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VARIAÇÃO TEMPORAL DO FEMTO- E PICOFITOPLÂNCTON MARINHO EM UMA ÁREA DE RESSURGÊNCIA COSTEIRA

ARRAIAL DO CABO / RJ

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Dissertação apresentada ao Instituto de Estudos do Mar Almirante Paulo Moreira e à Universidade Federal Fluminense, como requisito parcial para a obtenção do grau de Mestre em Biotecnologia Marinha.

Orientador: Dr. Lohengrin Dias de Almeida Fernandes

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"O que eu faço é uma gota no meio de um oceano.Mas sem ela, o oceano será menor".

Madre Teresa de Calcutá

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RESUMO

O picoplâncton marinho compõe-se de organismos entre 0,2-2μm, responsáveis por uma expressiva produção no ecossistema marinho. Somados ao femtoplâncton (<0,2μm), o qual é formado em sua maioria por vírus aquáticos (VLP), esses microorganismos destacam-se entre os demais devido a sua abundância, além de contribuir para o equilíbrio entre produtores e consumidores que afetam diretamente a produção primária dos oceanos e a alça microbiana.

 Dentro do picoplâncton estão presentes cianobactérias e picoeucariotos fotossintetizantes, que possuem uma significativa quantidade de pigmentos evidenciados durante o uso da técnica de citometria de fluxo, possibilitando a quantificação das diferentes populações baseadas em suas respectivas fluorescências. Técnica também utilizada para quantificação dos VLPs, que por sua vez foram corados com SYBR$^®$ Green I, corante que demonstra maior afinidade com o DNA.Para estudar a dinâmica de algumas populações picofitoplanctônicas e de VLPs relacionadas à área de Ressurgência costeira, foi utilizado um citômetro de fluxo BD Marine Influx para medir a abundância e distribuição de VLPs, *Synechococcus* (SYN) e picoeucariotos (PEUK)durante uma série temporal semanal de 10 meses em uma estação de coleta fixa. A distribuição da estrutura da comunidade foi numericamente dominada pelo virioplâncton (média: 2,25 x105 VLP/ml). SYN variou de 1,33x103 a 1,34x105 células /mL e a abundância de PEUK foi entre $9,09x10^2$a $5,09X10^4$ células/mL. Ambas as populações de SYN e PEUK foram mais bem previstas com uma defasagem de uma semana e foram fortemente relacionadas às mudanças no pH e NH$_4$. A abundância de VLP sem intervalo de tempo foi positivamente relacionada à concentração de clorofila a e negativamente relacionada ao conteúdo de feopigmentos.

Palavras-chave: Picofitoplâncton, Femtoplâncton, Citometria de fluxo, Séries temporais, Ressurgência

ABSTRACT

Marine picoplankton is composed by organisms between 0,2-2µm responsible of an expressive production in the marine ecosystem. Combined to the femtoplankton (<0,2 µm), formed mainly of virus-like particles (VLP), they contribute to the balance between producers and consumers affecting the net primary production and the microbial loop. Inside the picoplankton, photosynthetic cyanobacteria and picoeukaryotes' pigments are evidenced during the use of the flow cytometry technique, enabling the quantification of different populations based on their respective fluorescence. This technique was also used to quantify the VLPs, which in turn were stained with SYBR® Green I, a dye that demonstrates greater affinity with DNA. To study the dynamics of several picophytoplanktonic populations and VLP related to the coastal upwelling area, we used a BD Marine Influx flow cytometer to measure *Synechococcus,* picoeukaryotes (PEUK) and VLP abundance and distribution during a 10-month weekly time-series in a fixed station. The community structure distribution was numerically dominated by virioplankton (average: 2,25 $\times 10^5$ VLP/ml). *Synechococcus* ranged from $1,33 \times 10^3$ to $1,34 \times 10^5$ cells/mL and PEUK abundance was between $9,09 \times 10^2$ to $5,09 \times 10^4$ cells/mL. Both *Synechococcus* spp. and PEUK populations were better predicted with a one week lag and they were strongly related to changes in pH and NH_4. VLP's abundance with no time lag was positively related to chlorophyll a concentration and negatively related to phaeopigments content.

Key words: Picophytoplankton, Femtoplankton, Flow Cytometry, Time series, Upwelling

LISTA DE ABREVIATURAS E SIGLAS

IEAPM	Instituto de Estudos do Mar Almirante Paulo Moreira
PELD-RECA	Programa de Pesquisas Ecológicas de Longa Duração da Ressurgência de Cabo Frio
PEUK	Picoeucariotos (Picoeukaryotes)
DOM	Matéria orgânica dissolvida
POM	Matéria orgânica particulada
SYN	*Synechococcus*
UFF	Universidade Federal Fluminense
VLP	Virus-like particles

SUMÁRIO

1. INTRODUÇÃO

As espécies do plâncton abrangem vários reinos e filos taxonômicos, e várias ordens de magnitude no tamanho das células (<0,2 μm a200 cm)(CHASE et al., 2020), uma importante característica que influencia na absorção de nutrientes, taxas de crescimento, afundamento, mobilidade e interações com as demais espécies (LITCHMAN; KLAUSMEIER, 2008; SOMMER et al., 2017). Alguns estudos(HEDGES et al., 2004; KULASOORIYA, 2011)relatam que o primeiro organismo planctônico, uma cianobactéria marinha fotossintetizante, apareceu há aproximadamentetrês bilhões de anos. A liberação de oxigênio pelas cianobactérias mudou gradualmente a atmosfera redutora da terra primitiva para uma oxidante (OLSON, 2006)desencadeando uma evolução dramática da biodiversidade global.

O picoplâncton, classificação atribuída aos organismos planctônicos de tamanho compreendido entre 0,2-2μm, é composto em grande parte por cianobactérias,bactérias e eucariontes fototróficos. Cerca de metade da fotossíntese global e produção de oxigênio é realizada por tais organismos, que vivem na camada superior do oceano onde penetra luz suficiente para apoiar seu crescimento (DUFRESNE et al., 2003). As cianobactérias podem dominar a comunidade fitoplanctônica em termos de biomassa e abundância celular (PRZYTULSKA et al., 2016), e também contribuem para a base da rede trófica no processo da alça microbiana. Também conhecida por 'microbial loop', a alça microbiana consiste na produção de material orgânico dissolvido (DOM) e sua captação por bactérias heterotróficas. O conceito-chave é que as bactérias usam uma forma de material orgânico que não está disponível para outros organismos maiores (KIRCHMAN, 2018) e o recicla, devolvendo ao em forma de nutrientes para a manutenção do ciclo.

No picofitoplâncton, cianobactérias do gênero *Synechococcus* são amplamente distribuídas no Atlântico Sul subtropical, e também atuam nos ciclos do carbono (BUITENHUIS et al., 2012; LIU et al., 2020), juntamente ao gênero *Prochlorococcus,* os menores e mais abundantes organismos fotossintetizantes do planeta (YAN et al., 2020). Essas são algumas pequenas cianobactérias que somadas aos picoeucariotos dominam os sistemas costeiros, onde combinadas com bactérias heterotróficas, compõem o picoplâncton que ocupa a base da cadeia trófica estruturada por tamanho na plataforma do Atlântico Sul (MOSER et al., 2016).

Ainda menor que os organismos picoplanctônicos, encontra-se o femtoplâncton (<0.2μm), a menor fração do plâncton, composto em sua generalidade por partículas semelhantes a vírus (VLP), além de vários procariontes minúsculos chamados CPR (Candidate PhylaRadiation), DPANN (Diapherotrites, Parvarchaeota, Aenigmarchaeota,

Nanoarchaeota e Nanohaloarchaeota), ALN (aster-parecido com nanopartículas) (COLOMBET et al., 2020a).

Os vírus marinhos são os maiores componentes da cadeia trófica aquática, e as infecções virais são uma das principais formas de mortalidade, além de causar doenças em uma ampla escala de tamanho que atinge desde pequenos camarões abaleias (COLOMBET et al., 2020b).Concorrente a ingestão pelos níveis tróficos mais altos, o femtoplâncton, em sua maior parte vírus marinhos, também contribuipara os ciclos de nutrientes através da lise de hospedeiros autótrofos e heterótrofos e liberação de matéria orgânica dissolvida (DOM) e matéria orgânica particulada (POM), que podem ser subsequentemente utilizadas pelas células hospedeiras ("Viral shunt")(JOVER et al., 2014; YANG et al., 2019).

Entender a dinâmica temporal dos níveis tróficos basal, como o femto- e picofitoplâncton, é essencial para ampliar o conhecimento sobre a transferência de energia nos ecossistemas marinhos, bem como para prever como as mudanças ambientais podem influenciar o funcionamento do oceano.No entanto, embora nosso conhecimento sobre sua diversidade tenha crescido significativamente nas últimas décadas, os principais fatores que influenciam sua dinâmica temporal e a magnitude dessas relações permanecem muito mais elusivos (MOREIRA; LÓPEZ-GARCÍA, 2019), especialmente em regiões que sofrem a influência de fenômenos oceanográficos específicos, como a ressurgência em Arraial do Cabo, RJ.

A ressurgência induzida pelo vento é um fenômeno oceanográfico responsável pela mistura de massas de água, regulação das variações climáticas e da dinâmica dos ecossistemas marinhos, modificando a cadeia trófica local(GUENTHER et al., 2008; KÄMPF; CHAPMAN, 2016; WANG et al., 2015).Durante a ressurgência, as águas profundas, ricas em nutrientes, ascendem para zona eufótica na primeira fase.Em seguida, ocorre um aquecimento superficial, acompanhado do aumento da biomassa e da produção primária, simultânea a diminuição dos nutrientes.Por fim, a fase de afundamento, levando a uma situação oligotrófica como consequência da diminuição da biomassa fitoplanctônica devido à dispersão e esgotamento de nutrientes (GONZALEZ-RODRIGUEZ et al., 1992).

Além dos processos físicos, as interações bióticas também sãoreconhecidas como um importante condutor da dinâmica do plâncton (CHAFFRON et al., 2020). A dinâmica do femto- e picofitoplâncton em áreas de ressurgência costeira está extremamente associada a mudanças nas características ambientais, como enriquecimento de nutrientes na zona eufótica e processos físicos na camada mista (LIPS; LIPS, 2010; MADHU et al., 2021).

Compreendendo a relevância das populações picofito- e femtoplânctonicas e a complexidade em monitorá-las devido a seu pequeno tamanho, buscamos a citometria de

fluxo baseada na autofluorescência desses organismos, que possuem diversos pigmentos em seu interior.

As cianobactérias presentes no picofitoplâncton possuem em sua composição um significativo índice de ficobiliproteínas, pigmentos que captam energia luminosa e a transferem para o centro reativo da fotossíntese (CASTRO et al., 2016). As ficobiliproteínas podem ser divididas em quatro classes principais com base em seus máximos de absorção de comprimento de onda (PAGELS et al., 2019), na qual a ficocianina é a mais abundante nas cianobactérias, seguida pela ficoeritrina e pela aloficocianina (WILDMAN; BOWEN, 1974). Dessa forma, quando são excitados pelo comprimento de onda azul, as cianobactérias fluorescem amarelo (devido à ficoeritrina) ou vermelho-escuro (ficocianina), enquanto os eucariontes emitem uma luz vermelha devido à fluorescência da clorofila a (CALLIERI, 2008).

Em função da presença de pigmentos, o uso da citometria de fluxo faz uma análise da dispersão da luz e da fluorescência emitida a partir de células. Esse processo se dá à medida que elas fluem individualmente através de uma fonte de luz com foco intenso, a uma taxa de centenas a milhares de células por segundo, possibilitando a distinção de populações individuais e a quantificação das propriedades ópticas de cada uma (CAMPBELL, 2001).

O uso da citometria de fluxo para estudos ambientais aquáticos tem sido, em grande parte, direcionados às comunidades marinhas de organismos fluorescentes menores que 2 μm (picofitoplâncton) (SOSIK; OLSON; ARMBRUST, 2011) e aos de fluorescência induzida, como populações de bactérias heterotróficas e partículas semelhantes a vírus (VLPs). A citometria é um método que permite uma análise quantitativa muito mais rápida que a microscopia de luz convencional, pela sua capacidade de identificar várias células ou partículas diferentes em poucos segundos.

Portanto, para o presente estudo a citometria de fluxo foi utilizada em razão de sua eficiência, e devido à presença de cianobactérias e picoeucariotos fotossintetizantes no picoplâncton, que possuem uma significativa quantidade de pigmentos evidenciados durante o uso da técnica, possibilitando a quantificação das diferentes populações baseadas em suas respectivas fluorescências.

2. OBJETIVOS
OBJETIVO GERAL

A pesquisa "**Variação temporal do femtoplâncton e picofitoplâncton marinho em uma área de Ressurgência costeira**" é um projeto vinculado ao Programa de Pesquisas Ecológicas de Longa Duração da Ressurgência de Cabo Frio (PELD-RECA) (RC) [Proc. 441525/2016-4], que tem por macro objetivo o monitoramento de longo prazo da Ressurgência de Cabo Frio e parte do Programa "Horizon 2020 Mission Atlantic"[Grant Agreement No 862428], que visa melhorar a compreensão dos ecossistemas do Oceano Atlântico e dos fatores de mudança que afetam a biodiversidade marinha.

OBJETIVOS ESPECÍFICOS

- Realizar um prognóstico de oscilações em uma série temporal do picofitoplâncton e femtoplâncton no ecossistema marinho costeiro de ressurgência em Arraial do Cabo, RJ.

- Aprimorar protocolos de citometria de fluxo com o propósito de quantificar os parâmetros de abundância e biomassa das populações do picofitoplâncton e femtoplâncton.

3. **"Multi-scale temporal variation of marine femtoplankton and picophytoplankton: the role size and environment"**

Multi-scale temporal variation of marine femtoplankton and picophytoplankton: the role size and environment

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ABSTRACT

Femtoplankton and picophytoplankton organisms exert a major role in the balance between producers and consumers and are responsible for a large part of the net primary production in the ocean. However, despite their ecological importance, the magnitude and drivers of their temporal dynamics remain largely unexplored. To address this significant knowledge gap, we performed weekly sampling over ten months in a wind-driven coastal upwelling area in the subtropical South Atlantic Ocean. By investigating the temporal changes of both femto- and picophytoplankton at multiple temporal scales, we found that Virus-Like Particles (VLPs) were the most abundant femtoplanktonic organisms and responded faster (i.e., without a temporal lag) to environmental changes (mainlyrelated to chlorophyll-*a (chl-a)* and phaeopigment variations). On the other hand, picophytoplanktonic organisms showed as lower response to environmental changes, with positive responses to variation in pH and NH_4 concentrations after a one-week lag.Overall, our results demonstrate that the speed of response of planktonic organisms to environmental changes may be dependent on their size and highlight the importance of environmental variables and biological interactions as drivers of their temporal dynamics

KEYWORDS: FLOW CYTOMETRY, TIME SERIES, GENERALIZED ADDITIVE MODEL, UPWELLING

INTRODUCTION

Marine planktonic organisms compose the base of the size-structured marine food web and play a key role in ocean functioning (ANDERSEN et al., 2016; FUHRMAN, 2009; LITCHMAN et al., 2015; PIERELLA KARLUSICH; BOWLER; BISWAS, 2021). The smallest fractions, namely picophytoplankton (0.2-2μm) and femtoplankton (<0.2μm), comprise highly diverse assemblages (XIE et al., 2020) which have been intensively studied over the past two decades as molecular and microscopic techniques advanced (Colombet et al. 2000). Picoplankton comprises both autotrophic and heterotrophic unicellular organisms, with picocyanobacteria of the genera *Prochlorococcus* and *Synechococcus* usually dominating the autotrophic picoplankton(AL-OTAIBI et al., 2020). The femtoplankton, in turn, is composed of various tiny prokaryotes named CPR (Candidate Phyla Radiation), DPANN (Diapherotrites, Parvarchaeota,

Aenigmarchaeota, Nanoarchaeota, and Nanohaloarchaeota), ALN (aster-likenanoparticles), and VLPs (virus-like particles) (COLOMBET et al., 2020a).

These organisms exert a major role in biogeochemical cycles and are responsible for a large portion of the ecosystem's new production in the oceans (FLAVIANI et al., 2018; LI, 1994; PEDROTTI et al., 2017). Pico- and femtoplankton populations have a significant role in the microbial loop, a trophic pathway where dissolved organic carbon is incorporated into bacterial biomass and returned to higher trophic levels via the classical food chain: phytoplankton-zooplankton-nekton (AZAM et al., 1983; AZAM; MALFATTI, 2007). Understanding the temporal dynamics of pico- and femtoplankton is therefore essential to advance our knowledge on the transfer of energy in the marine ecosystems as well as to predict how environmental changes may influence the ocean's functioning. Nevertheless, while our knowledge of their diversity has grown significantly over the past decades, the key drivers influencing their temporal dynamics and the magnitude of these relationships remain much more elusive (MOREIRA; LÓPEZ-GARCÍA, 2019).

For decades, researchers have considered that planktonic organisms show periodical rhythms in their abundance following the "periodic plankton" concept (MOREIRA; LÓPEZ-GARCÍA, 2019). Yet, this idea was mainly based on the variation of planktonic animals, which have longer life cycles, and open ocean phytoplankton, whose variability is mainly related to the annual cycles of solar radiation (PAR) and atmospheric heat input (CLOERN; JASSBY, 2010). Phytoplankton variability in nearshore coastal waters, however, may be unpredictable as it is influenced by multivariate processes that propagate across their interfaces with land, ocean, atmosphere, and underlying sediments (CLOERN; JASSBY, 2010). The dynamics of the planktonic organisms in wind-driven coastal upwelling areas, for example, are eminently associated with local changes in environmental characteristics such as nutrient enrichments in the euphotic zone and physical processes in the mixed layer (LIPS; LIPS, 2010; MADHU et al., 2021). When wind-driven mixing processes deepen the mixed layer, the plankton benefits from the new nutrient input during the upwelling and generates a cascade effect up to the higher trophic levels (FERNANDES et al., 2012). Stochastic events such as storms and higher discharge of rivers associated with increased rainfall may also strongly influence the temporal dynamics of coastal phytoplankton (CLOERN; JASSBY, 2010).

Apart from physical processes, biotic interactions have also been recognized as an important driver of plankton dynamics (CHAFFRON et al., 2020). A growing body of studies has been showing that biological interactions, such as mortality processes related to the cellular lyses by VLPs may regulate biomass, community composition, and elemental cycling of microbial communities (BOLAÑOS et al., 2020; WEINBAUER; RASSOULZADEGAN, 2004; WILHELM; SUTTLE, 1999). Similarly, recent investigations (JOVER et al., 2014; YANG et al., 2019)showed that marine viruses may slow down the cascade effect by lysing autotrophic and heterotrophic hosts and thus returning dissolved organic matter (DOM) and particulate organic matter (POM) to the microbial loop (viral shunt).

These findings demonstrate that the drivers of phytoplankton fluctuations are likely to differ among different temporal scales, from seasonal (e.g., temperature and solar irradiance) to monthly and/or daily variations (e.g., biological interactions and physical drivers such as changes in salinity and turbulent mixing) (LIU; FENG; WANG, 2019). Thus, accurate predictions would depend on the investigation of temporal changes at multiple scales to disentangle the different impacts of various drivers. One alternative to predict phytoplankton fluctuations at a relevant timescale is to include time lags between the drivers and responses in predictive models (LIU; FENG; WANG, 2019).Yet, this would only be possible when adequate time-series data is available. As microorganisms exhibit relatively fast growth and population size fluctuations, even a monthly sampling strategy may miss part of the rapid plankton dynamics (MOREIRA; LÓPEZ-GARCÍA, 2019). In this regard, to better elucidate the dynamics and drivers of small-planktonic organisms, datasets should come from times series with a high sampling frequency (i.e., biweekly or higher). Unfortunately, to our knowledge, no study has investigated the temporal dynamics of femto- and picophytoplankton organisms with such high-frequency sampling for months.

In this study, we combined intensive fieldwork (i.e., weekly samplings), multi-color flow cytometry and statistical modeling to perform the first high-frequency assessment of the predictability and relative importance of factors governing the temporal dynamics of femto- and picophytoplankton at different timescales (no lag to two week lags). By conducting this intensive work, we expect to enhance our comprehension of the drivers of small planktonic organisms and their temporal changes, as well as to provide relevant information to better understand how future modifications in environmental conditions may influence our oceans.

METHODS

Location and sampling

Our study area is the Cabo Frio upwelling region, one of the most active planktivorous fishing areas (mainly sardines) along the Brazilian coast (FREIRE et al., 2021) due to the seasonal upwelling that boosts the energy transfer throughout the trophic chain (FERNANDES et al., 2012; FERNANDES; FAGUNDES NETTO; COUTINHO, 2017). Upwelling events in Cabo Frio usually last for a few days (GUENTHER et al., 2008) further highlighting the importance of high-frequency monitoring of planktonic organisms.

Weekly samplings of plankton and environmental variables were conducted from January to October 2020 at Cabo Frio Island (Fig.1) as part of the "Upwelling Long-Term Ecological Research" (PELD-RECA) and "EU Horizon 2020 Mission Atlantic" (Grant Agreement No 862428) programs. For environmental variables, sea surface temperature, salinity, and pH were estimated using a previously calibrated multiparameter probe (Model U-5000; HGS No. 7JETA790, Horiba) at approximately 1 meter deep. The concentration of macronutrients (ammonium, nitrite, nitrate, and phosphate) was evaluated according to the (STRICKLAND; PARSONS, 1972) protocol.
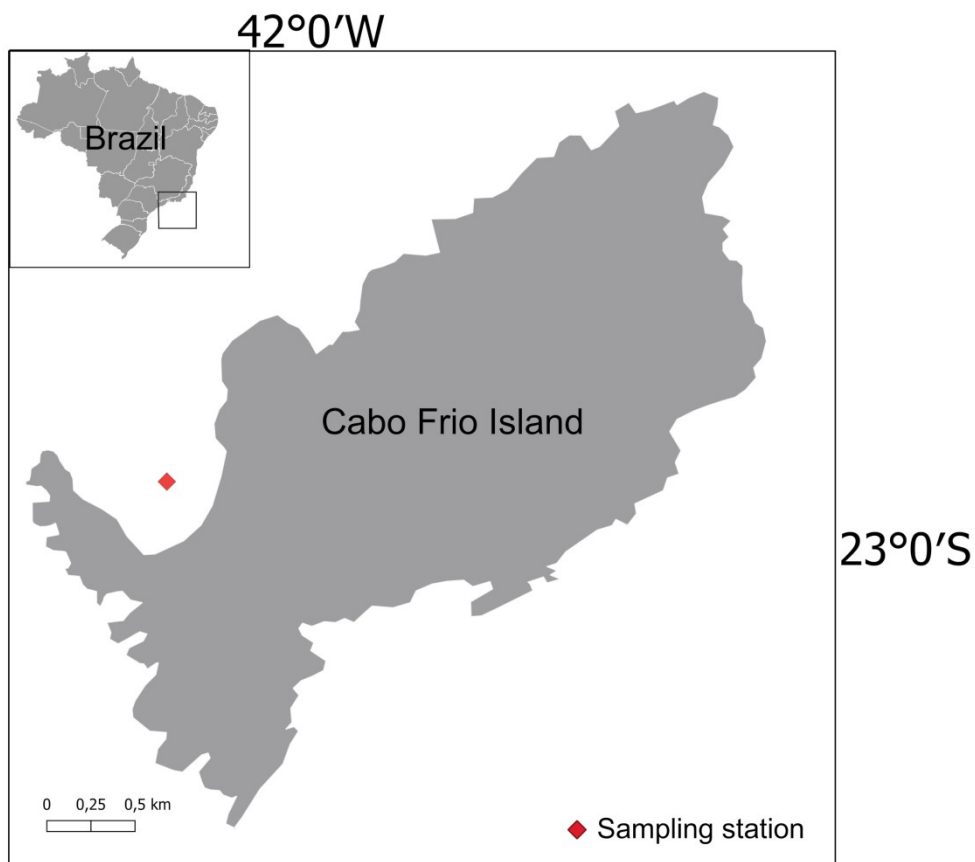

Figure 1 – Cabo Frio Island

Wind data were available in the Brazilian National Meteorological Institute (INMET) site, with hourly measurements done in a fixed automatic station located in Arraial do Cabo, Rio de Janeiro, Brazil (lat -22,98; long -42,02). The wind speed and direction distribution were calculated using the windrose graphic tool available in Python library (ROUBEYRIE; CELLES, 2018) and it were separated seasonally during the study time.

To account for the weekly change in the phytoplankton biomass, the concentration of chl-a, and total phaeopigments were estimated from seawater samples taken at the subsurface (~1 meter) with a 3-Liter Niskin bottle. In the laboratory, up to 2 liters of water were filtered on GFF membranes (Millipore), followed by cold (4°C) extraction in 90% PA acetone over 20 hours in the dark, and spectrophotometric analysis (JEFFREY S. W.; MANTOURA; WRIGHT, 1997). Sub-samples of 10 mL of seawater were fixed in 0.2%-1% volume-to-volume glutaraldehyde solution (final concentration), respectively, for femto-and picophytoplankton counting (GASOL, 1999; MARIE et al., 1999).

Flow cytometry counts

The abundance of picophytoplankton was estimated from 1 mL aliquots using a Marine Influx Cell Sorter (Becton Dickinson, San Jose, CA) equipped with a 488nm 200mWblue laser. *Synechococcus spp.* and picoeukaryotes(PEUK) were identified by the combination of cell size and fluorescence: red fluorescence (PMT 670/30 BP and PMT 750 LP) for all chlorophyll-bearing cells, either autotrophs or mixotrophs, and orange fluorescence (PMT 585/29 BP) for *Synechococcus*as a signal of phycoerythrin(COLLIER; PALENIK, 2003; GASOL, 1999). Virus-Like particles (VLPs) were enumerated as the dominant femtoplankton from 1 mL aliquots filtered through a 0.22 µm Cellulose Acetate membrane (Millipore®), diluted 100x in PBS buffer (Sygma-Aldrich), heated to 60ºC for 10 minutes, and stained with 2 µL of SYBR Green I (Thermo Fisher®) (MARIE et al., 1999). Cell size was estimated by the combination of side scatter (PMT SSC) and the polarized micro particle detector (PMT parallel and PMT perpendicular forward scatter – PA-FSC and PE-FSC) with the aid of 10µL of 1.35 µm microbeads (Spherotech®, 10^4beads.µL^{-1}) for *Synechococcus* and PEUK, and 5µL of 0.22µm ultrabeads (Spherotech®, 10^2beads.µL^{-1}) for VLPs, added as an internal reference standard. Enumeration was based on the average count of triplicates and each run stopped after acquiring 10,000 events. The cell concentration was corrected for the volume of sample processed in the flow cytometer by weighing the tube (1 mL≈1.03 mg) before and after each run (±0.01 mg, AUW-D220, Schimadzu Corporation, Japan). The acquisition was done using FACS™Sortware (Becton Dickinson, San Jose, CA) and the acquisition rate was kept under 200 events.sec^{-1}. Data analysis was done in Flowing Software® 2.5.1 (*Turku Bioscience* Centre, Finland), available at http://www.flowingsoftware.com.

Data analysis

Temporal variation in density of *Synechococcus*spp., PEUK and VLPs was predicted using Generalized Additive Mixed Models (GAMM) with REML smoothness selection (WOOD, 2017), based on relevant environmental data. The following environmental covariates were included: Sea surface temperature, bottom temperature, pH, salinity, pheopigments, chl-a, PO_4, NO_2, NO_3, NH_4. To account for possible biological interactions, VLP was included as a predictor of *Synechococcus* spp. and picoeukaryotes, whereas picoeukaryotes – strongly correlated (r = 0.89) with *Synechococcus*spp. – was included as a predictor of VLP. Autoregressive (AR) and moving average structures, with observation order as a covariate, were included in the models to account for the residual temporal autocorrelation. The optimal choice of AR(p) and MA(q) orders were performed with the auto ARIMA function, from the library *forecast* in R. Variables were checked for multicollinearity using the Variance Inflation Factor (VIF) > 2 as a cut-off value (ZUUR; IENO; ELPHICK, 2010), and for concurvity using the largest (worst) value > 0.7 as a cut-off. Thus, bottom water temperature (positively correlated to surface water temperature) and NO_2 and NO_3 (positively correlated to NH_4) were excluded from the analyses (ZUUR; IENO; ELPHICK, 2010). Models were based on a Gaussian distribution with significance assessed using the test criterion (P α=0.05) and backward elimination of covariates until all remaining terms in the model were significant (ZUUR; IENO; ELPHICK,

2010). All models were fitted using the 'mgcv' package (WOOD, 2017) in R statistical software (R Development Core Team, 2013).

Given the rapid response of small planktonic organisms, we considered that time intervals longer than two weeks would not result in direct effects on femtoplankton and picophytoplankton. Therefore, to assess the predictability of plankton fluctuations at different timescales, we compared the predictive performances of models (R^2) at three different forecasting time-lags: no lag (0), 1, and 2 weeks in advance. More formally, models were calibrated to predict the concentration of planktonic compartments for the predictor variables X_{t-n},

$$y_t = M_{(X_{t-n})}$$

where M is the specific model and n is a range of different time lags.

RESULTS

Physical and chemical characteristics

Over the 10 months of study, the sea surface temperature (SST) varied seasonally, ranging from 21.2°C to 25.6°C (average: 23.3°C ± 1.08°C), warmer waters occurring at the end of summer-autumn (February-April) and cold waters coincident with the upwelling season in the winter-spring (June-September). The wind variation during the study time (Fig.2) revealed highest frequency of easterly-northeasterly in austral spring and winter resembling to the summer conditions, correlated to cold surface waters. While the austral autumn displayed symbolic differences to the other seasons, with a considerable presence of west wind, concurrent to the wind-driven upwelling.
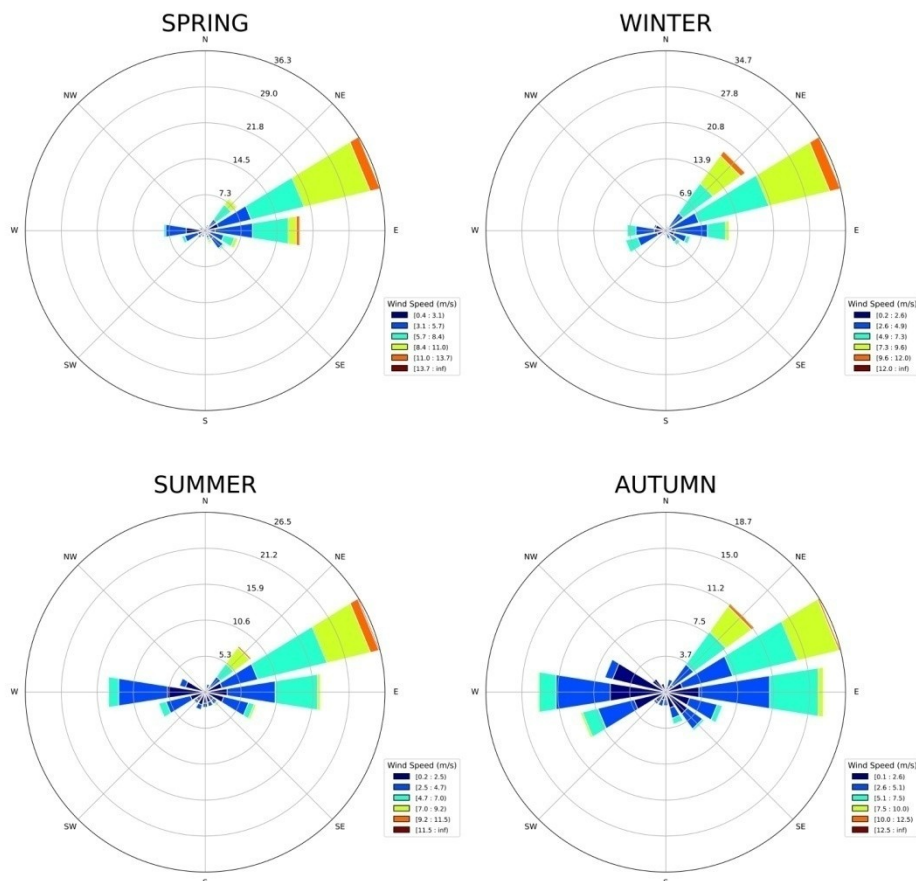

Figure 2 – Wind distribution during the study time

The salinity ranged from 27.1 in June to 38.3 in November (average: 36.1 ± 1.95) with the predominance of salty warmer Tropical Water (>36) most of the time. Macronutrients peaked seasonally, with the highest concentration of phosphate in June, while nitrate and ammonium increased in late August. Other occasional peaks were registered during the year, as seen in the nitrite curve (Fig 3). As expected, higher than average chl-a concentration (Chl-a > 0.56 mg/m^3) coincided with high-nutrient conditions during upwelling.
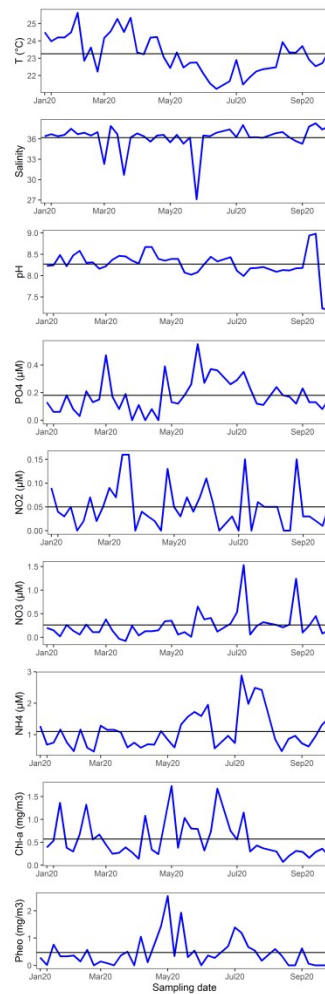

Figure 3 – Physical and chemical characteristics

Dynamics of aquatic virus-like particles (VLPs) and picophytoplankton

The planktonic community was numerically dominated by VLPs, followed by *Synechococcus* spp. and picoeukaryotes (PEUK) (Fig.4). The temporal dynamic of VLPs was highly variable, with extreme abundances occurring twice during the time series, firstly in the austral summer and later in the winter. *Synechococcus* spp. and PEUK, in contrast, peaked every two to three months, with higher abundances coincident with winter-spring (July to October).
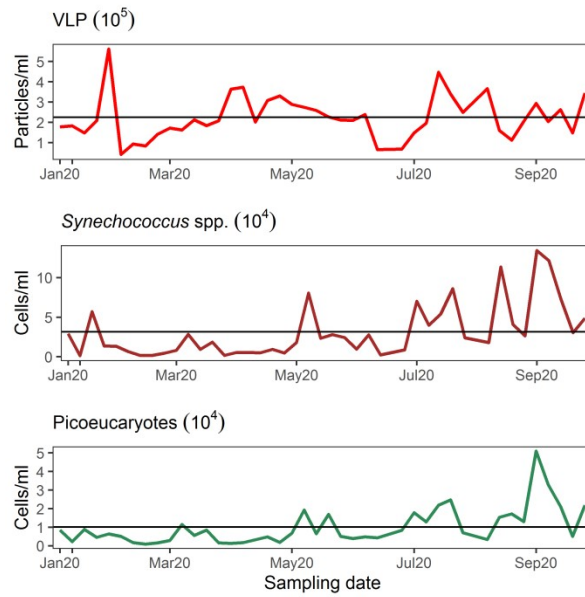
Figure 4 – Picophytoplankton and femtoplankton population abundances

The assessment of the variability of planktonic populations at multiple temporal scales revealed that *Synechococcus* spp. and picoeukaryotes were better predicted in the model with a one-week lag (R^2=64.7% for PEUK and 62.7% for *Synechococcus* spp.) whereas VLPs showed a faster response (no time lag, R^2=10.3%) (Table 1).The abundances of *Synechococcus* spp. and picoeukaryotes were strongly correlated to the changes in pH (Figs. 5a and 6a) and nutrients like NH_4 (Figs. 5b and 6b) and PO_4 with one-week lag (Fig. 5c), and to a lesser extent to the temperature. After two weeks, changes in pH were still positively correlated to the picoeukaryotes (Fig. 6c) but negatively to *Synechococcus* spp. The VLP abundance in turn was positively correlated to decreases in the chl-a concentration and increases in pheopigments at no time lag (Fig. 7a and 7b)
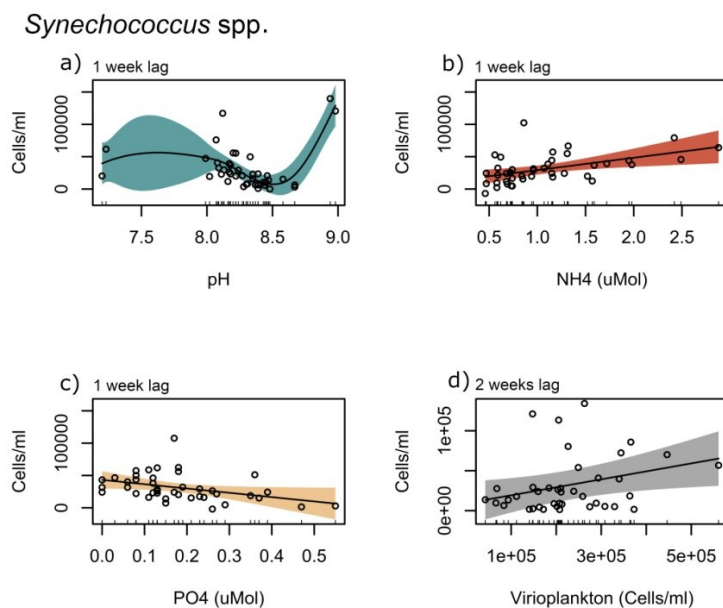

Figure 5 – Smoothers curves (S) showing the relationship (solid line) between the abundance of Synechococcus spp. and the variables selected (P < 0.05). Shaded areas indicate standard errors of the smooth curve. The 'rug plots' on the x-axis indicate the range of variables over which measurements were taken.
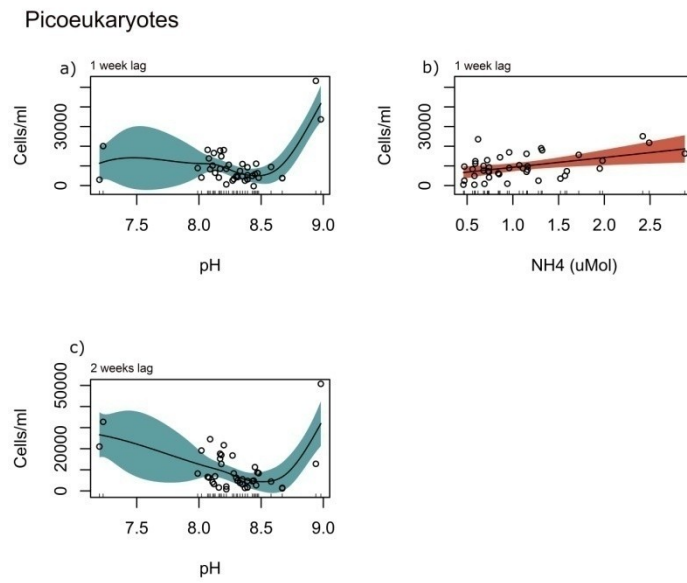
Figure 6 – Smoothers curves (S) showing the relationship (solid line) between the abundance of picoeukaryotes and the variables selected (P < 0.05). Shaded areas indicate standard errors of the smooth curve. The 'rug plots' on the x-axis indicate the range of variables over which measurements were taken.

Figure 6– Smoothers curves (S) showing the relationship (solid line) between the abundance of VLPs and the variables selected (P < 0.05). Shaded areas indicate standard errors of the smooth curve. The 'rug plots' on the x-axis indicate the range of variables over which measurements were taken.
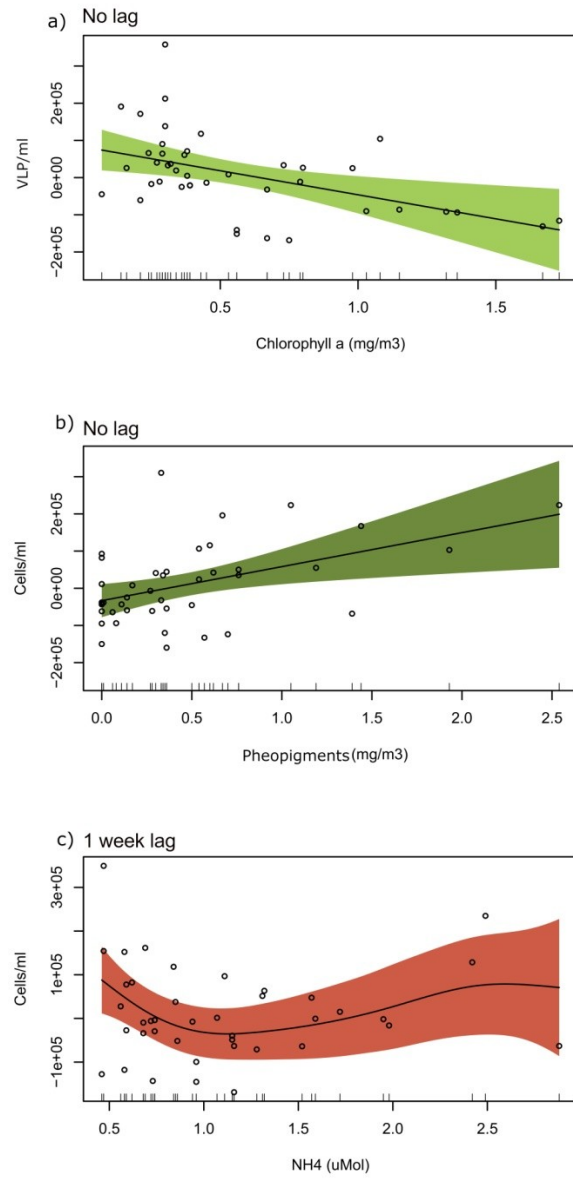
Source	Edf	F	P	R-sq.(adj)
Picoeukaryotes				
No lag				0
1 weeklag				64.70%
s(pH)	4.38	12.91	<0.001	
s(NH4)	1	7.1	<0.05	
2 weeklag				48.50%
s(pH)	3.97	9.03	<0.001	
***Synechococcus*spp.**				
No lag				0
1week lag				62.7%
s(pH)	4.12	9.97	<0.001	
s(NH4)	1	7.1	<0.05	
s(PO4)	1	4.31	<0.05	
2 weeklag				4.4%
s(virio)	1	6.23	<0.05	
Virioplankton				
No lag				17%
s(chla)	1	8.1	<0.01	
s(phae)	1	7.26	<0.05	
1 weeklag				10.30%
s(NH4)	3.16	2.84	<0.05	
2 weeklag				0%

Table 1. Generalized additive model outputs for the variation of planktonic populations.
Models were run using the 'mgcv' package and employed backward elimination of non-significant predictor variables. Virio = VLP, temp = temperature. Edf = estimated degrees of freedom, R-sq. (adj) = adjusted r squared.

DISCUSSION

Despite its fundamental role in the functioning of global ecosystems, few studies to date have addressed how environmental variables and biotic interactions shape the short-scale temporal dynamics of marine femtoplankton and picophytoplankton. By performing

weekly samplings over 10 months and addressing multi-temporal scales, we confirmed that the speed of response to environmental changes is dependent on the size of the organisms. VLPs, the most abundant group, (<0.2μm) promptly (no time lag) responded to the environmental changes while variations in *Synechococcus* spp. and picoeukaryotes abundance were better predicted after one week. While changes in VLPs abundance were mainly linked to variations in chl-a and pheopigments concentration, changes in the abundance of *Synechococcus* spp. and picoeukaryotes were mainly correlated to changes in nutrients and pH, a variable that may exert significant effects on the growth rate and ecology of marine phytoplankton but which has received little attention in ecological studies (Hinga et al. 2002).

VLPs, particularly the bacteriophages and cyanophages, are highly abundant entities in marine ecosystems and usually dominate the femtoplankton(MALITS et al., 2021; WOMMACK; COLWELL, 2000). In this study, their abundance was one-fold higher than the abundance of picophytoplankton populations, suggesting that they may exert a relevant role in the planktonic production and microbial loop. The observed relationship between VLPs' abundance and the concentration of chl-a and pheopigments, important indicators of the physiological status of the microalgal community, support this hypothesis. The negative relationship between VLP abundance and chl-a suggested that higher abundance of marine viruses may result in the lysing of their autotrophic and/or mixotrophic hosts. This hypothesis is further reinforced by the positive relation between VLPs' abundance and pheopigments, a proxy of chl-a degradation (PUSCEDDU et al., 2009; SATHISH; PATIL; ANIL, 2020; WIEKING; KRÖNCKE, 2005). Given the significant association between changes in VLP abundance, chl-a and pheopigments, our results indicate that changes in the pigments content may suggest an increased viral lysis of small-phytoplankton hosts.

Temporal and spatial changes in the environment can affect the picophytoplankton population at different scales, from dial to seasonal. Our results suggest that each temporal scale describing the distribution of *Synechococcus* spp. and picoeukaryotesis derived from a distinct driver. Under short-term oscillation, the observed two-week lagged correlation with the femtoplankton (mainly VLPs) fits into the expected virus-host relationship. The viral and subviral particles that dominate marine ecosystems are completely dependent on the host cells to replicate (COLOMBET et al., 2020a) and thus linked to the host lifecycle. Moreover, data analysis highlighted changes in pH with a one-week time lag as a meaningful environmental variable that regulates *Synechococcus* and picoeukaryotes.

Interestingly, pH variations were reported as a significant influence on the picophytoplankton abundance. Although a few studies have demonstrated the importance of pH on the dynamics of small phytoplanktonic organisms (e.g., Braak & Dame, 1989; Chen & Durbin, 1994), most studies do not consider this variable as a possible factor influencing the ecology of marine phytoplankton species, likely due the open-ocean idea of 'a constant pH of seawater' (Hinga, 2002). Yet, the pH of seawater responds to changes in different aspects like dissolved CO_2 concentration, concentration of nutrients and temperature, and may significantly varying coastal waters due to seasonality and ocean currents (HINGA, 2002; ISHIDA et al., 2021). In fact, the pH of seawater may reach values greater than 9 or lower than 7 in coastal environments (Hinga et al. 2002). In this study, we found that the abundance of picophytoplanktonic organisms was initially higher in waters with higher pH, however the opposite pattern was found after two weeks. Higher growth in elevated pH (i.e., >8) has been observed for the diatoms *Thalassiosira pseudonana, Stephanopyxis palmeriana, Coscinodiscus sp. and Ditylumbrightwellii. Pruder and Bolton(1979)*recorded that *T.pseudonana*grew constantly until pH 8.9 whereas (GOLDMAN, 1999) found that *S. palmeriana, Coscinodiscu*s sp. and *D. brightwellii* grew steadily when the pH increased from 8.1 to 8.5. Above these values, however, the growth rates of all the mentioned species decreased, suggesting that long-term exposure to extreme pH values may compromise the growth of most species. In our study, the exposure to high pH seems to initially favor the growth of picophytoplankton populations, however these extreme values seem to reduce their growth and reproduction after a two week period of exposure. These results are in accordance to Hinga (2002), which reported that the extreme values found in coastal environments (i.e., below 7 or over 9) compromise the growth of most phytoplanktonic species. Given that changes in the seawater

pH is expected under every climate change scenario (IPCC, 2021), our results suggest that increased emissions of CO_2 and the associated changes in pH seawater may not only affect the physical-chemical properties of the ocean but may also significantly influence marine photosynthetic organisms (GAO et al., 2019; HYUN et al., 2020). Considering the low number of investigations on the influence of pH variations on the biodiversity of costal ecosystems, furthers studies should expand our findings and investigate how changes in pH may influence planktonic organisms and marine trophic webs. Both laboratory experiments and long-term monitoring could provide important information to help us better understand this topic.

In parallel to the weekly changes, there was also strong seasonality in picophytoplankton population dynamics, with higher abundances in the second half of the study. This seasonality is mainly linked to the variability in nutrient concentration. Starting at the end of winter and lasting until the spring, the gradual rise of the deep nutrient-rich South Atlantic Central Water (SACW) shallows the Mixed Layer and fuels the local phytoplankton production at the study area (FERNANDES et al., 2012; GUENTHER et al., 2008). The highest peaks of *Synechococcus* and picoeukaryotes in the spring matched these high nutrients conditions during upwelling, mainly NH_4. The ecosystem metabolism linked to aquatic microorganisms is strongly based on the ammonia-ammonium ratio (NH_3:NH_4^+) and essentially ruled by the pH, that in turn affect the recycling of nitrogen by nitrification (KIRCHMAN, 2008).

The increase in nutrient concentration is also one of the effects of the wind-driven upwelling system in Cabo Frio. The predominant easterly-northeasterly winds during the study time, especially in spring and winter months, led to the onset of upwelling conditions, favoring a upward transport of the cold thermocline level towards the coast (CAMPOS et al., 2013; CASTELAO; BARTH, 2006; DE MAHIQUES et al., 2005). The Ekman's transport improved by the persistent winds intermediate water masses rich on nutrient, cold and less salty (OLIVEIRA et al., 2019), affecting the picophytoplankton growth and distribution, consequently the VLP's dynamics in the water column.

The picophytoplanktonic community living in the Cabo Frio upwelling system is also fueled either by the input of deep nutrient rich water or the recycling of coastal nutrients that respectively lead to an "herbivorous food web" or a "microbial food web" (GUENTHER et al., 2008; GUENTHER; VALENTIN, 2008). Under favorable northeastern winds that dominate during the winter-spring transition, the onset of upwelling that rises the nutrient-rich deep South Atlantic Central Water to the photic zone fuels the *Synechococcus* spp. and picoeukaryotes(CAMPOS et al., 2013; CASTELAO; BARTH, 2006; COELHO-SOUZA et al., 2012; DE MAHIQUES et al., 2005; OLIVEIRA et al., 2019). In contrast, the relaxing of upwelling during southwest winds generates a cascade effect combining cold fronts, rainy days, increased NH_4, and warm waters that acidifies the ecosystems and fuels the bacterioplankton(GUENTHER; VALENTIN, 2008). The nutrient contents and temperature variations are known to affect the plankton trophic structure in Cabo Frio (GUENTHER et al., 2008), but mainly the predominance of inorganic nutrients as ammonium that favors the small-sized phytoplankton (KUVALDINA et al., 2010; LIPS; LIPS, 2010; MADHU et al., 2021).

CONCLUSION

By performing weekly samplings of small-planktonic organisms and investigating their dynamic over multiple timescales, we found that the speed of response to environmental changes may depend on the size of the organisms. Whereas femtoplanktonic organisms (VLPs) responded fast (i.e., no time lag) to environmental changes, the influence of environmental variables on the abundance of picophytoplanktonic organisms (i.e., *Synechococcus* spp. and picoeukaryotes) was better perceived after a one week lag. Interestingly, we observed that VLPs seem to be more influenced by biological interactions as demonstrated by their relationship with chl-a and pheopigments concentration. On the other hand, variations in the abundance of *Synechococcus* spp. and picoeukaryotes were mainly related to changes in pH and nutrients. Overall, our results demonstrate the importance of high-frequency assessments to better understand the temporal dynamic of planktonic organisms and show that their response to environmental changes are better perceived at multiple temporal scales.

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4. CONSIDERAÇÕES FINAIS

As oscilações das populações picofitoplanctônicas e femtoplanctônicas durante uma série temporal de curta frequência realizada em uma área de ressurgência em Arraial do Cabo, RJ revelaram que:

- A comunidade planctônica é numericamente dominada por VLPs, seguida por *Synechococcus* spp. e picoeucariotos. A dinâmica temporal dos VLPs é altamente variável, com abundâncias extremas ocorrendo duas vezes durante a série temporal, primeiro no verão e depois no inverno;

- *Synechococcus* spp. e picoeucariotos atingiram o pico a cada dois ou três meses, com maiores abundâncias coincidentes com o inverno-primavera;

- A abundância de *Synechococcus* spp. e picoeucariotos são diretamente correlacionados com as mudanças no pH e na disponibilidade de nutrientes, como NH_4 e PO_4, com uma semana de atraso. A abundância do picoplâncton é correlacionada em menor grau com a temperatura;

- A abundância de VLP, por sua vez, é positivamente correlacionada com diminuições na concentração de clorofila e aumentos nos feopigmentos sem intervalo de tempo;

Para atingir os objetivos, foram realizadas coletas de plâncton semanais durante um período de 10 meses em uma área de ressurgência costeira impulsionada pelo vento, combinadas ao uso de citometria de fluxo e modelagem estatística.

A dinâmica populacional dos organismos planctônicos que compõem a cadeia trófica na região da ressurgência de Cabo Frio varia de acordo com o tamanho, limitação de nutrientes, fatores físico-químicos, como pH, temperatura, além do consumo dos detritos, das próprias comunidades entre elas ("grazing") e da lise viral. Nessa região, as oscilaçõesna temperatura e na disponibilidade de nutrientes têm efeito sobre o controle exercido pelo femtoplâncton sobre o picofitoplâncton.

De forma geral, os resultados forneceram novas informações sobre a dinâmica temporal do femto- e picoplâncton,e aumentaram a compreensão sobre os vetores dos organismos planctônicos, fornecendo assim dados relevantes para entender melhor como as mudanças futuras nas condições ambientais podem influenciar nossos oceanos.

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