

Research Article

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Microzooplankton community structure in a subtropical South-West Atlantic coastal site

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Abstract

The microzooplankton community structure (species abundance, biomass, diversity) was investigated at a coastal marine station on the South-West Atlantic Ocean (34°23'S–53°45'W, Uruguay). This is a hydrographically complex site within the Subtropical Convergence zone of the SW Atlantic where knowledge of the microzooplankton is particularly scant. The main goal was to perform a first characterization of that community and evaluate its association to environmental drivers along an annual cycle. Oceanographic variables (temperature, salinity, irradiance, nutrients, chlorophyll-a) and ciliates (aloricate and loricate), and dinoflagellates were recorded monthly from July 2019 to June 2020. Over 100 microzooplankton taxa belonging to approximately 30 families and 40 genera were identified, including several subtropical and subantarctic species. Community structure followed wide transitions at the seasonal scale – particularly between summer and winter as subtropical taxa alternated with euryhaline taxa from colder subantarctic waters. The core environmental variables (temperature, salinity and dissolved inorganic nitrogen [DIN]) explained overall variance in microzooplankton community assembly. During summer, high temperatures (20.3, 16.3–22.4°C) and low nutrients (DIN: 3.5, 0.7–6.7 µM; PO₄: 1.0, 0.8–1.5 µM) benefited the development of aloricate ciliates. A nutrient pulse in winter posed favourable stoichiometric conditions and the numerical abundance was dominated by dinoflagellates and loricate ciliates in the following months, while diversity remained highest (taxonomic richness: 36 [22–46]; Shannon–Wiener index: 2.5 [1.9–2.8]). Results suggested that the microzooplankton community at the study site is mainly structured by hydrographic variability linked to the seasonal replacement of offshore water masses that differed in thermohaline properties and nutrient levels, and local processes.

Introduction

Microzooplankton communities in the size range of 20–200 µm (Sieburth *et al.*, 1978) are mostly comprised of ciliates (loricate and aloricate), dinoflagellates and crustacean nauplii (Calbet, 2008). They encompass strict heterotrophs and mixotrophic protist plankton – mixoplankton – combining photo(auto)trophy and phago(hetero)trophy in a single organism (Flynn *et al.*, 2019). They constitute key components of marine food webs and rapidly synchronize productivity patterns due to their short generation time (Strom, 2002). Their grazing accounts for 60–75% of phytoplankton mortality across a spectrum of oceanic and coastal systems (Calbet and Landry, 2004).

Microzooplankton is sensitive to environmental variability and can respond rapidly to changes in meteorological-oceanographic conditions (Caron and Hutchins, 2013). Changes in species composition, abundance and size structure have been reported as responses to seasonal fluctuations in temperature (Antacli *et al.*, 2018; Urrutxurtu, 2004), salinity (Bojanić, 2001; Barría de Cao *et al.*, 2011), stratification (Kiørboe, 1993; Stoecker *et al.*, 2014), depth (Lavrentyev *et al.*, 2019; Romano *et al.*, 2021), trophic state (Bojanić *et al.*, 2012) and chlorophyll-a (Uye *et al.*, 1996). In coastal environments, plankton communities are further affected by tidal currents or river plumes (Acha *et al.*, 2020). However, disentangling responses to environmental variability is challenging as observed conditions represent the outcome of complex interactions between hydrography and plastic, community-level interactions (Caron and Hutchins, 2013; López-Abbate, 2021).

The Subtropical Convergence in the South-West Atlantic (SWA) between 30 and 40°S is dominated by the Brazil–Malvinas Confluence (BMC), which spans over the southern Brazilian, Uruguayan and northeastern Argentinean shelves, as well as the adjacent ocean basin. The confluence moves latitudinally with the seasons; thus, a transitional region is alternatively affected by the warmer and saltier tropical water (TW) transported poleward by the Brazil current during summer (December–March), and by the cold and diluted subantarctic water (SAW) of the Malvinas equatorward flow during winter (July–September) (Piola *et al.*, 2018). Uruguayan shelf waters (35–38°S) are at the core of the Subtropical Convergence where hydrographic conditions result from the interplay of water masses advected from adjacent zones and modified by continental runoff (Matano *et al.*, 2010).



Low salinity Río de la Plata plume (RPP) waters (ca. 25000 m³ s⁻¹; Hoffmeyer *et al.*, 2018) are advected beyond the estuary's mouth and reach maximum northeastern penetration (up to 1000 km) during winter (Piola *et al.*, 2008). This buoyant plume adds hydrographic complexity and makes this an important biogeographic boundary between communities of coastal-brackish, oceanic subtropical and subantarctic origins (Boltovskoy *et al.*, 1999).

Plankton assemblages in the Subtropical Convergence change with prevailing water masses. As a general trend, small diatoms, nanoflagellates and cyanobacteria dominate in oligotrophic TW, while bigger diatoms and dinoflagellates are more abundant in nutrient-rich SAW. Intermediate areas affected by subtropical shelf waters that result from the mixing of tropical and coastal waters (Piola *et al.*, 2018) share features from both water masses and may be dominated by nanoflagellates (Carreto *et al.*, 2008; Gonçalves-Araujo *et al.*, 2012). In coastal areas, the winter intrusion of RPP is associated with high satellite chlorophyll-*a* (García *et al.*, 2004; García and García, 2008; Ciotti *et al.*, 2010), and is a relevant driver of phytoplankton (Carreto *et al.*, 2008), copepod and ichthyoplankton communities (Muelbert *et al.*, 2008). Such studies suggested that water masses dramatically influence trophic dynamics by favouring the development of distinct plankton assemblages that may impact the top-down control of microzooplankton communities.

Knowledge of regional microzooplankton derives from investigations further south in Argentinean waters (Thompson *et al.*, 1999; Thompson and Alder, 2005; Santoferrara and Alder, 2009), including the Patagonian shelf (Antacli *et al.*, 2018), the Beagle Channel (Barria de Cao *et al.*, 2013) and the Bahía Blanca estuary (Barria de Cao *et al.*, 2005, 2011; Pettigrosso and Popovich, 2009; López-Abbate *et al.*, 2015, 2019). Further observations are available for Brazilian coastal and shelf waters (Eskinazi-Sant'Anna and Björnberg, 2006; Islabão and Odebrecht, 2011; Gonçalves-Araujo *et al.*, 2018; Menezes *et al.*, 2019), and Patos Lagoon estuary (Jesus and Odebrecht, 2002). Information regarding the microzooplankton at the core area along Uruguayan waters, however, is still very limited. Early studies dealt with specific taxa (i.e., *Ceratium*, Vaz-Ferreira, 1943; loricate ciliates, Balech, 1948, and Souto, 1970), with a special focus on toxic or potentially toxic species (i.e., *Gymnodinium catenatum*; Méndez and Ferrari, 2003; see also Wells and Daborn, 1997). The occurrence of dinoflagellates is frequently reported as part of phytoplankton assemblages in studies focused on primary producers (e.g., Calliari *et al.*, 2005, 2009), but a comprehensive analysis of the microzooplankton assemblage has not been addressed so far, and information is particularly scant for groups lacking protective structures (i.e., aloricate ciliates). Also, and in spite of their relevance in both trophic dynamics and carbon fluxes, microzooplankton's responses to environmental conditions have been so far overlooked.

The present paper provides a first characterization of the species abundance, biomass and diversity of the microzooplankton community at a marine coastal site in Uruguay in all seasons, and analyses its relationship with the environmental variability. Given the hydrographic context within the Subtropical Convergence, it can be expected that Uruguayan waters host a diverse microzooplankton assemblage comprised of temperate and subtropical taxa, subjected to strong seasonal variability. The hypothesis is that the periodic fluctuation of water masses over the continental shelf and the adjacent ocean basin drives environmental variability (i.e., temperature, salinity, nutrients, light) and microzooplankton assemblages at this SWA site. To test the hypothesis, the seasonal and vertical patterns in species abundance, biomass and diversity were analysed along with environmental variables on a monthly basis during an annual cycle. The results provide new insights into the environmental influence

on natural microzooplankton communities, which in turn affects energy flow in the marine food web.

Materials and methods

Study area, data collection and samples processing

Cabo Polonio is a tombolo resulting from the deposition of sandy sediments by littoral currents during the Quaternary on the coast of Uruguay (Panario *et al.*, 1993). The original rocky island is currently connected to the mainland thus defining two large beach arcs: La Calavera, northwards of Cabo Polonio proper (facing east), and Playa Sur (facing south-east). The sampling station is nearly 2 nautical miles offshore La Calavera beach arc at a depth of 12 m on the Uruguayan coast (34°23'S-53°45'W) (Figure 1).

Environmental conditions were recorded and microzooplankton samples taken monthly over a full year (from 11 July 2019 to 9 June 2020). For simplicity, in the present study, seasons are defined as beginning on the following days: winter = 1 July 2019; spring = 1 October 2019; summer = 1 January 2020; autumn = 1 April 2020. On every occasion, a Conductivity-Temperature-Depth profiler (CTD, SBE 19plus V2, Seabird Electronics, USA) was used to record the vertical distribution of temperature, salinity, the fluorescence of chlorophyll-*a*, turbidity and photosynthetically active radiation (PAR). Using a 5 L Hydrobios bottle, samples were collected from three discrete standard depths: surface at 0 m, intermediate at 3.5 m and near-bottom at 8.5 m, and analysed for nutrients, chlorophyll-*a* and microzooplankton taxonomy, abundance and biomass. For inorganic nutrient analyses, 0.5 L aliquots were filtered (Munktell GF/F equivalent, 47 mm diameter), frozen in clean acid-washed bottles and measured following standard protocols in a Thermo Evolution 60 spectrophotometer for nitrite, nitrate, ammonium, phosphate and dissolved reactive silicates. For subsequent analyses, nitrites, nitrates and ammonium were summed and presented as dissolved inorganic nitrogen (DIN). For chlorophyll-*a* concentration, 0.25–0.45 L aliquots were filtered under a low vacuum and stored at –20°C until analysis, usually 1–2 weeks later. Retained material was extracted in 96% ethanol at 4°C in the dark and, after 24 h, its fluorescence was quantified using a Turner Designs fluorometer (Model No. Trilogy 040) with a non-acidification module (Welschmeyer, 1994).

Microzooplankton community was analysed in samples from the surface and 3.5 m, i.e., the depth at which fluorescence maxima usually occur (own unpublished data, Calliari *et al.*). Two litres of seawater were concentrated to 100 mL with utmost care by slowly and gentle sieving through a 20 µm-mesh and subsequently preserved with Bouin's solution (10% final concentration). Filtering and preservation of microzooplankton samples may lead to underestimation in microzooplankton numbers due to loss of fragile components (especially aloricate ciliates), and those with an individual size similar and smaller to sieve's pores; also changes in cells morphology may occur due to handling and the action of fixatives. Thus, routine analysis of filtered and preserved samples was complemented by a qualitative observation of fresh unfiltered samples. Bouin's solution is a suitable preservative for taxa devoid of protective structures such as naked ciliates and dinoflagellates as it minimizes cell loss and deformation, thus facilitating proper identification and quantification (Alder and Morales, 2009). In summary, current results provide information on medium-sized and larger components of the whole microzooplankton assemblage. A total of 24 samples were collected representing 12 dates and two depths. Microzooplankton was identified to the lowest possible taxonomic level (i.e., species or genus level, whenever possible) based on

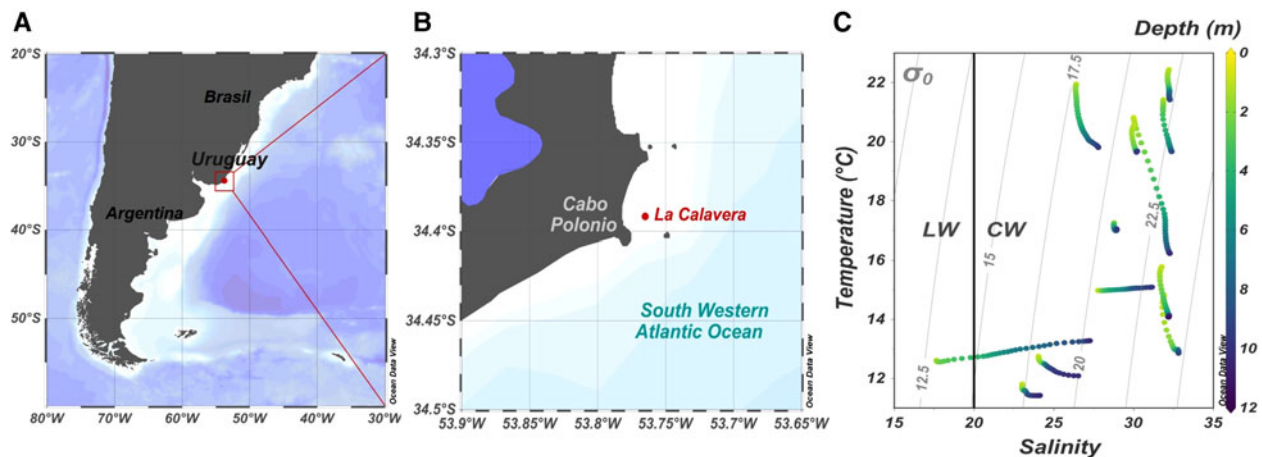


Figure 1. (A–B) Map of the study area: La Calavera beach, Cabo Polonio, Uruguay, South-West Atlantic Ocean; (C) Temperature–salinity diagram of monthly CTD casts performed between 11 July 2019, and 9 June 2020. The straight line indicated the thermohaline limit of water masses: littoral waters (LW) and coastal waters (CW). Isopycnals lines connecting points of constant density are shown.

morphological characteristics using bibliographic sources and taxonomic keys (e.g., Balech, 1988; Montagnes and Lynn, 1991; Barría de Cao, 1992; Lynn and Small, 2002). A special focus was made on dinoflagellates since they represent the most abundant group in the study area, and on ciliates (both, loricate and aloricate) as their study remains elusive. For enumeration, 3–6 replicate ~3 mL subsamples for each date and depth were taken after thorough homogenization of the 100 mL samples, settled in Utermöhl chambers, and analysed under an inverted microscope at 400 \times and 1000 \times magnification. Microzooplankton individual cell volumes (V ; μm^3) were calculated by assigning standard geometric configurations to each taxon (Sun and Liu, 2003), and biomass was calculated in terms of biovolume ($\mu\text{m}^3 \text{L}^{-1}$).

Data analysis

Environmental data

To characterize the structure of the water column a $\{\text{month} \times \text{depth}\}$ environmental data matrix of CTD variables was used to construct TS diagrams using the Ocean Data View software (Schlitzer, 2021), and to profile CTD variable's temperature, salinity and fluorescence. Identification of water masses followed Guerrero and Piola (1997) and Calliari *et al.* (2009). The light environment was represented by turbidity, and percentage irradiance (%PAR, with surface PAR as reference) and expressed by the diffuse attenuation coefficient for downward irradiance (K_d) by fitting the Beer–Lambert equation. Further analyses and comparisons relied on a subset of that data matrix comprising depth intervals closest to bottle depths, i.e., 0, 3.5 and 8.5 m. All replicates were averaged to obtain monthly values prior to analyses, when necessary. Differences between 'season' and 'depth' for nutrient concentration were assessed by two-way analysis of variance (ANOVA).

Biological data

Trends in $\{\text{month} \times \text{depth}\}$ community composition data (e.g., abundance and biomass of different taxa) were assessed for microzooplankton as a whole (i.e., without taxonomical discrimination), and also for meaningful trophic categories. Ciliates were subdivided by size (maximum width), which is closely linked with the oral diameter (Hansen *et al.*, 1994). For loricate ciliates, the lorica oral diameter was used as the hallmark feature (Dolan *et al.*, 2013). The oral diameter is highly conservative and determines the size of ingested particles, thus defining functional groups with similar prey types (Fenchel, 1980). Modal oral

diameter and modal maximum width were used to define small and large categories, with threshold values of 40 and 55 μm , for loricate ciliates ($\text{LC} < 40$, $\text{LC} > 40$) and aloricate ciliates ($\text{AC} < 55$, $\text{AC} > 55$), respectively. No size delimitation was employed for dinoflagellates as they perform diverse feeding strategies and are thereby considered relatively non-selective feeders (Hansen *et al.*, 1997). Instead, dinoflagellates were classified into heterotrophic (HD) and mixotrophic dinoflagellates (MD) according to extant published literature.

Differences in microzooplankton abundance and biomass between 'season' and 'depth' were tested using factorial ANOVA followed by post-hoc Tukey analyses. The taxonomic structure of the microzooplankton along the annual cycle was compared by non-metric Multi-Dimensional Scaling (nMDS; Field *et al.*, 1982). The analysis was based on a Bray–Curtis distance matrix of untransformed abundance data using the R-library vegan (R Core Team, 2020). Significant differences in microzooplankton structure between 'season' and 'depth' were calculated by one-way analysis of similarities (ANOSIM) at a significance level of 5% and R statistic > 0.5 . SIMilarity PERcentage analysis (SIMPER) was used to assess the contribution of each species to average between-group Bray–Curtis dissimilarity. Rare species were dropped to avoid over-fitting; only those species present in at least ~8% of the samples were included. Alpha diversity was estimated for each month and depth as (i) taxonomic richness (S'), (ii) Shannon–Wiener index (H') and (iii) Pielou's evenness index (J').

Microzooplankton community structure and environmental variability

Spearman's rank correlation (ρ) analysis was used to explore the links between diversity indexes and environmental conditions. To identify those environmental variables driving community structure, a multivariate redundancy analysis (RDA; ter Braak and Prentice, 1988) was performed. First, a detrended correspondence analysis was carried out with the *decorana* R-function to confirm the suitability of an RDA for the present dataset (Leps and Smilauer, 2003). The microzooplankton abundance data were square root-transformed and the environmental variables were standardized and normalized. The explanatory power was assessed as the percentage of explained constrained variance (adjusted R^2) and its significance was tested with one-way ANOVA and permutation tests. The importance of each explanatory variable was evaluated with a 'forward' selection procedure considering the corresponding inflation factor. In addition, to

evaluate interaction among functional groups and the environment, the association of coexisting environmental variables with the monthly abundance of MD, HD, LC < 40, LC > 40, AC < 55 and AC > 55 were estimated using Pearson's correlation with the R package corrplot visual exploratory tool.

Results

Water column, nutrients and chlorophyll-*a*

Coastal waters (CW, $S < 33.4$) dominated during most of the study period, except in winter when colder littoral waters (LW, $S < 20$, $T < 13^\circ\text{C}$) were observed in the first 4 m of the water column (Figure 1C). The vertical structure was subtle (Figure 2). In winter 2019 and autumn 2020, temperature and salinity profiles were nearly homogeneous except in July 2019 when a strong halocline was present ($\delta S \sim 10$ over 6 m). A weak thermocline was observed below 2 m depth in spring 2019 and summer 2020. On most occasions, a subsurface fluorescence maximum was present at around ~ 3.5 m. Also, during most of the study period, turbidity was low

(mean 9 Nephelometric Turbidity unit (NTU), range 2–40 NTU at depth < 7.5 m) except near the bottom where high turbidity (mean 32, range 1.6–133 NTU) and often also high fluorescence suggested bottom re-suspension of fine sediments and senescent phytoplankton.

Descriptive statistics of CTD-derived variables are summarized in Table 1. Briefly, temperature varied between 11.4 (August, winter) and 22.4°C (March, summer). Salinity also fluctuated seasonally between 17.6 (July, winter) and 32.8 (October, spring). Fluorescence and turbidity ranged between 0.95 and 5.88 Relative Fluorescence units (RFU), and between 1.61 and 133.39 NTU, respectively, without clear seasonality. PAR% was always < 1% at 8.5 m, while at 3.5 m varied between 4 and 30% throughout the year. The K_d varied between 0.79 and 1.84 m^{-1} , both extreme values were recorded in autumn, and average values were similar in all seasons. Nutrient concentrations varied throughout the annual cycle and were generally higher in winter and lower in summer and spring (Figure 3). SiO_4 (range: 9–40 μM) concentration was highest in winter, lower in summer and lowest in spring ($F[3] = 3.88$, $P < 0.01$). No significant differences

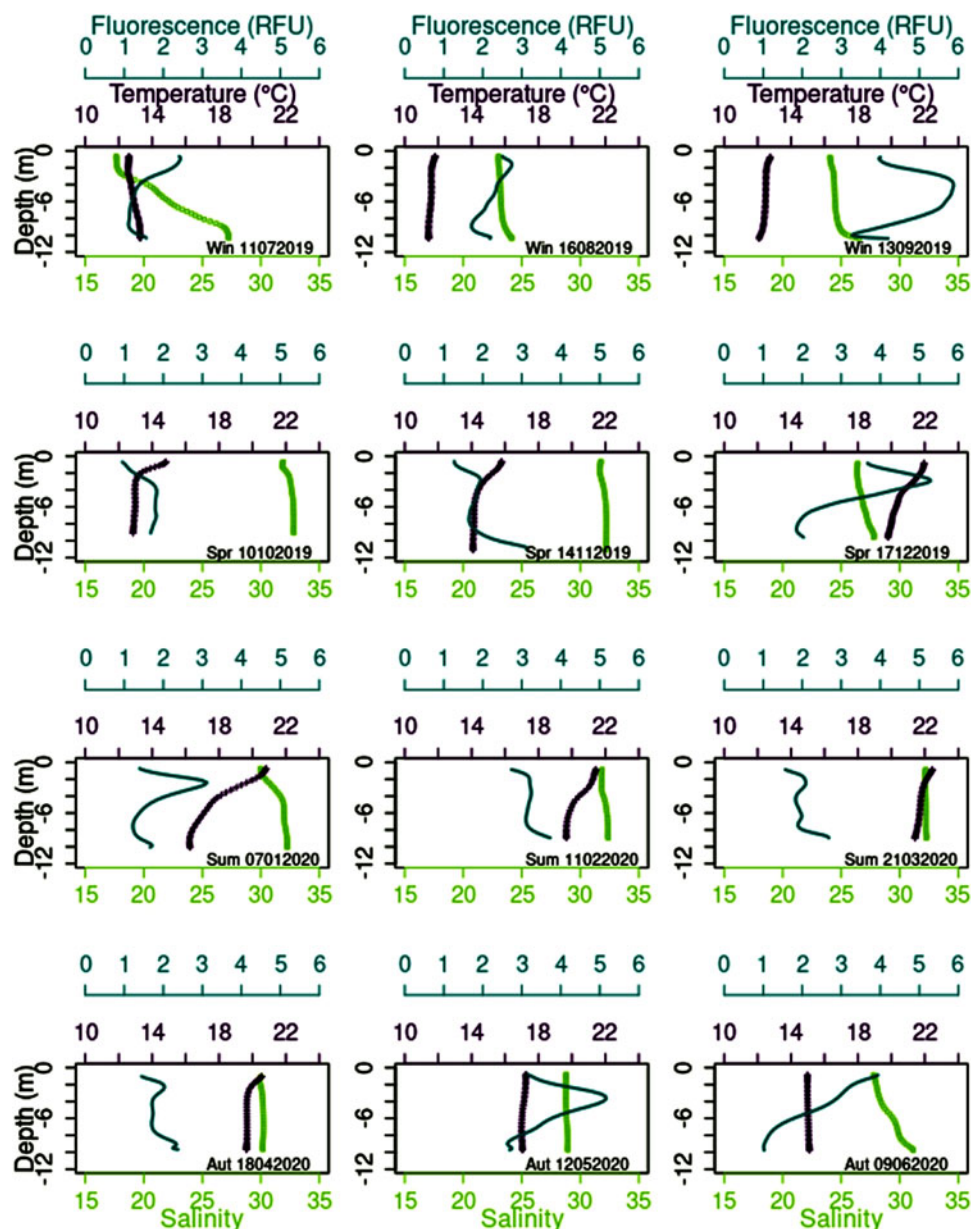


Figure 2. Monthly vertical variability of CTD-derived environmental variables shown by season (from top to bottom, the first three panels correspond to the winter (Win), the next three to spring (Spr), then to summer (Sum) and the last three to autumn (Aut)) and date (day-month-year) at the study site.

Table 1. Monthly mean and range of the environmental variables sampled at the study site; and mean and standard deviation (SD units) by season.

Date	Season	Temperature °C	Salinity	Fluorescence RFU	Turbidity NTU	PAR %	K_d m^{-1}
11/07/2019	Winter	12.91 12.56–13.28	22.11 17.64–27.23	1.57 1.11–2.44	19.31 10.56–55.45	20 0–100	0.97
16/08/2019	Winter	11.57 11.42–11.81	23.31 23.01–24.14	2.26 1.71–2.74	6.52 5.04–15.42	25 0–100	1.36
13/09/2019	Winter	12.45 12.09–12.75	24.60 24.04–26.53	4.78 3.24–5.88	9.59 2.98–46.44	24 0–100	1.15
	Mean SD	12.32 0.60	23.31 2.42	2.84 1.50	11.93 10.32	23 35	1.16 0.20
10/10/2019	Spring	13.32 12.85–14.83	32.49 31.80–32.81	1.55 0.95–1.83	7.28 4.07–9.67	23 0–100	1.34
14/11/2019	Spring	14.58 14.08–15.78	32.03 31.64–32.23	1.90 1.27–3.15	33.50 7.19–133.39	14 0–100	1.07
17/12/2019	Spring	20.66 19.80–21.95	26.91 26.36–27.79	3.22 1.85–5.29	11.84 3.36–41.12	16 0–100	1.27
	Mean SD	16.48 3.34	30.26 2.62	2.29 1.10	17.91 26.87	17 26	1.23 0.14
07/01/2020	Summer	17.95 16.23–20.81	31.49 30.00–32.27	1.78 1.22–3.12	12.44 1.92–30.57	28 0–100	1.69
11/02/2020	Summer	20.40 19.66–21.42	32.11 31.83–32.38	3.20 2.74–3.73	22.11 10.35–48.55	15 0–100	0.92
21/03/2020	Summer	21.82 21.42–22.44	32.21 32.13–32.27	2.01 1.57–2.68	21.99 9.78–66.99	14 0–100	0.85
	Mean SD	20.00 1.98	31.92 0.61	2.29 0.72	18.61 14.92	19 30	1.15 0.47
18/04/2020	Autumn	19.81 19.64–20.55	30.13 29.89–30.23	1.90 1.45–2.38	31.68 6.44–69.40	16 0–100	0.79
12/05/2020	Autumn	17.10 16.98–17.25	28.83 28.76–28.94	3.65 2.63–5.16	4.85 1.61–12.51	18 1–100	1.84
09/06/2020	Autumn	15.02 14.98–15.09	29.11 27.77–31.15	2.26 1.01–3.94	8.15 3.92–27.33	24 0–100	1.64
	Mean SD	17.31 1.95	29.35 0.81	2.62 1.08	14.71 16.62	19 28	1.42 0.56

PAR, irradiance; K_d , light attenuation coefficient.

were detected in PO₄ (0.4–2 µM) ($F[6] = 0.18$, $P = 0.98$) and DIN (0.7–18 µM) ($F[6] = 0.23$, $P = 0.96$) between seasons. No significant differences were detected between depths for PO₄, DIN and SiO₄ (ANOVA, $P > 0.05$). In July, inorganic nutrients showed the highest concentration and were closest to the Redfield ratio (N:P = 8:1, N:Si = 0.45:1). Chlorophyll-a ranged between 0.2 (July) and 21.9 mg m⁻³ (January) (Figure 3), and was highest in autumn and summer (mean 4.7 mg m⁻³, $n = 27$) and lower in winter (3.9 mg m⁻³, $n = 21$) ($F[3] = 2.82$, $P < 0.03$) although a peak was observed in September (14.5 mg m⁻³). In addition, chlorophyll-a was higher at the surface and 3.5 m (5.4 mg m⁻³; SD = 3.8, $n = 64$) compared to 8.5 m (2.5 mg m⁻³; SD = 2.0, $n = 32$) ($F[2] = 11.07$, $P < 0.001$).

Microzooplankton abundance and biomass

Microzooplankton absolute abundance ranged from 1 indL⁻¹ (July) to 8800 indL⁻¹ (May); the annual average abundance was 350 indL⁻¹ for dinoflagellates and 100 indL⁻¹ for ciliates (both loricate and aloricate). Total biovolume varied between 566 µm³ L⁻¹ (July) and 7.1 × 10⁸ µm³ L⁻¹ (August) (leaving aside *Noctiluca scintillans* whose average diameter was ca. 410 µm). The annual average biovolume was 1.9 × 10⁷ µm³ L⁻¹ for dinoflagellates, 1.0 × 10⁷ µm³ L⁻¹ for loricate ciliates and 1.7 × 10⁶ µm³ L⁻¹

for aloricate ciliates. Monthly variation in the abundance of microzooplankton groups is shown in Figure 3. It is worth mentioning that estimates corresponding to total counts <10 indL⁻¹ are subject to potentially substantial errors and should be taken with caution. Microzooplankton abundance and biovolume changed seasonally (complete ANOVA tables are provided in Supplementary Table S1). Total abundance was higher in autumn (total [mean, range]: 6.8 × 10⁴, 385 [5–8800] indL⁻¹) and lower in summer (2.6 × 10⁴, 159 [5–5370] indL⁻¹) and spring (3.2 × 10⁴, 156 [2–2555] indL⁻¹). Total biovolume followed a similar seasonal trend but without significant differences. The abundance of dinoflagellates (MD and HD) was also higher in autumn (total [mean, range]: 5.2 × 10⁴ [680, 5–8780] indL⁻¹ for MD and 9.3 × 10³ [206, 5–1685] indL⁻¹ for HD) and lower in summer (1.8 × 10⁴ [380, 5–5370] indL⁻¹ for MD and 2.0 × 10³ [60, 5–525] indL⁻¹ for HD). HD biovolume also varied between seasons (autumn > summer). In summer, AC < 55 abundance and biovolume were highest (total [mean, range]: 3.1 × 10³ [138, 7–570] indL⁻¹ and 1.0 × 10⁷ [4.6 × 10⁵, 1.5 × 10⁴–2.4 × 10⁶] µm³ L⁻¹, respectively); a peak was observed in June (i.e., autumn) associated with a *Strombidium* species of 35 µm maximum width. The biovolume of AC > 55 was highest in winter (2.6 × 10⁷ [3.3 × 10⁶, 3.0 × 10⁵–8.3 × 10⁶] µm³ L⁻¹), and as in the small aloricate ciliates, it did not differ between depths either. Yet, while the

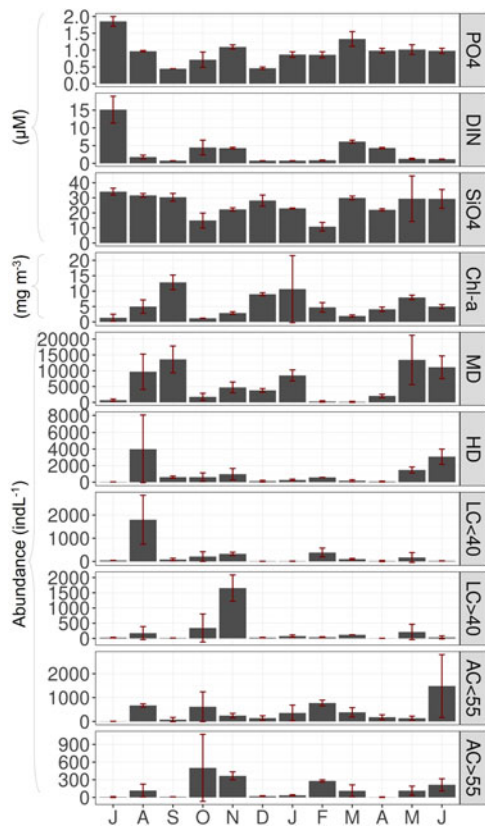


Figure 3. Monthly variability of nutrient concentration (μM), chlorophyll-a (Chl-a; mg m^{-3}) and microzooplankton abundance (indL^{-1}) (MD, mixotrophic dinoflagellate; HD, heterotrophic dinoflagellate, small and large loricate ciliates: LC < 40 and LC > 40 and aloricate ciliates: AC < 55 and AC > 55) registered in the first 3.5 m of the water column at the study site.

depths selected for microzooplankton analysis were within the mixed layer, some differences were detected for heterotrophic dinoflagellates and loricate ciliates between the upper and intermediate layers. Abundance and biovolume were highest at 3.5 m for HD (total: $1.5 \times 10^4 \text{ indL}^{-1}$, and $1.7 \times 10^9 \mu\text{m}^3 \text{ L}^{-1}$, respectively) and for LC > 40 ($3.7 \times 10^3 \text{ indL}^{-1}$ and $7.2 \times 10^8 \mu\text{m}^3 \text{ L}^{-1}$, respectively).

Dinoflagellates (both MD and HD) were numerically dominant during most of the study period and represented >65% of total abundance and biovolume, in all seasons (Supplementary Figure S1). The most significant numerical contribution of ciliates occurred in spring for loricates (~20%) and in summer for aloricates (~15%). In certain summer months (i.e., February–March), ciliates dominated microzooplankton abundance (~63–67%, respectively) associated mainly with the presence of *Strombidium* spp. and AC < 55, and biovolume (>75%) associated mainly with loricate ciliates. In spring, and particularly in October–November, ciliates reached a second peak that surpassed 40–30% of total abundance and 30–55% of total biovolume, associated mainly with large loricates *Tintinnopsis gracilis* and *Tintinnidium* spp. During late spring–early summer (i.e., December–January), MD biovolume total contribution was the highest (>90%), while in winter HD biovolume contribution exceeded 40%.

Microzooplankton composition and diversity

A total of 100 taxonomic groups of dinoflagellates and ciliates (loricate and aloricate) were identified in the sample collection, mostly belonging to classes Dinophyceae and Oligotrichea. The complete list is shown in Supplementary Text S1; 68% were identified at the species level, 27% at the genus level and 5% at

the order level. The class Dinophyceae consisted of eight orders and 15 families, of which the Proto-peridiniaceae and Ceratiaceae (in particular, *Proto-peridinium* and *Tripos* genera) contributed the most to morpho-species diversity. Dinoflagellates also included several potentially harmful species from the genera *Alexandrium*, *Dinophysis*, *Gymnodinium*, *Gonyaulax* and *Prorocentrum*. Loricate ciliates (Choreotrichids) were spread in seven families of which Codonellidae was the most representative. *Tintinnopsis* was the most diversified genus, comprising ~55% of the total recorded species with agglutinated lorica. Aloricate ciliates comprised five orders and nine families, of which Strombidiids were the most species-rich oligotrichous ciliates. Other, rarer taxa present at low abundance were radiolaria, rotifers, cladocerans of the genus *Evadne* and *Podon*, copepodid and nauplii stages.

The nMDS ordination based on microzooplankton abundance illustrated large overlapping in samples from different depths but clear seasonal segregation, especially between winter and summer (stress = 0.17) (Figure 4). Microzooplankton assemblages differed between seasons (ANOSIM, $P = 1 \times 10^{-4}$, global $R = 0.58$): unidentified nanociliates, mixotrophic ciliates (e.g., *Lohmaniella oviformis*, *Strombidium* spp.) and marine *Tintinnopsis* species occurred mainly in summer, while species from *Tripos* and *Dinophysis* genera did so mainly in winter; still, dinoflagellates were dominant throughout the year. No differences in community composition between depths were found (ANOSIM, $P = 0.81$, global $R = 0.10$). SIMPER analysis identified dinoflagellates as the dominant group responsible for the biotic characterization of each season (Supplementary Table S2). The species that contributed most to the dissimilarity between seasons were *Kryptoperidinium cf. triquetrum* and *Scrippsiella cf. acuminata* in winter (total 1.8×10^4 and $7.1 \times 10^3 \text{ indL}^{-1}$, respectively, corresponding to 28 and 11% of total abundance) together with the hyaline loricate ciliate *Eutintinnus* sp. ($2.7 \times 10^3 \text{ indL}^{-1}$ only recorded in August). In summer, the taxa that contributed most to dissimilarity were nanociliates and the unarmoured dinoflagellate *Akashiwo sanguinea* (that surpassed $7.7 \times 10^3 \text{ indL}^{-1}$ and accounted for 42% of total abundance in January), while in autumn–spring were *Prorocentrum* spp. and *Pseliodinium* sp. (accounting for 20 and 14%, respectively). Overall, the dissimilarity between microzooplankton communities was highest (~90%) between winter and summer and lowest (~78%) between spring and autumn.

Taxonomic richness (S') ranged between 17 and 48 (in April and August, respectively), Shannon index (H') between 1.3 and 2.9 (in April and March, respectively) and evenness (J') between 0.5 and 0.9 (in April and March, respectively). Spring was characterized by the highest values in the three metrics, while in autumn H' and J' and in summer S' were the lowest (ANOVA, all $P < 0.05$; $F[3] = 2.89$ for S' ; $F[3] = 3.68$ for H' ; $F[3] = 2.99$ for J') (Supplementary Figure S2). Differences between depths were detected only for S' , which was higher at 3.5 m ($F[1] = 1.47$, $P = 0.02$).

Microzooplankton community structure and environmental conditions

Environmental variables affected microzooplankton abundance and composition. S' and H' were negatively correlated to temperature ($\rho = -0.75$, $P < 0.001$ and $\rho = -0.47$, $P < 0.01$, respectively). In addition, S' was negatively correlated with turbidity ($\rho = -0.37$, $P < 0.05$). In turn, H' and J' were negatively correlated with chlorophyll-a ($\rho = -0.50$ and $\rho = -0.60$, respectively, $P < 0.05$).

RDA (Figure 5) identified three core environmental variables (temperature, salinity and DIN) which statistically explained overall variance in microzooplankton composition ($R^2 = 0.32$,

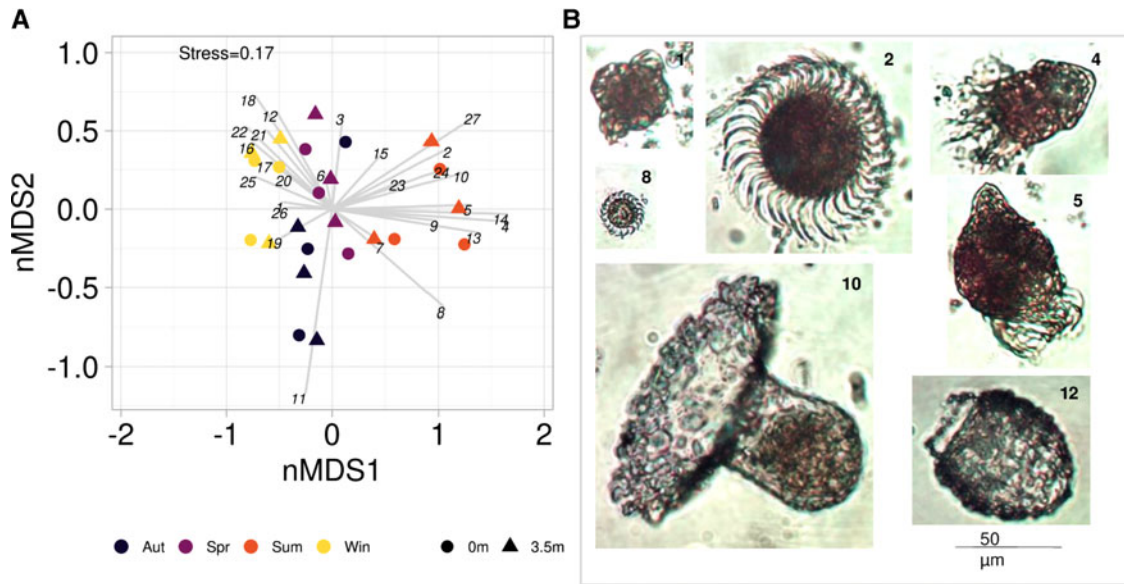


Figure 4. (A) Non-metrical multidimensional scaling (nMDS) at two standard depths (0 and 3.5 m) at the study site. Stress value indicates the goodness of representation of differences among samples. Win, winter; Spr, spring; Sum, summer; Aut, autumn. (1) *Mesodinium rubrum*, (2) *Pelagostrobilidium spirale*, (3) *Strombidium cf conicum*, (4) *Strombidium cf epidemum*, (5) *Strombidium* spp., (6) *Paratontonia gracillima*, (7) Ciliate 2, (8) Nanociliate, (9) Ciliate 3, (10) *Tintinnopsis buetschlii* var *mortensenii*, (11) *Tintinnopsis radix*, (12) *Stenosemella* sp. 3, (13) *Tintinnopsis cylindrica*, (14) *Tintinnopsis* sp. 3, (15) *Tintinnopsis* sp. 4, (16) *Tintinnidium balechi*, (17) *Triplos dens*, (18) *Triplos fusus*, (19) *Triplos cf horridum*, (20) *Triplos muelleri*, (21) *Dinophysis acuminata*-complex, (22) *Dinophysis triplos*, (23) *Kryptoperidinium cf triquetrum*, (24) *Polykrikos kofoidii*, (25) *Katodinium* sp., (26) *Protooperidinium depressum*, (27) *Protooperidinium grande*. (B) Microzooplankton from Cabo Polonio, inverted microscope images at total magnification of 400x; numbers identify each species/genus and correspond to those of panel A.

$P < 0.001$). The first two axes (RDA1 and RDA2) accounted for 35 and 20% of the total variance, respectively. Seasonal patterns were clearer along RDA1, as the summer months were located on the positive side of the first axis, while winter months were mainly on the negative side, reflecting warm- and cold-water microzooplankton assemblages. Although several species were dispersed near the centre of the triplot, thus suggesting lower correlations with the first two axes, first-order variability in microzooplankton communities corresponded to a seasonal temperature–salinity gradient, with higher ciliate abundances in summer months and at salinity >32 during spring (i.e., October–November). A second-order effect on microzooplankton community structure was driven by DIN concentration. Correlation matrix analysis revealed significant associations among environmental variables and microzooplankton

functional groups (Figure 6; Supplementary Table S3). $LC < 40$ and HD were positively related and both responded negatively to temperature. Aloricate ciliates were related to salinity (positive), and to SiO_4 (negative). MD was related to chlorophyll-a (positive), and to turbidity and temperature (both negative). Nutrients (DIN and PO_4) were negatively associated with the concentration of dinoflagellates. In addition, correlation analysis relating specific microplankton genera of dominant ecophysiological groups (sensu Mitra *et al.*, 2016) and environmental variables (Supplementary Table S4) revealed that non-constitutive generalist mixotrophic species (i.e., *Strombidium* spp.) thrived under high salinity, while constitutive mixotrophs (i.e., *Triplos* spp.) and specialists non-constitutive (SNC, i.e., *Dinophysis* spp., *Mesodinium rubrum*) were positively associated with fluorescence and chlorophyll-a.

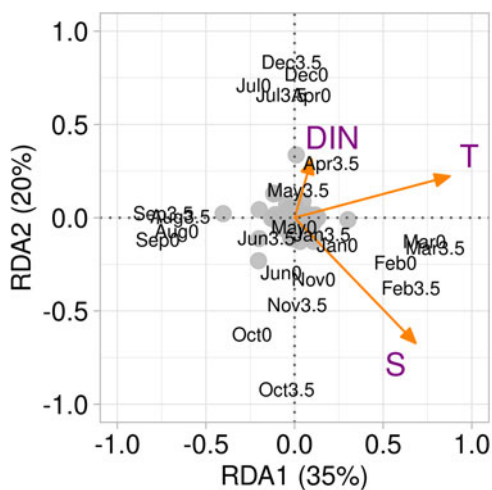


Figure 5. RDA triplot showing only significant vectors ($P < 0.05$). Species are represented as points, environmental variables as arrows (DIN, dissolved inorganic nitrogen; T, temperature; S, salinity), and each date with the abbreviation of each month (i.e. Jul for July, etc.) followed by 0 or 3.5 indicating depth level.

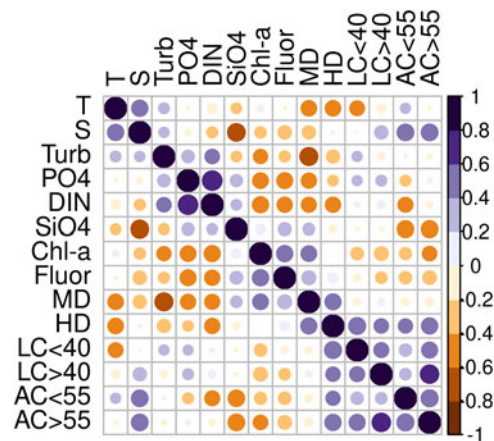


Figure 6. Pearson's correlations matrix among study site microzooplankton trophic groups abundance (small ciliates: $AC < 55$ and $LC < 40$, large ciliates: $AC > 55$ and $LC > 40$, hetero- and mixotrophic dinoflagellates: HD and MD, respectively) and environmental variables (temperature: T, salinity: S, turbidity: Turb, nutrient concentration: PO_4 , DIN, SiO_4 , chlorophyll-a: Chl-a, fluorescence: Fluor).

Discussion

Temporal sampling at the coastal sea of Cabo Polonio allowed to cover a wide range of environmental conditions (e.g., temperature, salinity, nutrients) in a region subjected to ample hydrographic variability linked to the dynamics of the SWA Subtropical Convergence and under the influence of the nearby land mass. A rich microzooplankton community included subtropical and subantarctic taxa typically found further north or south of the study area, respectively. Briefly, microzooplankton community structure evidenced significant seasonal variability coherent with the evolution of hydrographical conditions.

Environmental variability

Environmental conditions at the study site changed throughout the year cycle in response to regional hydrodynamics and local forcing. In the SWA, slope and open ocean areas at latitudes ca. 35°S are subjected to Brazil–Malvinas currents; that influence extends also over the shelf by the coastal penetration of both western boundary currents (Piola *et al.*, 2018). The observed temperature cycle in Cabo Polonio (that yields a thermal range of 11°C) was consistent with an alternating influence of modified subtropical waters in summer and subantarctic in winter. However, evidence is inconclusive as it is difficult to set the boundaries between such influence and the typical mid-latitude seasonal warming/cooling cycle over shallow seas (Mann and Lazier, 2006). Hence, consideration of salinity variability can aid in the identification of regional forcing on local hydrology. In the SWA, CW ($6.5 < T < 21^{\circ}\text{C}$ and $S < 33.4$) results from the mixing of oceanic water masses and freshwater from the continental drainage (Thomsen, 1962; Guerrero and Piola, 1997). For shallower areas near the coast, it is useful to extend that classification for a broader salinity regime. For instance, within this same region and for the full salinity spectrum (0–36), Calliari *et al.* (2009) defined Río de la Plata (RP) waters as those with $S < 20$. At the study site, salinity varied over a wide range (>15) and during winter low values prevailed ($18 < S < 27$, mean = 23.3), i.e., water subjected to strong dilution but still above the estuarine range quoted formerly. These low salinity LW likely resulted from further local dilution of the already low salinity subantarctic shelf water. Despite the absence of significant freshwater point-sources in the vicinity of the study area, important diffuse groundwater discharges are expected (Windom *et al.*, 2006). Also, winter penetration of the RPP likely contributed to the lower salinity. In turn, the highest salinity recorded during summer months (ca. 32) suggests the presence of modified subtropical shelf waters subjected to lower dilution by local freshwater sources. Nutrients and chlorophyll-*a* concentrations mean values at the study site were higher than those typically reported for shelf waters in the vicinity area (Gonçalves-Araujo *et al.*, 2012, 2018), similar to that in coastal areas affected by the RPP (Ciotti *et al.*, 1995; Garcia *et al.*, 2004; Carreto *et al.*, 2008), and lower than those within the RP estuary (Calliari *et al.*, 2018). Thus, although similarities regarding hydrology, nutrients and chlorophyll-*a* can be identified in comparison to other areas of the Subtropical Convergence, some particularities emerged likely related to the proximity to land of the sampling site.

Microzooplankton abundance and community structure

The absolute abundance of microzooplankton (total [mean; SD]: 1.8×10^5 [245; 740] indL⁻¹) at the study site was in agreement with the reports for the coastal areas of the SWA in Brazil (Gonçalves-Araujo *et al.*, 2012; Menezes *et al.*, 2019) and Argentina (Antacli *et al.*, 2018; Santoferrara and Alder, 2009).

The range of abundance and mean biovolume by taxa were also similar to those observed in the region (e.g., for loricate ciliates: at 38°S, Barria de Cao *et al.*, 2005; and at 23°S, Eskinazi-Sant’Anna and Björnberg, 2006). Abundance and biovolume were mostly dominated by thecate dinoflagellates, with maxima in autumn and considerable relative contribution of HD in winter. In turn, ciliates encountered more favourable conditions in spring and summer months. In areas with no clear recurrent primary productivity patterns, the dominance of dinoflagellates over loricate and aloricate ciliates may be favoured by more flexible trophic requirements, along with their ability to sustain latent populations during periods of low food supply by resorting to reserves (Sherr and Sherr, 2007). The annual pattern of MD mirrored that of the chlorophyll-*a*, suggesting that this group is an important contributor to total pigment concentration. That agrees well with observations of dinoflagellates as co-dominant contributors to total pigment concentration along with diatoms in coastal and shelf areas of the Subtropical Convergence (Carreto *et al.*, 2008). Vertically, the predominance of HD and LC > 40 at 3.5 m, matching peaks in chlorophyll-*a* and fluorescence, may reflect the capability of dinoflagellates to control their vertical aggregation at preferred depths (Islabão *et al.*, 2017).

Microzooplankton at the coastal sea of Cabo Polonio was a taxonomically rich coastal marine association comprising a large set of species, most of which are widely distributed in estuarine, temperate, coastal and shelf waters in Brazil (Eskinazi-Sant’Anna and Björnberg, 2006; Menezes *et al.*, 2019), Argentina (Antacli *et al.*, 2018; Barria de Cao *et al.*, 2003; Santoferrara and Alder, 2009) and Uruguay (for dinoflagellates: e.g., Balech, 1988; Ferrari and Vidal, 2006). During the observed period, a core group of taxa (~20% of the total) with a frequency of occurrence $>50\%$ was constituted by widespread species in the SWA known for their ample environmental tolerance (e.g., dinoflagellates: *Prorocentrum* spp., *Dinophysis acuminata*-complex and *Protooperidinium depressum*; ciliates: *L. oviformis*, *Tintinnidium balechi* and *Tintinnopsis gracilis*). Instead, several other taxa occurred with low or moderate frequency throughout the year (~30% of species occurred $<10\%$ of the time) under more specific conditions associated with given seasons (e.g., *Tintinnopsis tocaninensis*, *Leprotintinnus nordqvistii* and *Paratontonia gracillima* in summer, *Strombidium cf capitatum* and *S. cf emergens* in autumn, *Stenosemella* species in spring and several unidentified *Tintinnopsis* and *Protooperidinium* species mainly in winter). Those taxa were the main contributors to the differentiation of the assemblages at different times of the year.

Environmental drivers of microzooplankton community structure

Environmental variability impacted microzooplankton structure through the seasonal evolution of water masses with contrasting thermohaline properties and nutrients. Distinct assemblages occurred in summer and winter, when subtropical taxa alternated with euryhaline groups associated with colder SAW. The winter season (lowest salinity, highest nutrients concentration and stoichiometry closest to Redfield’s) was characterized by high species richness (mean = 38) and the dominance of dinoflagellates, specially pigmented mixotrophic species. In that season, the structure resembled neritic assemblages found in the SWA shelf under the influence of continental runoff and low salinity (e.g., *K. cf triquetum*, *S. cf acuminata*, *D. acuminata*-complex, *Dinophysis caudata*, *Tripes furca* and *Tripes muelleri*) (Ciotti *et al.*, 1995), together with brackish loricate ciliates like *Tintinnopsis fimbriata* and *Codonellopsis lusitanica* and the eutrophic water species *Tintinnopsis uruguayensis* (Sivasankar *et al.*, 2018). Those features

indicate the importance of LW and the influence of the buoyant RPP on the coast of Cabo Polonio during that season.

In contrast, during summer under higher temperatures, salinity and turbidity, microzooplankton species richness was the lowest (mean = 27), and ciliates were better represented. Typically, marine loricate ciliates *L. nordqvistii*, *T. tocantinensis*, *Tintinnopsis radix* and *Tintinnopsis buetschlii* occurred exclusively during this season.

Seasonality impacted also the functional structure of the microzooplankton assemblage, i.e., during summer non-constitutive generalist mixotrophs (i.e., *Strombidium* spp.) were well represented in terms of numbers and biomass levels, while in winter the same was valid for constitutive mixotrophs (i.e., *Tripos*) and SNC mixotrophs (i.e., *Dinophysis* spp., *Mesodinium rubrum*). Seasonal alternation between functional groups was likely related to changes in key environmental attributes. For instance, lowest nutrient concentrations in summer may have favoured the former, as they are better adapted to subsist by predation and are less dependent on photosynthesis. In contrast, the latter groups were favoured under the short-day lengths of the cold season, when nutrients and average fluorescence levels were higher, and turbidity lower. A relevant consequence of the seasonal alternation of functional groups is increased resilience of microzooplankton at the community level regarding its role as a shunt for chemical energy between primary producers and higher trophic levels in the mesozooplankton and micronekton communities, despite significant environmental variability.

Results from community-based ecological parameters (e.g., species richness, diversity and evenness) were complementary to those discussed so far: they mostly captured differences between transition seasons spring and autumn, which were otherwise least different in terms of actual species composition. Differences in diversity metrics resulted from a sharp decrease in the abundance of several species of loricate and aloricate ciliates in autumn compared to spring months; instead, the composition and abundance of mixotrophic dinoflagellate species were rather similar between both periods. So far, it is unclear which processes could have specifically affected loricate and aloricate ciliates, but not mixotrophic dinoflagellates, between spring and autumn. That is a matter that deserves further attention in next investigation.

Top-down control by mesozooplankton predators, particularly copepods, can be a relevant structuring process for microzooplankton assemblages. Copepods are able to predate on actively swimming prey (Jonsson and Tiselius, 1990; Tiselius *et al.*, 2013), and they frequently select those over traditional phytoplankton (e.g., diatoms) due to enhanced hydromechanical perception of moving prey and their generally higher nutritive quality (Calbet and Saiz, 2005). As a result, heterotrophic prey may constitute >50% of copepods' daily carbon intake (Calliari *et al.*, 2009). It was beyond the scope of the present study to characterize top-down regulation on microzooplankton, but preliminary results on copepods species and abundance in samples taken concurrently (main taxa: *Acartia tonsa*, *Paracalanus* spp., *Temora turbinata*, *Oithona* spp.; total copepods abundances $\sim 10^4$ – 10^5 ind m⁻³; own unpublished data) suggest they may actually represent a relevant factor for microzooplankton dynamics in Cabo Polonio.

Finally, microzooplankton assemblages can be expected to shift in response to large-scale regional processes, particularly those linked to climate change. The SWA is undergoing rapid warming. Increased instabilities and eddy generation in the western boundary current that transports heat to higher latitudes lead to enhanced poleward penetration of the Brazil current (Li *et al.*, 2022), directly impacting our study area. Also, in recent years, marine heatwaves in the SWA between 32°S and 38°S have

increased in frequency, duration and intensity (Manta *et al.*, 2018). Several actual and projected consequences of climate change have been identified for the SWA ecosystem (Franco *et al.*, 2020), including among the former an intensification of harmful dinoflagellates blooms along Uruguayan coasts (Méndez and Carreto, 2018).

Conclusions

This study provides the first comprehensive assessment of the microzooplankton within a broad area of the SWA, contributing to fill critical information gaps on ciliates and dinoflagellates communities in Uruguayan waters. Current results for Cabo Polonio suggest the existence of coherent patterns in the variability of environmental conditions and that of the microzooplankton. Within that community, a core group of dinoflagellates and ciliates widely distributed in SWA were frequently found along the year cycle under varying environmental conditions, and their abundance was often linked to chlorophyll-a, nutrients and probably also driven by internal regulation mechanisms (e.g., competition, predation). On top of those, several other taxa occurred with a more marked seasonality under narrower environmental conditions, supporting a hypothesized hydrographic modulation on the microzooplankton community structure in this subtropical coastal site.

Supplementary material. The supplementary material for this article can be found at <https://doi.org/10.1017/S0025315423000358>

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Competing interest. None.

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