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THE ECOLOGICAL ROLE OF VIRUS IN TROPICAL COASTAL LAGOONS

PEDRO CIARLINI JUNGER SOARES

Dissertation submitted to the Postgraduate Program in Ecology of the Federal University of Rio de Janeiro in partial fulfillment of the requirements for the degree of Masters in Ecology.

Supervisor: Dr. Vinicius Fortes Farjalla

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“If I could do it all over again, and relive my vision in the

twenty-first century, I would be a microbial ecologist…”

Edward O Wilson

“Y cuando por fin consiguió hablar, temblando, tartamudeando,

pidió a su padre: –¡Ayúdame a mirar!”

Eduardo Galeano

“Para mim, é muito melhor compreender o universo como ele realmente é do que

persistir no engano, por mais satisfatório e tranquilizador que possa parecer”

Carl Sagan

“Deixai que os fatos sejam fatos naturalmente

Sem que sejam forjados para acontecer

Deixai que os olhos vejam

os pequenos detalhes lentamente”

Corpo de Lama, **Chico Science**

“Pretending to know everything closes the door to finding out what’s really there”

Neil deGrasse Tyson

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TABLE OF CONTENTS

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ABSTRACT

Viruses are the most abundant biological entity in nature and play key ecological, evolutionary and biogeochemical roles in aquatic ecosystems. Even though our knowledge on virus ecology has improved over the last two decades, there is very limited information on virus abundance, diversity and ecological importance in tropical ecosystems. This dissertation firstly issues an overview on viruses regarding their abundance, diversity, replication strategies and impacts on aquatic microbial communities. Scientometrics data prove that most studies have focused on virioplankton of temperate marine systems, yet there has been an increasing number of studies towards the tropics over the last 5 years. In this study, virus and bacterial parameters and their response to limnological characteristics were examined in 20 tropical coastal lagoons. Very high abundances were recorded, especially in the hypersaline lagoons. Regression models pointed that salinity is the main environmental variable positively driving bacterial and viral abundances. Virus production was mainly explained by bacterial production and virus abundance, revealing an apparent paradox of high abundance but low production in hypersaline lagoons. This pattern is likely observed because of both the nonexistence of bacteria's predators and the decreasing bacterial diversity in highly salt aquatic systems.

Keywords: microbial food webs, virioplankton, viral loop, bacterial metabolism, shallow lagoons, aquatic ecosystems

RESUMO

Os vírus são a entidade biológica mais abundante na natureza e desempenham papéis ecológicos, evolutivos e biogeoquímicos essenciais nos ecossistemas aquáticos. Apesar do nosso conhecimento sobre a ecologia dos vírus ter avançado nas duas últimas décadas, ainda existe pouca informação sobre a sua abundância, diversidade e importância ecológica em ecossistemas tropicais. Primeiramente, esta dissertação disponibiliza informações gerais sobre abundância, diversidade e estratégias de replicação dos vírus, além de seus impactos sobre as comunidades microbianas aquáticas. Dados cienciométricos comprovam que a vasta maioria dos estudos procurou investigar virioplâncton em sistemas marinhos temperados, embora haja um número crescente de estudos em ecossistems trópicos e sub-tropicais nos últimos 5 anos. No presente estudo, diversos parâmetros bacterianos e virais foram medidos e a suas respostas às características limnológicas foram avaliadas em 20 lagoas costeiras tropicais. Valores altos de abundância foram registrados nestes sistemas, especialmente nas lagoas hipersalinas. Os modelos de regressão desenvolvidos neste estudo confirmam que a salinidade é a principal variável ambiental influenciando positivamente as abundâncias bacterianas e viral. A produção viral estava relacionada com a produção bacteriana e abundância viral, revelando um aparente paradoxo da elevada abundância elevada, porém baixa produção em lagoas hipersalinas. Este padrão é provavelmente observado devido à inexistência de predadores de bacterias e uma diversidade bacteriana decrescente em sistemas aquáticos com altíssima concentração de sal.

Palavras-chave: redes tróficas microbianas, virioplâncton, alça viral, metabolismo bacteriano, lagos rasos, ecossistemas aquáticos

ABBREVIATIONS

BCD	Bacterial carbon demand
BP	Bacterial production
BR	Bacterial respiration
Bs	Burst Size – the amount of phages that are released upon lysis of a cell.
TEM	Transmission Electron Microscopy
VBR	Virus-bacterium ratio
VLP	Virus like particles
VP	Virus production (VLP mL^{-1} h^{-1})

GENERAL INTRODUCTION

GENERAL INTRODUCTION

Viruses are the most abundant biological entity in nature and are found wherever there is life (Suttle 2005). They are a sort of life classified as capsid-encoding organisms, which is in contrast with a second group called ribosome-encoding organisms, or simply, all cellular life forms (Koonin and Dolja 2014). Viruses fully rely on hosts' cellular apparatus to replicate themselves and can infect several types of microorganisms such as eukaryotic algae (Brussaard 2004a, Short 2012), cyanobacteria (Suttle 2000a) and bacteria (Wilhelm and Suttle 1999). They are called virions when in an extracellular form, which is basically a packet of genetic material (DNA or RNA) folded by a protein involucre. This biologically inactive state of viruses is usually between 10-300 nm in length (Prescott 1999) and move only by Brownian motion, which means that they depend on randomly contacting a suitable host to initiate infection. Owing to that, their relative concentration to a host has an important role to determine viral infection in a microbial community (Wommack and Colwell 2000).

Virus ecology has advanced considerably over the last two decades both in marine and freshwater ecosystems (Suttle 2007, Wommack and Colwell 2000). Many studies have provided very relevant information on the virus ecological importance such as for microorganisms' mortality, food web interactions, biogeochemical cycles, genetic exchange and evolution (Fuhrman 1999, Wilhelm and Suttle 1999, Suttle 2000b, Buckling and Rainey 2002, Brussard 2004, Anesio and Bellas 2011). Plenty of these investigations are about virus-bacterium interactions because virus' main hosts are bacteria (Middelboe et al. 2008) and most of them have been carried out in marine environments (Figure 1C). Even though there has been an increasingly effort to examine the ecological role of viruses in freshwater ecosystems (Wilhelm and

Matteson 2008, Jacquet et al. 2010), those located in tropical regions are still poorly understood (Figure 1A). Further details on virus abundance, diversity, replication strategies and ecological importance are discussed in the following sections.

VIRUS ABUNDANCE AND DISTRIBUTION

Viruses are the most numerous biological entities in nature and are estimated to be 10^{31} on Earth (Breitbart and Rohwer 2005). The first report to suggest that viruses were very abundant in marine systems was published in the late seventies (Torrella and Morita 1979) and was then confirmed about one decade later (Bergh et al. 1989). The first estimations made by transmission electron microscopy (TEM) technics were inaccurate and better results were only possible when new methods using epifluorescence microscopy and flow cytometry (Figure 2) were developed over the following years (Brussaard 2004b), which revealed that viruses were nearly 15 times more abundant than Bacteria and Archaea (Bettarel et al. 2010). Viruses can reach an abundance of 10^{9} to 10^{11} viruses particles per litre in surface waters (Wommack and Colwell 2000) and from 10^{7} to 10^{10} g^{-1} of dry sediment in aquatic ecosystems (Danovaro et al. 2008). Their abundance accounts to 90% of the total planktonic particles' abundance, however, viruses are very small (30–89 nm) and thus stand for only 10% of the total carbon biomass (Suttle 2007), although their C biomass has been estimated to be nearly 25 times larger than the carbon in protists (Peduzzi and Luef 2009).

Virus abundance tends to increase in nutrient-rich environments and therefore freshwater ecosystems have generally more viruses than marine ones (Wilhelm and Matteson 2008). This increasing virus abundance within a productivity gradient is also evidenced in marine environments once coastal areas are also more virus

abundant than offshore zones as well as surface waters are in comparison to deep waters (Weinbauer et al. 1993). This variation in virioplankton abundance is, however, observed in one to two orders of magnitude, while planktonic biomass increases more than three orders of magnitude within trophic gradients such as those observed in the same environment (Wilhelm and Matteson 2008). Even though long-term studies are still needed to further understand virus abundance fluctuation over time, it has been shown in temperate regions that freshwater ecosystems present stronger seasonal variability in virus abundance than marine systems (Wilhelm and Matteson 2008). This pattern would happen because freshwaters, especially lentic systems, are more susceptible to temporal fluctuations in environmental factors (Wetzel 2001) already proven to affect virus such as temperature and nutrient concentration (Mathias et al. 1995, Wommack and Colwell 2000).

VIRUS DIVERSITY

Viruses may be classified by several different approaches. One of the most common ways of phenotypically classifying them is based on their forms directly observed by transmission electron microscopy (TEM). This method has revealed that viruses from aquatic samples may be divided basically into two groups: tailed and untailed phages. Even though 96% of overall bacteriophages have been described to be tailed particles (Ackermann 2007), non-tailed viruses dominate (50–90%) natural aquatic samples and have been recently suggested to be the most ecologically important viral group in aquatic microbial communities (Brum et al. 2013).

Environmental viruses are expected to present a large genetic diversity due to their extremely high abundance and morphological diversity all over the world. Indeed, viruses represent the largest genetic reservoir on Earth (Hambly and Suttle

2005). However, most of this genomic diversity has been for a long time unknown since only viruses from isolated hosts (about 1%) could be studied (Hugenholtz et al. 1998). More recently, the development of new molecular approaches has allowed microbial ecologists to sequence uncultured natural communities (Radford et al. 2012, Adriaenssens and Cowan 2014, Brum and Sullivan 2015, Brum et al. 2015), by that means gradually making progress toward a better understanding of the global viral genomic diversity and its geographical distribution. Despite this current scientific advancement to characterize viral assemblages and separate them into protein clusters, there is still no global estimate of the percentage of already sequenced aquatic viruses, but it has been suggested that there would be at most 3.9 M protein clusters in the global virome (Ignacio-Espinoza et al. 2013).

Overall, virus diversity is driven by environmental factors affecting their hosts' diversity (Brum et al. 2015). This massive global survey has recently shown that the oceans virus community is mainly determined by local environmental variables such as temperature and oxygen concentration (Brum et al 2015). This virus community structure response to environmental variables follows the same pattern observed for the microbial community structure (Sunagawa et al. 2015). There is additional evidence that virus diversity changes over time and space in aquatic ecosystems. For instance, a recent metagenomic study carried out in the Pacific Ocean found that virus functional richness decreased along a transect from open-ocean to near-shore waters, as well as from deep to surface samples (Hurwitz and Sullivan 2013). However, a recent study with a larger dataset found that virioplankton community is not structure by depth (Brum et al. 2015). Virus diversity is affected by seasonality since it was functionally richer during the winter period rather than the summer (Hurwitz and Sullivan 2013). There has been only one study that compared virus diversity between

two temperate lakes (Roux et al. 2012). This survey firstly found that freshwater virus communities contrast with those from marine systems. The authors also demonstrated that a mesotrophic lake had higher virus richness and diversity than an oligotrophic one (Roux et al. 2012), which is in agreement with Brum et al. (2015) who found an influence of nutrient concentration on virus diversity This is an expected outcome since a trophic state effect on diversity is also observed for virus' hosts such as bacteria (Boucher et al. 2006). Interestingly, an Antarctic lake was found to have greater virus richness than any other aquatic environment, which generally contradicts higher organisms' distribution trend of having a decreasing diversity from the tropics towards the poles (Fuhrman et al. 2008).

VIRUS LIFE STRATEGIES

Viruses have basically three different mechanisms of infecting their hosts: lytic infection, lysogeny (Figure 3) and chronic infection. In lytic infection the virus genetic material is injected into the host cell where it replicates itself by using the cell biosynthetic apparatus, lysing the cell and thereby releasing the new viruses to the surrounding environment. In the lysogenic pathway, the inserted virus genome is attached as a prophage in the bacterial genome. This prophage can remain dormant for many generations while being copied as the bacteria divide into new daughter cells, up until the time it is induced to a lytic pathway. It has been shown that this switch from lysogenic to lytic infection is activated by several factors such as UV radiation and nutrient concentration (Weinbauer and Suttle 1996; Fuhrman 1999). Interestingly, this process can be easily confounded with a phenomenon known as pseudolysogeny (Ripp and Miller 1998). This event would be a virus life strategy only observed when resource conditions are unfavorable and is basically

characterized by a delay in the virus latent period, which resembles lysogeny once host cells remain alive, yet presenting an increasing infection and a low amount of viruses released from a lysis event, which is named in the literature as burst size (*Bs*). Finally, chronic infection is an unusual mechanism in aquatic microbial organisms; it is commonly performed by filamentous phages and has been more frequently reported in marine protist (Mackinder et al. 2009) and green algae (Thomas et al. 2011; Clerissi et al. 2012). However, there have been studies detecting potential chronic cycles in Archaea (Porter et al. 2007) and Bacteria (Bettarel et al. 2011; Bellas et al. 2013) living in extreme environments. This kind of virus cycle does not cause the hosts's lysis whilst using their cell apparatus, but constantly releases progeny phage particles by budding or extrusion, which may worsen the bacteria fitness.

There also seems to be a habitat control on virus-bacterium interactions. Even though a strong relationship between *Bs* and bacterial production (BP) has not been detected across different systems (Parada et al. 2006), bacterial metabolic activity determines cell size and morphology and should then be indirectly linked to *Bs* (Simek et al. 2001). Some studies have indeed found that higher bacterial activity leaded to larger bacterial burst sizes (i.e.: Hwang and Cho 2002, Bettarel et al. 2004) and thus, virus hosts are expected to yield a higher amount of phage progeny in favorable environments. On the other hand, environments under poor conditions should have low hosts quality, which in turns lead to a lower virus production due to an increasing latent period of lytic phages (Proctor et al. 1993; Middelboe 2000). For instance, environmental factors such as nutrient limitation (Wilson et al. 1996) and light availability (Bratbak et al. 1998) were found to reduce *Bs* of phytoplankton cells. Additionally, bacteria had their usually rapid lysis delayed under experimentally limited glucose conditions (Los et al. 2003), although this effect may also be

conditional on the phage strain (Moebus 1996). Also, Ripp and Miller (1998) accounted in their experiment an increase in the incidence of infected cells from 13.2% in nutrient enriched conditions to 45% in nutrient limited treatments, with a reduction of 40% in the occurrence of lysis.

EVOLUTIONARY IMPORTANCE

Viruses are also important for microbial life evolution. Besides representing a sort of parasite pressure on their host populations, they have been proved to be able to transfer genetic material between hosts through a process known as transduction (Koonin and Wolf 2008; Rohwer and Thurber 2009). This sort of lateral gene transference, which happens independently of reproduction, takes place very frequently in natural waters and has been estimated to move nearly a thousand genes per year in the ocean worldwide (Rohwer and Thurber 2009).

Viruses may also supply their hosts with genes of their interest and their presence may be essential for generating strains and ecotypes (Rodriguez–Valera et al. 2009). For instance, it has been estimated that 10% of total global photosynthesis could be carried out as a result of genes originated from phages (Rohwer and Thurber, 2009). They may also be of major importance to bacterial adaptation in extremely cold environments such as cryoconite holes (Bellas et al. 2013). This long-term association between viruses and their specific hosts may either reduces their fitness or represent a mutualistic symbioses that can confer competitive advantages to hosts (Roossinck 2011).

ECOLOGICAL INTERACTIONS AND MICROBIAL COMMUNITIES REGULATIONS

Viruses and grazers usually compete for the same prey in aquatic ecosystems (Bratbak et al. 1992). For instance, viruses kill about 20-40% of the marine bacterial population in a daily basis (Suttle 2005), which is a quite similar rate observed in grazing pressure exerted by predators (Fuhrman and Noble 1995). In some extreme environments such as cryoconite holes (water-filled depressions on glaciers' surface), where grazers are not abundant, viruses are probably the main cause of bacterial mortality (Säwström et al. 2008). When lysogeny or pseudolysogeny occur, new metabolic traits may be provided by viral genomes that stay within host cells, which in turns can confer both an increasing host's fitness due to an improved immunity and the phage's survival, which characterize a mutualistic interaction between viruses and their hosts. This sort of interaction is in fact expected to be common since 3–10% of bacterial genomes are composed by DNA from prophages (Brüssow and Hendrix 2002).

A novel research has recently shown that viruses were responsible for triggering a bottom-up cascade effect in an African lake (Peduzzi et al. 2014). In this study, viruses caused a decline of cyanobacteria (*A. fusiformis*) population, the main food source of the Lesser Flamingo (*P. minor*), which in turns had their population collapsed.

The microbial community can be regulated by selective virus infection. For instance, when certain bacteria specie's abundance increases, the bacteria likelihood to contact their associated viruses becomes higher. This growth leads to an increasing viral-induced bacterial mortality, which in turns controls that dominant bacteria population (Bouvier and del Giorgio 2007), avoiding a possible exclusion of other

species. This control over bacteria species composition in a microbial community has been called as “killing the winner” (Thingstad and Lignell 1997). Indeed, a recent experimental work has demonstrated that viruses thwarted the dominance of competitively superior bacteria taxa in the Mediterranean Sea and suggested there would be a higher bacterial diversity at intermediate virus production to bacterial production (VP:BP) ratio (Motegi et al. 2013). Nonetheless, other authors have proposed that this hypothesis might be wrong in some cases, since it has been proved that the same virus type may infect bacteria from completely different environments (Sano et al. 2004), which implies that they are not necessarily as host specific as suggested before.

Virus-mediated mortality may also indirectly affect microbial composition and community structure by altering the composition and bioavailability of ambient organic substrates and nutrients through the release of organic matter to the environment (Sime-Ngando 2014). A bacterial community submitted to both grazers and viruses pressure are supposed to have their relative abundance, production and species richness increased, which was in fact described for bacterial phyla such as *Flectobacillus* or *Actinobacteria* (Šimek et al. 2007).

VIRUS EFFECT ON ECOSYSTEM FUNCTIONING

Virus pressure may require a resistance reply from their hosts, which has an energetic cost that generally lead to a decrease in the host’s fitness and growth rate (Thomas et al. 2011). This is observed, for instance, when bacteria produce proteins and extracellular matrices that disguise the phage receptor (Bohannan and Lenski 2000). Virus-infected cells have indeed been shown to be physiologically different from non-infected ones (Ankrah et al. 2014), however, there is also evidence that

infected cells have their metabolism active until cell lysis and bacterial growth is similar in infected and non-infected cells from a given bacterial population (Middelboe 2000). Further studies are still needed to come to a conclusion on this topic.

Viruses may be very relevant for ecosystems functioning as they directly affect the activity of two crucial players on the carbon cycle: bacteria and phytoplankton. Bacterial metabolism is known to be a crucial factor for ecosystems functioning (Cotner and Biddanda 2002). Bacterial respiration (BR) has been shown to increase by 33% under viral lysis activity when compared to hypothetical virus-free systems in a modeling scenario where half of the bacterial mortality was virus-mediated (Fuhrman 1999). Another study has experimentally showed that the virus effect on respiration of bacteria, microplankton and single species of phytoplankton was actually very variable and not very conclusive (Eissler and Quiñones 2003). Nevertheless, there is also experimental evidence that BR increases while BP decreases under virus presence, which then magnifies the bacterial role as organic matter oxidizers or, in other words, as CO_2 producers and nutrients (N, P or Fe) remineralizers (Bonilla-Findji et al. 2008). Additionally, BP suppression by virus activity has been suggested to happen due to a viral lyses effect on total number of bacteria, which represent a top-down control (Middelboe and Lyck 2002).

Viruses may affect the carbon and nutrient cycle in food webs by lysing both prokaryotic phytoplankton and bacteria and thereby reintroducing fixed carbon into the system. This short-circuit, also known as viral loop (Bratbak et al. 1992), decreases the amount of organic carbon that is shifted to higher trophic levels as well as increases the carbon and nutrient availability to photosynthetic and heterotrophic microorganisms in the bottom of the food web (Figure 4). The amount of organic

carbon that flows through this "viral shunt" has been estimated to be 25% of the total in marine systems (Wilhelm and Suttle 1999) and viruses have been estimated to release 10^8–10^9 tons of carbon day^{-1} to the world's ocean (Suttle 2007), demonstrating how significant they are for aquatic biogeochemical cycles. This process is believed to be of major importance in nutrient limited ecosystems due to the release of limiting nutrients, such as N and P, to the surrounding environment (Fuhrman 1999). Viruses have also been demonstrated to be responsible for releasing more than 10% of ambient iron (Fe) concentration in marine environments (Gobler et al. 1997). This virus-mediated release of Fe would be enough to meet the metabolic need of marine phytoplankton (Poorvin et al. 2004). Virus may also affect dissolved organic matter composition. For instance, a recent laboratory study showed that virus's activity changed the DOM composition by increasing the contribution of labile components in batch cultures (Lønborg et al. 2013). A recent modelling study has shown that virus increases the abundance of organic and inorganic nutrients, which in turns increase the heterotrophic bacteria and autotrophic total biomass in marine surface waters (Weitz et al. 2014).

VIRUS IN THE TROPICS: STATE OF THE ART AND PERSPECTIVES

Aquatic virus ecology has been much less studied in tropical and sub-tropical systems than in temperate ones (Figure 1A). However, their relative number has consistently increased throughout the last half-decade (Figure 5). We here claim that aquatic virus might have an important role in tropical and sub-tropical aquatic carbon cycling and should have its ecology more investigated towards the tropics due to several factors. Firstly, tropical ecosystems have higher bacterial production (BP) than temperate ones (Amado et al. 2013). Secondly, tropical aquatic systems are more

efficient in producing phytoplankton biomass on a given nutrient base (Lewis 1996), but such production is less efficiently passed to the highest trophic levels. These two primarly factors together lead to a larger amount of carbon flowing through tropical microbial food webs (Sarmento 2012) and therefore the quantity of DOC and nutrients released by virus impact on their main hosts, bacteria and phytoplankton, is expected to be higher in tropical aquatic ecosystems.

Tropical aquatic ecosystems are very different from temperate ones in several ways. Their main difference regards the average solar irradiance that directly affects other environmental variables such as temperature and nutrient concentration (Sarmento 2012). These environmental factors are critical for bacterial activity (Del Giorgio and Cole 1998; Farjalla et al. 2002, López-Urrutia and Morán 2007, Sarmento and Gasol 2012) and would in turn affect virus dynamics since bacteria are their main host as indicated by their usually strong correlation (Anesio et al. 2007). Bacterial metabolic rates (bacterial production - BP, bacterial respiration - BR and bacterial carbon demand - BCD) have been shown to be higher in tropical inland aquatic ecosystems than in temperate ones (Amado et al. 2013). For that reason, COD release by the virus impact on bacteria should be higher in tropical aquatic ecosystems. These systems should also present accelerated cell lysis and increasing *Bs* rates because fast growing cells are supposed to provide more resources for phage formation (Parada et al. 2006). Alternatively, *Bs* might also be lower in tropical regions due to the high average solar irradiation impact on the virus host's metabolism. These adverse conditions could also induce the lysogenic cycle, which would decrease the bacterial mortality mediated by virus and as a result reduce the amount of nutrients released to the environment. In general, tropical regions do not present the same pronounced seasonal cycles mainly regulated by temperature

observed in temperate regions (Sarmento 2012). Seasonality is actually more linked to temporal changes in precipitation that regulate mechanisms such as nutrient and organic matter entrance as well as plankton dilution in water bodies. Thus, virus abundance and its ecological impacts may present distinct temporal variability.

Tropical regions present a wide range of potentially interesting aquatic systems that may have alternative patterns of virus-host interactions. Many tropical freshwater ecosystems are already eutrophic or hypereutrophic and numerous others are likely to become eutrophic due to the economic growth of countries located in tropical regions (Sarmento 2012). For instance, semi-arid man-made lakes are mostly hypereutrophic or eutrophic (Bouvy et al. 2000, Sousa et al. 2008) and may represent a potential virus hotspot since productive aquatic systems seem to have higher VBRs due to an increasing relative abundance of non-bacteriophage viruses (Bettarel et al. 2003) and higher *Bs*, contact and infection rates (Sime-Ngando 2014). Even giant viruses might be found in these eutrophic environments once these sort of recently discovered viruses have already been found in eutrophic reservoirs (Sommaruga et al. 1995). Furthermore, tropical coastal lagoons present a wide range of characteristics regarding salinity, humic substances and dissolved organic carbon (DOC) concentration (Suhett et al. 2012). Even extreme environments, such as hypersaline lagoons (70–375‰), may be found in these locations and endemic extremophiles microorganisms could be discovered (Cardoso et al. 2011) once other extreme environments such as deserts (Prigent et al. 2005, Prestel et al. 2008) and deep-dark permanently anoxic volcanic lake sediments (Borrel et al. 2012) have already be shown to present unique types of viruses.

Virobenthos have been much more understudied than virioplankton in all systems (Figure 1D), but such difference is more remarkable in tropical and

subtropical regions. There has been only one single viriobenthos study carried out in the tropics (Senegal) and three others performed in sub-tropical regions, which together accounted for only 7% of all viriobenthos studies. Least of the tropical and subtropical studies (34%) were conducted in inland waters. Most of these studies were carried out in lentic ecosystems, but tropical regions are also very rich in rivers and streams, important carbon processing hotspots, which have recently been shown to be understudied in terms of virus ecology (Peduzzi 2015). For instance, in tropical forests such as the Amazon, there is a massive amount of recalcitrant, terrestrial, dissolved organic matter entering a large number of rivers and floodplains, making them one of the most important locations for C cycling. Viruses probably play an important role to accelerate this proccess by repetitively infecting and lysing their hosts (mainly bacteria), constantly releasing labile material to the environment. This remineralization mediated by bacteria–virus loop is expected to increase at each turn of the loop (Pollard and Ducklow 2011) and may be even more relevant in tropical regions once remineralization is faster in warmer systems (Amado et al. 2013). Besides, the virus-mediated bacterial lysis would represent a permanent source of labile organic matter that might potentially speed up the degradation of recalcitrant organic material by means of a process called priming effect (Guenet et al. 2010). There have been only two studies in the Amazon (Barros et al 2010, Almeida et al. 2015) and we still know almost nothing about how virus might be affecting all these C-related processes.

GENERAL OBJECTIVE

This dissertation aims to investigate virus-bacterium interactions and their response to environmental variables in tropical aquatic ecosystems, which have been

understudied in comparison to temperate systems. In order to achieve this goal, virus/bacteria abundance and production were determined alongside with limnological parameters in tropical coastal lagoons. We used these shallow lagoons as natural model tropical aquatic systems because they are relatively close to each other while presenting a wide range of environmental characteristics. To the best of our knowledge, this is the first study to investigate such responses in a large set of ecosystems combining widely varying environmental factors. Further details about the reasons, procedures and hypothesis of this investigation are provided in chapter 1.

Figures

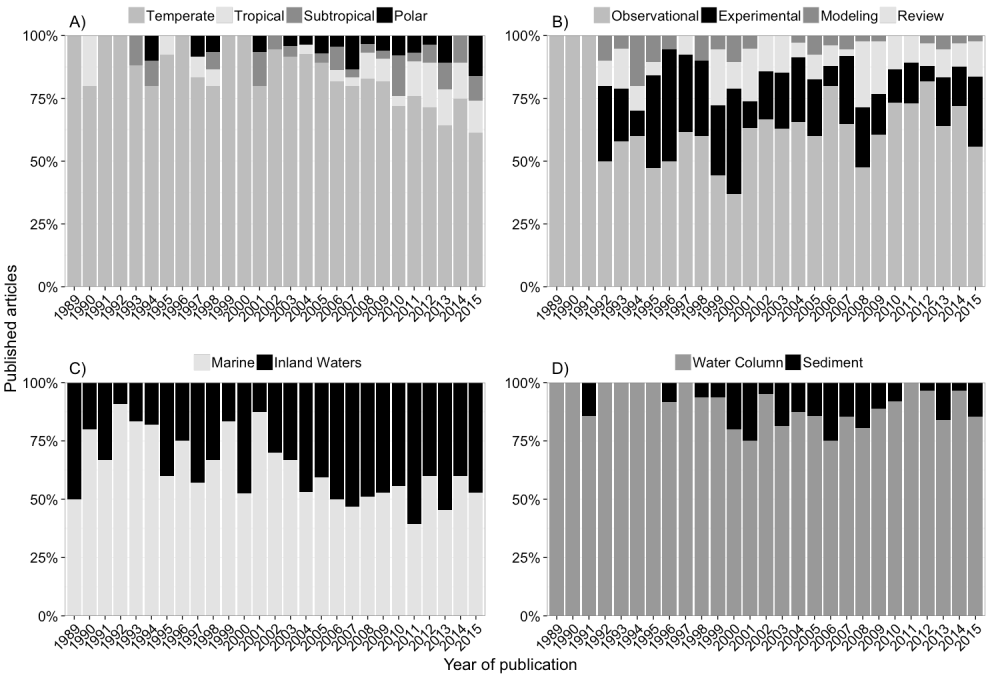

Figure 1. Relative contribution of different research subjects within aquatic virus ecology. A) Climate; B) Approach; C) Ecosystem; D) Compartment.

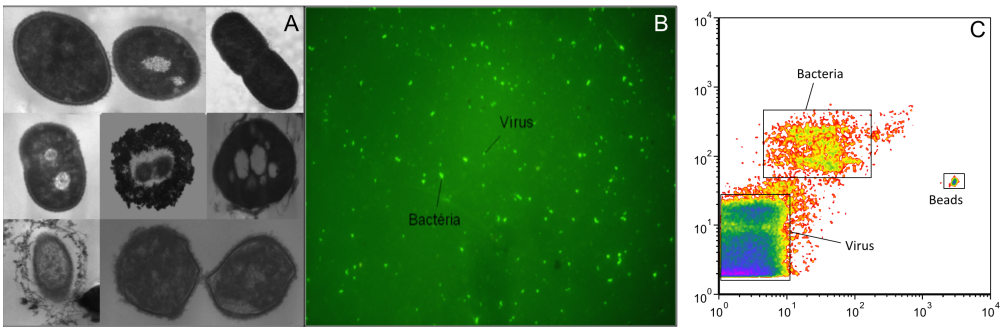

Figure 2. Three different methods available for enumerating aquatic virus: A) transmission electron microscopy (TEM) (photo: Nathan Barros); B) epifluorescence microscopy (EFM) (photo: Nathan Barros) and C) flow citometry (FC), x-axis is sidescatter/SSC; y-axis is green-fluorescence/FL-1 (cytogram generated using FSC Express 4: Anderson Cabral).

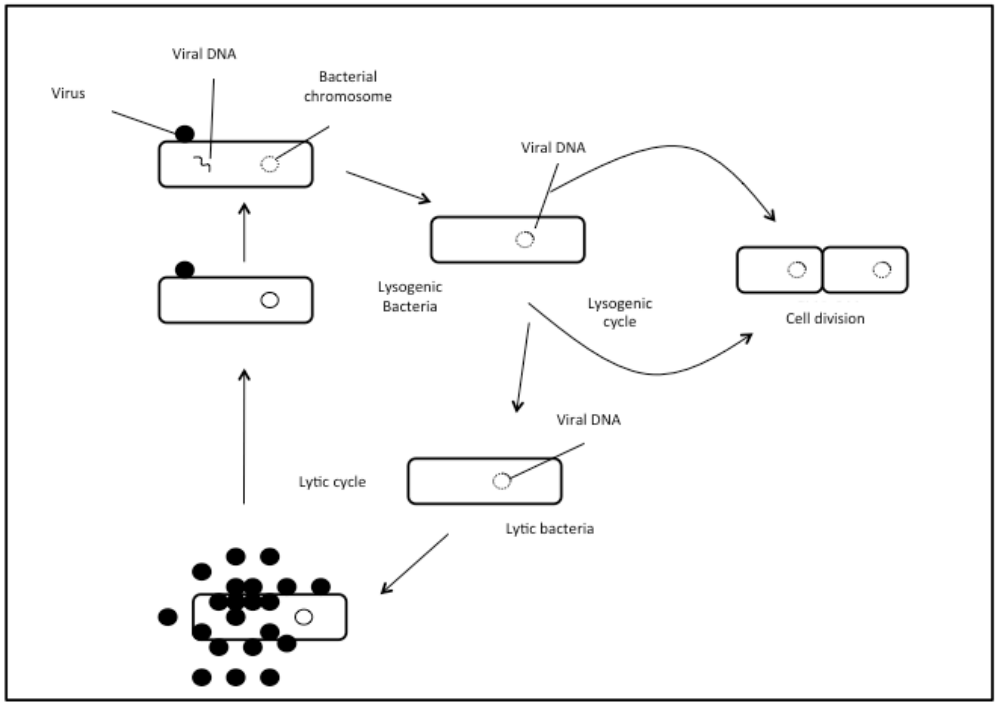

Figure 3. Simplified scheme showing the two main virus replication strategies: lytic and lysogenic cycles.

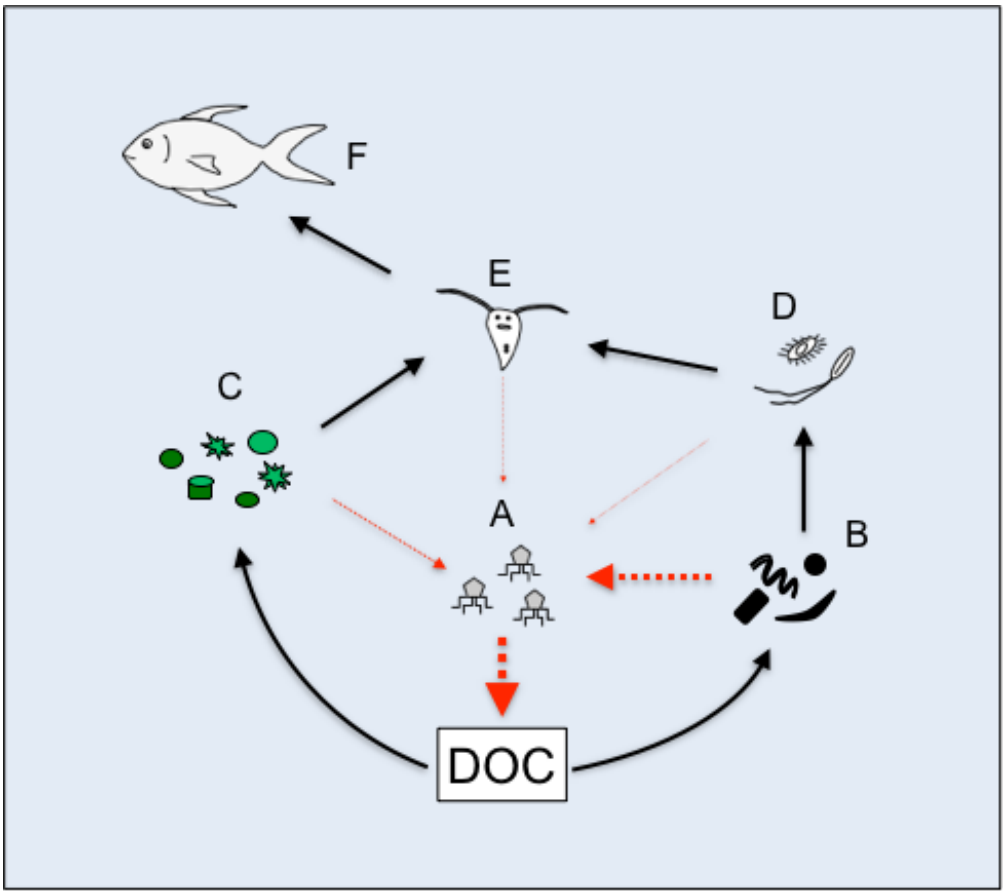

Figure 4. Simplified scheme showing how virus impact the carbon flow in planktonic food webs of aquatic ecosystems. Virus (A) may infect all organisms, but their main hosts are bacterioplankton (B) and phytoplankton (C), respectively. Virus cause their hosts'cells lysis, whereby releasing dissolved organic carbon (DOC) of high liability to the water. The viral loop decreases the amount of DOC that reaches higher trophic levels, such as microzooplankton (flagellates and cilliates – D), macrozooplankton (rotifers, cladocerans and copepods – E) and predators (i.e: fish – F) and increases the pool of DOC available for the bottom (B and C) of both the classic (C, D, F) and the microbial (B, D, E) food webs. Black arrows represent carbon flow through the food chain. Red dashed arrows stand for virus-mediated carbon release and their thickness roughly depict their magnitude.

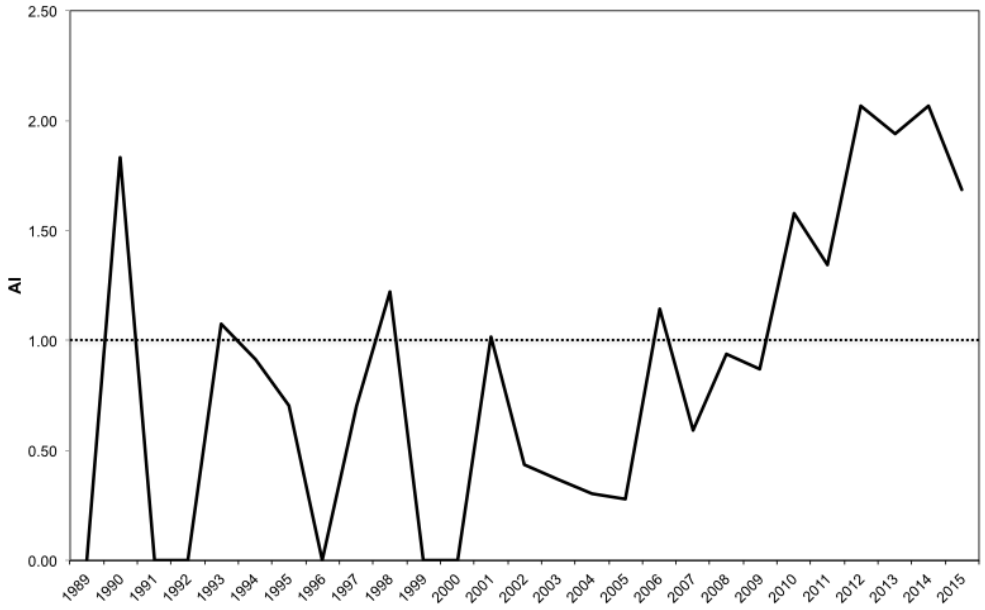

Figure 5. Temporal trends of published articles on virus ecology conducted in tropical and subtropical regions for the period from 1989 to 2015. The solid line stands for the activity index (*AI*). When $AI > 1$ (horizontal dashed line), the papers carried out in tropical and subtropical systems were published at a high relative frequency compared to those conducted in all aquatic systems (for more details see methods in supplementary material).

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SUPPLEMENTARY MATERIAL

Scientometrics data extraction

To evaluate the progress of scientific publication in aquatic virus ecology research, we used ISI Web of Science to survey for all articles published from August 1989 (first publication found) to December 2015 using the following searching key words: TOPIC ((viruses OR bacteriophages OR cyanophages) AND ("aquatic environments" OR "aquatic *systems")) *OR* TOPIC**:** (virioplankton OR viriobenthos). This search resulted in a total of 1292 articles, but many of them were still related to other research areas (i.e.: biotechnology, medical and veterinary sciences), were conducted in environments not related to our study (i.e.: wastewater or soil) or did not present any virus data. These studies were manually excluded from our analysis and, at the end, we reached a final number of 595 articles that explicitly studied virus ecology in natural aquatic ecosystems and thus were taken into account in our study. These studies were grouped according to year of publication and then clustered into categories based on the aquatic ecosystem studied (marine or inland waters freshwater), the compartment under study (water column or sediment), the climate (temperate, subtropical, tropical, or polar) and the methodological approach employed (observational, experimental, modeling or review).

Marine ecosystems were considered to be studies performed in the ocean and estuaries. Inland waters ecosystems consisted of rivers, wetlands, lakes and lagoons (including brackish, saline and hypersaline systems). Water column studies were considered all those that studied virioplankton. Sediment studies were those that investigated virus in sediments (viriobenthos), microbial mats and other substrates. Observational studies were those that carried out field measurements. Experimental studies were those in which the authors conducted laboratory or mesocosm

experiments in order to manipulate conditions. Modeling studies were those including mathematical or conceptual models. Review studies were those that only provided either narrative review from the literature or meta-analytical reviews.

Data analysis

We used the Activity Index (*AI*) to properly evaluate the quantitative trends of published articles on virus ecology conducted in tropical and subtropical regions in comparison to published articles in all aquatic systems over the period analyzed. This index has been used frequently in scientometrics studies (Kumari 2006) in order to avoid wrong conclusions from evaluating absolute number of articles, which might be influenced by the numerical trends of the overall literature. *AI* is calculated as follows: $AI = (CY/CT)/(TY/TT)$; where CY stands for the number of virus ecology articles in tropical and subtropical systems published in a respective year (y); CT is the total number of virus ecology articles in tropical and subtropical aquatic systems published for all years studied; TY is the number of virus ecology articles published in all aquatic systems in a year (y); and TT is the number of virus ecology articles in all aquatic systems published for all years studied. When $AI = 1$, the papers carried out in tropical and subtropical systems were published at the same relative rate as those conducted in all aquatic systems. If $AI > 1$, the papers carried out in tropical and subtropical systems were published at a high relative frequency compared to those conducted in all aquatic systems. And if $AI < 1$, the papers carried out in tropical and subtropical systems were published at a low relative frequency compared to those conducted in all aquatic systems.

Descriptive results

Tropical and sub-tropical regions represented nearly the same percentage of all published articles with 6% and 5%, respectively. Virus ecology studies in tropical and

subtropical regions have together clearly increased over the last decade (Figure 4B) and although *AI* widely varied throughout the first twenty years, it presented a consistent growth ($AI > 1$) since 2010 (Figure 5). Studies in temperate regions were the most abundant and accounted for 72% of all studies. Polar regions were the least studied in the virus ecology literature, representing 5% of all studies published since 1989.

The main approach recorded over time was the observational, representing 61% of all articles. Experimental studies represented 13% and those that combined both observational and experimental data accounted to 9% of all studies. Review and modelling studies represented 13% and 5%, respectively. Observational studies were the majority in most of the years and none of the approach categories presented an evident increasing trend over time (Figure 4B). From a total of 64 tropical and subtropical studies, 78% were observational, 6% experimental and 16% included both approaches experiments. There hasn't been neither modelling nor review studies that specifically approached virus ecology in tropical and subtropical systems.

Nearly half of the studies (52%) were conducted in marine systems, whilst those carried out in inland waters represented 34% of all articles. 8% of the studies were performed simultaneously in both marine systems and inland waters. The percentage of studies in inland waters greatly oscillated over the first decade (1989-2003), but showed a trend of being more equalized or sometimes surpass marine studies from 2003 onwards. Most of the tropical and subtropical studies (70%) were conducted in marine systems whilst 30% were in inland waters. Virioplankton was studied in 90% of the articles, while only 10% of them studied viriobenthos. Although the first study conducted in sediments was only recorded in 1991, they peaked between 2001 and

2006, when they reached about 25% of all studies but then they deacresed back to 10%. Only 4 (6%) out of 64 tropical and subtropical articles investigated sediments.

CHAPTER 1

SALINITY AS THE MAIN ENVIRONMENTAL DRIVER OF VIRIOPLANKTON IN TROPICAL COASTAL LAGOONS

Salinity as the Main Environmental Driver of Virioplankton in Tropical Coastal Lagoons

Target journal: Microbial Ecology

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Abstract

Viruses are abundant and important components of microbial food webs in inland waters. Humic substances, nutrient concentration, salinity, temperature and turbidity have already been proven to impact virioplankton in several aquatic systems. However, a vast majority of these studies were carried out in temperate systems combining a limited range of environmental variables. In this study, virus and bacterial abundance and production were determined and their response to limnological variables was assessed in 20 neighbouring shallow tropical coastal lagoons that combine wide environmental gradients of factors such as water colour (1.82–92.49 m^{-1}), dissolved organic carbon (0.71–88.6 mM), turbidity (2.32–571 NTU), salinity (0.13–332.1‰) and chlorophyll-a (0.28 to 134.5 µg L^{-1}). Here, we recorded virus abundance varying from 0.37 $\times 10^8$ to 117 $\times 10^8$ VLP mL^{-1} and production from 0.68 $\times 10^7$ and 56.5 $\times 10^7$ VLP mL^{-1} h^{-1}. We conclude from our regression models that salinity is the main environmental variable positively driving bacterial and viral abundances. This pattern is likely observed because of both the nonexistence of bacteria's predators and the decreasing bacterial diversity in highly salty aquatic systems. Total nitrogen was also an important variable explaining virus abundance. Virus production was mainly explained by bacterial production and virus abundance, but these variables did not respond accordingly to salinity, depicting an apparent paradox of high abundance but low production in hypersaline lagoons.

Introduction

Viruses have been widely recognized as the most abundant component 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 freshwater than marine ones [2]. They play important roles such as regulating microbial community composition [3] and altering ecosystem functioning and biogeochemical cycles by killing phytoplankton [4] and, namely, bacteria [5]. Most viruses are bacteriophages and, hence, their abundance is always strongly correlated with bacterial abundance in aquatic systems [6]. Thus, environmental factors that significantly impact bacterial productivity and density, in general, also have an effect on virus production and distribution [2].

Many studies have shown that virus may be strongly affected, directly or indirectly, by several environmental factors in aquatic systems. High-molecular-weight dissolved organic matter (HMW DOM) such as humic substances have been demonstrated to negatively impact virus abundance by either binding and destroying virus particles or decreasing virus infectivity and replication rates in freshwater ecosystems [7]. However, there are also some studies providing evidence of higher virus infection, yet lower virus abundance, in humic rather than clearwater lakes [8, 9]. Salinity has been described as an important virus regulator as high salt concetrations may drastically decrease the prokaryotic community diversity [10] and impede microzooplankton (e.g.: flagellates and cilliates), that compete with virus for bacteria, to live in hypersaline systems [11]. Virus is also affected by the system trophic status and there is evidence that eutrophic systems tend to have higher virus abundance than oligotrophic ones [2, 12]. On top of that, chlorophyll-*a* has been described to correlate with virus abundance in several aquatic environments such as

temperate lakes [13] and marine systems [14]. DOC concentration has also been shown to be both positively [12, 15] and negatively related to virus abundance [7]. Physical factors such as temperature [16, 17] and UV radiation [18] have also been suggested to strongly affect virus distribution in aquatic systems. Finally, high turbidity has been pointed to affect virus abundance by reducing the availability of labile autochthonous organic matter, due to lower primary production, for bacterioplankton in amazonian freshwater systems [19].

In spite of these investigations about virus responses to different environmental factors in inland aquatic systems, a very few of them have studied systems with co-occurring widely ranged environmental gradients. Moreover, a vast majority of these studies were conducted in temperate systems, although bacteria comparatevely present higher production and respiration in tropical freshwater ecosystems [20] and, therefore, different patterns of virioplankton abundance and activity might comparatively emerge from these systems. We here studied tropical coastal lagoons as model aquatic systems to investigate how virus abundance and production are affected by environmental factors. These shallow lagoons are relatively close to each other and thus are submmited to similar broad-scale regional factors such as climate and closeness to the sea [21]. However, they combine widely ranging environmental characteristics that make these lagoons ideal model aquatic ecosystems to conduct an investigation into how environmental variables drive virus-bacterium interactions. In this study, virus and bacterial abundance and production were determined in 20 tropical coastal lagoons ranging from oligotrophic to hypereutrophic, with salinity varying from 0.13 to 332‰, water colour ranging between 1.82 and 92.5 m^{-1}, DOC concentration varying from 0.71 to 88.3 mM and turbidity ranging between 2.3 and 571 NTU.

This survey aims to investigate virus-bacterium interactions in tropical coastal lagoons by determining their abundance and production as well as estimating virus-mediated mortality and the amount of carbon released from bacteria lyses by the virus shunt. As far as we know that is the first time flow cytometry has ever been used to enumerate virus and bacteria in such systems. The present study also intends to assess virus-bacterium abundance and activity responses to major environmental variables that have already been shown to importantly drive virus and bacteria in aquatic systems. This is also the first study to investigate such responses in ecosystems combining such wide environmental gradients. It was hipothesized that water colour and DOC concentration would have negative impacts, while trophic state and salinity would positively impact virus and bacterial abundance. We here found that salinity overrides any other environmental variables to be the main driver of bacterial and viral abundance. However, bacterial and virus production were not similarly affected by higher salinities, 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 mainly marked by sparce shrub-like vegetation covering parallel nutrient-poor sand dunes. The amid-dunes areas are dominated by both seasonally flooded forest areas and perennial or temporary coastal lagoons in the lower parts of the terrain. These shallow lagoons are located within a maximum distance of 73 km from each other, present nearly the same distance to the sea and are

submmited to similar climate factors [21]. Nevertheless, they present a wide range of limnological features such as dissolved organic carbon, chlorophyll-a (chl-a) and nutrient (N and P) concentrations, salinity and water colour (Table 1), which combined make these lagoons interesting model ecosystems to investigate how such factors drive microbial abundance and activity. Water, nutrients and organic matter inputs to these lagoons are mainly from the water table, the ocean and small streams, however these sources' relative importance greatly depend on each lagoon and may also vary over a seasonal scale [22]. Cabiúnas, Carapebus, Comprida, Preta, Paulista, Amarra-Boi and Atoleiro are humic, mostly oligotrophic systems with high concentrations of total DOC namely derived from the surrounding impermeable soil plentiful in organic matter [23]. Amarra-Boi and Atoleiro are usually oligotrophic [24] but were here recorded with high chl-*a* and nutrients concentrations (Table 1), probably due to their current drastic decreasing volume driven by drought. Salgada and Iriry are humic lagoons that receive nutrient inputs from both diffusive (agriculture activity) and punctual (sewage run-off from a nearby city) sources. Carapebuzinho, Carcará and Pretinha are distinguished tiny lagoons occasionally formed during dry periods near Carapebus, Comprida and Preta lagoons, respectively. Imboassica lagoon is fully inserted in an urban area and receives high nutrient loads from direct sewage discharges. Itapebussus and Encantada are clear brackish lagoons while Visgueiro, Catingosa, Ubatuba, Garças and Garcinhas are saline lagoons that have become hypersaline ponds (from 43 up to 332‰ – Table 1) because of their reduced volume in response to the persistent drougth periods lately observed in the studied region. The regional weather in the region is warm, with an average air temperature of 22.6°C, and the annual precipitation is 1,165 mm. Additional details

about the local climate as well as the limnological and morphological traits of these lagoons are found in Esteves et al. [21] and Caliman et al. [22].

Sampling

Sub-surface water samplings and in-situ measurements were carried out in a near-shore sampling station of each lagoon during a dry period in January 2015. Water temperature and dissolved oxygen were computed in-situ using specific probes (YSI-30 and YSI-61, respectively) while water samples were collected in 5L polyethylene flasks previously washed with HCl 10% and rinsed with distilled water. Bacterial, virus and autotrophs duplicate aliquots (1mL), pre-filtered through a 50-µm membrane mesh to remove large particles, were immediately added into 2 mL-cryotubes containing P+G (paraformaldehyde 10% + glutaraldehyde 0.5%), glutaraldehyde (0.5%) and paraformaldehyde (10%), respectively. These samples were fixed for 15-30 min at 4ºC and flash frozen in liquid N_2 in the field and then stored at -80ºC. The remaining water sample was taken to the laboratory for limnological and bacterial production measurements as well as for the time-course experiment set up to determine virus parameters. Turbidity, pH and water colour measurements were undergone in the laboratory within 2 hours after sampling, while chl-*a* and salinity were measured after one week (see limnological analysis section below).

Time-course experiment

Virus production (VP), virus decay (VD), virus turnover (VT), burst size (*Bs*) and virus mediated mortality (VMM) were determinate through the dilution technique in a laboratory time-course experiment [25], as adapted by Hewson et al. [26]. This technique has been widely used and considered to present the least complex design, yet providing consistent inter-comparison data [27]. 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 (Figure S3). First, we obtained virus-free water for every lagoon by filtering water samples (previously filtered through a 50 µm membrane mesh) from the original environment through a 0.02 µm filter backed by a GF-D filter. Second, 300 ml of the primary water samples were vacuum filtered (< 250 mm Hg) through 0.2 µm pore-size polycarbonate filters (47 mm diameter, Millipore). In the course of 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 while 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. This final 300 ml of diafiltered sample was separated into 100-mL polycarbonate flasks (previously autoclaved, washed with HCl 10% and rinsed with Milli-Q water) triplicates that were kept in a dark incubator (25 ± 1ºC) for a period of 10 hours. Virus-bacterium density sub-samples (1 mL) were collected from the 100-mL every 2 h and 30 min and then fixed and stored for flow cytometric measurements as described in the section below. Three bacterial production sub-samples (1.2 mL) were also performed after 2 h and 30 min of the incubations, when the first virus abundance (VA) increase is usually observed.

VP rates were determined from the mean slope of first order regressions of VA against time for each incubation replicate. VD was also obtained from the first order regression when virus started decreasing just after the main VA increase. VT, which is 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. The virus mediated mortality (VMM) of bacterial cells (cells lysed mL^{-1} h^{-1}) was calculated by

dividing VP values by the viral burst size (*Bs*) calculated from the division of the VP by the bacteria decrease registered during the first VA increase.

$$VMM = VP/Bs$$

VMM was further divided by new the bacterial cells production (NCP) 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.

$$VIBM\ (\%) = VMM/NCP \times 100$$

Finally, estimates of the amount of organic carbon (ng C mL^{-1} h^{-1}) released by viral lysis of infected bacterial hosts were calculated by multiplying VMM by the mean carbon content per cell of 20 fg C $cell^{-1}$ [28].

Flow citometric measurements: virus and bacteria enumeration

Flow citometry (FCM) was used to evaluate virus and bacteria numbers [29]. 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 PBS buffer (0.22 µm filtered just prior use) was used as sheath fluid (supplied by the manufacturer). The aspiration and optical systems were cleaned between samples with sterile Mili-Q water (18,2 mΩ) for 2 min. We analyzed the fluorescence emitted by the samples using the forward scatter (FSC) and the side scatter at 90º (SSC). Fluorescence signals were collected by three different photomultipliers: FL1 (530 ± 15 nm); FL2 (585 ± 21

nm); FL3 (661 ± 10 nm). Counts were made in triplicate at low flow mode for 30 s, and data were acquired in logarithmic mode. Fluorescent latex beads (Fluoresbrite YG carboxilate 1.58 μm, ref 17687, 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. The bead standard concentration was determined by epifluorescence microscopy, following for accurate and precise counting [30].

Firstly, natural samples were diluted (Table S1) in sterile TE-buffer, pH 8 (10 mM Tris-hydroxymethyl-aminomethane, Roche Diagnostics; 1 mM ethylenediaminetetraacetic acid, Sigma-Aldrich) when needed to reach an ideal working event rate (200 – 800 events s^{-1}) that avoids electronic coincidence in counts. Diluted viral and bacterial samples were promptly stained with SYBR Green I (Invitrogen) at final concentrations of 0.5 x 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 80ºC for 10 min and at room temperature for additional 5 min [31]. A yellow-green fluorescent latex microspheres solution (1μL) was added to each tube as an internal reference. Viruses were detected and had their abundance and groups determined by their signature in a plot of side scatter (SSC, X-axis) versus green fluorescence (FL1, Y-axis, green fluorescence from SYBR Green I related to nucleic acid content and virus size) [31]. Bacterial samples were kept in the dark until cytometer readings were taken (between 15 and 30 min). Total bacterial abundance was determined by their signature in a plot of side scatter (SSC, 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 [29]. Phototrophic bacteria groups were detected and

had their cellular abundance determined by their specific autofluorescence properties and signature in a plot of orange fluorescence (FL2) versus red fluorescence (FL3) [32]. Heterotrophic bacterial abundance was determined from difference between total bacterial abundance and phototrophic bacterial abundance. Inorganic particles (IP) were differed from biological particles and estimated as "particles-like events" from their signature in a plot of SSC against FL2 (high scatter and low fluorescence), and a standard gate was used for a relative comparison between samples in terms of presence of inorganic particles [33]. All data were obtained and analyzed by Cell Quest software (B-D) using a Macintosh computer.

Bacterial production

Bacterial production (BP) was measured by using the ^{3}H-leucine incorporation method [34] and trichloroacetic acid (TCA) protein extraction [35], as modified by Miranda et al. [36]. An intracellular isotopic dilution factor of 2 was used in calculations [37]. 1.2 ml of water sample was incubated in microcentrifuge tubes (25 ± 1 ºC) in the dark for 45 minutes 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 (unpublished data). Killed negative controls were set up by adding 90 µL of TCA before starting the incubations. After the incubation, ^{3}H-leucine incorporation was stopped by the addition of 90 µL TCA. Bacterial protein was extracted by washing with 5% TCA and 80% ethanol. After protein extraction, liquid scintillation cocktail (EcoLite(+)^{TM}) was added to each sample, which was radio-assayed by liquid scintillation counting (Beckman LS – 6500) after 2 days in the dark to reduce the chemiluminescence. The obtained values in disintegrations per minute (DPM) were converted to bacterial biomass using a ratio incorporation of 0.86, according to Wetzel and Likens [38].

Limnological variables

Water pH was obtained using a pH meter (Analion PM 608). Turbidity was measured using a turbidimeter (Hanna HI 98703). Salinity was evaluated by chlorinity method [39]. Dissolved organic carbon (DOC) and total dissolved nitrogen (TN) water samples 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 one week) for chl-*a* concentration analysis. Chl-*a* was extracted from these filters with 90% ethanol and absorbance was measured by spectrophotometry at 665 nm [40]. DOC and TN concentrations were determined using a Pt-catalyzed high-temperature combustion method with a Total Organic Carbon Analyser (TOC-5000 with a TNM-L unit; Shimadzu Scientific Instruments). Unfiltered water samples were autoclaved and total phosphorus (TP) was spectrophotometrically determined through formation of phosphorus molybdate [41]. Water colour was estimated by the absorbance at 440 nm and expressed as the absorption coefficient calculated according to Hu et al. [42]. All spectrophotometric analyses were performed in a 1-cm quartz cuvette (distilled water as a blank) using a Beckman® DU520 spectrophotometer.

Data analysis

Pearson's correlations were firstly conducted to verify relationships and redundancy between log-transformed (except pH) variables (Table S2). Regression models were developed in order to understand which environmental factors best explain virus abundance and activity in these ecosystems. Based on the correlation factors, we chose virus abundance and virus production as the most relevant virus parameters to be included in our models as response variables and selected which variables would be imputed in our models as explanatory variables. In models with virus abundance as the response variable, heterotrophic bacterial abundance, salinity,

total nitrogen, dissolved organic carbon as well as their interactions were set as explanatory variables. In models with virus production as the response variable, virus abundance, bacterial production, total phosphorus, salinity and their interactions were used as explanatory variables. For each response variable, the best models were selected using Akaike's information criterion (AIC). This model selection analysis basically retains the best-predicting model by balancing the number of variables and the explanatory power added to the model [43]. AIC is independent of the order of computation and provides consistent results (AICc values) that are used to calculate differences (ΔAICc) between the best-fitting model and all other models [43]. ΔAICc < 2 indicates substantial evidence for alternative models; when ΔAICc is between 3 and 9, alternative models have relatively less support, and ΔAICc > 10 points that alternative models are very unlikely [43]. All statistical analyses were carried out using RStudio statistical software (R Development Core Team, 2014).

Results

VA varied from 0.37 $\times 10^8$ to 117 $\times 10^8$ VLP mL^{-1}, while HBA varied from 0.14 $\times 10^7$ to 509 $\times 10^7$ $cells^{-1}$ mL^{-1} (Table 2). VP ranged between 0.68 $\times 10^7$ and 56.5 $\times 10^7$ VLP mL^{-1} h^{-1}, whereas BP ranged from 0.26 to 20.87 μM C h^{-1} (Table 2). VD varied between 0.46 and 39.6 $\times 10^7$ VLP mL^{-1} h^{-1}, whilst VT ranged from 0.15 to 2.1 h^{-1} (Table 2). 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 6 of the lagoons (Table 3). *Bs* varied from 3.7 to 861 VLP $lyse^{-1}$, VMM ranged from 2.27 $\times 10^6$ to 37.6 $\times 10^6$ cells lysed mL^{-1} h^{-1} and VIBM varied from 16.2 to 1680.8 % (Table 3). We estimated that viruses are responsible for releasing from 45.4 to 751.2 μg C L^{-1} h^{-1} of DOC through bacterial

cells lyse in surface waters of tropical coastal lagoons (Table 3), which represent about 35% to 550% of the bacterial production.

IP varied from 0.1 $\times 10^4$ to 305 $\times 10^4$ PL mL^{-1} h^{-1} among lagoons. Chl-*a* ranged from 0.28 to 134.5 µg L^{-1} (Table 1). Water colour was recorded between 1.82 and 92.49 m^{-1} (Table 1). DOC varied from 0.71 to 88.6 mM, while TN and TP concentrations varied between 0.77 to 156 µM and from 40.3 to 8730.4 µM, respectively (Table 1). Turbidity was recorded between 2.32 to 571 NTU and pH ranged from 3.22 to 7.59 (Table 1). Temperature was between 23 and 35.2 °C and salinity ranged from 0.13 to 332.1‰ (Table 1). DO varied from 1.2 and 181.6% (Table 1). Some remarkable correlations were observed amongst environmental variables in these lagoons (Table S2). DOC was positively correlated to water colour (r=0.63, $p<0.005$), TP (r=0.72, $p<0.005$), TN (r=0.91, $p<0.005$) and turbidity (r=0.70, $p<0.005$) as well as negatively correlated to DO (r= –0.66, $p<0.005$). TP was positively correlated to TN (r=0.81, $p<0.005$). TN was positively correlated to turbidity (r=0.71, $p<0.005$) and salinity (r=0.66, $p<0.005$) while negatively correlated to DO (r= –0.63, $p<0.005$).

VA and HBA presented a significantly positive relationship with salinity (Fig. 2). HBA and salinity were shown to be together the most important explanatory variables for VA. Their interaction made up the most parsimonious candidate model for positively predicting the VA in these coastal lagoons (Table 4). Adding TN to the previous model as an explonatory variable, interacting with HBA, slightly improved the prediction of VA but also increased complexity and, thus, this model was selected only as the second-best one (Table 4). VP and BP were closely positively linked (Fig. 3). VA and BP were selected as the best explanatory variables for predicting VP. They comprised the only significant models that explained VP (Table 4). Having

either single or interactive variables added 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 environmental factors were most important to drive virus-bacterium abundance and production in tropical coastal lagoons. Among many environmental factors, salinity has been clearly shown to be the main driver of HBA and VA in the studied coastal lagoons as evidenced by our best predicting model (Table 4). VA and HBA were closely linked (Table S2) and sharply increased with salinity and reached their highest numbers in the hypersaline systems (Table 1, Fig. 2), which is in agreement with several previous studies [10, 44–46]. Conversely, BP slightly decreased in saltier lagoons (Table S2, Fig. S2) exposing an apparent contradicting scenario of higher abundances despite a trend of decreasing production in increasingly salty systems. Nutrient concentration was demonstrated to be the second most important variable explaining virus-bacterium interactions in these systems. However other usually strongly determinant variables like humic substances, DOC and Chl-*a* concentration, temperature and turbidy were all irrelevant for determining virus parameters in our study.

Overall, the virioplankton abundance in most of the lagoons was within the previously reported ranges of values for inland waters with similar environmental features. We recorded higher VA than those found in other tropical freshwater ecosystems [19, 47–49]. Hence, the hypothesis that virus would be less abundant in tropical than temperate systems due to higher UV radiation [19] is not supported by our data. Instead, VA would be determined by a combination of several internal environmental factors, without regard to latitude, that affect virus' hosts. The lowest VA values were found in freshwater humic lagoons such as Atoleiro, Cabiúnas,

Comprida and Carcará, which presented values in the order of magnitude of 10^7 viruses per milliliter (Table 2). These values are within the range reported for surface water of temperate meso- oligotrophic humic lakes [7–9] and is possibly a result of virus absorbance by humic substances [50]. However, some freshwater humic lagoons studied here presented relatively higher VA, ranging from 1.86 to 6.4×10^8. This outcome is likely owing to the fact that these lagoons were also either eutrophic (Amarra Boi, Salgada and Iriry) or brackish (Carapebus and Preta), which are conditions already described to positively affect HBA and, consequently, VA [10, 13]. Among the nonhumic lagoons, which all presented higher VA than humic systems, those with eutrophic and hypereutrophic status (Carapebuzinho and Imboassica, respectively) were about threefold more abundant in virus (Table 2). These results hint that trophic status does play a role to determine virus and bacterial abundance in these lagoons, which corroborates the positive correlations found between nutrient concentration (TN and TP) and virus-bacterium abundances (Table S2). Such trend of higher VA in eutrophic rather than oligotrophic systems has been found both in marine [51] and freshwater ecosystems [9, 12, 52]. Yet, neither nutrients nor chl-*a* were pointed as main drivers of VA by our regression models as salinity was indicated to represent the strongest environmental regulator (Table 4). The extremely high VA found in all hypersaline systems is in line with previous studies that recorded VA of up to 10^9 virus mL^{-1} in hypersaline lakes [10, 45, 46], solar salterns [11] and crystallizer ponds [44]. However, we here recorded a remarkable maximum VA of 1.17 10^{10} virus mL^{-1} in the Catingosa hypersaline lagoon (Table 2), which is, to the best of our knowledge, the highest virioplankton abundance ever documented for aquatic systems in the literature.

The increasing VA and HBA within a salinity gradient is basically explained by two possible factors. First, salinity contrains microzooplankton (i.e.: nanoflagellates) presence [11, 53], whereby releasing bacterial populations from a very relevant predation pressure [54]. Secondly, this pattern might be observed due to lower inter-specific competition as a result of a decreasing diversity [10] since a few bacterial groups are capable of surviving in such harshly osmotic conditions [55]. Many studies have shown that the prokaryotic community composition drastically shifts from freshwater to hypersaline conditions [56]. The main change is observed with salt concentrations above 150‰, when the prokaryotic assemblage starts being dominated by Archaea instead of the tipically dominant Eubacteria [10]. Other relatively rare groups such as the *Bacteroidetes* have been reported to have their abundance increased in extremely salty systems [10]. Finally, the higher increase of VA in comparison to BA in hypersaline systems is probably observed because of a higher resistance of halophages to environmental virucidal agents [10] and, to a lesser extent, due to a lack of virivorous nanoflagellates or ciliates [11].

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 [26] and tropical estuaries [10, 57] as well in temperate lakes [52] and marine systems [27, 58]. Our results show that VP is significantly positively related to BP in these lagoons (Table 4; Table S2). Similarly, several studies have demonstrated that VP increases within a trophic gradient as a result of higher BP rates [26, 58]. Thus, eutrophic systems usually present higher BP and, in turn, VP rates than oligotrophic ones [52], which is endorsed by our observations (Table 2).

VP was mainly explained by virus abundance and bacterial production (Table 4). Likewise, previous studies have shown a VP coupling with both BP and VA as

response to increasing nutrient concentration [59, 60]. Furthermore, we found a significantly positive correlation between VP and TP, although TP was not related to BP (Table S2), which suggests that P-limitation is pottentially an important direct regulator of virus activity. Phosphorus has indeed been described as a limiting nutrient for virus proliferation since this element is a key component of DNA [61]. The decreasing BP under high salt concentrations, yet also nutrient-rich environments (Table 1), is in agreement with previous studies [11, 56]. This pattern is observed because prokaryotes need to protect themselves from the osmotic stress by using several energy-costing strategies [62]. For instance, halobacterial groups have adaptations to equalize their cytoplasmic salt concentration (KCl) with that of the environment [63]. Other halophilic bacterial groups accumulate glycine betaine and other compatible solutes in order to avoid water loss through osmotic instability of their cytoplasm [63, 64]. Such expensive mechanisms negatively impact the bacterial physiological state and growth rate [10]. Since smaller and stressed bacteria produce fewer viruses [65, 66], VP rates tend to decrease when salinity increases. Other possible explanation for a decreasing VP with increasing salinity is the potentially lower virulence of halophages on account of slow adsorption processes [67] and hampering of virus attachment to their hosts' cells [68].

In general, calculated *Bs* values were mostly recorded to be similar to the range of values previously found in inland waters worldwide [16, 52, 65, 69]. Salgada was the only coastal lagoon with an extremely high average value (Table 3) and greatly differed from any other system with no apparent reason. High *Bs* values are usually supported by larger cell biovolumes [65] and growth rates [66]. 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 [52].

Nevertheless, no consistent pattern regarding trophy seems to emerge about the *Bs* variability among the systems where *Bs* could be calculated (Table 3). The lack of effect of environmental variables on *Bs* has already been observed in another study carried out in temperate lakes [9]. Similarly, there was no apparent environmental variable driving VIBM (%) values (Table 3). VIBM was recorded to be above 100% of bacterial production in 3 out of 6 lagoons where VIBM could be calculated (Table 3). This result is very likely to be unrealistic and TEM analysis would be needed to further investigate this matter. Garcinha presented a VIBM of 16%, which is nearly the same percentage observed in many temperate systems [16, 52, 60, 69], but it is still at least twofold higher than recorded in a tropical inland waters [57, 70]. Salgada and Carapebuzinho presented VIBM of >50% (Table 3). This is even higher than observed for tropical systems, but it's realistic if compared to a study conducted in an eutrophic temperate lake [71]. It is important to recall that because BP is high in tropical systems [20], VIBM may be of relatively greater importance for the carbon cycling in these ecosystems.

The fact that VP was not recorded in Itapebussus and Garças incubations (Table 1) indicates that lysogeny may be prevalent over lytic infection in these systems. This observation contrasts with the high BA recorded in these systems since high host abundances usually favour lytic infection due to higher contact rates [2]. We recorded VA increase without HBA decrease in the time-course incubations of 8 out of 16 lagoons (Table 2 and 3), which suggests that VP occured without bacterial lysis. This phenomenon of continuous virus release without cell lysis has been described as chronic infection [72] and considered to be a potential mechanism of virus replication in harsh environments [10]. We could not find an association of these virus life strategies with any specific environmental condition. Such high frequency of chronic

infections in tropical lagoons might be due to high intensities of UV radiation, which has been widely shown to damage bacterial cells [73]. However, we do not have substantial empirical data to confirm this assumption and further investigations combining TEM and VP techniques are required to come to a conclusion on which virus life strategies prevail 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 environmental conditions. We found very high virus abundance and production, indicating that virioplankton perform a substantial role in the biogeochemical cycles of tropical coastal lagoons. We conclude that salinity is by far the strongest environmental factor to positively impact virus and bacterial abundance. There is consistent evidence from the literature showing that saline and hypersaline systems are very abundant in virus and bacteria because of a decreasing microbial diversity (lower interspecific competition) and microzooplankton density (lower bacterial predation). Conversely, virus production was not affected by salinity and was mainly controlled by bacterial production and virus abundance, besides presenting a positive coupling with total phosphorus concentration.

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Figures

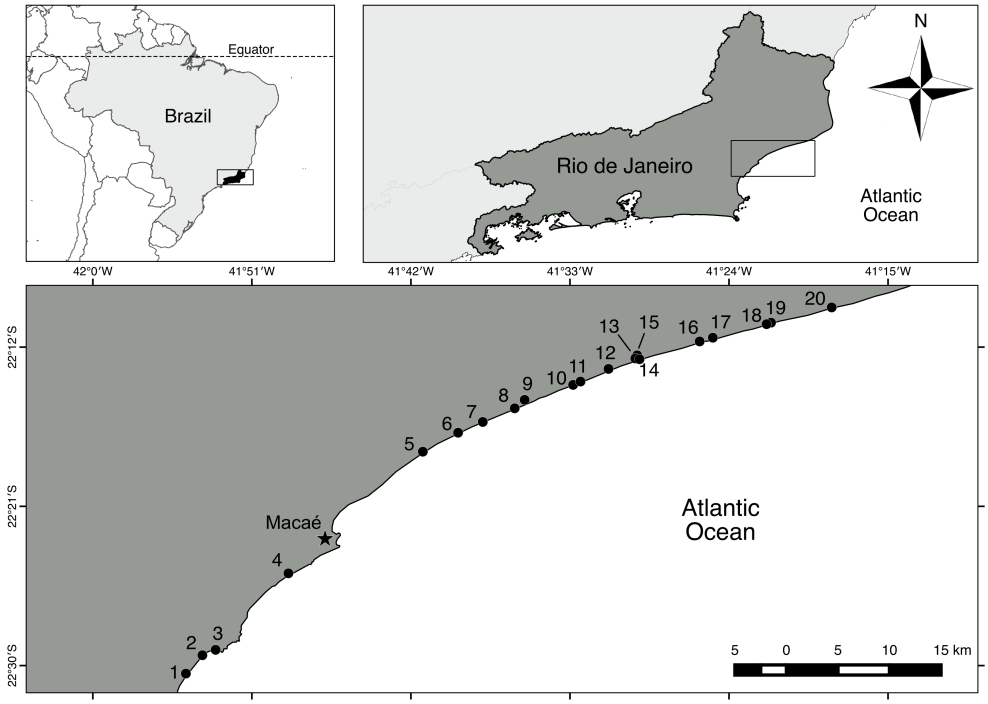

Figure 1. Map of the study area with the geographical location of all coastal lagoons (black dots) sampled in this study. Each lagoon is numbered as follows: 1) Iriry, 2) Salgada, 3) Itapebussus, 4) Imboassica, 5) Cabiunas, 6) Comprida, 7) Carcará, 8) Carapebuzinho, 9) Carapebus, 10) Encantada, 11) Paulista, 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.

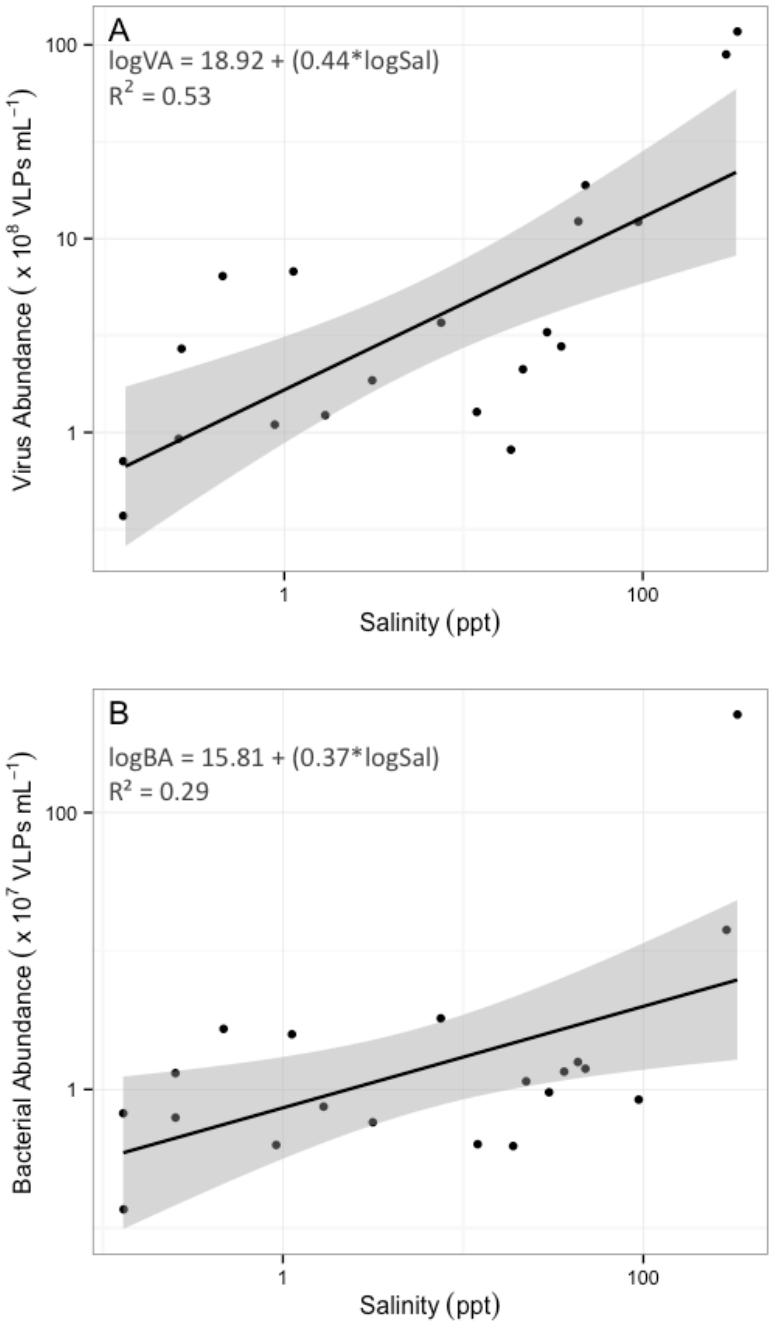

Figure 2. Linear regression between A) virus abundance and salinity; and B) bacterial abundance and salinity. The shadow area around the regression line represents 95% confidence interval for the linear regression. Note that both axes are in log-scale. The relationships were significant ($p < 0.05$).

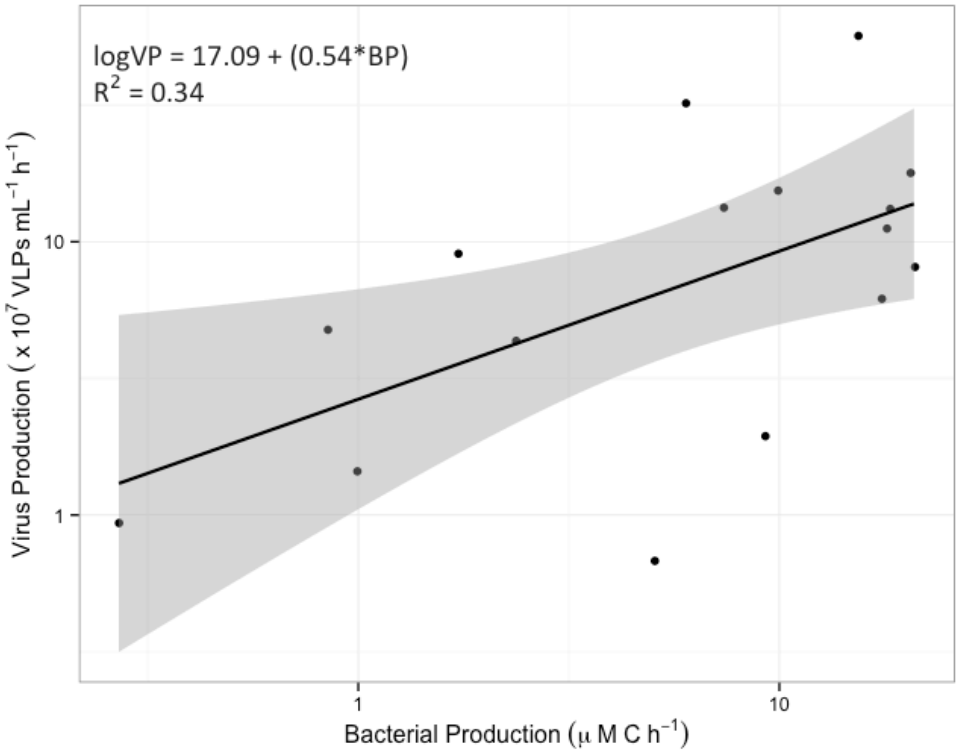

Figure 3. Linear regression between virus production and bacterial production. The shadow area around the regression line represents 95% confidence interval for the linear regression. Note that both axes are in log-scale. The relationship was significant ($p < 0.05$).

Tables

Table 1. Physicochemical parameters measured at the 20 tropical coastal lagoons in Rio de Janeiro state (Brazil), January 2015.

Lagoon	IP ($\times 10^4$ PL mL^{-1})	Chl-*a* ($\mu g\ L^{-1}$)	DOC (mM)	Water colour (m^{-1})	TP (μM)	TN (μM)	pH	DO ($mg\ L^{-1}$)	DO (%)	Turb (NTU)	T °C	Sal (‰)
Amarra Boi	304.94	16.65	45.72	92.49	37.47	1248.10	3.22	5.51	72.7	571	29.20	3.1
Atoleiro	6.45	37.72	4.90	51.98	1.32	112.79	3.33	0.73	8.9	26.2	23.40	0.3
Carapebuzinho	2.82	28.42	1.21	1.95	2.29	82.21	5.38	7.09	91.2	9.31	26.80	18.6
Cabiúnas	0.71	2.69	0.74	4.34	0.77	40.29	5.19	7.11	92.2	4.88	27.60	0.3
Carapebus	2.43	2.48	1.05	3.53	1.57	75.17	5.27	6.17	83	6.35	27.40	7.4
Comprida	1.50	3.52	1.14	36.45	0.90	75.46	6.19	6.15	79.7	5.43	28.00	0.1
Carcará	6.44	8.99	1.23	24.43	1.92	59.68	5.57	6.83	92	18.3	30.20	0.1
Encantada	0.10	0.28	1.17	1.82	0.79	89.76	4.52	5.53	79.6	2.32	26.80	22.1
Imboassica	143.96	134.53	1.08	2.17	9.25	238.05	5.76	6.29	78	51.5	24.90	1.1
Iriry	7.92	14.53	1.48	14.00	16.21	128.56	6.14	11.04	140	0.89*	25.90	0.5
Itapebussus	12.96	0.93	2.13	2.62	1.23	143.33	6.63	8.1	112	0.4*	27.20	12.0
Paulista	0.89	2.04	0.71	4.94	2.63	69.28	4.08	7.19	91	4.45	26.10	1.7
Preta	5.00	3.83	8.34	10.81	3.21	302.07	6.97	6.82	101.7	23.9	26.70	29.9
Pretinha	5.37	0.98	2.70	7.67	38.00	131.87	7.1	8.42	131.7	2.56	27.00	35.5
Salgada	3.38	7.35	2.23	21.81	2.75	92.33	4.93	8.04	101.5	0.99*	26.70	0.9
Catingosa	11.16	NA	88.63	53.21	156.01	8730.39	5.7	0.08	1.2	392	34.00	332.1
Garças	4.94	3.27	4.70	4.84	2.63	296.10	7	5.3	114	8.15	23.00	93.2
Garcinha	13.96	1.42	1.35	4.92	2.27	237.02	7.59	8.12	94.4	2.8	25.20	46.9
Ubatuba	5.50	NA	2.43	4.79	11.47	361.47	5.67	10.96	181.6	73.3	25.80	43.4
Visgueiro	0.88	11.59	17.93	12.41	39.25	1950.96	6.45	3.16	71.9	69.9	35.20	289.8

IP – inorganic particles; DOC – Dissolved Organic Carbon; TN – total nitrogen; TP – total phosphorus; DO – dissolved oxygen; Turb – turbidity; T – temperature; Sal – salinity. *Turbidity values measured from samples (kept at 4 ºC) 2 months after sampling.

Table 2. Virus and bacteria abundance and acitivity parameters measured in 20 tropical coastal lagoons from Rio de Janeiro state, Brazil.

Lagoon	VA ($\times 10^8$ VLPs 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})	BP ($\mu M\ C\ h^{-1}$)
Amarra Boi	1.86	66.51	29.65	3.85	9.04 ± 1.25	0.83 ± 0.19	1.15 ± 0.18	0.58	1.73 ± 0.10
Atoleiro	0.93	36.23	58.94	4.83	4.76 ± 0.93	3.28 ± 2.40	0.81 ± 0.19	0.62	0.85 ± 0.13
Cabiúnas	0.81	48.52	45.59	5.89	0.94 ± 0.29	0.46 ± 0.21	0.28 ± 0.10	0.39	0.27 ± 0.01
Carapebus	2.71	68.44	26.97	4.59	13.30 ± 4.40	2.84 ± 0.96	1.45 ± 0.70	1.31	7.40 ± 1.34
Carapebuzinho	3.69	59.17	33.53	7.30	6.17 ± 2.74	18.40 ± 5.38	0.40 ± 0.17	3.25	17.54 ± 0.17
Carcará	0.37	36.40	50.99	12.61	1.94 ± 0.39	NA	0.86 ± 0.22	0.67	9.29 ± 0.21
Comprida	0.71	43.17	51.51	5.32	1.45 ± 0.62	1.95 ± 1.13	0.52 ± 0.24	0.14	1.00 ± 0.07
Encantada	2.12	81.28	17.39	1.33	4.34 ± 0.54	9.86 ± 2.46	0.27 ± 0.05	1.15	2.37 ± 0.22
Imboassica	6.78	50.97	39.72	7.22	19.70 ± 14.2	NA	0.47 ± 0.50	2.48	18.45 ± 0.54
Iriry	6.40	39.25	51.46	8.60	11.20 ± 4.94	12.60 ± 6.43	0.48 ± 0.21	2.72	18.04 ± 1.69
Itapebussus	1.28	63.11	35.64	1.25	NA	1.38 ± 0.56	NA	0.40	10.42 ± 1.62
Paulista	1.23	63.88	33.50	1.46	0.68 ± 0.31	5.92 ± 0.43	0.15 ± 0.07	0.75	5.04 ± 0.27
Preta	3.30	73.67	20.85	5.49	8.10 ± 3.66	0.88 ± 0.06	0.82 ± 0.38	0.96	20.87 ± 0.39
Pretinha	2.77	89.73	8.20	2.08	56.50 ± 3.69	39.60 ± 13.4	1.27 ± 0.87	1.34	15.35 ± 3.26
Salgada	1.10	41.22	52.53	5.01	32.10 ± 14.6	16.10 ± 5.38	2.10 ± 1.13	0.40	6.04 ± 0.65
Catingosa	117.00	58.06	35.81	6.12	NA	NA	NA	509.25	0.26 ± 0.12
Garças	12.20	74.50	19.54	5.96	NA	6.62 ± 3.02	NA	0.85	8.84 ± 0.84
Garcinha	18.90	76.33	22.30	1.37	15.40 ± 2.51	31.10 ± 19.4	0.72 ± 0.10	1.40	9.89 ± 0.28
Ubatuba	12.30	68.14	27.00	4.86	17.80 ± 4.34	21.60 ± 8.19	0.19 ± 0.05	1.58	20.50 ± 0.77
Visgueiro	89.10	92.85	6.78	0.37	NA	NA	NA	14.17	3.53 ± 0.87

VA – virus abundance; V1, V2, V3 – virus cytometric groups; VP – virus production; VD – virus decay; VT – virus turnover; HBA – heterotrophic bacteria abundance; BCP – bacterial carbon production

Table 3. 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})
Carapebuzinho	6.92 ± 1.85	84.52 ± 26.16	8.47 ± 1.7	138.42 ± 36.93
Encantada	7.92 ± 2.56	1680.8 ± 642.2	5.87 ± 1.21	158.48 ± 51.24
Iriry	10.50 ± 2.78	168.43 ± 58.82	17.09 ± 12.38	209.85 ± 55.57
Salgada	2.74 ± 1.90	65.25 ± 43.12	860.9 ± 676.6	54.84 ± 37.94
Garcinha	2.27 ± 0.77	16.17 ± 5.66	80.86 ± 38.40	45.40 ± 15.36
Ubatuba	37.60 ± 10.8	287.48 ± 17.89	3.70 ± 0.73	751.16 ± 64.03

VMM – virus mediated mortality, VIBM – virus-induced bacterial mortality, Bs – burs size

Table 4. Best-fitting linear regression models describing virus and bacterial abundance and production. VA = virus abundance; HBA = heterotrophic bacterial abundance; Sal = Salinity; TN = total nitrogen; VP = virus production; BP = bacterial production; TP = total phosphorus.

Response Variable	Model	AICc	Δ AICc	R^2	*p*-value
Virus Abundance	*log*VA = 5.48 + (0.85**log*HBA) + (0.07*Sal) - (0.003**log*HBA*Sal)	48.48	**0**	0.86	**< 0.0001**
	*log*VA = 5.06 + (8.63*l*og*HBA) + (1.52*Sal) - (0.0004*TN) - (0.008**log*HBA*Sal) + (0.00006**log*HBA*TN)	53	4.53	0.89	**< 0.0001**
Virus Production	*log*VP = 4.94 + (0.68**log*VA)	52.61	**0**	0.35	**< 0.05**
	*log*VP = 17.09 + (0.54**log*BP)	52.72	**0.11**	0.34	**< 0.05**
	*log*VP = 17.61 + (0.05*TP)	55.23	2.63	0.23	> 0.05

Supplementary material

Table S1. Geographical coordinates from all sampling stations and dilution factors used for flow cytometric measurements of virus and bacterial sambles.

Lagoon	Latitude	Longitude	Virus dilution factor	Bacterial dilution factor
Amarra Boi	22°13'13.91"S	41°30'49.29"W	1000×	50×
Atoleiro	22°12'27.87"S	41°29'11.85"W	500×	500×
Cabiúnas	22°17'54.75"S	41°41'19.92"W	100×	10×
Carapebus	22°14'58.93"S	41°35'34.13"W	300×	30×
Carapebuzinho	22°15'27.55"S	41°36'7.92"W	500×	50×
Carcará	22°16'13.87"S	41°37'56.33"W	100×	10×
Comprida	22°16'50.40"S	41°39'20.16"W	100×	5×
Encantada	22°14'8.13"S	41°32'49.58"W	200×	20×
Imboassica	22°24'47.27"S	41°48'56.35"W	1000×	100×
Iriry	22°30'27.33"S	41°54'44.54"W	3000×	30×
Itapebussus	22°29'6.82"S	41°53'3.52"W	100×	10×
Paulista	22°13'56.82"S	41°32'24.40"W	100×	10×
Preta	22°10'36.93"S	41°21'37.26"W	2000×	50×
Pretinha	22°10'42.12"S	41°21'52.48"W	3000×	100×
Salgada	22°29'25.15"S	41°53'48.57"W	300×	30×
Catingosa	22°11'28.17"S	41°24'55.00"W	5000×	5000×
Garças	22°12'38.54"S	41°29'18.34"W	1000×	20×
Garcinha	22°12'40.97"S	41°29'4.00"W	1500×	20×
Ubatuba	22° 9'45.39"S	41°18'11.35"W	1000×	100×
Visgueiro	22°11'40.73"S	41°25'39.74"W	4000×	500×

Table S2. Pearson's correlation relationships between all parameters estimated in the surface waters of the 20 coastal lagoons. Statistically significant correlations (p<0.005) are shown in boldface.

	VA	VP*	VD*	VT*	HBA	BP	IP	Chla	DOC	Abs	TP	TN	pH	DO	O%	Turb	Temp	Sal
VA	**1**																	
VP*	**0.59**	**1**																
VD*	**0.64**	**0.62**	**1**															
VT*	-0.16	**0.56**	-0.06	**1**														
HBA	**0.82**	0.47	**0.57**	-0.16	**1**													
BP	0.03	**0.58**	**0.58**	0.09	-0.18	**1**												
IP	0.11	0.30	-0.03	0.26	0.01	0.04	**1**											
Chla	-0.15	-0.13	-0.36	0.16	-0.17	-0.17	**0.49**	**1**										
DOC	**0.53**	0.27	-0.31	0.38	**0.57**	-0.39	0.38	0.04	**1**									
Abs	-0.01	0.03	-0.35	**0.54**	0.17	-0.35	0.32	0.23	**0.63**	**1**								
TP	**0.65**	**0.49**	0.25	0.08	**0.66**	-0.14	**0.45**	0.10	**0.72**	0.39	**1**							
TN	**0.78**	0.37	-0.02	0.07	**0.75**	-0.27	0.39	-0.01	**0.91**	0.41	**0.81**	**1**						
pH	0.39	0.34	0.30	0.12	0.16	**0.46**	-0.01	-0.27	-0.08	-0.32	0.10	0.11	**1**					
DO	-0.43	0.20	0.25	-0.14	**-0.72**	**0.64**	-0.08	0.01	**-0.66**	**-0.49**	-0.41	**-0.63**	0.21	**1**				
O%	-0.34	0.23	0.25	-0.16	**-0.67**	**0.65**	-0.11	-0.02	**-0.60**	**-0.50**	-0.35	**-0.56**	0.27	**0.99**	**1**			
Turb	0.41	-0.08	-0.41	-0.08	**0.44**	-0.40	0.42	0.30	**0.70**	**0.46**	**0.58**	**0.71**	-0.30	**-0.53**	**-0.49**	**1**		
Temp	0.37	-0.23	-0.43	0.18	**0.53**	-0.39	-0.09	-0.12	**0.52**	0.36	**0.51**	**0.55**	0.02	-0.40	-0.35	0.41	**1**	
Sal	**0.73**	0.29	0.31	-0.28	**0.54**	-0.06	-0.05	-0.42	**0.50**	-0.25	**0.48**	**0.66**	0.43	-0.27	-0.16	0.25	0.27	**1**

VA – virus abundance; VP – virus production; VD – virus decay; VT – virus turnoever; HBA – heterotrophic bacteria abundance; BP – bacterial carbon production; IP – inorganic particles; Chla – Chlorophyll-*a*; DOC – dissolved organic carbon; Abs – water colour; TP – total phosphorus; TN – total nitrogen; DO – dissolved oxygen; Turb – Turbidity; Temp – Temperature; Sal – Salinity.

* These virus paramenters were determined in 16 lagoons (N=16)

GENERAL CONCLUSIONS

GENERAL CONCLUSIONS

Virioplankton has been increasingly studied worldwide. However, this abundant and diverse component of microbial food webs has been neglected from microbial ecology studies conducted in sub-tropical and tropical regions. This lack of data contrains our ability to understand virus importance in such systems, where bacteria communties have been shown to be very productive and, therefore, a higher amount of carbon is expected to be diverged from higher trophic levels by the virus shuntting effect on bacteria. The recent increasing efforts to investigate virus in tropical aquatic ecosystems have focused on enumerating virus abundance, which represents an important advancement, but it is still not enough to determine the real effect of virus on bacterial communities and nutrient cycling. Furthermore, there have not been studies carried out in a large number of systems submited to the same local climate, but also with largely different limnological characteristics such as the shallow lagoons found in the northern coast of Rio de Janeiro state, Brazil. The dilution technique and flow cytometry have been used for the first time to determine virus production and virus/bacteria abundances, respectively, in tropical coastal lagoons. Here, I investigated the virioplankton and bacterioplankton response to environmental variables in 20 tropical coastal lagoons. In summary, I conclude that:

1. **Virus and bacterial abundance widely vary between systems but, in general, reach very high abundances in tropical coastal lagoons;**
2. **Salinity is the main environmental driver of virus and bacterial abundances in the water column of tropical coastal lagoons as they sharply increase within a salinity gradient;**

3. **Nutrient concentration is the second most important environmental variable positively driving virioplankton and bacterioplankton abundance;**
4. **Virus and bacterial production widely range among systems and are positively related to each other;**
5. **Virus production was not affected by salinity and was mainly controlled by bacterial production and virus abundance, besides presenting a positive coupling with total phosphorus concentration;**
6. **Viruses play an important ecological role by controlling bacterial populations and releasing high amounts of labile organic carbon to the water column of tropical coastal lagoons.**

This dissertation shows that these tropical coastal lagoons are successful model systems to test the effects of environmental variables on virus and bacterial parameters in aquatic ecosystem. They allowed me to understand that salinity clearly overrides any other environmental variable to determine virus and bacterial abundance. Additional studies are necessary to shed light on which mechanisms are behind this pattern. However, based on previous studies, I hypothesized that increasing salinity leads to a growing virus-bacterium abundance because of a decreasing in both microbial diversity and microzooplankton density.

I here argue that virioplankton does play an important role in microbial food webs and are responsible for releasing a high quantity of carbon from bacterial cells to the water column of tropical aquatic ecosystems. I suggest that future studies aiming to understand the ecological importance of virus in tropical aquatic systems should thus focus on measuring parameters such as virus producion, virus-mediated

mortality, virus-induced host mortality and burst size. These measurements are essential to properly estimate the amount of carbon and nutrients released by virus activity. Additionally, experimental and modelling studies are also needed to further understand the importance of virus in such systems and predict how climate change will affect virus-related process. I expect that this dissertation boost microbial ecologists' interest in investigating every aspects of virus ecology in aquatic environments from tropical and sub-tropical regions.

APPENDIX

Additional Figures

Additional figures

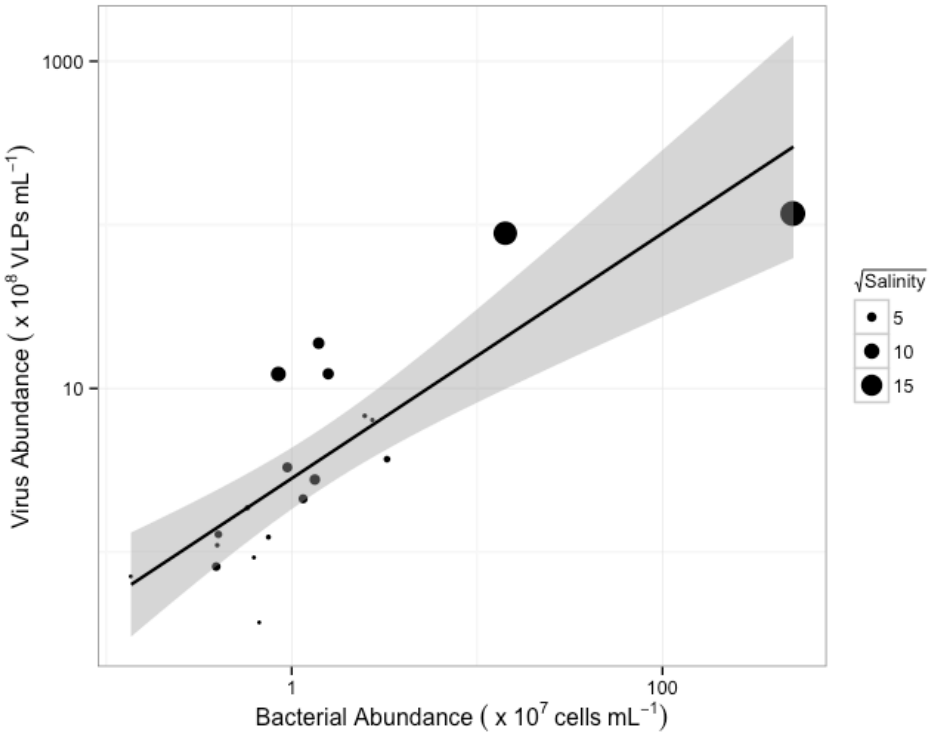

Figure S1. Linear regression between A) viral and bacterial abundance; and B) virus and bacterial production. The points' size depicts salinity of the sampling station. The shadow area around the regression line represents 95% confidence interval for the linear regression.

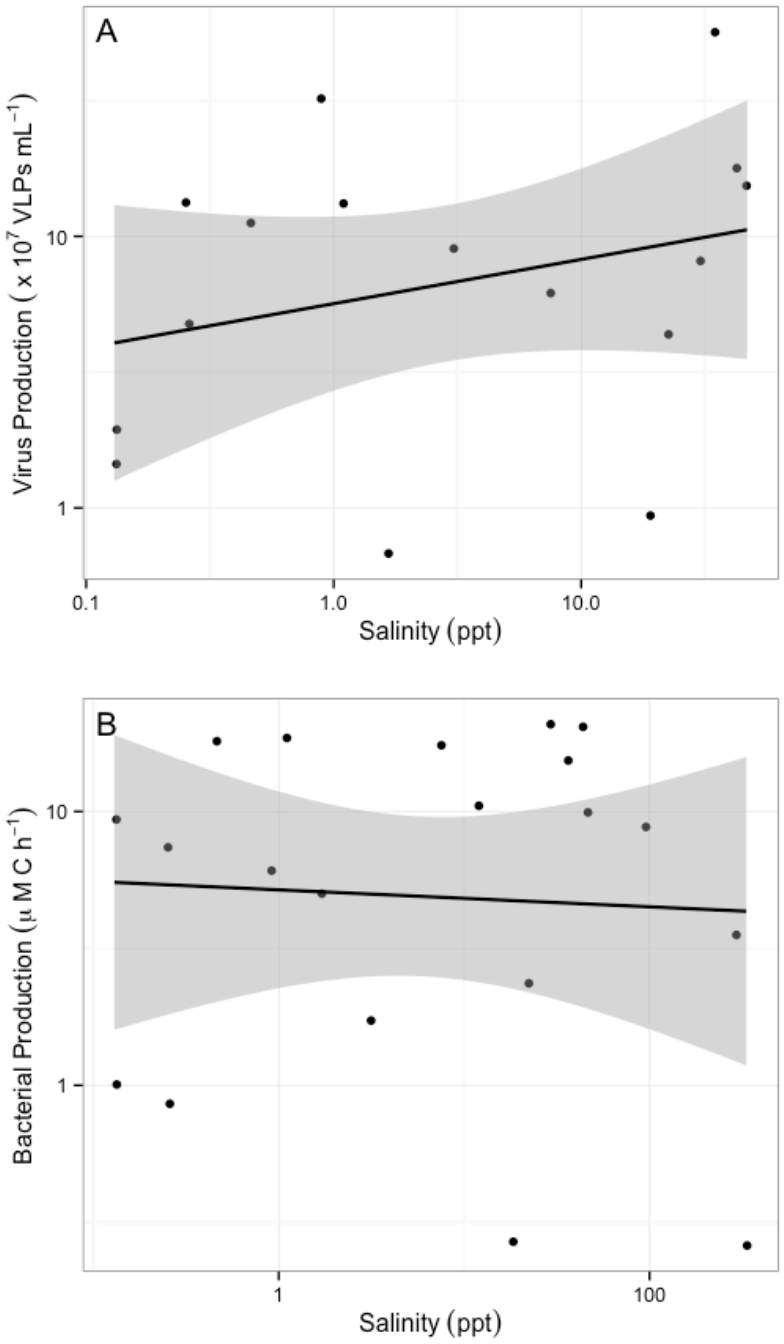

Figure S2. Linear regression between A) virus production and salinity; and B) bacterial production and salinity. The shadow area around the regression line represents 95% confidence interval for the linear regression. Note that both axes are in log-scale.

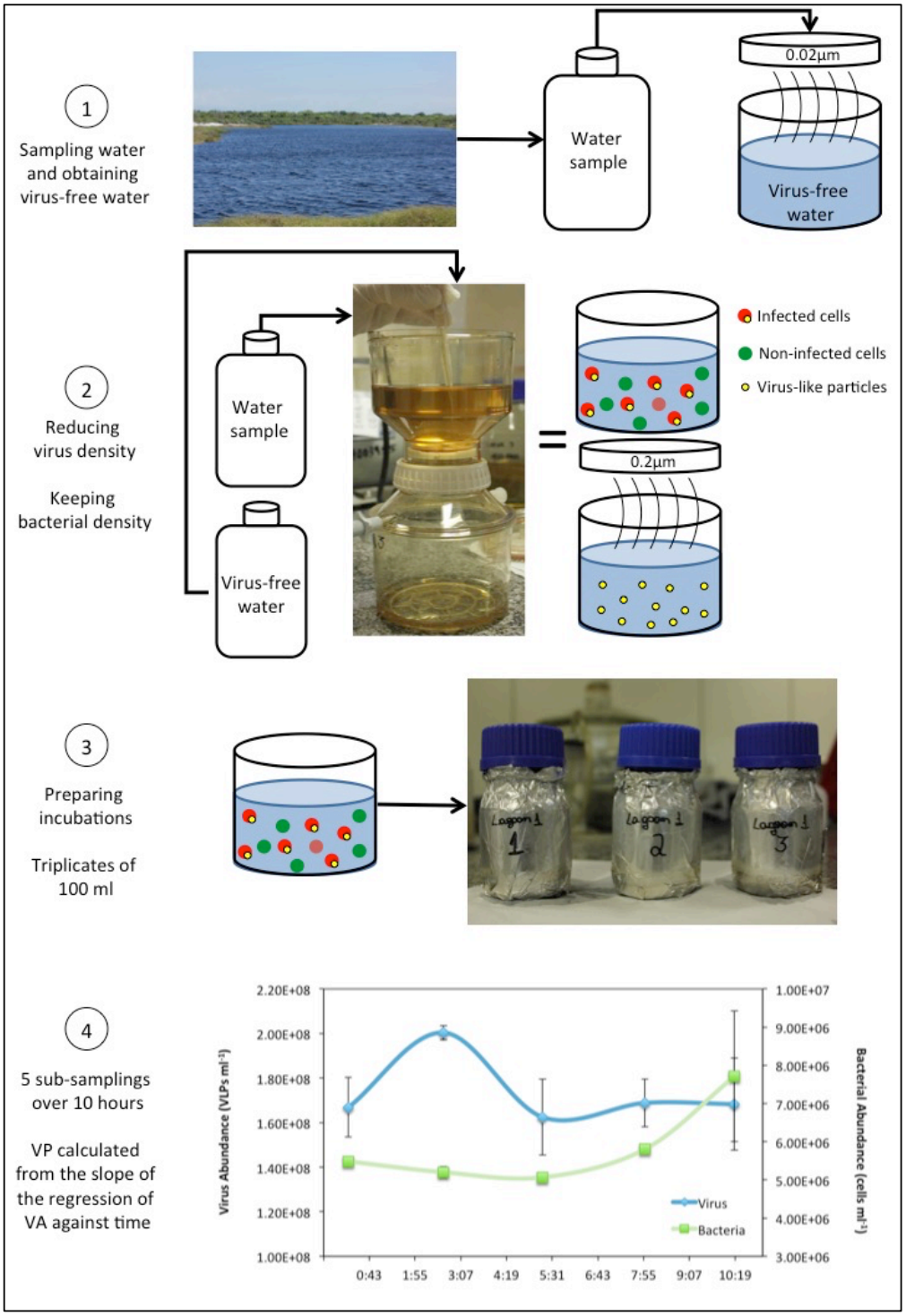

Figure S3. Schematic figure of the dilution technique procedures used to measure virus production and other virus parameters in the water column of the coastal lagoons. For a detailed description see methods of chapter 1.