Antarctic coastal nanoplankton dynamics revealed by metabarcoding of desalination plant filters: detection of short-term events and implications for routine monitoring

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Abstract:

One of the main requirements of any sound biological monitoring is the availability of long term and, possibly, temporal data with a high resolution. This is often difficult to be achieved, especially in Antarctica, due to a variety of logistic constraints, which make continuous sampling and monitoring activities generally unfeasible. Here we focus on the 5µm filters used in the desalination plant of the Italian research base "Mario Zucchelli" in the Terra Nova Bay area (Ross Sea, Antarctica) to evaluate intra-annual coastal nanoplankton dynamics. These filters, together with others of larger mesh sizes, are used to decrease the amount of organisms and debris in the input seawater before the desalination processes take place, hence automatically collect the plankton present in the water column around the desalination system intake. We have used a DNA metabarcoding approach to characterize the communities retained by filters' sets collected in January 2012 and 2013. Intra-annual dynamics were disclosed with an unprecedented detail, that would not have been possible by using standard sampling approaches, and highlighted the importance of extreme, stochastic events such as katabatic wind pulses, which triggered dramatic, short-term shifts in coastal nanoplankton composition. This method, by combining a cost-effective sampling and molecular techniques, may represent a viable solution for long-term monitoring programs focusing on Antarctic coastal communities.

Keywords:

Antarctica, Ross Sea, Terra Nova Bay, coastal plankton, monitoring, desalination plant, DNA metabarcoding

1.1 Introduction:

In the last decades, fine-scale studies on plankton diversity have acquired an increasing importance and attention (Moreira and López García, 2019). Notwithstanding the fact that we are aware that major changes are affecting oceans' functioning, we still lack an effective and internationally coordinated strategy to better detect the effects of these changes (Bindoff et al., 2019). The biggest obstacles are due to the intrinsic variability of spatial and temporal plankton dynamics, coupled with a plethora of methodologies available for plankton biodiversity monitoring. These two aspects exert a synergistic negative effect, overall causing a limited effectiveness in our capability to draw meaningful conclusions on ocean ecosystems state and evolution (Navarro et al., 2017; Buttigieg et al., 2018). Moreover, regardless of the chosen sampling design, biodiversity monitoring programs may be also hampered by

logistic constraints, often driven by financial shortcomings, especially when sampling takes place in remote areas (Lacoursière-Roussel et al., 2018).

Traditional methods relying on morphological identification have failed to provide an appropriate solution to these issues (Gast et al., 2006; Chain et al., 2016; Zhang et al., 2018). These methods, in fact, require a lengthy period of sample processing time and, in consequence, are generally used on a local-scale, thus leading to higher costs and a magnification of all the above issues (Baird and Hajibabaei, 2012). They are also affected by low precision and reproducibility (Baird and Hajibabaei, 2012). One solution proposed to overcome this problem relies on the use of High Throughput Sequencing (HTS), which gained more attention in the last decade due to its high reproducibility, short period of processing time and steadily decreasing costs of the analyses (e.g. Taberlet et al., 2012; Valentini et al., 2016; West et al., 2020).

In the Southern Ocean major drivers such as the increase of temperatures, ocean acidification and altered sea ice dynamics are expected to be the most important factors influencing the future biological communities (Convey and Peck, 2019). Here, therefore, there is an increasing need of long-term monitoring programs, especially in a multidisciplinary setting (Convey and Peck, 2019), where fine-scale approaches would be useful to track changes at high resolution in biological communities.

This task, however, is not easy due to the uncomfortable environmental settings typically occurring in polar areas, where temperatures exceed the freezing point exclusively during the summer months, sea ice conditions may abruptly change in a short time and sampling activities may be severely hampered by weather conditions. On top of this there is also the higher cost of maintaining personnel in these remote areas. Thus, operating in Antarctica, the fulfilment of one of the most important requirements of a sound monitoring program, i.e. a high sampling frequency, it is generally difficult to be achieved (Proença et al., 2017).

This is even more exacerbated in the case of studies of the Antarctic plankton, which is characterized by an intrinsic extreme dynamism, with composition and vertical carbon export changing in a matter of weeks to days (Bathmann et al., 1991; Di Tullio et al., 2000; Smith et al., 2003) or even hours, with variations between daytime and night (Celussi et al., 2009). Moreover, a variety of other local, stochastic factors may further sustain this high dynamism, such as water column instability driven by strong winds, that may even suppress the development of phytoplanktic blooms (Moline and Prezelin, 1996) or, in the opposite case, the stratification of the water column in a time frame of days or even hours due to absence of wind-induced mixing (Brandini, 1993). Also coastal pack-ice dynamics can introduce further local variability by moving the location of the sea ice marginal zone and hence the seeding of phytoplanktic blooms

(Mangoni et al., 2009), with effects varying at the regional spatial scale and at the seasonal time scale (Dayton et al., 2013). The availability of high resolution time series for Antarctic plankton is thus a crucial point and, at the same time, one of the most difficult research and monitoring tasks, always requiring a great effort to be achieved.

A possible solution or improvement, for achieving high-resolution time series of Antarctic coastal plankton, could be the analysis of samples automatically collected by research base desalination plants. These facilities were already used in a number of ecological studies as an auxiliary sampling methodology for the collection of additional planktic samples, for the investigation of seasonal variations in the phytoplankton, bacteria and picoplankton (Balzano et al., 2015), for the monitoring of harmful algal blooms in the proximity of desalination plants (Villacorte et al., 2015), or to collect invertebrate larvae (Heimeier et al., 2010a, 2010b). Desalination plants are employed wherever freshwater availability is limited and rely on the use of different pre-treatment filters that intercept water-carried particles and organisms and prevent system clogging, before the final reverse osmosis process (Wolf et al., 2005; Veerapaneni et al., 2007). Regardless of the possible technical differences existing in different desalination plants, all these systems employ filters (usually in form of “bags” and “cartridges”) with decreasing mesh sizes, which are replaced whenever the pressure inside the filter housing increases, i.e. when they start to clog. Since the freshwater is constantly needed by research base activities, desalination plants operate continuously, drawing seawater throughout the entire research base opening season, hence representing a potential source of planktic samples constantly collected.

As far as we know, the earliest Antarctic studies of desalination filters were authored about ten years ago by Sewell and Jury (2009, 2011) and were done at the New Zealand’s “Scott Base” (McMurdo Sound, Ross Sea). In these studies, desalination plant “primary filters” (100 µm mesh size) successfully collected representative samples of zooplankton (even without damaging the most delicate larval forms) and disclosed the year-round temporal dynamics of the Antarctic meroplankton (Sewell and Jury 2009, 2011). These studies were also supported by a qualitative comparison with standard net tows samples collected during the same days in the vicinity of the base, revealing a similar composition between desalination plant filter samples and a more traditional sampling strategy (Sewell et al., 2006; Sewell and Jury, 2009). Sewell and Jury (2009) recognized the many advantages observed by the application of this method, from the opportunity of sampling regardless of weather and sea ice conditions, to the large amount of seawater filtered by the desalination plant (Sewell and Jury, 2009, 2011). The high filtered-water quantity also enabled the collection of rare species that could have been overlooked by using standard plankton net sampling (Sewell et al., 2006; Sewell and Jury, 2009, 2011).

Here, we explore the usefulness of samples obtained from the desalination plant filters of a research base ("Mario Zucchelli" station, Terra Nova Bay, Ross Sea) combined with highly reproducible molecular metabarcoding analysis (which further reduce sample processing time, increase data precision and expand the study target to smaller ranges of planktic organisms' sizes) to disclose possible changes in the composition of nanoplanktic communities.

This research represents a proof-of-concept study, where we have specifically: i) looked for a correspondence between levels of particulate matter in the seawater and the filter replacement rate, ii) explored the composition and short-term dynamics of the nanoeukaryotic and particle-attached bacterioplankton communities collected by 5 µm mesh cartridge filters during the Antarctic summer in 2012 and 2013, iii) addressed some of the potential issues on the sampling and extraction protocol with the final, future aim of achieving a standardized protocol to be applied on a more general scale.

1.2 Materials and methods:

1.2.1 Timeframe of the study and of the considered data sets

The first two objectives of this study employed analyses with a different timeframe. In the first case, all the available satellite data, as well as the logbook data from the electronic logbook of the desalination plant, from October to February of 2002 to 2019, were included in the analyses, with the only exception of the 24th expedition (2008/2009) for which no logbook data were available. In the second case, sampling of the desalination plant filters was carried out in January and February of 2012 and 2013 corresponding to a total activity period of the filters examined spanning from the 25th of January to the 4th of February of 2012 and from the 8th to the 25th of January of 2013. Automatic Weather Station (AWS) hourly data were downloaded from mid-October to the end of February for both 2012 and 2013, but only those corresponding to the same timeframe of the sampled filters activity time were used. Satellite data, AWS and 5 µm filter activity time (from the desalination plant logbook) for the entire research base opening season (mid-October to end of February) of the 2011-2012 and 2012-2013 Italian Antarctic Expeditions (XXVII and XXVIII) are showed in the supplementary material (Fig. S1 and S2).

1.2.2 Description of the desalination facility

The Italian research base "Mario Zucchelli" station, hereafter "MZS", is located in Terra Nova Bay (Fig. 1) and provides facilities and support for 85 people on average (between research and logistic personnel) operating during the Austral summer from mid-October to the beginning of February. One of the main facilities is the desalination plant (Fig. 2),

which is composed of different pre-filtration steps, leading to the main and final filtration operated by ceramic filters (Fig. 3). Since MZS operates only during the Austral summer, the desalination plant is closed each year at the end of the expedition (around middle of February) by pumping air in all pipes and valves in order to prevent freezing during the Antarctic winter. At the beginning of each season (around mid-October), pipes are therefore fully clean, with no remaining water from the previous season. The entire MZS desalination plant processes 3.5-4 m^3/h on average. Only part of this water enters the true desalination pipeline where the filters operate. Given the total volume of the pipeline from the intake to the filters (0.24 m^3) it is possible to estimate that this water mass is replaced approximately 15 times per hour, i.e. once every 4 minutes.

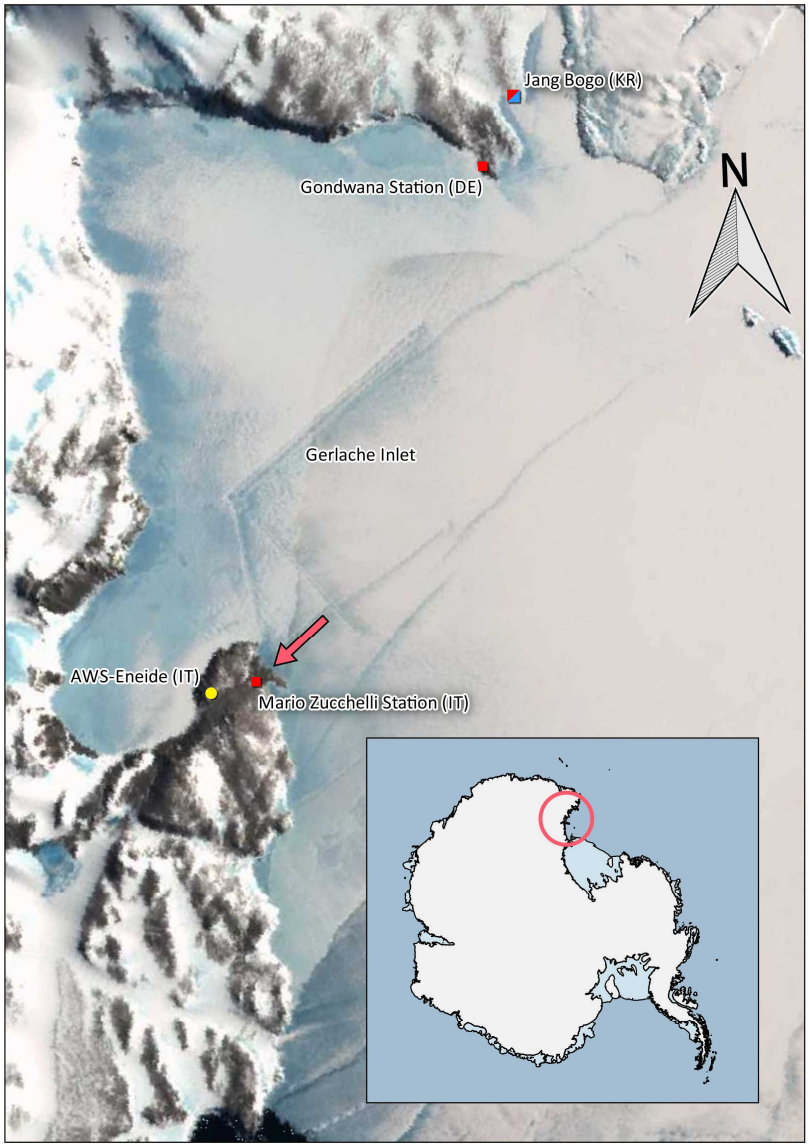
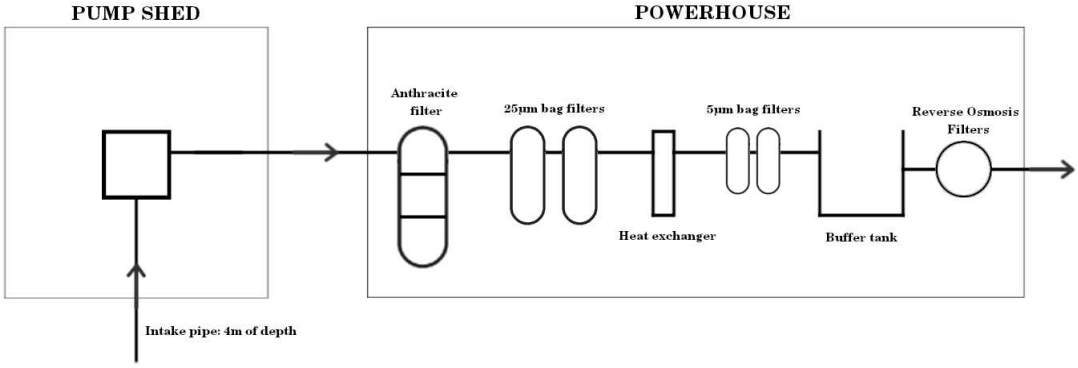


Fig. 1. *Overview on Gerlache Inlet (Terra Nova Bay, TNB) showing the three research stations operating in TNB: Mario Zucchelli Station (IT=Italy), Gondwana Station (DE=Germany) and Jang Bogo Station (KR=Republic of Korea). The red squares indicate the research stations operating only during the summer, whereas the red and blue square indicate the only all year-round operating research station (Jang Bogo). The map was produced using the collection of datasets "Quantarctica" (Matsuoka et al., 2018) and the 2.18 version of QGIS (QGIS Development Team, 2020). The map depicts the coastline orientation before the desalination plant seawater intake pipe (red arrow) in the locality of Punta Stocchino and of the Automatic Weather Station (AWS) "Eneide" (yellow circle).*

Fig. 2. *Desalination plant of Mario Zucchelli station. (a) View of the plant pump shed in the locality of Punta Stocchino. (b) 25 µm (left) and 5 µm (right) filter housings in the desalination plant powerhouse. (c) New cartridge filters (5 µm) just replaced before the closure of the lid of the filter housing.*

Fig. 3. *Simplified diagram of MZS desalination plant.*



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127 The desalination facility starts with the seawater intake pipe (-74.6936°, 164.1185°), opening at a depth of 4 meters in
128 the locality of "Punta Stocchino". From there, a series of pipes (diameter of 2 inches) and valves allow the water to
129 flow directly to the main powerhouse, distant approximately 120 meters from the intake pump shed. Here the first
130 steps of filtration are obtained through a filter packed with anthracite, followed by polyester bag filters of 25 µm mesh
131 size, a heat exchanger (which brings the seawater temperature to 10° C to maximize the efficiency of the final ceramic

filters) and a final set of filters made by polypropylene cartridges of 5 µm mesh size, which were the focus of the present analysis.

The electronic logbook of the desalination plant was inspected to gather all the available historical records for cartridge and bag filters activity and replacement, as well as the amount of consumed water at the research base. All the timings for the filter replacements, together with the activation and turn-off of the desalination plant for technical purposes, are recorded in the logbook. Thus, it is possible to obtain the exact number of hours each filter has been filtering before its replacement, done in order to avoid reaching the clogging limit.

Differently from Sewell and Jury (2009), where 100 µm filters are "reusable" and regenerated after having been in use the same amount of time, at MZS Station, the 25 µm bag and 5 µm cartridge filters are disposable, hence discarded after use. Their smaller mesh size, in fact, makes any potential regeneration process unsuitable. Collected plankton samples for analyses are thus not obtained by washing the filters as in Sewell and Jury (2009), but only by the disruption of the filter structure (see below). In our case each filter is changed when the pressure inside the cartridge filter housing reached high levels, meaning that similar levels of plankton biomass and particulate matter are collected, regardless the amount of filtered seawater or time of activity, although this datum is always recorded in the logbook.

1.2.3 Sampling and laboratory procedures

Sampling was carried out in January and February of 2012 and 2013 enabling the collection of a total of eleven 5 µm cartridge filters, five in 2012 and six in 2013. The starting day for the two time ranges refers to the day in which the first filter was installed, differently from the sample name, which identifies the day it was sampled. For example, the filter "30_1_12" sampled the 30th of January of 2012 was installed 115 hours earlier, thus the 25th of January is the starting day for the time range investigated during 2012. The volume of water treated by the filters, based on data from the desalination plant electronic logbook, ranged from a minimum of 12.7 to a maximum of 64.8 m^3, with an average of approximately 23.41 m^3 per filter. The sea was in ice-free conditions from at least ten days before our sampling (Illuminati et al., 2017; Monti et al., 2017 for 2012; Schiaparelli personal communication for 2013). As soon as the pressure inside the cartridge filter housing reached high levels, the desalination plant technician informed one of the authors (SS) of the imminent replacement and let all the remaining seawater in the housing to flow "downstream" to the next desalination step. At this point filters were removed from the housing using lab gloves, placed in a sterile plastic bag and then stored at -20° C. These filters (Fig. 4a), measuring 50.8 cm of length and 6.4 cm

161 of diameter, were kept at -20° C until summer 2018, when they were processed for the molecular analyses. Three
162 replicates were obtained from each filter (one at the top, one in the middle and one at the end of the filter in order to
163 cover all its length, see Fig. 4a), for a total of 33 replicates.

Fig. 4. *(a) A frozen cartridge filter sampled on February 4th after having filtered ~22.5 hours. The three replicates were sampled from both extremities and the centre (blue arrows). (b) Layers of polypropylene extracted using a cork borer and a pair of heat-sterilized tweezers prior to the DNA extraction. Successively, the layers were cut in half and then in stripes of 1 mm of width.*

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165 A metal, cylindrical, autoclave-sterilized cork borer of 26.25 mm in diameter was used to carve a circular cut on the
166 surface of the cartridge filter. Different subsampling protocols were attempted on unused filters weeks before
167 processing the filters used for this study, and tested by evaluating the amount and quality of the extracted DNA.
168 During this optimization of the subsampling protocol, the deepest layers were found to yield a low amount of DNA.
169 The most exterior layer of the filter (< 1 mm) was peeled off using a pair of heat-sterilized tweezers, in order to avoid
170 any potential risk of post-sampling contamination, and discarded. Molecular analyses were thus performed on the
171 immediately lower layer of the filter, and multiple cuts were performed for each replicate on different sides of the
172 filter, enabling the extraction of the appropriate amount of sample weight required by most DNA extraction kits (i.e.
173 at least the 0.3 g for the DNeasy PowerSoil Kit), also optimizing the amount of recoverable DNA.

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175 **1.2.4 Molecular analyses**

176 Filter layers from each replicate were cut into small stripes (<1 mm) and then placed in the PowerBead Tubes
177 provided by the DNeasy PowerSoil Kit (QIAGEN). DNA was extracted following the manufacturer's instructions, with
178 the exception of an additional incubation step with the C1 solution in a thermostatically controlled water bath (70° C
179 for 10 minutes) and a final elution with 50 µl (instead of 100) of the C6 solution, in order to increase the DNA
180 concentration. PCR amplification and sequencing of fragments of the 16S rRNA and 18S rRNA genes, for bacteria and

eukaryotes respectively, were performed by IGA Technology (Udine, Italy, https://igatechnology.com/). The primers used for the V3 and V4 regions of 16S rRNA gene (approximately 450 bp) were chosen from Herlemann et al. (2011) and have the following sequences (Illumina adapters underlined): 341F - 5' TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG 3' and 805R - 5' GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC 3'. The primers used for the V4 and V5 regions of the 18S rRNA gene (approximately 550 bp) were selected from Hugerth et al. (2014) and have the following sequences (Illumina adapters underlined): 574*F – 5' TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCGGTAAYTCCAGCTCYV 3' and 1132R – 5' GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCCGTCAATTHCTTYAART 3'. The PCR mix was the same for both markers and consisted in 12.5 µl of 2x KAPA HiFi HotStart ReadyMix (Kapa Biosystems, Woburn MA, USA), 5 µl of each primer and 2.5 µl of microbial DNA at a concentration of 5 ng/µl. The amplification conditions were: 95° C for 3 minutes, 25 cycles of 95° C for 30 seconds, 55° C for 30 seconds and 72° C for 30 seconds, followed by a final step at 72° C for 5 minutes.

A PCR clean-up step was performed using AMPure XP beads (Beckman Coulter) to purify from free primers and primer dimer species. This was followed by an indexing step using the Nextera XT Index (Illumina), to attach dual indices and Illumina sequencing adapters. The PCR program was the same of the amplicon PCR, except for the number of cycles set to 8 instead of 25. Another PCR clean-up step was performed prior to the quantification, normalization and sequencing using Illumina MiSeq v3 reagents on a 300 bp paired end reads MiSeq platform.

The PCR amplicons of the 16S rRNA region were sequenced on two different MiSeq runs to reach the minimum number of agreed sequences, which was 200,000 paired-end reads per replicate. All fastq files generated in this study are available in Mendeley Data (http://dx.doi.org/10.17632/89xmbhsgvc.1).

1.2.5 Bioinformatic analyses

Raw 18S rRNA sequences, after demultiplexing, were quality checked using FastQC and paired-end reads were merged using Vsearch (Rognes et al., 2016), excluding merged products with more than 1 ambiguous base and more than 3 differences in the alignment. Primers were removed using Cutadapt (Martin, 2011), allowing only one error in the alignment. Mothur (Schloss et al., 2009) was adopted to remove sequences with homopolymers greater than 8 bases, whereas Vsearch was used to remove all sequences with a maximum expected error of 1, for length filtering (max 580 bp and min 490 bp) and for the dereplication. After the dereplication, the UNOISE2 algorithm (Edgar, 2016)

implemented within USEARCH (Edgar, 2010), using the command "unoise3", was used to check for chimeras and remove singletons, generating the Zero-radius Operational Taxonomic Units (ZOTUs) fasta file. Vsearch was used again for the creation of a count table (command "usearch_global") using a global pairwise alignment with id equal to 1. The taxonomic assignment was conducted using the "Wang method" (naïve Bayesian classifier; Wang et al., 2007) implemented in Mothur and using version 4.12.0 of the PR^2 database (Guillou et al., 2013).

Raw 16S rRNA sequences were processed with the same programs as for 18S rRNA, but with the following differences: the maximum differences allowed for merging were set to 10 (due to the longer alignment region for that primers), concatenation of the fastq files of the two different runs for each replicate, maximum expected error set to 0.5, length filtering set to 430 and 400 of maximum and minimum length respectively and the original (i.e. not modified) mothur-formatted version of the Silva database (release 132) for the taxonomic assignment (Quast et al., 2012).

The following bioinformatics analyses were all undertaken in R (version 3.6.3, R Core Team, 2020) and Qiime2 (Bolyen et al., 2019). A variance stabilizing transformation, implemented in the R package DESeq2 (Love et al., 2014) was applied to account for differences in the number of sequences, without prior merging of all the replicates. This stabilization was introduced as an alternative to the more common rarefaction method (McMurdie and Holmes, 2014). Negative values, which in the context of a variance stabilizing transformation indicate that in the original count table those values were more likely to be zero, or in any case negligible, were approximated to 0, as suggested by the phyloseq authors (McMurdie and Holmes, 2013) (https://www.bioconductor.org/packages/release/bioc/vignettes/phyloseq/inst/doc/phyloseq-FAQ.html#negative-numbers-in-my-transformed-data-table, last access on October 07 2020). This approximation allowed the calculation of Bray-Curtis distances for the ordination plot generated through a Non-metric Multidimensional Scaling (NMDS) using phyloseq. The Mantel test for evaluating a correlation between the distance matrices of 18S rRNA and 16S rRNA datasets was performed using qiime2. Heatmaps were produced using the phyloseq R package, following the phyloseq-specific implementation of the NeatMap approach (Rajaram and Oono, 2010), adopting an ordination method instead of a hierarchical cluster analysis. Both heatmaps were calculated on Bray-Curtis distances and with a NMDS ordination. The heatmap for the 18S rRNA dataset was produced after reducing the count table to the 50^{th} most abundant ZOTUs sorting samples by chronological order, from the 30^{th} of January to the 5^{th} of February of 2012 and from the 11^{th} to the 25^{th} of January of 2013. The heatmap for the 16S rRNA dataset was produced after reducing the count table to the 1000^{th} most abundant ZOTUs, agglomerating them at the order level (fourth taxonomic level of

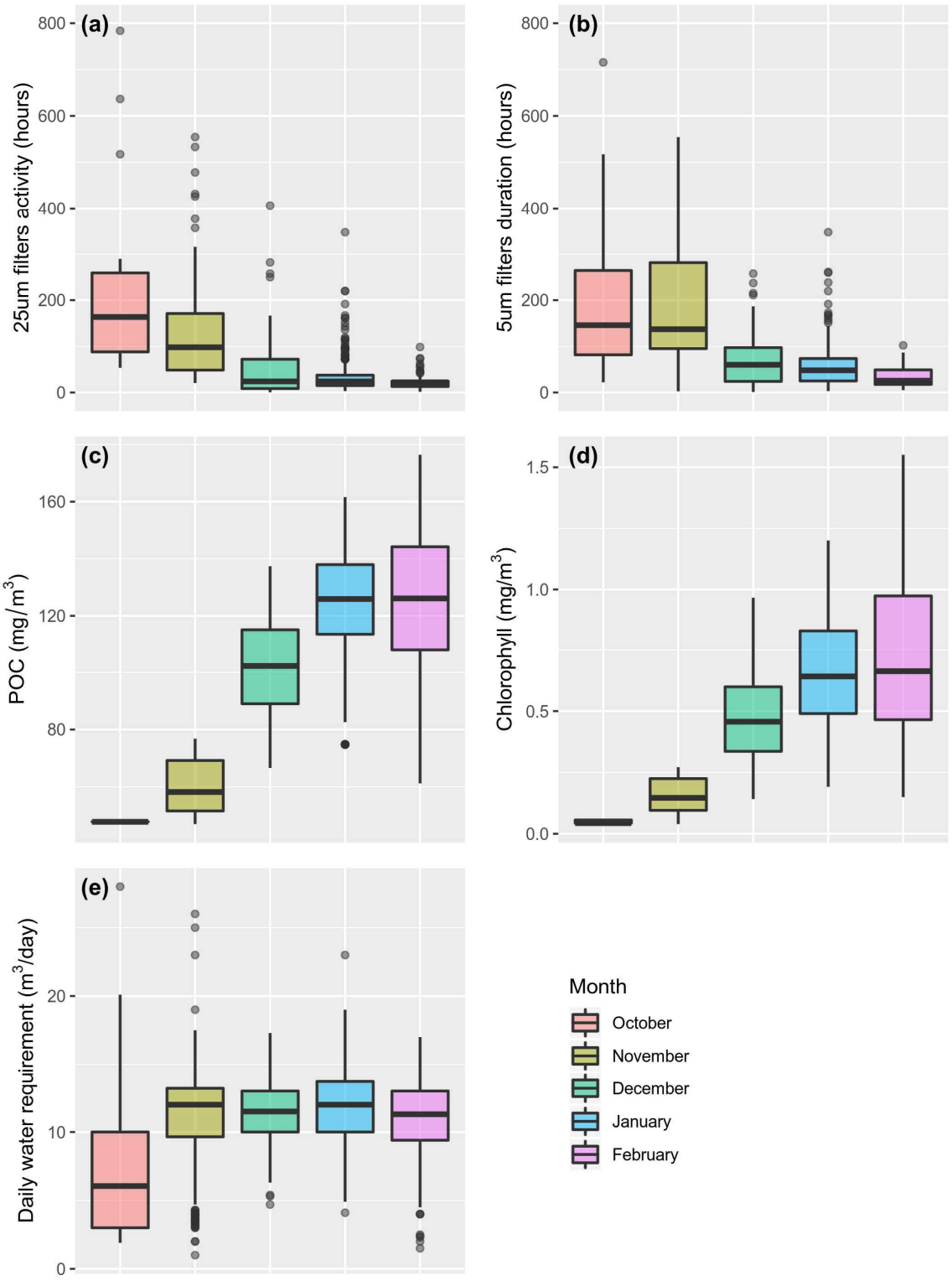
238 the Silva Database) and sorting samples by chronological order. Taxa barplots were generated using phyloseq from the
239 original, not transformed, count table after collapsing together all the replicates in the respective samples.
240
241 **1.2.6 Environmental data: air temperature, wind and chlorophyll**
242 AWS data on surface air temperature and wind direction and velocity were obtained from the "MeteoClimatological
243 Observatory at MZS and Victoria Land" of PNRA (www.climantartide.it), for the AWS "Eneide" (-74.6959°, 164.0921°),
244 located approximately 820 meters from the desalination plant pump shed. Data were processed in R using the
245 packages "oce" (Kelley and Richards, 2019), "signal" (signal developers, 2013), "tsibble" (Wang et al., 2019), "dplyr"
246 (Wickham et al., 2019) and "cowplot" (Wilke 2019).
247 Satellite data on chlorophyll (Ocean Biology Processing Group 2018a) and POC (Ocean Biology Processing Group
248 2018b) concentrations were obtained from NASA's OceanColor Web site using the level-3 browser to extract daily and
249 monthly climatology data (from October to February of each year). Data were extracted choosing the "Standard"
250 product at a 4 km resolution grid and for the area with the following latitudinal and longitudinal bounding box: -74.5°,
251 -75°; 163.5°, 165°. The downloaded mapped files were converted from the format NetCDF to "csv" (comma separated
252 values) using GDAL (Geospatial Data Abstraction Library, GDAL/OGR contributors 2020) and processed in R using the
253 "ggplot2" package (Wickham 2016).

1.3 Results and Discussion

1.3.1 Particulate matter and filter replacement rate

Logbook data on filtering activity for filter cartridges (5 µm) and bags (25 µm) from 2002 to 2019 showed a consistent decrease in filtering activity hours from October to February, resulting in a higher rate of filter replacement towards the end of the summer (Fig. 5a and b). This observed higher rate of filter replacement since the end of the summer could be due to two different reasons: i) an increase of the desalination plant activity because of the intensification of the logistic activities in the research station, or ii) a progressive increase of the particulate matter present in the seawater. However, it is clear that the decreased filtering time in summer is not due to the logistic and scientific activities as the daily water requirement shows no particular trend (Fig. 5e) while, on the contrary, there is a clear increase of chlorophyll and POC from October to February (Fig. 5c and d). A more detailed overview on the temporal dynamics of filter replacement rate, with hourly and daily recordings of environmental variables throughout the 2011-2012 and 2012-2013 opening seasons, is provided in the supplementary material (Fig. S1 and S2).

Fig. 5. *Boxplots of log-diary and satellite data from 2002 to 2019. Upper boxplots refer to (a) filter activity hours for bag filters (25 µm mesh size) and (b) cartridge filters (5 µm mesh size). Boxplots in the middle refer to (c) Particulate Organic Carbon (POC) and (d) Chlorophyll concentration measured in milligrams per cubic meter. Lower boxplot (e) refers to the total monthly cubic meters of water consumed by the research station. All data has been gathered based on month of registration and ordered from October to February. Satellite data for Chlorophyll and POC in October are less abundant than for the other months, as most of the area is usually covered in sea-ice during that period. Filters were assigned to month on the base of their installation time.*



This means that when the phytoplanktic bloom takes place, the increased amount of biomass in the seawater progressively and comparably determine an increase in the filter replacement rate, with a dramatic transition from weeks of activity of a single filter to peaks of multiple changes of filters per day. This situation takes place every year during Antarctic summer, in conjunction with the sea ice retreat and the occurrence of phytoplanktic blooms triggered by sympagic communities (Mangoni et al., 2009; Saggiomo et al., 2017). The distribution of blooms is rather patchy, being influenced by the seasonal extension and shape of the marginal ice-zone. This determines a mosaic of different planktic communities in the water column, each one characterized by a different taxonomic composition (Nuccio et al., 2000). Other environmental drivers, such as winds, may introduce other sources of variability, further affecting community dynamics (Brandini, 1993; Moline and Prézelin, 1996; Fitch and Moore, 2006). The effect of wind is especially important in Antarctica due to the existence of high-energy winds, i.e. katabatic winds, whose pulses can be considered extreme events.

Thus, due to the high community patchiness and the presence of major environmental drivers, the availability of a higher sampling frequency is mandatory in order to unravel intra and inter-annual planktic dynamics, especially when it is know that rapid short-term variations have a high probability of occurrence as also shown by our data.

1.3.2 Community composition, diversity and dynamics of nanoplankton revealed by DNA metabarcoding

Bioinformatics analyses produced a total of 603 ZOTUs for 18S rRNA and 3,914 ZOTUs for 16S rRNA. Final abundance values add up to 1,219,853 and 1,726,680 sequences, corresponding to ~30% and ~38% of the total “raw” sequences for the 18S rRNA and 16S rRNA datasets, respectively.

The NMDS (Fig. 6) showed the ability of amplicon sequencing to differentiate nanoplanktic communities investigated during similar seasons of two consecutive years and to track short-term changes in community composition taking place just in a few days (Fig. 6). For both years the ordination showed a clear distinction between the first days and the following ones, meaning that the investigated time frame was characterized by a transition of the community composition from a particular state to another one. The same transition has been recorded both in the 16S rRNA and 18S rRNA datasets (Fig. 6), suggesting that the different environmental and biological conditions similarly influenced both communities, with a very neat and strict positive correlation between the two Bray-Curtis distance matrices (Pearson r=0.90387, p=0.001) (Fig. 7). Thus, any change in community composition can be tracked by DNA metabarcoding using alternatively 16S rRNA or 18S rRNA, which provide highly overlapping metrics.

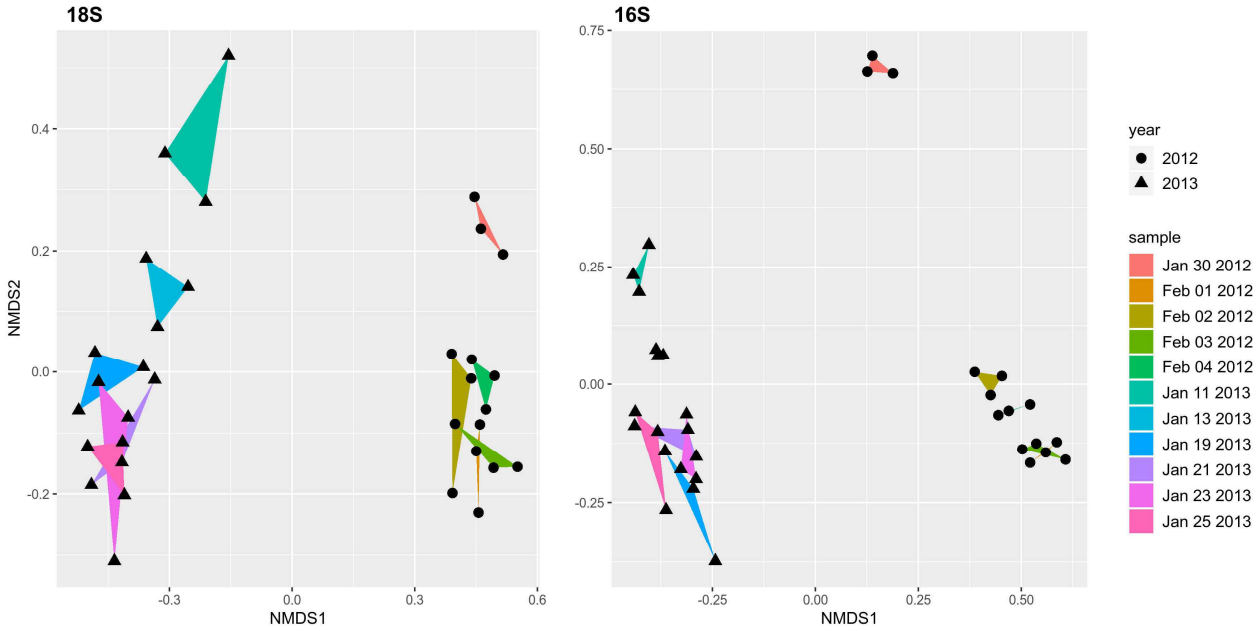


Fig. 6. *Non-metric Multidimensional Scaling of 16S and 18S based on Bray-Curtis distances. Colours refer to the replicates of the same filter, thus corresponding to the same day of sampling. Dates in the legend are ordered in temporal succession. Triangles refer to 2012 samples and circles to 2013 samples.*

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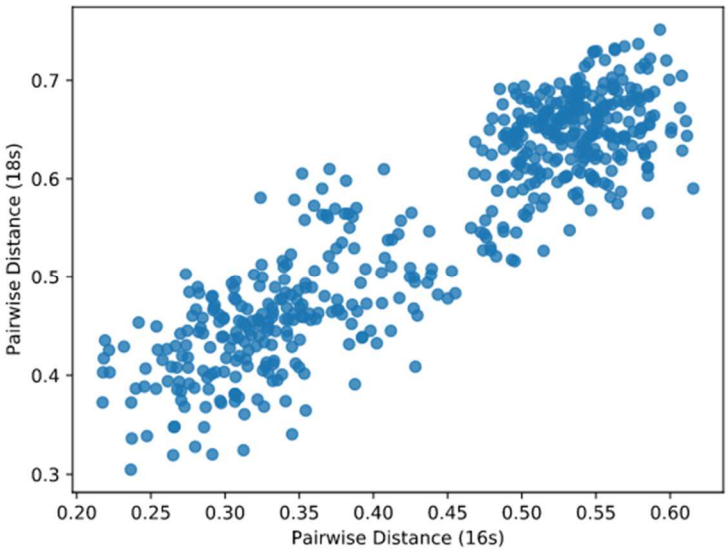
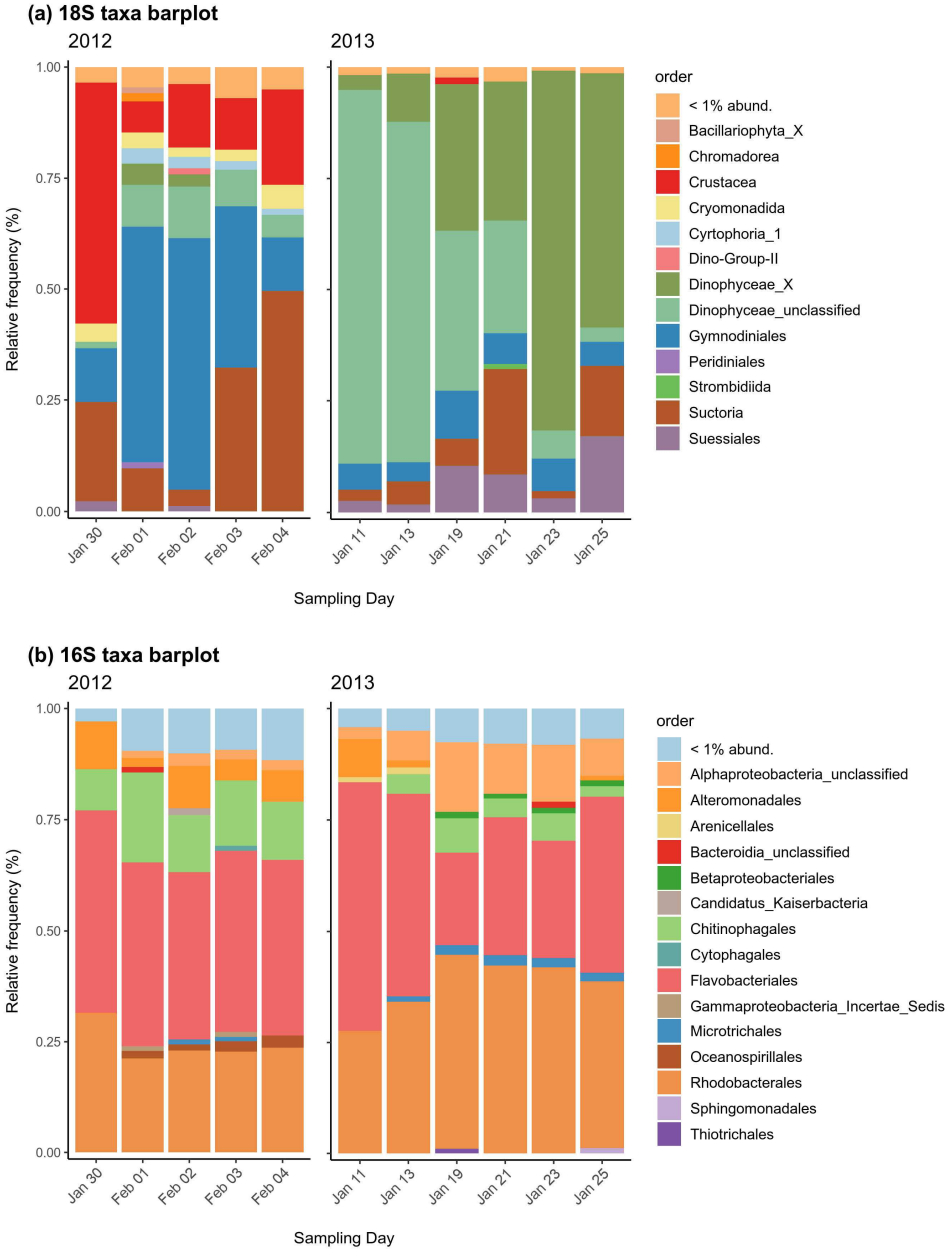


Fig. 7. *Scatterplot showing the correlation between the two different matrices of Bray-Curtis distances for 18S and 16S.*

297

298 The nanoeukaryotic community here investigated showed a marked presence of different taxonomic groups of
299 Dinophyceae in both years. The 2012 dataset was characterized by the presence of Gymnodiniales and a more
300 relevant incidence of Metazoa (Arthropoda, Maxillopoda) and Suctoria (Ciliophora, Phyllopharyngea), while in January
301 2013 two unidentified groups of Dinophyceae resulted to be the most abundant taxa (Fig. 8a). The 16S rRNA dataset
302 showed a community resembling the typical composition of surface waters Antarctic copiotrophic prokaryotes, being
303 dominated by the classes Bacteroidia, Alphaproteobacteria and Gammaproteobacteria, already evidenced in previous
304 studies (Celussi et al., 2010; Lo Giudice et al., 2012; Lo Giudice and Azzaro, 2019) (Fig. 8b). However, due to the mesh
305 size of the cartridge filters, the bacterioplankton community here investigated should not be referred to free-living
306 bacteria, but rather to particle-attached prokaryotes.

Fig. 8. *(a) Taxa barplots for 18S of 2012 (left) and 2013 (right). (b) Taxa barplots for 16S of 2012 (left) and 2013 (right).*



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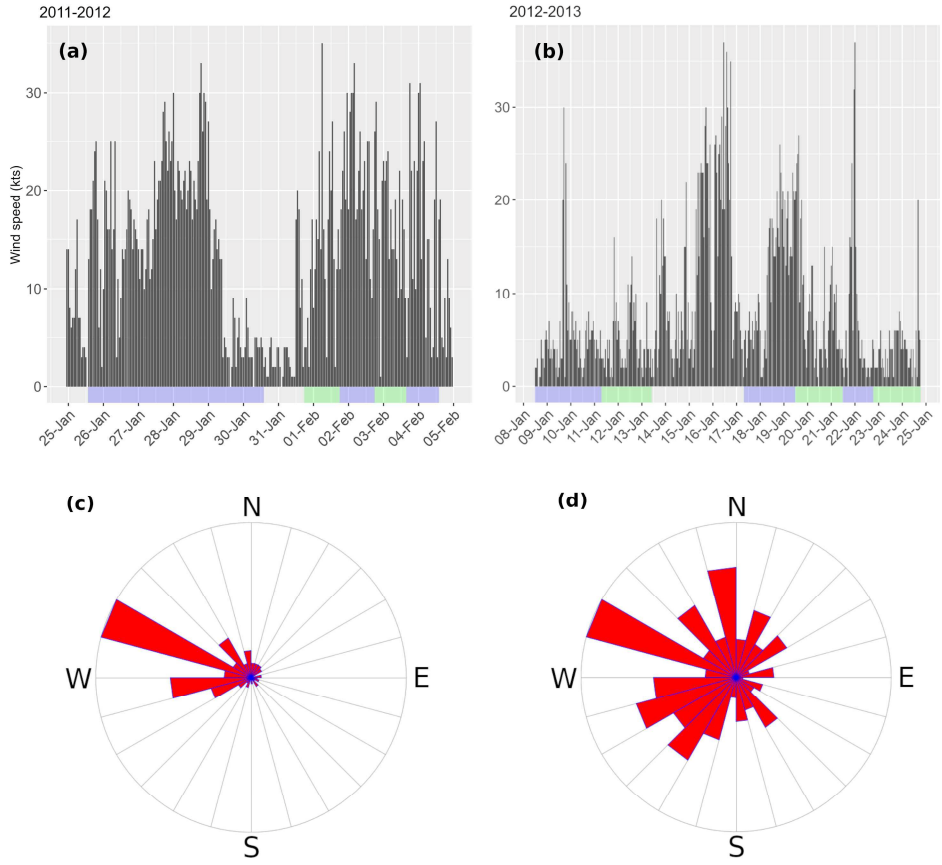
The two different time ranges investigated also indicate different intra-annual dynamics: in 2012 there was a temporary (lasting only three days, from the 1th to the 3th of February) increase of the relative frequency of Gymnodiniales and other orders of Dinophyceae over Maxillopoda and Suctoria, while in 2013 there was a clear shift between two distinct groups of Dinoflagellates, represented in particular by the class Dinophyceae (Fig. 8a).

The abrupt change in the community composition detected in 2012 may be the result of a water column instability induced by katabatic wind pulses that, as shown by the AWS "Eneide" data (Fig. 9a and c), characterized two distinct periods of high wind intensity separated by an interval of 3 days with low-intensity winds (January 29th - February 1th). During this brief period of calm weather and water column stability there was an increase of different groups of Dinoflagellates, but this did not lead to a monospecific bloom, which was likely disrupted by the second katabatic event.

Fig. 9. *Barplots of hourly wind speed recordings (a,b) and wind roses (c,d) for the two different time ranges investigated during 2012 and 2013. (a) The wind intensity (knots) and c) wind rose at the bottom for the 2012 series, (b) and (d) for the 2013 series. Blue and green bands below the barplots indicate activity time ranges for the individual filters sampled for this study, whereas the grey areas represent the activity time of filters that couldn't be sampled for this study.*



318

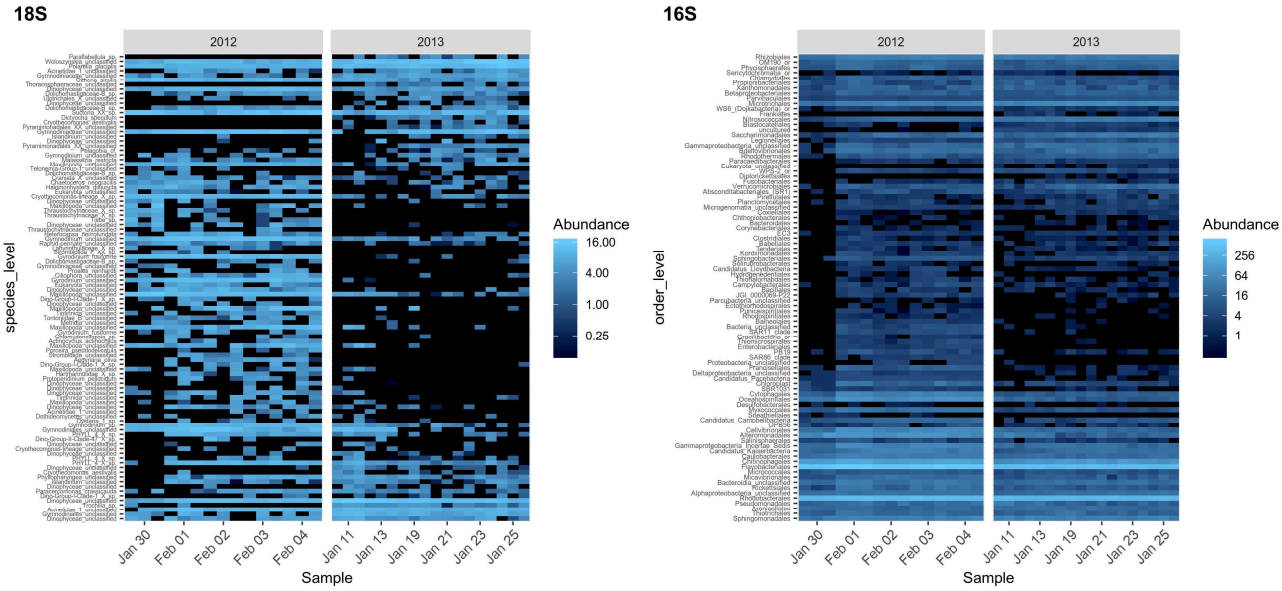
319 The high presence of Maxillopoda sequences registered during periods of high wind intensity should not be
320 considered as indicative of the presence of crustacean adults on the cartridge filter itself, but as a possible result of
321 spawning or molting events or even from disrupted body parts originating from individuals intercepted from upstream
322 components of the desalination plant. The latter would be more likely, especially if we consider the equally high
323 presence of Suctorian sequences, which are the most widespread symbiotic group in the phylum Ciliophora and can
324 be found as facultative ectosymbionts on crustaceans (Lynn, 2008) or even on phytoplankton (Sazhin et al., 2007).

On the other hand, no katabatic event was recorded by the AWS during the 2013 time series, resulting in a relatively calm period with just sporadic peaks of wind intensity from different directions (Fig. 9b and d). These more stable conditions may have favoured a progressive shift between two different Dinoflagellate groups, without abrupt changes as those observed in the 2012 series.

Regarding the bacterioplankton community, the distinction in the community composition (Fig. 6) is likely to be determined by an increase in the relative abundance of Rhodobacterales (Alphaproteobacteria) and an unidentified group of Alphaproteobacteria, mostly to the detriment of Gammaproteobacteria and of Flavobacterales (Bacteroidia).

For the 2012 series, no evident temporal dynamics at higher taxonomic levels can be appreciated and the major difference inferred by the NMDS is likely to be the result of an abrupt increase in alpha diversity between the first day (January 30th) and the following ones (Fig. 10). The orders Chitinophagales (Bacteroidia) and Alteromonadales (Alphaproteobacteria) were the only ones showing a slight increase and decrease (Fig. 8b) in percentage, respectively, probably reflecting the increase in phytoplanktic activity and algal-derived polymeric substrates (Wilkins et al., 2013) of both dinoflagellates and diatoms.

Fig. 10. *Heatmap of 18S 50 most abundant ZOTUs (left) and 16S 1,000 most abundant ZOTUs (right), the latter agglomerated by taxonomic order (fourth level from the highest of the Silva database taxonomy). Abundances values refer to those given after the variance stabilizing transformation. The x-axis is sorted in chronological order, from the oldest to the most recent filter, with the 2012 series on the left and the 2013 on the right, for both heatmaps.*



The correlation between low wind activity and the development of phytoplanktic blooms has already been recognized, not only in Antarctic coastal planktic communities (e.g. Brandini, 1993, Moline and Prezelin, 1996), but also in Antarctic offshore areas (e.g. Sallée et al., 2015; Kanta et al., 2017; Park et al., 2019), as well as in non polar areas (e.g. Nieblas et al., 2009; Qu et al., 2020). High wind activity has a direct effect on the water column structure, being capable of mixing it and inducing upwelling phenomena, thus hampering bloom occurrences (Tripathy and Jena, 2019).

Our data showed a sudden temporal response of these communities after the reduction in wind intensity, which may have allowed a temporary condition of stability that, in turn, enabled the start of a water column stratification process. This was reflected in the increase in dinoflagellate abundance, which, however, couldn't last more than three days due to the occurrence of a second katabatic event.

Unfortunately, most of the research based on HTS methodologies conducted in Terra Nova Bay (and especially near the Italian Research station "MZS") focused on prokaryotic communities only (Lo Giudice and Azzaro, 2019) and not on the eukaryotic ones. Consequently, the absence of an in depth knowledge of coastal eukaryotic communities studied through metabarcoding hampers a critical comparison with our results. Nonetheless, the data obtained in this study showed a clear dominance of dinoflagellates in the nanoplanktic community, in accordance to what is known from previous study focusing on deeper water strata (Zoccarato et al., 2016) or on the sea ice (Torstensson et al.,

2015), suggesting that these gruops may play a very important and general role in Antarctic ecosystems (Liu et al., 2020). The absence of other protists, such as Radiolaria, Hacrobia and Excavata (which, apart from the latter, are nonetheless represented by some ZOTUs in the dataset), may be due to the difference in the size range investigated, wider in the aforementioned studies or simply to the intrinsic differences in the water masses examined or in the timing of sampling.

Several highly represented taxa in our results have never or only just rarely been documented in this area before. This is the case of some groups of eukaryotes such as: i) Cryomonadida (Cercozoa; Filosa-Thecofilosea), which graze on bacteria and may also parasitize phytoplankton (Zoccarato et al., 2016); ii) Cyrtophoria (Ciliophora; Phyllopharyngea), typically found in biofilms or as facultative or obligate symbionts on the body surfaces of invertebrates, such as crustaceans (Lynn, 2008); and iii) Suctoria (Ciliophora; Phyllopharyngea), this latter one representing the third most abundant taxon in the entire 18S rRNA dataset. The absence of diatoms in high number is a more surprising result, and will be discussed later in the next section.

However, regarding the comparison of inter-annual dynamics, it has to be stressed that no conclusions can be drown despite the sampling activities occurred in the same season and with a similar timing. In fact, the investigated time ranges are too short and it is not possible to assess whether or not the two observed situations represent “typical” seasonal dynamics, just shifted in time. A more comprehensive analysis, embracing the whole Austral spring and summer months of the Antarctic field season 2018-2019 is currently under study (Cecchetto et al. unpublished results) and will be of help in understanding these dynamics.

1.3.3 Advantage of the method and possible implementations

Despite only few filters were available for this study, also limited only to the 5 µm mesh size fraction, our molecular approach enabled a high-resolution analysis of intra-annual dynamics of Terra Nova Bay plankton. This was obtained through a cost-effective method (no funds were needed to set up the filtering system as it is part of the research base) and, especially, without the need of personnel at sea for continuous samplings, which is logistically unfeasible especially during extreme weather conditions that characterize katabatic wind events. Several new taxa were also recorded for the first time and future studies will enable clarifying if these are regular occurrences in the area.

Unfortunately, not enough filters have been studied so far in order to address the capacity of recovering rare taxa based on the different amount of filtered seawater, as most of the filters were in use for a similar amount of hours (see materials and methods). Sewell and Jury (2009) stated that the system is capable of recovering most of the rare

taxa, but their methodology allowed a sampling frequency based on the quantity of seawater filtered, whereas in our study this approach would be logistically unfeasible. Only a couple of filters (the first of the two series) had significantly higher values of filtered seawater but, as mentioned earlier, they also were those with the lower numbers of taxa recorded.

Moreover, as the amount of particulate present in the input seawater does not necessarily correspond to higher biomass, uncertainties in the interpretation of actual bloom events may arise. This issue could be easily resolved by monitoring also other environmental and biological parameters (e.g. turbidity and chlorophyll concentration) by establishing an *in situ* monitoring station located in the vicinity of the seawater intake pipe to obtain environmental data *in continuum*. The availability of these data will enable a more precise interpretation of the community changes disclosed by metabarcoding.

It has also to be considered that in metabarcoding studies the abundances of taxa are always difficult to be estimated in "absolute" terms for a variety of reasons (Taberlet et al., 2018), above all the well-known issues regarding primer amplification biases (Jovel et al., 2016; Piñol et al., 2018). The adoption of different methodologies, such as metagenomics (adopting shotgun sequencing techniques), which don't rely on amplification enrichment, would certainly reduce the impact of these issues (Bohmann et al., 2014). In this context, biodiversity monitoring using filters from desalination plants, and its usefulness in detecting short-term dynamics in coastal communities, would greatly benefit from the potentials of methodologies such as metatranscriptomics. In general, further and specific research would be required to validate the applicability of different methodologies, also according to the taxonomic group of interest, the project goals, and the availability of *in situ* lab facilities.

Some eukaryotes recorded in this study are typically found growing on biofilms, such as Cryomonadida, which has already been documented in water treatment systems (Angell et al. 2020, Fried et al. 2000) and whose abundance could potentially result overestimated (Henthorne and Boysen, 2015). However, the dynamics of eukaryotic communities inside desalination plants are largely unknown and very few papers deal with this issue (e.g. Belila et al., 2017), the main focus of seawater pre-treatment studies having been bacterial biofilm eradication to prevent membrane clogging (e.g. Bar-Zeev et al., 2009). Nonetheless, due to the long period of inactivity of the Italian research station "MZS" during the winter, as well as the frequent replacement of different pre-treatment filters during most of the summer, the impact of potential biofilm growth should be minimal. As stated before, in fact, the desalination plant is also closed at the end of each expedition by pumping air in all pipes and valves, completely removing the amount of liquid seawater at the end of each expedition. This cleaning practice, together with the high amount of

seawater usually filtered daily through the entire desalination plant (which is fully replaced every 4 minutes) suggests as this contribution, although not quantifiable, should be really negligible. Thus, the data reported should really reflect what is present in the water column.

Surprisingly, diatoms, despite being usually reported as one of the main components of the phytoplanktic blooms (Pabi and Arrigo, 2006; Mangoni et al., 2009), were not abundant in our samples. Another survey, carried out during the austral summer 2011-2012, before and immediately after our sampling time frame at an offshore site, roughly 1 Km far from the desalination plant intake pipe, showed a dominance of diatoms, both in terms of cell abundances and biomass, while Dinoflagellates represented only a minor group (Illuminati et al., 2017). However, the method adopted by Truzzi et al. (2015) and Illuminati et al. (2017) involved a completely different protocol based on a quali-qualitative methodology, and not based on a selective filtration process. For this reason, the absence of abundant diatom sequences in the 5 µm "cartridge" filters may simply be due to the retention of most diatom species by the 25 µm "bag" filters located upstream. On the other hand, this apparent incongruence could also simply be due to the well-known patchy distribution of plankton communities in Terra Nova Bay, where areas dominated by diatom blooms are intermixed with others mainly dominated by dinoflagellates and other flagellates, also forming strong inshore-offshore gradients (Nuccio et al., 2000). Another reason could be related to the sub-sampling protocol and DNA extraction we have adopted. The chosen primers (Hugerth et al., 2014) should theoretically amplify 18S rRNA from Ochrophyta, as running an *in silico* PCR on the Silva SSU RefNR Database (Klindworth et al., 2013), allowing only two mismatches, reports 98% of coverage for that group. However, since the first layers of the filters were discarded and no aggressive steps, such as the mechanical lysis of diatom cell wall (frustules), were adopted during DNA extraction (Vasselon et al., 2017), it is possible that the diatom component in the total DNA extract was potentially reduced.

A general aspect to consider for the proposed method would also be the storage conditions for the samples which, in this case, correspond to a storage at -20° C for some years. It is known that, despite being one of the most widespread techniques for storing samples used for molecular analyses, freezing at -20° C could be optimal for short periods of time, whereas, on the long-term, -80° C would be preferable (Straube and Juen, 2013). Other storage conditions were also proposed in literature, each one with different pros and cons that may condition the results of a study (e.g. Ransome et al., 2017) thus hampering comparisons between studies that adopt different storage protocols. In our case however, as samples were stored and processed under the same conditions, the comparison of observed dynamics are valid, potential biases being exactly the same for the two sets of samples.

An implementation of the method (which was already tested in the field during the Austral summer 2018-2019 with another set of samples) is the adoption of a subsampling procedure done immediately after filters' collection. This step greatly reduces the size of the samples (i.e. small cores instead whole filters have to be preserved) and also allows adopting different storage procedure (e.g. medium-based instead frozen). This simple step surely facilitates the storage and shipping of samples by greatly reducing their physical volume. Thanks to the increasing availability, portability and cost-efficiency of new molecular technologies (Gilbert, 2017; Johnson et al., 2017) all these analyses could also be ideally done in the field, thus completely eliminating storage-related potential issues or biases.

1.4 Conclusions:

The HTS methodologies applied to the desalination plant filters, regardless of any technical peculiarity of a given desalination plant or mesh size considered, could represent a turning point in the always-increasing need of detailed and fine-scale data about the structure of phyto- and zooplankton inhabiting Antarctic coastal waters. Despite the need of further calibrations and the possible existence of issues that will require attention in the future, the availability of filters from a desalination plant offers unprecedented research opportunities at a more than achievable cost. Data shown here represent a great leap in our knowledge of coastal plankton communities for the study area, highlighting previously unknown dynamics, such as the short-term and abrupt changes in coastal nanoeukaryotic communities' composition triggered by katabatic winds pulses, and finding groups of organisms never recorded before. This approach also overcomes most of the constraints linked to the logistic of sampling activities in a harsh environment and provides precise and fine-scale data that would simply not be achievable by using standard monitoring approaches based on the collection of water samples taken in the field, e.g. from the pack-ice or a boat. By imagining a long term approach, where data of this type are collected each year at a given research station, it is out of doubts that the spatial and temporal dynamics of Antarctic coastal plankton will be revealed at unprecedented level of detail.

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Figure Captions:

Fig. 1. Overview on Gerlache Inlet (Terra Nova Bay, TNB) showing the three research stations operating in TNB: Mario Zucchelli Station (IT=Italy), Gondwana Station (DE=Germany) and Jang Bogo Station (KR=Republic of Korea). The red squares indicate the research stations operating only during the summer, whereas the red and blue square indicate the only all year-round operating research station (Jang Bogo). The map was produced using the collection of datasets "Quantarctica" (Matsuoka et al., 2018) and the 2.18 version of QGIS (QGIS Development Team, 2020). The map depicts the coastline orientation before the desalination plant seawater intake pipe (red arrow) in the locality of Punta Stocchino and of the Automatic Weather Station (AWS) "Eneide" (yellow circle).

Fig. 2. Desalination plant of Mario Zucchelli station. (a) View of the plant pump shed in the locality of Punta Stocchino. (b) 25 µm (left) and 5 µm (right) filter housings in the desalination plant powerhouse. (c) New cartridge filters (5 µm) just replaced before the closure of the lid of the filter housing.

Fig. 3. Simplified diagram of MZS desalination plant.

Fig. 4. (a) A frozen cartridge filter sampled on February 4th after having filtered ~22.5 hours. The three replicates were sampled from both extremities and the centre (blue arrows). (b) Layers of polypropylene extracted using a cork borer and a pair of heat-sterilized tweezers prior to the DNA extraction. Successively, the layers were cut in half and then in stripes of 1 mm of width.

Fig. 5. Boxplots of log-diary and satellite data from 2002 to 2019. Upper boxplots refer to (a) filter activity hours for bag filters (25 µm mesh size) and (b) cartridge filters (5 µm mesh size). Boxplots in the middle refer to (c) Particulate Organic Carbon (POC) and (d) Chlorophyll concentration measured in milligrams per cubic meter. Lower boxplot (e) refers to the total monthly cubic meters of water consumed by the research station. All data has been gathered based on month of registration and ordered from October to February. Satellite data for Chlorophyll and POC in October are less abundant than for the other months, as most of the area is usually covered in sea-ice during that period. Filters were assigned to month on the base of their installation time.

Fig. 6. Non-metric Multidimensional Scaling of 16S and 18S based on Bray-Curtis distances. Colours refer to the replicates of the same filter, thus corresponding to the same day of sampling. Dates in the legend are ordered in temporal succession. Triangles refer to 2012 samples and circles to 2013 samples.

Fig. 7. Scatterplot showing the correlation between the two different matrices of Bray-Curtis distances for 18S and 16S.

Fig. 8. (a) Taxa barplots for 18S of 2012 (left) and 2013 (right). (b) Taxa barplots for 16S of 2012 (left) and 2013 (right).

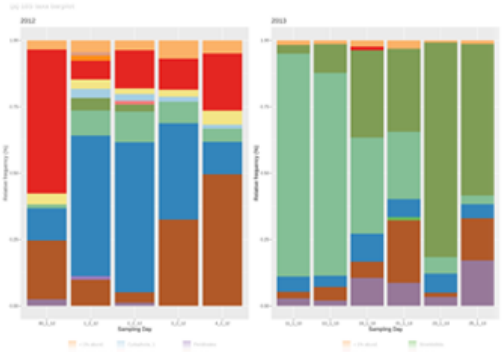
779 Fig. 9. Barplots of hourly wind speed recordings (a,b) and wind roses (c,d) for the two different time ranges
780 investigated during 2012 and 2013. (a) The wind intensity (knots) and c) wind rose at the bottom for the 2012
781 series, (b) and (d) for the 2013 series. Blue and green bands below the barplots indicate activity time ranges for
782 the individual filters sampled for this study, whereas the grey areas represent the activity time of filters that
783 couldn't be sampled for this study.
784 Fig. 10. Heatmap of 18S 50 most abundant ZOTUs (left) and 16S 1,000 most abundant ZOTUs (right), the latter
785 agglomerated by taxonomic order (fourth level from the highest of the Silva database taxonomy). Abundances
786 values refer to those given after the variance stabilizing transformation. The x-axis is sorted in chronological
787 order, from the oldest to the most recent filter, with the 2012 series on the left and the 2013 on the right, for
788 both heatmaps.

Desalination plant cartridge filters (5 µm mesh size)

nanoplankton collection

Metabarcoding

Intra- and inter-annual dynamics



Link to environmental drivers