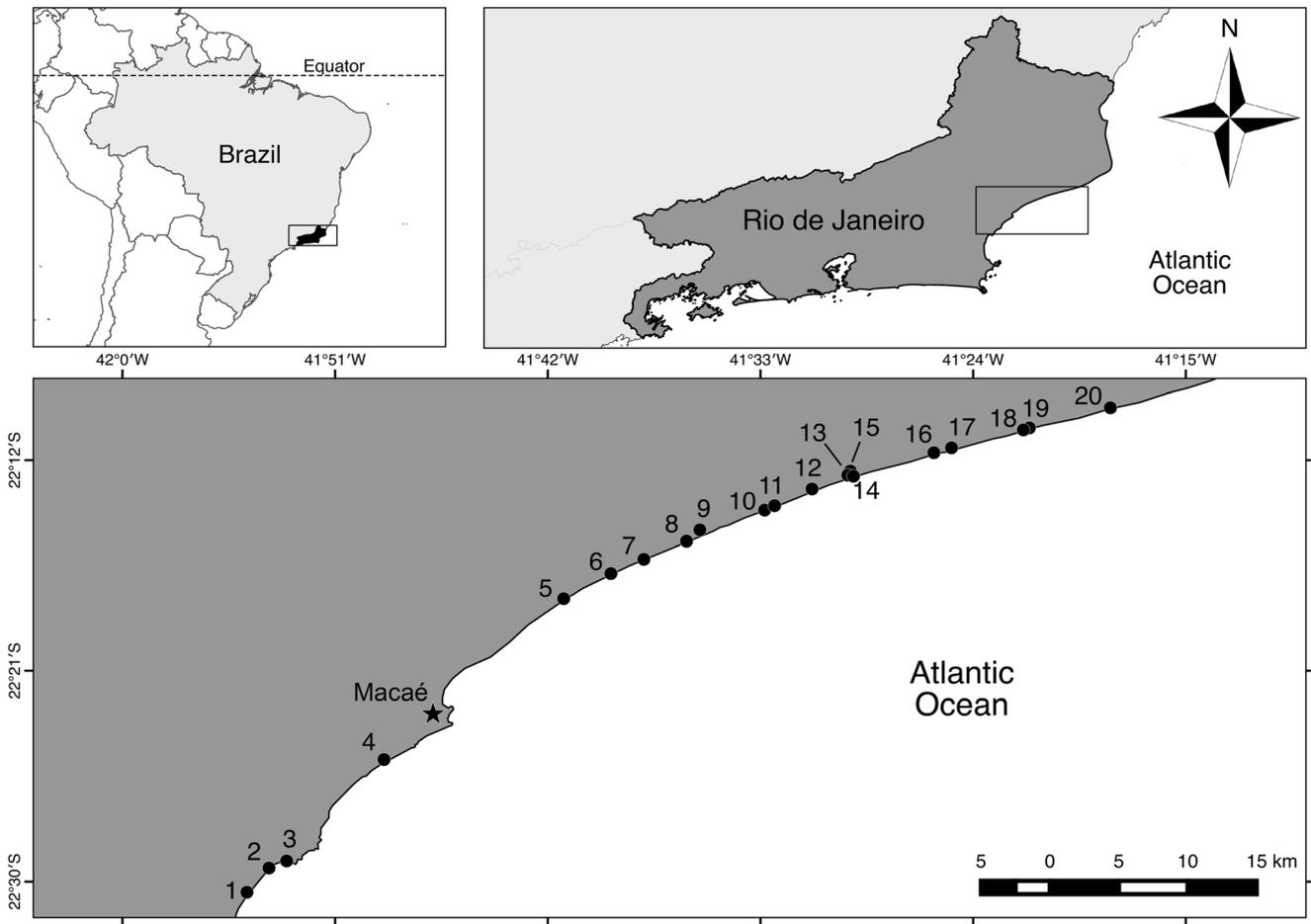
Fig. 1 Map of the study area with the geographical location of all coastal lagoons (*black dots*) sampled in this study. Each lagoon is numbered as follows: *1* Iriry, *2* Salgada, *3* Itapebussus, *4* Imboassica, *5* Cabiunas, *6* Comprida, *7* Carcará, *8* Carapebuzinho, *9* Carapebus, *10* Encantada, *11* Paulista, *12* Amarra Boi, *13* Garças, *14* Garcinha, *15* Atoleiro, *16* Visgueiro, *17* Catingosa, *18* Pretinha, *19* Preta, *20* Ubatuba. The *black star* stands for the city of Macaé, the largest urban area of the northern coast of Rio de Janeiro state, Brazil

respectively). Two water samples (1 mL each) for density evaluation of virus, bacteria, and phototrophic bacteria were pre-filtered through a 50-μm membrane mesh to remove large particles. These samples were fixed for 15–30 min at 4 °C into 2-mL cryotubes with paraformaldehyde (1%) and glutaraldehyde (0.05%), or glutaraldehyde (0.5%), or paraformaldehyde

Fig. 2 Historical average of the monthly-accumulated precipitation (*bars*) and the monthly-accumulated precipitation of the year (2014) previous to our sampling in January 2015 (*dotted line*). The precipitation accumulated during this year was 676 mm. Dataset provided by the Instituto Brasileiro de Meteorologia, INMET
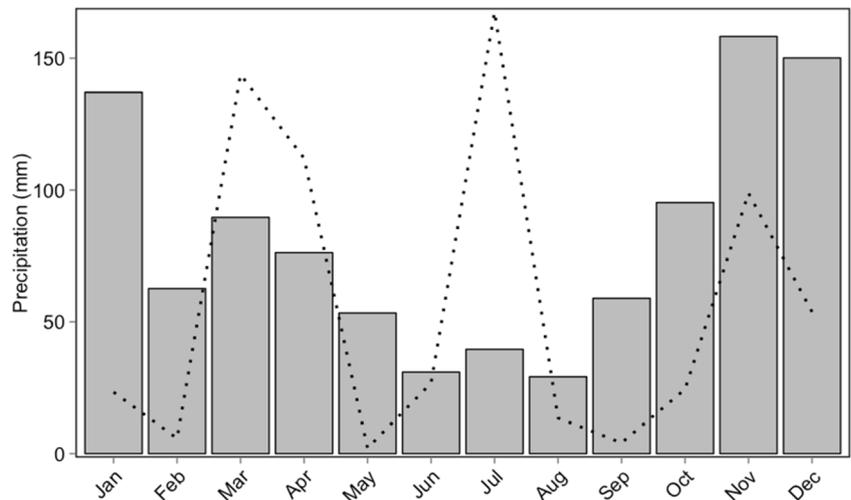
(1%), respectively. The fixed samples were then flash frozen in liquid N_2 in the field and then stored at −80 °C in the laboratory. Water samples were also collected in polyethylene flasks previously washed with HCl 10% and rinsed with distilled water. These samples were taken to the laboratory within 2 h after sampling for limnological and bacterial production measurements as well as for the time-course experiment set up to determine virus parameters.

Flow Cytometric Measurements: Virus and Bacteria Enumeration

Flow cytometry was used to evaluate virus, bacteria, and phototrophic bacteria numbers [20, 21]. These enumerations were performed separately using a FACSCalibur (BD) Flow Cytometer with standard laser and optics as follows: air-cooled argon ion laser emitting at 488 nm (power at 20 mW), fixed laser alignment, and fixed optical components. A 70-µm nozzle aspirated the sample, and sterile Mill-Q water (18,2 mΩ) was used as sheath fluid. We analyzed the fluorescence emitted by the samples using the forward scatter and the side scatter at 90°. Fluorescence signals were collected by three different photomultipliers: FL1 (530 ± 30 nm), FL2 (585 ± 42 nm), and FL3 (670 LP). Counts were made in triplicate at flow rate of ca. 40 µL min^{-1}, and data were acquired in logarithmic mode. Fluorescent latex beads (Fluoresbrite YG carboxylate 1.0 µm, Polysciences) were added at a known abundance to each sample for calibration of side scatter and green fluorescence signals and as internal standards for cytometric counts and measures.

Fixed viral and bacterial (heterotrophic + phototrophic bacteria) samples were promptly stained with SYBR Green I (Invitrogen) at final concentrations of 0.5×10^{-4} of the commercial stock solution and then mixed in a vortex apparatus for 15 s. Viral samples were then incubated in the dark at 80 °C for 10 min and at room temperature for additional 5 min [21]. All samples were highly concentrated and, hence, they were diluted (Table S1) in sterile TE buffer, pH 8 (10 mM Tris-hydroxymethyl-aminomethane, Roche Diagnostics; 1 mM ethylenediaminetetraacetic acid, Sigma-Aldrich), to reach an ideal working event rate (200–800 events s^{-1}) that avoids electronic coincidence in counts [21].

Viruses were detected, identified, and counted by their signature in a plot of side scatter (X-axis) versus green fluorescence (FL1, Y-axis, green fluorescence from SYBR Green I related to nucleic acid content and virus size) [21]. Bacterial samples were kept for 15 min in the dark until cytometer readings were taken. Total bacterial abundance was determined by their position in a plot of side scatter (X-axis and indicative of cellular size) versus green fluorescence (FL1, Y-axis, related to nucleic acid content) and red fluorescence (FL3) in order to detect different groups of heterotrophic bacterioplankton [20]. Phototrophic bacteria groups were detected by their specific autofluorescence properties and signature in a plot of orange fluorescence (FL2) versus red fluorescence (FL3) [22]. Heterotrophic bacterial abundance (HBA) was determined from difference between total bacterial abundance and phototrophic bacterial abundance. All data were obtained and analyzed by Cell Quest software (BD) using a Macintosh computer.

Bacterial Production

Bacterial production (BP) was measured by using the ^{3}H-leucine incorporation method and trichloroacetic acid (TCA) protein extraction [23], as modified by Miranda et al. [24]. An intracellular isotopic dilution factor of 2 was used in calculations [25]. An aliquot of 1.2 mL of the water sample was incubated in microcentrifuge tubes (25 ± 1 °C) in the dark for 45 min with 20 nM of ^{3}H-leucine (specific activity 58.8 Ci mmol^{-1}). The concentration of 20 nM of ^{3}H-leucine was previously established from saturation curves of bacterial leucine uptake in some of the coastal lagoons studied here (unpublished data). Negative controls were set up by adding 90 µL of TCA to kill microbes prior to starting the incubations. At the end of the incubation, 90 µL of TCA was added to each tube in order to stop ^{3}H-leucine incorporation by bacteria. Bacterial protein was extracted by washing with 5% TCA and 80% ethanol. After protein extraction, liquid scintillation cocktail (EcoLite(+)™) was added to each sample, which was radioassayed by liquid scintillation counting (Beckman LS 6500) after 2 days in the dark to reduce the chemiluminescence. The obtained values in disintegrations per minute were converted to bacterial biomass using a ratio incorporation of 0.86, according to Wetzel and Likens [26].

Time-Course Experiment

Virus production (VP), virus decay (VD), virus turnover (VT), burst size (Bs), and virus-mediated mortality (VMM) were determined for 16 lagoons through the dilution technique in a laboratory time-course experiment [27], as adapted by Hewson et al. [50]. This technique has been widely used and considered the simplest method, while still providing consistent inter-comparison data [28]. This method decreases the virus-bacteria meeting rate, thereby preventing new viral infection, by reducing the amount of viruses in the sample while keeping the original bacterial concentration [27]. First, we obtained virus-free water for every lagoon by filtering water samples (previously filtered through a 50-µm membrane mesh) through a 0.02-µm filter backed by a 2.7-µm filter (25 mm; GF/D Whatman). Second, 300 mL of the unfiltered water samples was vacuum filtered (<250 mmHg) through 0.2-µm pore size polycarbonate filters (47 mm diameter, Millipore). During this filtration procedure, the sample was constantly mixed up by pumping water in and out from a

sterilized Pasteur plastic pipette in order to avoid bacteria attachment to the filter. Also during the filtration procedure, 270 mL of the previously obtained virus-free water was gradually added to maintain the initial volume (300 mL) and result in a 1:9 virus dilution. The final 300 mL of diafiltered sample was separated into triplicates of 100-mL polycarbonate flasks and was kept in a BOD chamber (25 ± 1 °C) for a period of 10 h. The polycarbonate flasks used for the incubation were previously autoclaved, washed with HCl 10%, and rinsed with Milli-Q water. Virus-bacterium density sub-samples (1 mL) were collected from each polycarbonate flasks every 2 h and 30 min and then fixed and stored for flow cytometric measurements as described in the section above. Three bacterial production sub-samples (1.2 mL) were also performed after the first 2 h and 30 min of the incubations, when a rapid increase in the virus abundance (VA) is usually observed [29].

VP rates were determined from the mean slope of linear regressions of VA against time for each incubation replicate. VD was obtained from the first-order regression when virus started decreasing just after the main VA increase. VT—the fraction of the viral population that is replaced every hour—was calculated by dividing VP rates by initial abundances of viruses in each replicate. Bs is the amount of virus-like-particles (VLPs) released from a cell lysis event (VLPs lyse^{-1}) and was calculated by dividing the first VA increase by the first HBA decrease of the incubations. The VMM of bacterial cells (cells lysed mL^{-1} h^{-1}) was calculated by dividing VP values by the Bs (VMM = VP / Bs) [29]. VMM was further divided by the new bacterial cell production rates (cells mL^{-1} h^{-1}) calculated from ^{3}H-leucine incorporation, to give the virus-induced bacterial mortality (VIBM), expressed as a percentage of bacterial production (VIBM (%) = VMM / NCP × 100) [29]. Finally, estimates of the amount of organic carbon (ng C mL^{-1} h^{-1}) released by viral lysis of infected bacterial cells were calculated by multiplying VMM by the mean carbon content per cell of 20 fg C cell^{-1}, which is a conversion factor widely used in the literature [30].

Limnological Variables

Water pH was measured using a pH meter (Analion PM 608), turbidity with a turbidimeter (Hanna HI 98703), and salinity with the chlorinity method [31]. Water samples for dissolved organic carbon and dissolved nitrogen evaluation were filtered through 0.7-μm glass fiber filters (47 mm; GF/F Whatman) and the filters were kept frozen in the dark (up to 1 week) for chlorophyll-a concentration analysis. Chlorophyll-a was extracted from these filters with 90% ethanol and absorbance was measured by spectrophotometry at 665 nm [32]. Dissolved organic carbon and dissolved nitrogen concentrations were determined using a Pt-catalyzed high-temperature combustion method with a Total Organic Carbon Analyzer (TOC-5000 with a TNM-L unit; Shimadzu Scientific

Instruments). Unfiltered water samples were autoclaved and total phosphorus was spectrophotometrically determined through formation of phosphorus molybdate [33]. Water color was estimated by the absorbance at 440 nm and expressed as the absorption coefficient calculated according to Hu et al. [34]. All spectrophotometric analyses were performed in a 1-cm quartz cuvette (distilled water as a blank) using a Beckman® DU520 spectrophotometer.

Data Analysis

We used linear models to evaluate the main and interactive effects of abiotic (e.g., salinity, temperature, nutrient concentration) and biotic (e.g., chlorophyll-a concentration, HBA, BP) factors on VA and VP using the package *nlme* [35] in the R Statistical Software (version 3.2.2, <www.r-project.org>). Other viral parameters calculated in this study (e.g., VMM, Bs) were not statistically tested due to the small number of results (see "Results" section). We log-transformed VA and VP to meet normality and homoscedasticity assumptions. We built models containing all variables and used an averaging procedure to identify the best-fitting model (functions *aictab* and *evidence* in the *Aiccmodavg* package; [36]). This model selection procedure retains the best-predicting model by balancing the number of variables and the explanatory power added to the model [37]. Best-predicting models were those that had the smallest AICc scores [37]. We also conducted Pearson's correlations to test relationship and identify multicollinearity among log-transformed (except pH) environmental variables. For this test, an alpha of 0.05 indicated statistical significance.

Results

VA varied by three orders of magnitude from 0.37×10^8 to 117×10^8 VLP mL^{-1}. The lowest VA values were found in humic lagoons such as lagoons 5, 6, 7, and 16 (Table 1). Eutrophic and hypereutrophic lagoons (lagoons 8 and 4, respectively) were about threefold more abundant in virus than the humic lagoons (Table 1). The highest VAs were recorded in the hypersaline systems (lagoons 13, 14, 16, 17, 20), where VA reached a remarkable maximum of $1.17 \cdot 10^{10}$ virus mL^{-1}. HBA varied from 0.14×10^7 to 509×10^7 cells^{-1} mL^{-1} and the virus-to-bacteria ratio (VBR) ranged from 2.3 to 144 (Table 1). VP varied by two orders of magnitude, between 0.68×10^7 and 56.5×10^7 VLP mL^{-1} h^{-1}, whereas BP ranged from 0.26 to 20.87 μM C h^{-1} (Table 1). VD varied between 0.46 and 39.6×10^7 VLP mL^{-1} h^{-1}, whereas VT ranged from 0.15 to 2.1 h^{-1} (Table 1). From a total of 16 lagoons, 10 did not present a bacterial decay alongside the first virus increase recorded in the time-course incubations and, therefore, Bs, VMM, and VIBM were only calculated for six of the lagoons

Table 1 Virus and bacteria abundance and activity parameters measured in 20 tropical coastal lagoons from Rio de Janeiro state, Brazil

Lagoon	VA ($\times 10^8$ VLP mL^{-1})	V1%	V2%	V3%	VP ($\times 10^7$ VLPs mL^{-1} h^{-1})	VD ($\times 10^7$ VLPs mL^{-1} h^{-1})	VT (h^{-1})	HBA ($\times 10^7$ cells mL^{-1})	VBR	BP (μM C h^{-1})
1	6.40	39.25	51.46	8.60	11.20	12.60	0.48	2.72	24	18.04
2	1.10	41.22	52.53	5.01	32.10	16.10	2.10	0.40	27	6.04
3	1.28	63.11	35.64	1.25	NA[a]	NA	NA	0.40	32	10.42
4	6.78	50.97	39.72	7.22	19.70	13.70	0.47	2.48	27	18.45
5	0.81	48.52	45.59	5.89	0.94	0.46	0.28	0.39	21	0.27
6	0.71	43.17	51.51	5.32	1.45	1.95	0.52	0.14	52	1.00
7	0.37	36.40	50.99	12.61	1.94	0.93	0.86	0.67	6	9.29
8	3.69	59.17	33.53	7.30	6.17	18.40	0.40	3.25	11	17.54
9	2.71	68.44	26.97	4.59	13.30	2.84	1.45	1.31	21	7.40
10	2.12	81.28	17.39	1.33	4.34	9.86	0.27	1.15	18	2.37
11	1.23	63.88	33.50	1.46	0.68	5.92	0.15	0.75	16	5.04
12	1.86	66.51	29.65	3.85	9.04	0.83	1.15	0.58	32	1.73
13	12.20	74.50	19.54	5.96	NA[a]	NA	NA	0.85	144	8.84
14	18.90	76.33	22.30	1.37	15.40	31.10	0.72	1.40	135	9.89
15	0.93	36.23	58.94	4.83	4.76	3.28	0.81	0.62	15	0.85
16	89.10	92.85	6.78	0.37	NA[a]	NA	NA	14.17	63	3.53
17	117.00	58.06	35.81	6.12	NA[a]	NA	NA	509.3	2	0.26
18	2.77	89.73	8.20	2.08	56.50	39.60	1.27	1.34	21	15.35
19	3.30	73.67	20.85	5.49	8.10	0.88	0.82	0.96	35	20.87
20	12.30	68.14	27.00	4.86	17.80	21.60	0.19	1.58	78	20.50

VLP virus-like particles; *VA* virus abundance; *V1, V2, V3* virus cytometric groups; *VP* virus production; *VD* virus decay; *VT* virus turnover; *HBA* heterotrophic bacteria abundance; *VBR* virus-bacteria ratio; *BP* bacterial carbon production; *NA* not available

[a] Time-course incubations were not conducted for lagoons 3, 13, 16, and 17

(Table 2). Bs varied from 3.7 to 861 VLP lyse^{-1}, VMM ranged from 2.27×10^6 to 37.6×10^6 cells lysed mL^{-1} h^{-1}, and VIBM varied from 16 to 1681% (Table 2). We estimated that viruses were responsible for releasing from 45.4 to 751.2 μg C L^{-1} h^{-1} of carbon through bacterial cells lyse in surface waters of the tropical coastal lagoons (Table 2).

The environmental conditions in each lagoon during viral and bacterial samplings are shown in Table 3. Chlorophyll-*a* concentration ranged from 0.28 to 134.5 μg L^{-1}. Carbon concentration varied from 0.71 to

16.7 mM, while nitrogen and phosphorus concentrations varied between 40.3 and 8730.4 μM and from 0.77 to 156 μM, respectively. Water color was recorded between 1.82 and 92.49 m^{-1}. Turbidity was recorded between 2.32 and 571 NTU and pH ranged from 3.22 to 7.59. Temperature was between 23 and 35.2 °C and salinity ranged from 0.13 to 332.1‰. Oxygen saturation in the water column varied from 1.2 and 181.6%.

We observed strong correlation among physical-chemical variables in these lagoons (Table S2). Carbon concentration

Table 2 Average (± standard error) of calculated virus parameters and the amount of DOC released from bacterial cells lysis in six coastal lagoons

Lagoon	VMM ($\times 10^6$ cells lysed mL^{-1} h^{-1})	VIBM (%)	Bs (VLP lyse^{-1})	DOC released (μg C L^{-1} h^{-1})
1	10.50 ± 2.78	168.43 ± 58.82	17.09 ± 12.38	209.85 ± 55.57
2	2.74 ± 1.90	65.25 ± 43.12	860.9 ± 676.6	54.84 ± 37.94
8	6.92 ± 1.85	84.52 ± 26.16	8.47 ± 1.7	138.42 ± 36.93
10	7.92 ± 2.56	1680.8 ± 642.2	5.87 ± 1.21	158.48 ± 51.24
17	2.27 ± 0.77	16.17 ± 5.66	80.86 ± 38.40	45.40 ± 15.36
20	37.60 ± 10.8	287.48 ± 17.89	3.70 ± 0.73	751.16 ± 64.03

These parameters could not be calculated for the other lagoons due to the lack of bacterial decrease together with the first virus increase in the time-course incubations

VMM virus-mediated mortality, *VIBM* virus-induced bacterial mortality, *Bs* burst size

Table 3 Physical-chemical parameters measured at the 20 tropical coastal lagoons in Rio de Janeiro state (Brazil), in January 2015

Lagoon	Chl-a (μg L^{-1})	DOC (mM)	Water color (m^{-1})	TP (μM)	DN (μM)	pH	DO (%)	Turb (NTU)	Temp (°C)	Sal (‰)
1	14.5	1.48	14.0	16.2	129	6.1	140	0.9[b]	25.9	0.5
2	7.4	2.23	21.8	2.7	92	4.9	101.5	1.0[b]	26.7	0.9
3	0.9	2.13	2.6	1.2	143	6.6	112	0.4[b]	27.2	12.0
4	134.5	1.08	2.2	9.3	238	5.8	78	51.5	24.9	1.1
5	2.7	0.74	4.3	0.8	40	5.2	92.2	4.9	27.6	0.3
6	3.5	1.14	36.4	0.9	75	6.2	79.7	5.4	28.0	0.1
7	9.0	1.23	24.4	1.9	60	5.6	92	18.3	30.2	0.1
8	28.4	1.21	1.9	2.3	82	5.4	91.2	9.3	26.8	18.6
9	2.5	1.05	3.5	1.6	75	5.3	83	6.4	27.4	7.4
10	0.3	1.17	1.8	0.8	90	4.5	79.6	2.3	26.8	22.1
11	2.0	0.71	4.9	2.6	69	4.1	91	4.5	26.1	1.7
12	16.7	>16.7[a]	92.5	37.5	1248	3.2	72.7	571	29.2	3.1
13	3.3	4.70	4.8	2.6	296	7.0	114	8.2	23.0	93.2
14	1.4	1.35	4.9	2.3	237	7.6	94.4	2.8	25.2	46.9
15	37.7	4.90	52.0	1.3	113	3.3	8.9	26.2	23.4	0.3
16	11.6	>16.7[a]	12.4	39.3	1951	6.5	71.9	69.9	35.2	289.8
17	NA	>16.7[a]	53.2	156.0	8730	5.7	1.2	392	34.0	332.1
18	1.0	2.70	7.7	38.0	132	7.1	131.7	2.6	27.0	35.5
19	3.8	8.34	10.8	3.2	302	7.0	101.7	23.9	26.7	29.9
20	NA	2.43	4.8	11.5	361	5.7	181.6	73.3	25.8	43.4

DOC dissolved organic carbon concentration, *DN* dissolved nitrogen concentration, *TP* total phosphorus concentration, *DO* dissolved oxygen, *Turb* turbidity, *Temp* water temperature, *Sal* salinity

[a] Values above calibration curve

[b] Turbidity values measured from samples (kept at 4 °C) 2 months after sampling

was positively correlated to phosphorus ($r = 0.72$) and nitrogen ($r = 0.91$) concentrations, as well as nitrogen and phosphorus concentrations were positively correlated ($r = 0.81$). Carbon concentration was also positively correlated to water color ($r = 0.63$), indicating the strong influence of humic-rich water table to these lagoons. Both carbon and nitrogen concentrations were negatively correlated to oxygen saturation ($r = -0.60$ and $r = -0.56$, respectively). All nutrient concentrations were positively correlated to turbidity ($r = 0.71$, for nitrogen; $r = 0.58$, for phosphorus; $r = 0.70$, for carbon) and salinity ($r = 0.66$, for nitrogen; $r = 0.48$, for phosphorus; $r = 0.50$, for carbon).

HBA, salinity, and nitrogen concentration were shown to be together the most important explanatory variables for VA (Table 4 and Fig. 3). HBA, salinity, and their interaction constituted the most parsimonious candidate model for positively predicting the VA in these coastal lagoons (Table 4). Adding the interaction of nitrogen concentration and HBA slightly improved the prediction of VA but also increased complexity of the model (Table 4). BP and phosphorus concentration were selected as the best explanatory variables for VP; these variables comprised the only significant models that explained VP (Table 4 and Fig. 4). Adding either single or interactive

variables to the model only enhanced complexity but did not significantly improve prediction of VP (Table 4).

Discussion

Our main aim in this study was investigating which bacterial and environmental factors were most important to drive virus abundance and production in tropical coastal lagoons. Among many environmental factors, HBA and salinity have been clearly shown to be the main drivers of VA in the studied coastal lagoons as evidenced by our best-predicting models (Table 4). VA and HBA were strongly related (Fig. 3); both sharply increased with salinity and reached their highest numbers in the hypersaline systems (Table 3 and Fig. 3), which is in agreement with several previous studies [11, 38–40]. Conversely, VP and BP did not increase within a salinity gradient, revealing an apparent paradox of higher abundance despite unaffected production in increasingly salty systems. Nutrient concentration was demonstrated to be also an important factor explaining virus-bacterium interactions in these systems. Other usually important environmental virus regulators in aquatic ecosystems, such as water temperature and

Table 4 Best-fitting linear regression models describing virus abundance ($N = 20$) and production ($N = 16$)

Response variable	Model	AICc	Δ AICc	R^2	p value
Virus abundance	$\log_{10}VA = 3.55 + (0.68 \times \log_{10}HBA) + (0.68 \times \log_{10}Sal) - (0.06 \times \log_{10}HBA \times \log_{10}Sal)$	22.56	**0**	0.79	**<0.0001**
	$\log_{10}VA = -2.63 + (1.41 \times \log_{10}HBA) + (2.55 \times \log_{10}TN) - (0.28 \times \log_{10}HBA \times \log_{10}TN)$	22.94	**0.38**	0.78	**<0.0001**
	$\log_{10}VA = 3.20 + (0.75 \times \log_{10}HBA)$	24.61	**2.04**	0.68	**<0.0001**
	$\log_{10}VA = 23.72 - (2.26 \times \log_{10}HBA) - (10.43 \times \log_{10}Sal) - (10.81 \times \log_{10}TN) + (1.37 \times \log_{10}HBA \times \log_{10}Sal) + (1.57 \times \log_{10}HBA \times \log_{10}TN) + (5.93 \times \log_{10}Sal \times \log_{10}TN) - (0.78 \times \log_{10}HBA \times \log_{10}Sal \times \log_{10}TN)$	24.61	**2.05**	0.92	**<0.0001**
	$\log_{10}VA = 0.83 + (1.08 \times \log_{10}HBA) + (2.20 \times \log_{10}DOC) - (0.28 \times \log_{10}HBA \times \log_{10}DOC)$	26.32	5.44	0.73	**<0.0001**
Virus production	$\log_{10}VP = 7.42 + (0.54 \times \log_{10}BP)$	26.03	**0**	0.34	**<0.05**
	$\log_{10}VP = 7.56 + (0.47 \times \log_{10}TP)$	28.34	**2.31**	0.24	**<0.05**
	$\log_{10}VP = 7.36 + (0.35 \times \log_{10}BP) + (0.20 \times \log_{10}TP) + (0.18 \times \log_{10}BP \times \log_{10}TP)$	30.72	4.7	0.46	**<0.05**

Column indicates the delta AICc value that is used to select the best-fitting models. The significance of the models are shown in the last column with the p values (all in bold indicating significance) VA virus abundance, HBA heterotrophic bacterial abundance, VP virus production, BP bacterial production, Sal salinity, TN total nitrogen, DOC dissolved organic carbon, TP total phosphorus

concentrations of humic substances, carbon, and chlorophyll-a, were irrelevant for determining virus parameters in our study.

On the one hand, the VA in most of the lagoons was within the previously reported ranges of values for temperate inland waters with similar environmental features [5, 6, 11, 41]. On the other hand, we recorded higher VA than those found in other tropical freshwater ecosystems [16, 42–44]. Hence, the general hypothesis that virus would be less abundant in tropical than temperate systems due to higher UV radiation [16] is not supported by our data. We suggest VA would be determined by a combination of several internal environmental factors, without regard to latitude, that affect virus' hosts. The extremely high VA found in all hypersaline systems is in line with previous studies that recorded VA of up to 10^9 viruses mL^{-1} in hypersaline lakes [11, 38, 39], solar salterns [12], and crystallizer ponds [40]. However, we here recorded a remarkable maximum VA of $1.17\ 10^{10}$ virus mL^{-1} in the hypersaline lagoon 17 (Table 1), which is, to the best of our knowledge, the highest virioplankton abundance ever documented for aquatic systems.

The increasing VA, as a response to higher HBA, within a salinity gradient can be explained by two related factors. First, salinity constrains microzooplankton (i.e., nanoflagellates) presence [12, 45], releasing bacterial populations from predation pressure [46], which should increase HBA. Second, this pattern might be observed due to lower inter-specific competition as a result of a decreasing diversity [11] since a few bacterial groups are capable of surviving in such harshly osmotic conditions [47]. Some studies have shown that the prokaryotic community composition drastically shifts from freshwater to hypersaline conditions [48]. In fact, the bacterial community composition of the lagoons studied here has already been shown to greatly change as salinity increases [49]. The main change is observed with salt concentrations above 150‰, when the prokaryotic assemblage starts being dominated by Archaea instead of the typically dominant Eubacteria [11]. Finally, the higher increase of VA in comparison to HBA in hypersaline systems was probably related to a higher resistance of halophages to environmental virucidal agents [11] and, to a lesser extent, due to a lack of virivorous nanoflagellates or ciliates [12].

This is the first study to measure VP in tropical coastal lagoons. VP was found 1–2 orders of magnitude higher than recorded in sub-tropical [50] and tropical estuaries [51] and coral reefs [52] as well in temperate lakes [53], estuaries [54], and marine systems [27, 28, 55, 56]. VP was mainly explained by BP and phosphorus concentration (Table 4, Fig. 4). Several studies have demonstrated that higher VP is indeed associated to increasing BP rates [53, 55, 57], which is reasonable since the viral development depends on the metabolic state of their hosts [58]. Eutrophic systems usually present higher VP rates than oligotrophic ones [50, 53] because nutrient amendments
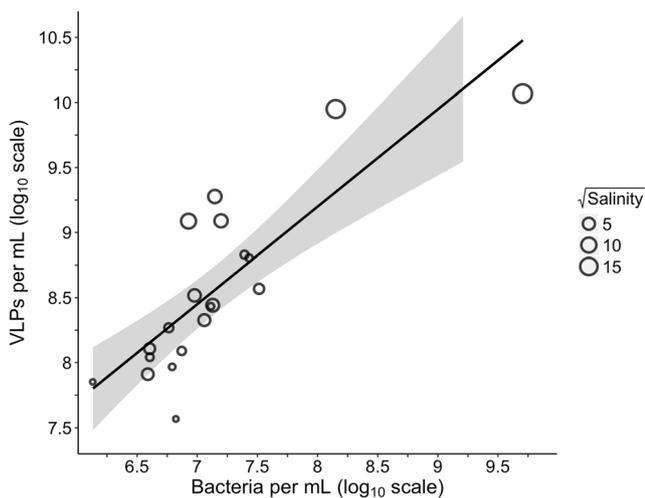
Fig. 3 Linear regression between virus abundance and heterotrophic bacterial abundance within salinity gradient. The *size of the circles* represents square-rooted salinity values. The *shadow area* around the regression line represents 95% confidence interval for the linear regression. The relationship is significant ($p < 0.05$)

enhance VP rates [57], which is endorsed by our observations (Table 1). However, the relationship between VP and phosphorus concentration is not mediated by changes in the bacterial metabolism (Fig. 4 and Table 4), which is supported by the
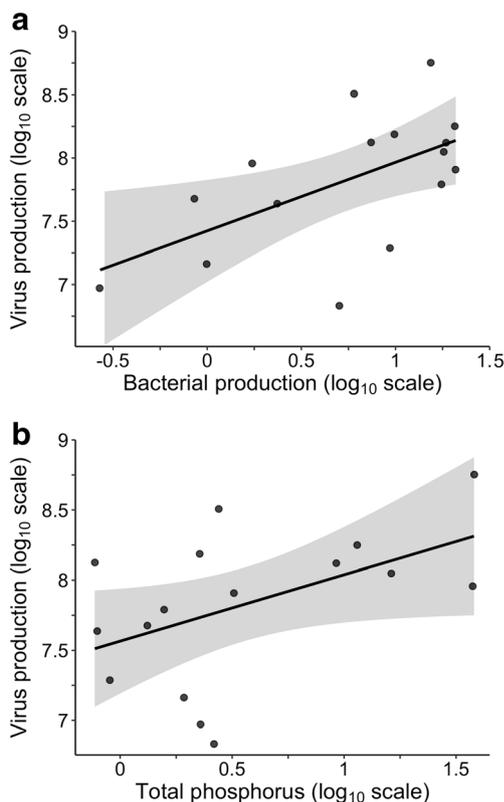
Fig. 4 Linear regression between **a** virus production and bacterial production and **b** virus production and total phosphorus concentration. The *shadow area* around the regression line represents 95% confidence interval for the linear regression. Relationships are significant ($p < 0.05$)

fact that phosphorus concentration was not correlated to BP (Table S2). This result contradicts previous findings that bacteria mediate the phosphorus effect on virus production [55] and suggests that P-limitation is potentially an important direct regulator of virus activity. Phosphorus has indeed been described as a direct limiting nutrient for virus proliferation since this element is a key component of DNA [59]. Moreover, the VP coupling with phosphorus concentration could be linked to phytoplankton not bacterial populations as a recent experimental study found higher VP from isolated picoautotrophs (*Micromonas pusilla*) under P-enriched conditions [60].

The lower BP found in highly salty, yet also nutrient-rich environments (Table 3), is in agreement with previous studies [12, 48]. This pattern is observed because prokaryotes need to protect themselves from the osmotic stress by using several energy-costing strategies [61]. For instance, halobacterial groups present several mechanisms to tolerate the high environmental salinities, such as increasing cytoplasmic salt concentration with or accumulating glycine betaine and other compatible solutes in order to avoid water loss through osmotic instability of their cytoplasm [62]. Such expensive mechanisms may negatively impact the bacterial physiological state and growth rate [11] and, in turn, affect the VP rates since smaller and stressed bacteria produce fewer viruses [63, 64]. Salinity may also constrain VP by slowing down or hampering the virus adsorption processes to their host cells [65]. These results point that VA and HBA increase within a salinity gradient regardless of VP and BP, suggesting that the lack of top-down control on bacterial and viral populations is indeed an important mechanism behind the high abundances in the saltiest ecosystems.

In general, calculated Bs values were mostly recorded to be similar to the range of values previously found in inland waters worldwide [7, 53, 63, 66]. Lagoon 2 was the only coastal lagoon with an extremely high average Bs value (Table 2) and greatly differed from any other system without apparent reason. High Bs values are usually supported by larger cell biovolumes [63] and growth rates [64]. Therefore, Bs was expected to increase with the trophic state of the lagoons, since bacterial cell size and growth rate are commonly larger in eutrophic than in oligotrophic systems [53]. Nevertheless, no consistent pattern regarding lagoon trophic status seems to emerge about the Bs variability among the systems where Bs could be calculated (Table 2). The lack of effect of environmental variables on Bs has already been observed in another study carried out in temperate lakes [6].

There was also no apparent environmental variable driving VIBM (%) values (Table 2). VIBM was recorded to be above 100% of bacterial production in three out of six lagoons where VIBM could be calculated (Table 2). This result is very likely to be overestimated and TEM analysis would be needed to further investigate it. Lagoon 14 presented a VIBM of 16%, which is nearly the same percentage observed in many

temperate systems [7, 53, 66, 67], but it is still at least twofold higher than recorded in a tropical inland waters [44, 51]. Lagoons 2 and 8 presented VIBM of >50% (Table 2). This is even higher than observed for tropical systems, but it is realistic if compared to a study conducted in an eutrophic temperate lake [68]. It is worth noticing that because BP is higher in tropical systems [17], VIBM may be of relatively greater importance for the carbon cycling in these ecosystems.

The amount of dissolved organic carbon estimated to be released from bacterial cells lysed by virus (Table 2) represents about 35 to 550% of the bacterial secondary production in the surface waters of these systems. These estimates need to be interpreted with caution since average bacterial carbon content may vary under different environmental conditions [69]. For instance, our calculations could be underestimated because bacteria living in highly productive coastal systems tend to have higher carbon content per cell (30 fg C cell^{-1}) [69]. Here we used a more conservative conversion factor (20 fg C cell^{-1}), and we found high percentages of bacterial production being controlled by virus activity. We thus suggest that viruses have a great impact on the bacterial community carbon cycling in the tropical coastal lagoons. This viral loop would decrease the amount of carbon that reaches higher trophic levels and increases the pool of dissolved carbon in the water column of these lagoons.

The fact that HBA decrease was not recorded in the time-course incubations of 10 out of 16 lagoons indicates that lysogeny is probably prevalent over lytic infection in these microbial abundant systems. This observation is in line with the recently proposed Piggyback-the-Winner model that predicts that lysogeny becomes more frequent with high microbial abundances (>10^6 microbes mL^{-1}) and growth rates [2]. We also recorded VA increase without HBA decrease in some of the time-course incubations (Table 1), which suggests that VP could have occurred without bacterial lysis. This phenomenon of continuous virus release without cell lysis is known as chronic infection [70], and it has been considered a potential mechanism of virus replication in harsh environments [11]. We could not find an association of these virus life strategies with any specific environmental condition, which is still a research challenge in the field of virus ecology [71]. Further investigations combining TEM, metagenomics, and VP techniques would lead to better insights into the virus life strategies in these systems.

Conclusion

This study provides novel data on virioplankton abundances and production from a large number of tropical coastal lagoons that present a wide range of co-occurring environmental conditions. We found very high virus abundance and production, indicating that virioplankton performs a substantial role in the biogeochemical cycles of tropical coastal lagoons. We conclude that salinity is the strongest environmental factor to positively impact virus abundance by affecting the abundance of bacterial populations. Virus production was mainly controlled by bacterial production and total phosphorus concentration. The uncoupling between virus abundance and production would only be possible because of a decreasing microzooplankton density and, consequently, lower grazing on bacterial and virus populations, in extremely salty ecosystems.

It is worth noticing that wetlands are currently undergoing salinization worldwide because of anthropogenic impacts such as wastewater effluent disposal, irrigation, applications of de-icing salts, and climate change-driven processes such as sea-level rise and shifts in precipitation patterns [72]. Our findings hint that coastal wetlands subjected to these changes could present a future with increasing virus abundances despite unaltered virus production. It is key for future research to experimentally investigate the interactive effects of salinization and eutrophication on the virus-bacterial dynamics in aquatic ecosystems.

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