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Multi-scale temporal variation of marine femtoplankton and picophytoplankton: the role of 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 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. We combined this intensive fieldwork with multi-color flow cytometry and statistical modeling to investigate the temporal changes of both femto- and picophytoplankton at multiple temporal scales. We found that femtoplanktonic organisms (including virus-like particles) responded faster (i.e., without a temporal lag) to environmental changes, mainly related to chlorophyll-*a* (chl-*a*) and phaeopigment variations. On the other hand, picophytoplanktonic organisms showed a slower response to environmental changes, with positive responses to variation in pH and NH4 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, which highlights the importance of environmental variables and biological interactions as drivers of their temporal dynamics.

Descriptors: Flow cytometry, Time series, Generalized additive model, Upwelling, Virus-like particles.

INTRODUCTION

Marine planktonic organisms compose the base of the size-structured marine food web and play a key role in ocean functioning (Fuhrman, 2009; Litchman et al., 2015; Andersen et al., 2016; Pierella Karlusich et al., 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. 2020). 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., 2020).

These organisms exert a major role in biogeochemical cycles and are responsible for a large portion of the ecosystem's new production in the

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oceans (Li, 1994; Pedrotti et al., 2017; Flaviani et al., 2018). 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 and 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 and López-García, 2019).

For decades, researchers have considered that planktonic organisms show periodic rhythms in their abundance following the "periodic plankton" concept (Moreira and 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 and 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 and 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 and 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 and 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 (Wilhelm and Suttle, 1999; Weinbauer and Rassoulzadegan, 2004; Bolaños et al., 2020). 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, thus returning dissolved organic matter (DOM) and particulate organic matter (POM) to the microbial loop (viral shunt). Also, many of the important metabolic processes of planktonic species are size-dependent (Platt & Denman, 1977; Edvardsen et al. 2002), indicating that environmental changes may result in faster or slower responses of planktonic organisms according to their size.

These findings demonstrate that the drivers of phytoplankton fluctuations are likely to differ among biological groups and 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 et al., 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 et al., 2019).Yet, this would only be possible when adequate time-series data is available. As microorganisms exhibit fast growth and population size fluctuations, even monthly sampling may miss part of the rapid plankton dynamics (Moreira and 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.

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). Specifically, we (1) tested whether the speed of response of planktonic organisms to environmental changes is dependent on their size, and (2) investigated the influence of environmental variables and biotic interactions on these responses. 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, 2017). Upwelling events in Cabo Frio usually last 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” 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 and Parsons, 1972) protocol.

Wind data were available on the Brazilian National Meteorological Institute (INMET) site, with hourly measurements made in a fixed, automatic station located in Arraial do Cabo, Rio de Janeiro,

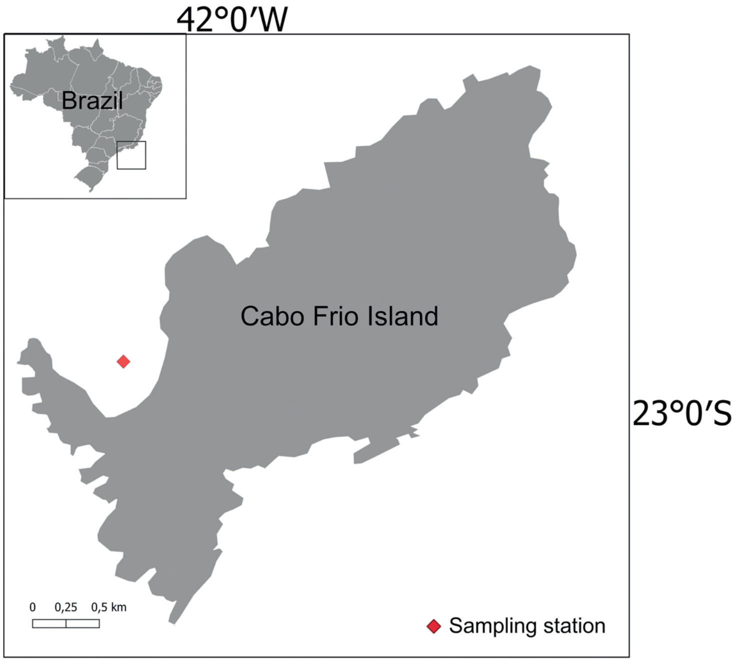

Figure 1. Sampling site (42°0´W, 23°0´S) in the Cabo Frio upwelling region (Brazil).

Brazil (lat -22,98; long -42,02). Wind speed and direction distribution were calculated using the windrose graphic tool available in the Python library (Roubeyrie and Celles, 2018) and were separated seasonally during the study time.

To account for the weekly change in phytoplankton biomass, the concentration of chl-*a* and total phaeopigments were estimated from seawater samples taken at the subsurface (~1 meter) using 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. et al., 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 (Gasol, 1999; Collier and Palenik, 2003). 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®) at a final concentration of 5X 10^{-5} of the commercial stock solution (Gasol, 1999; Marie et al., 1999; Brussaard, 2004)(Fig.2b), and promptly analyzed. Since staining procedures of femtoplankton and picoplankton are thermosensitive (Brussaard. 2004), any potential bias on the time series analysis was removed by data normalization (Zar, 2010). 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. The small particle option of the Influx system improves the FSC detection, with the help of the pinhole and

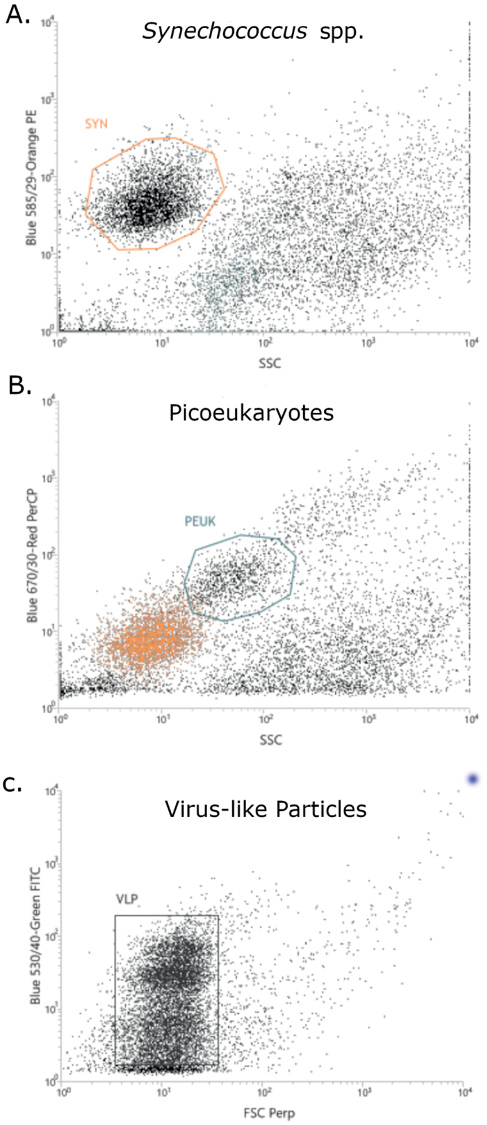

Figure 2. Representative cytograms of *Synechococcus* spp. (A) and picoeukaryotes (B), using the natural fluorescence of phycoerythrin (orange) and chlorophyll (red); and VLPs (C) according to their induced green fluorescence.

a photomultiplier (PMT). For this reason, it was used as a trigger for the VLP's population.

Manual gates in specific regions (Fig. 2) were standardized and used to compare all the picophytoplankton and VLP's samples (Fig.2 A-C) (Gasol and Morán, 2015). 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, and 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 et al., 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 et al., 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 et al., 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

Environmental conditions

The wind variation during the study revealed a highest frequency of easterly-northeasterly in austral spring (Fig.3a) and winter (Fig.3b), resembling the summer conditions correlated to the upwelling occurrence. While the austral autumn (Fig.3d) displayed symbolic differences to the other seasons, with a considerable presence of west wind, concurrent to the wind-driven upwelling.

Over the 10 months of study, the sea surface temperature (SST) (Fig.4a) varied seasonally, ranging from 21.2°C to 25.6°C (average: 23.3°C ± 1.08°C), with warmer waters occurring at the end of summer-autumn (February-April) and colder waters coincident with the upwelling season in winter-spring (June-September).The salinity (Fig.4a) ranged from 27.1 in June (rainy period) to 38.3 in November (average: 36.1 ± 1.95), with the predominance of salty warmer Tropical Water (>36) most of the time. The pH (average: 8.27 ± 0.32) (Fig.4c) was generally higher from January to May and lower from May to September. A slight decrease to 8.0 coincided with the rainy season

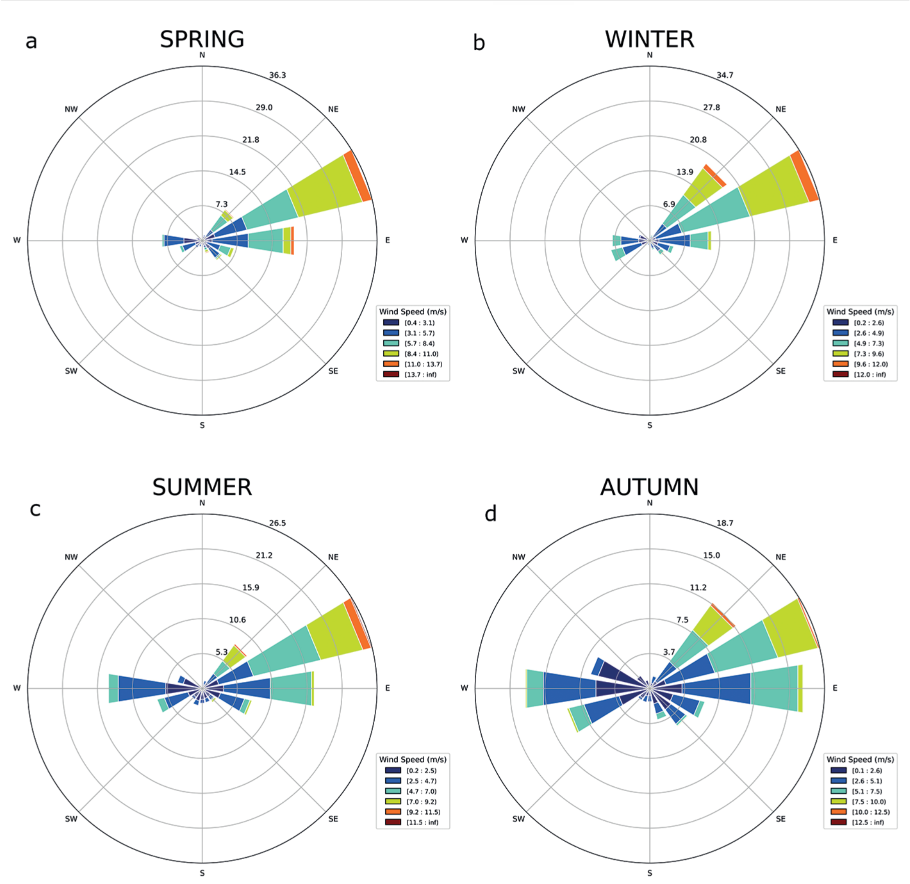

Figure 3. Wind roses during the study period: (a) spring; (b) winter; (c) summer; (d) autumn.

in June, when continental runoff is expected to increase. An unexpected, but significant, oscillation was observed at the end of the time series, in October (Fig. 4c), when the highest (~9) and lowest (~7.5) values were recorded in sequence. Macronutrients peaked seasonally (Figs. 4d-g), with the highest concentration of phosphate (average: 0.46 ± 1.79) in June coincident with rainy inputs to the coast, while nitrate (average: 0.26 ± 0.29µM) and ammonium (average: 1.30 ± 1.43 µM) increased in late August during upwelling. Other occasional peaks were registered during the year, as seen in the nitrite (average: 0.05 ± 0.04 µM) curve (Fig.4f). As expected, higher than average chl-*a* (Fig. 4h) concentration (Chl-*a* > 0.56 mg/m^3) coincided with high-nutrient input (PO$_4^{3-}$ ≥0.55µM;NO$_2$–≥0.15µM;NO$_3$-≥1.5µM; NH$_3$ ≥ 2.5µM) during the upwelling (Table S1).

DYNAMICS OF FEMTOPLANKTON AND PICOPHYTOPLANKTON

The planktonic community was numerically dominated by femtoplankton as VLPs (max of 5.61x10^5 particles mL^{-1}), followed by *Synechococcus* spp. (max of 1.34 x 10^5 cells mL^{-1}) and picoeukaryotes (PEUK) (max of 5.09 x 10^4 cells mL^{-1}) (Table S2).The temporal dynamic of VLPs was highly variable (Fig. 5a), with extreme abundances occurring twice during the time series, first in the austral summer and last in the winter. *Synechococcus* spp. and PEUK (Fig.5b-c), in contrast, peaked every two to three months, with higher abundances coincident with winter-spring (July to October).

The assessment of the variability of planktonic populations at multiple temporal scales revealed that *Synechococcus* spp. and picoeukaryotes were better predicted by the model with

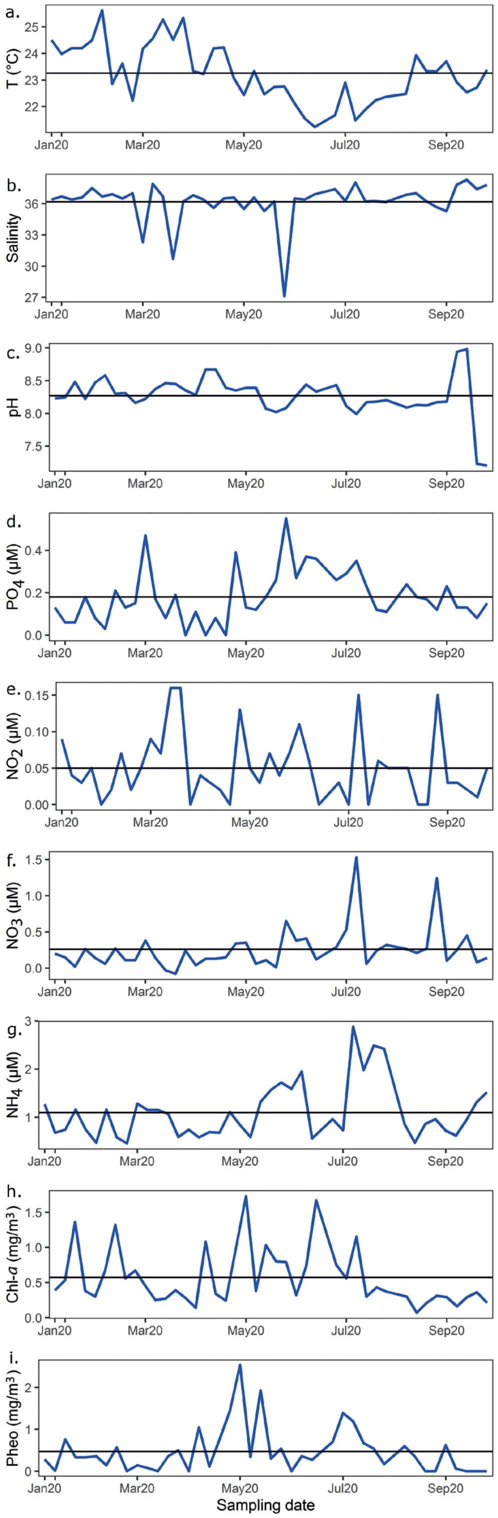

Figure 4. Environmental conditions during the study time. Temperature (a), salinity (b), pH (c), phosphate (d), nitrite (e), nitrate (f), ammonium (f), chlorophyll-*a* (h), pheopigments (i). The straight line corresponds to the average value registered throughout the sampling period.

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. (Fig.6a-d) and picoeukaryotes (Fig.6e-g) were strongly correlated to changes in pH and nutrients such as NH_4 and PO_4, with one-week lag. After two weeks, changes in pH were still positively correlated to the picoeukaryotes, but negatively to *Synechococcus* spp. The VLP abundance, in turn, was positively correlated to decreases in chl-*a* concentration (Fig.7a) and increases in pheopigments, at no time lag (Fig.7b).

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 (<0.2µm), the most abundant group, promptly (no time lag) responded to environmental changes, while variations in *Synechococcus* spp. and picoeukaryotes (>0.2µm) 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.

VLPs, particularly the bacteriophages and cyanophages, are highly abundant entities in marine ecosystems and usually dominate the femtoplankton (Wommack and Colwell, 2000; Malits et al., 2021). In this study, the VLPs abundance was one-fold lower than that previously found by Pereira et al. (2009), but one-fold higher than the abundance of picophytoplankton populations, suggesting that they may exert a significant role in the planktonic production and microbial loop. The observed relationship between VLP abundance and chl-*a* and pheopigments concentration, important indicators of the physiological status of the microalgal community, support this hypothesis.

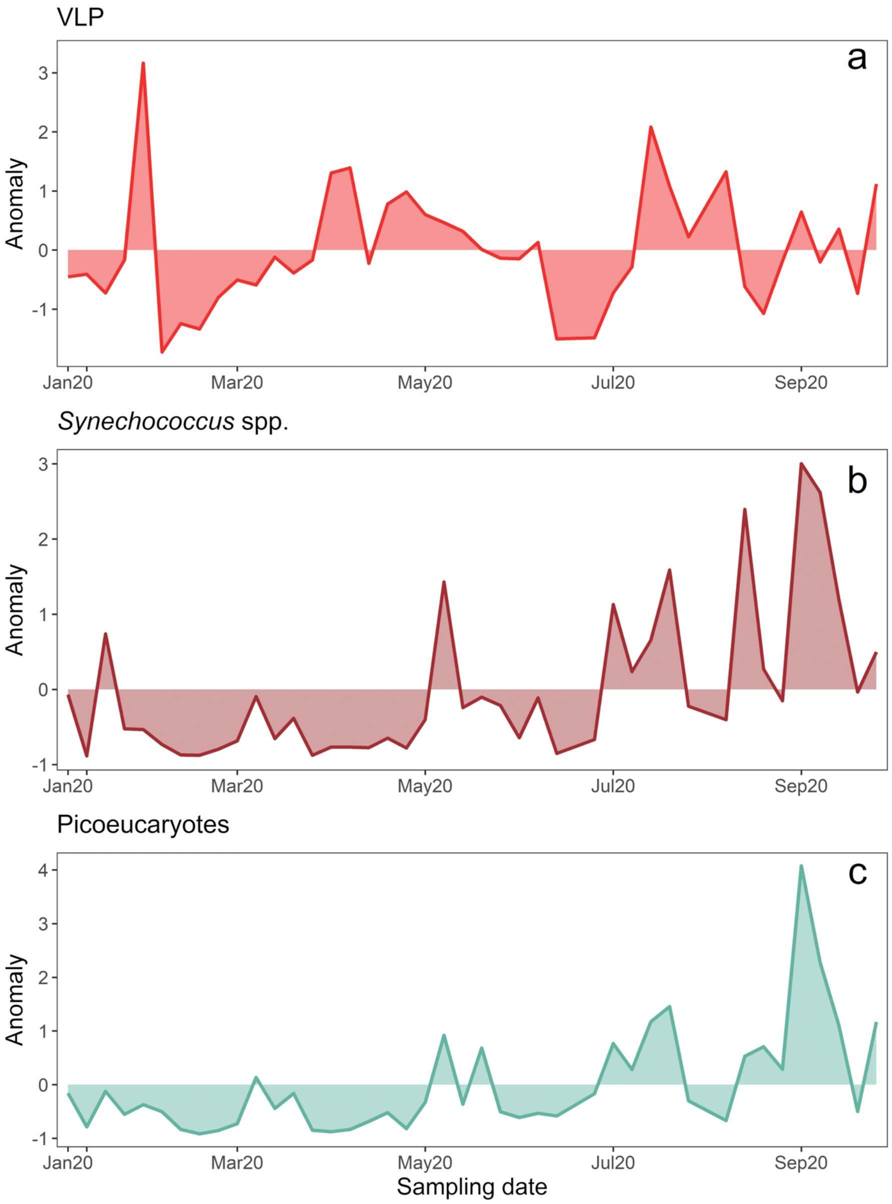

Figure 5. Standardized anomalies of VLP (a), *Synechococcus* spp. (b) and picoeukaryotes (c).

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 VLP abundance and pheopigments, a proxy of chl-a degradation (Wieking and Kröncke, 2005; Pusceddu et al., 2009; Sathish et al., 2020). Given the significant association between changes in VLP abundance, chl-a, and pheopigments, our results indicate that changes in the pigment content may suggest an increased viral lysis of small-phytoplankton hosts.

Temporal 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 picoeukaryotes is derived from a distinct driver. Under short-term oscillation, the observed two-week lagged correlation with the femtoplankton (mainly VLPs) fits the expected virus-host relationship. The VLPs that

Table 1. Generalized additive model outputs for the variation of planktonic populations.

Source	Edf	F	P	R-sq.(adj)
Picoeukaryotes				
No lag				0
1 week lag				64.70%
s(pH)	4.38	12.91	<0.001	
s(NH_4)	1	7.1	<0.05	
2 week lag				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(NH_4)	1	7.1	<0.05	
s(PO_4)	1	4.31	<0.05	
2 week lag				4.4%
s(virio)	1	6.23	<0.05	
Virioplankton				
No lag				17%
s(chl-*a*)	1	8.1	<0.01	
s(phae)	1	7.26	<0.05	
1 week lag				10.30%
s(NH_4)	3.16	2.84	<0.05	
2 week lag				0%

Models were run using the ‘mgcv’ package and employed backward elimination of non-significant predictor variables. Virio = VLP, Edf = estimated degrees of freedom, R-sq.(adj) = adjusted r squared.

dominates marine ecosystems are completely dependent on host cells to replicate (Colombet et al., 2020) and are 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.

Variations in pH were reported as a significant influence on the dynamics of small phytoplanktonic organisms (e.g., Braak & Dame, 1989; Chen & Durbin, 1994).The pH of seawater responds to changes in different aspects such as dissolved CO_2 concentration, concentration of nutrients, and temperature, and may significantly vary in 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 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 Ditylum brightwellii.* 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 pH increased from 8.1 to 8.5. Above these values, however, the growth rates of all mentioned species decreased, suggesting that long-term exposure to extreme pH values may compromise the growth of most species. Accordingly, Xu et al. (2012) found that micronutrient uptake rates by phytoplankton decreased about 30% as pH decreased from 8.5 to 7.7, whereas Shi et al. (2010) showed that ocean acidification

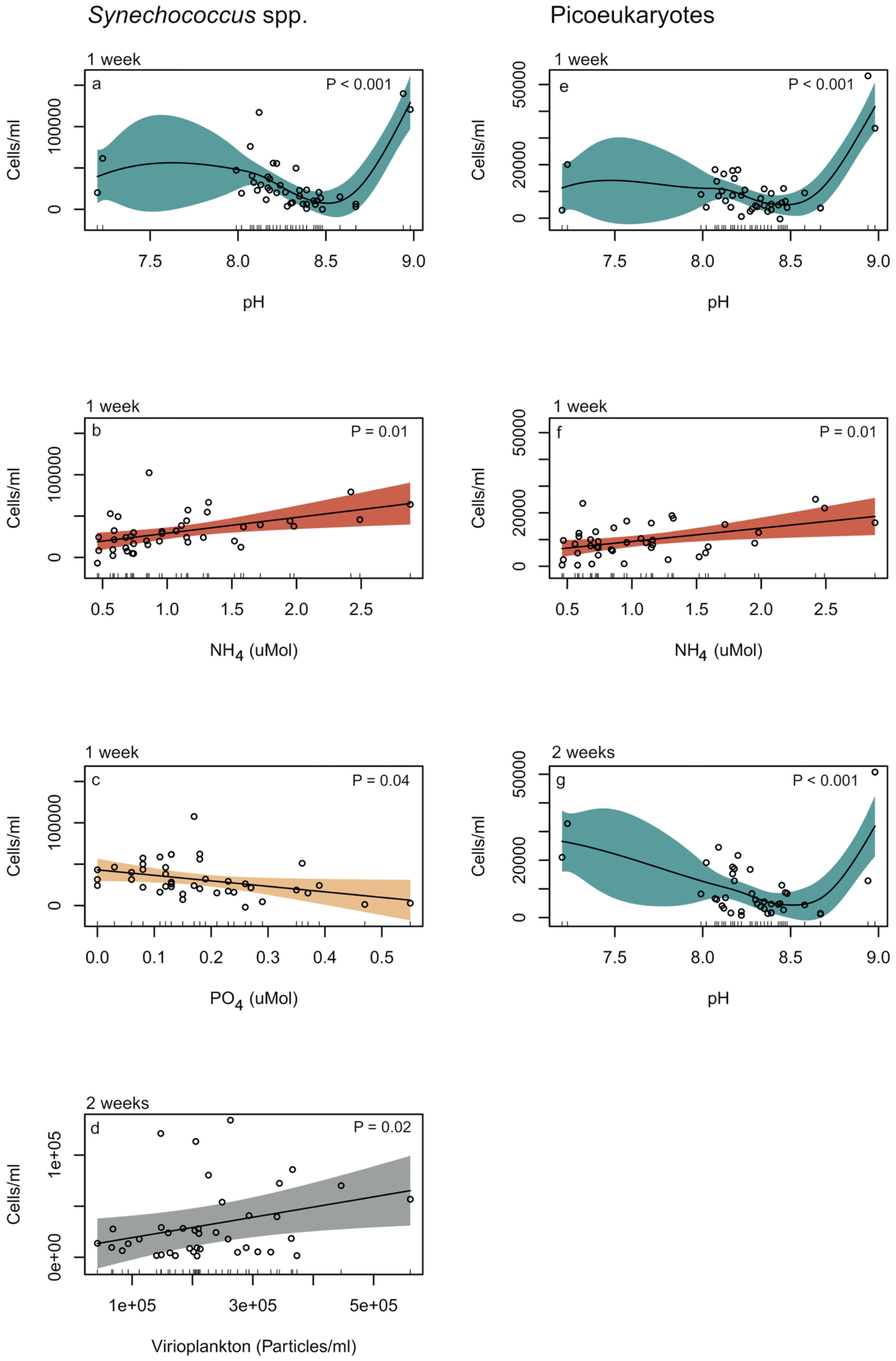

Figure 6. Smoothers curves (S) showing the relationship (solid line) between the abundance of *Synechococcus* spp., picoeukaryotes and the variables selected ($P < 0.05$). Shaded areas indicate standard errors of the smooth curve. The 'rug plots' on the x-axis indicate the range of variables over which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

leads to a decrease in the rate of iron (Fe) uptake by phytoplankton. In our study, the exposure to high pH seems to initially favor the growth of picophytoplankton populations, then reducing their growth and reproduction after a two-week exposure. These results are in accordance with Hinga

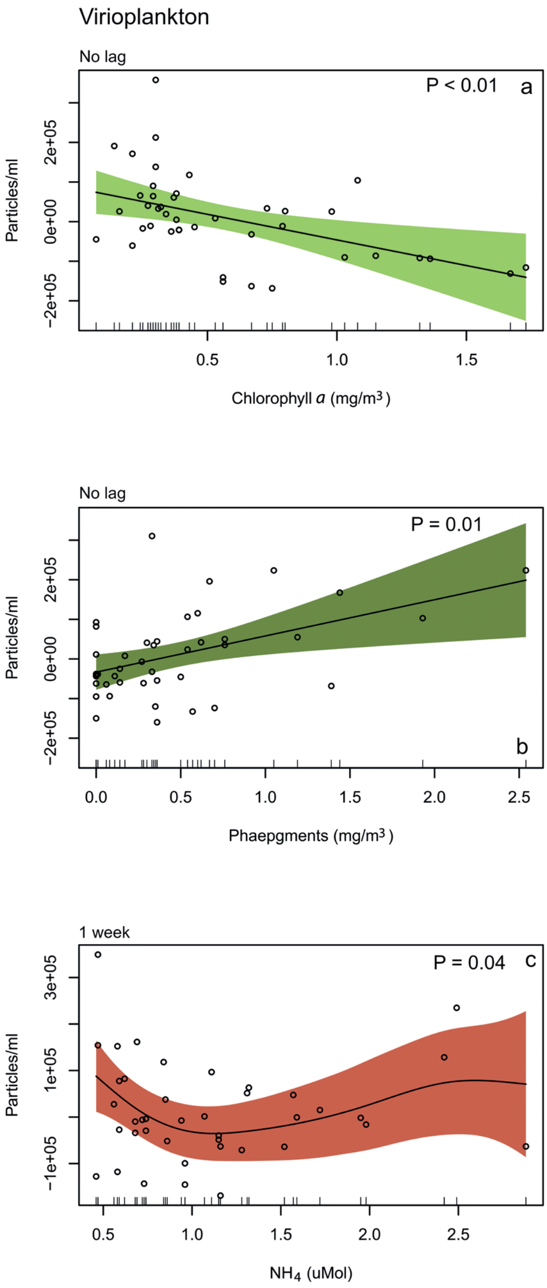

Figure 7. Smoothers curves (S) showing the relationship (solid line) between the abundance of VLPs and the variables selected ($P < 0.05$). Shaded areas indicate standard errors of the smooth curve. The 'rug plots' on the x axis indicate the range of variables over which measurements were taken.

(2002) and Rai and Rajashekhar (2014), which reported that extreme values found in coastal environments (i.e., below 7 or over 9) compromise the growth of most phytoplanktonic species. Given that changes in 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).

In parallel to the weekly changes, there was strong seasonality in picophytoplankton population dynamics, with higher abundances in the second half of the study. This 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 phytoplankton production at the study area (Guenther et al., 2008; Fernandes et al., 2012). The highest peaks of *SYN* and PEUK in the spring matched these high nutrients conditions during upwelling, mainly NH_4. Similar results were found by Bergo et al. (2017), which showed that picoplankton carbon biomass patterns reflect the strong effect of the SACW intrusion on the Southeastern Brazilian continental shelf, inducing the dispersion of dissolved organic carbon (DOC) below the euphotic zone in an effective biological pump.

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 an upward transport of the cold thermocline level towards the coast (De Mahiques et al., 2005; Castelao and Barth, 2006; Campos et al., 2013). The Ekman's transport improved by the persistent winds intermediate water masses rich on nutrient, cold and less salty (Oliveira et al., 2019), affecting picophytoplankton growth and distribution, and thus the VLPs 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 and Valentin, 2008; Guenther et al., 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 (De Mahiques et al., 2005; Castelao and Barth, 2006; Coelho-Souza et al., 2012; Campos et al., 2013; Oliveira et al., 2019). Similarly, Ribeiro et al. (2016) showed that the uplifting of nutrient rich waters seemed to induce an abundance increase in SYN and PEUK populations.

In contrast, the relaxing of upwelling during southwest winds generates a cascade effect, combining atmospheric cold fronts, rainy days, increased NH_4, and warm waters that acidifies ecosystems and fuels bacterioplankton (Guenther and Valentin, 2008). Both nutrient content and temperature variation are known to affect plankton trophic structure in Cabo Frio (Guenther et al., 2008), but mainly the predominance of inorganic nutrients, such as ammonium, that favors small-sized phytoplankton (Kuvaldina et al., 2010; Lips and 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 organisms. Whereas femtoplanktonic organisms (VLPs) responded quickly (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 one week. 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 of small femtoplankton and picoplankton size-classes to better understand the temporal dynamic of the whole planktonic assemblage, and show that their response to environmental changes are better perceived at multiple temporal scales.

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AUTHOR CONTRIBUTIONS

C.R.: Sampling; Analysis, Investigation; Writing - original draft &editing;

G.C.: Formal Analysis; Investigation; Writing - review & editing;

L.F.: Supervision; Resources; Project Administration; Funding Acquisition; Writing - review & editing.

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