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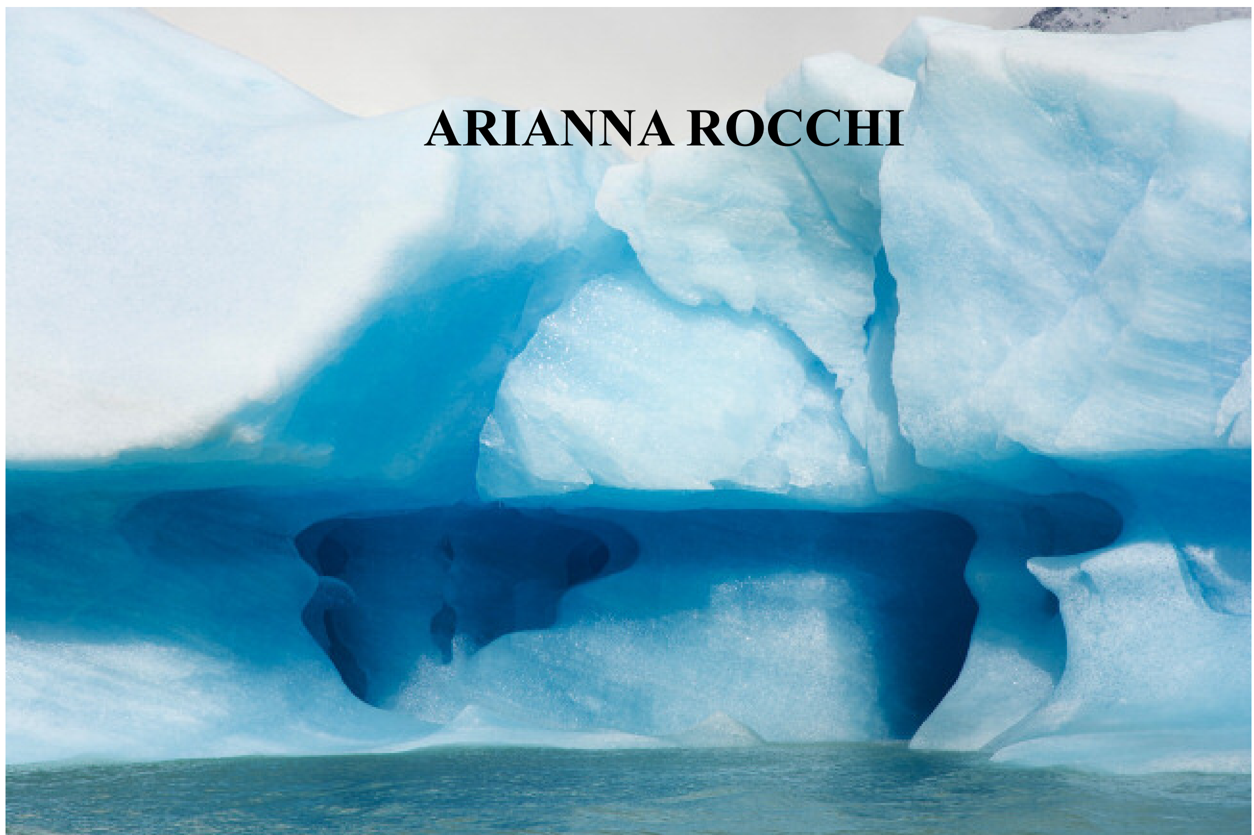

ARIANNA ROCCHI

La actividad viral del hielo marino antártico aumenta la aerosolización primaria

A actividade viral do xeo mariño da Antártida aumenta a aerosolización primaria

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ABSTRACT

The ocean covers 71% of the surface of our planet Earth and viruses are extremely abundant. Marine viruses play a key role in modulating several biogeochemical cycles. Still - very little is known about their role in the production of aerosol, clouds and climate change.
A recent interest on the marine viruses' contribution to the marine aerosols is growing, revealing that - after viral infection - phytoplankton (nanoflagellates) and prokaryote (bacteria) cells release organic matter to the water. The new released organic matter can contribute at making primary marine aerosol - produced at the sea surface through interaction between wind and waves, and subsequent bubble bursting. Aerosol particles affect the hydrological cycle because they act as cloud condensation nuclei (CCN) thereby influencing the formation and development of clouds.

Therefore, the aim of this thesis is to test if the lysis of prokaryotes (bacteria) and eukaryotes (heterotrophic and phototrophic nanoflagellates) - produced by viruses in melted sea ice - affects the production of primary organic marine aerosols. To achieve this goal, we carried out melted sea ice - atmosphere experiments in a marine controlled and bubble-bursting aerosol generation chamber in the laboratory during the Spanish 2018-2019 Antarctic campaign. This thesis is presented into two main sections: 1) results produced in this thesis (mortality experiments and biological measurements) and 2) results discussed with complementary data produced by other colleagues.

Preliminary results are promising, showing an increase in viral abundance and production, rate of lysed prokaryotes and eukaryotes and the organic carbon released from these lysed cells when viral concentrate is added, followed by a rise in the number of atmospheric aerosols produced within primary aerosol chamber. In a nutshell, this study points to the direction on which the fresh organic matter released in the water from melted sea ice is an important source of Antarctic marine aerosols.

RESUMEN

El océano cubre el 71% de la superficie de nuestro planeta Tierra y los virus son extremadamente abundantes (10^{30}). Los virus marinos desempeñan un papel clave en la modulación de varios ciclos biogeoquímicos, todavía se sabe muy poco sobre su papel en la producción de aerosoles, nubes y cambio climático.
Un interés reciente en la contribución de los virus marinos a los aerosoles marinos está creciendo, revelando que - después de la infección viral - fitoplancton (nanoflagelados) y células procariotas (bacterias) liberan materia orgánica al agua. La nueva materia orgánica liberada puede contribuir a la producción de aerosol marino primario - producido en la superficie del mar a través de la interacción entre el viento y las olas, y posterior formación de burbujas. Las partículas de aerosol afectan al ciclo hidrológico porque actúan como núcleos de condensación de nubes (CCN).

Por lo tanto, el objetivo de mi tesis es probar si la lisis de procariotas (bacterias) y eucariotas (nanoflagelados heterotróficos y fototróficos) - producida por infección viral en el hielo marino derretido - afecta la producción de aerosoles marinos orgánicos primarios. Para lograr este objetivo, realizamos experimentos de hielo marino fundido - atmósfera en un tanque de generación de aerosoles en laboratorio durante la campaña española 2018-2019 en la Antártida. Esta tesis se presenta en dos secciones principales: 1) resultados producidos en esta tesis (experimentos de mortalidad y mediciones biológicas) y 2) resultados discutidos con datos complementarios producidos por otros colegas.

Los resultados preliminares son prometedores, mostrando un aumento en la abundancia y producción viral, la tasa de procariotas y eucariotas lisadas y el carbono orgánico liberado de estas células lisadas cuando se añade el concentrado viral, seguido de un aumento del número de partículas de aerosol producidas en el tanque empleado para para la realización de los experimentos con muestras de hielo. En pocas palabras, este estudio confirma como la materia orgánica liberada del hielo marino derretido a la columna de agua es una fuente importante de aerosoles.

ACRONYMS

BAE	Antarctic Spanish BAE Juan Carlos I
BS	Burst Size
C	Carbon
CCN	Cloud Condensation Nuclei
CHL-A	Chlorophyll-*a*
CSP	Coomassie Stainable Particles
DOC	Dissolved Organic Carbon
DOM	Dissolved Organic Matter
DMS	Dimethyl Sulfide
DMSP	Dymethylsulfopropionate
HNF	Heterotrophic Nanoflagellates
ICM-CSIC	Institute of Marine Science
JCI	Juan Carlos I
PI-ICE	Polar atmosphere-ice-ocean Interactions: Impact on Climate and Ecology
POA	Primary Organic marine Aerosol
POC	Particulate Organic Carbon
PNF	Phototrophic Nanoflagellates
RLC	Rate of Lysed Cells
SI	Sea Ice
SOA	Secondary Organic marine Aerosol
SSA	Sea Spray Aerosol
SW	Sea Water
TEP	Transparent Exopolymer Particles
VA	Viral Attack
VP	Viral Production
VPL	Viral Lytic Production
VPLyso	Viral Lysogenic Production
WAP	Western Antarctic Peninsula

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1 INTRODUCTION

Sea Ice

Almost 10% of the world's ocean is covered by sea ice at least once per year, which makes it one of the largest biomes on Earth (Dieckmann and Hellmer, 2010). Although being a cold and harsh environment, sea ice is full of life. Specialized microorganisms live inside brine channels and pockets that are formed during freezing conditions, when salts and nutrients from the seawater become concentrated between the ice crystals (Thomas and Dieckmann, 2002).

Sea ice Biology

Brine remains a liquid inside the ice, due to its high salinity, and this makes it a suitable habitat for the sea-ice microbial community, which comprises phytoplankton, mainly diatoms, protists, bacteria, archaea and their viruses (Figure 1) (Maranger, Bird and Juniper, 1994; Mock and Thomas, 2005; Arrigo, Mock and Lizotte, 2010; Deming and Collins, 2017).

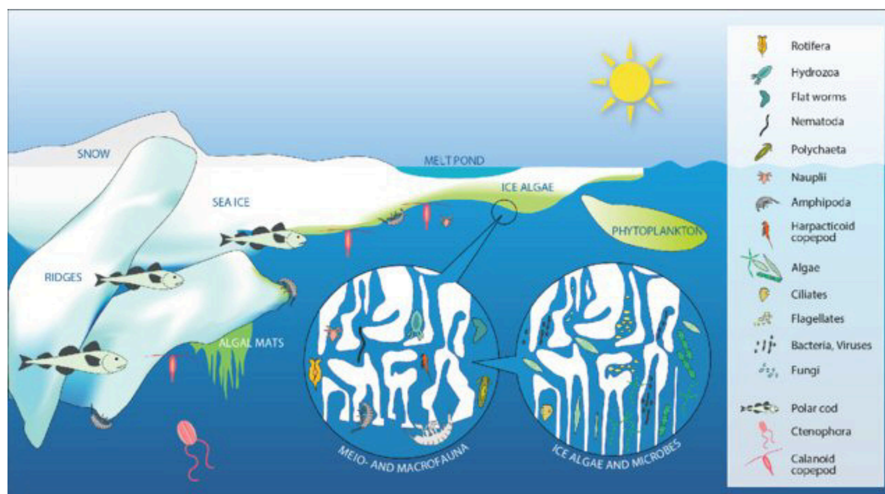

Figure 1. Sea ice provides a wide range of microhabitats for diverse biota including microbes (virus and bacteria), single-celled eukaryotes, multicellular meiofauna, larger under-ice fauna, as well as polar cod. Modified from Bluhm et al. (2017).

Microbes affect the biogeochemical properties of the sea ice, gas exchange between the ocean and atmosphere, and provide food for ice-associated animals (Arrigo and Thomas, 2004).

Virus

Viruses are the most abundant biological entities in the sea (10^{30} in the whole ocean) and play important roles in the biogeochemical cycles of the oceans, and driving microbial

diversity (Fuhrman, 1999; Suttle, 2007). The highest viral proportion of viruses are bacteriophages (i.e. viruses infecting bacteria) since these are the highest abundant potential hosts (10^{29} bacteria in the whole ocean) (Weinbauer, 2004; Suttle, 2007). Viruses can multiply only within their host cells; hence, their activity is dependent on the abundance and activity of their hosts (Maranger, Bird and Juniper, 1994; Marchant et al., 2000). Due to their relatively host specificity, they are crucial to the control of bacterial community composition and activity (Proctor and Fuhrman, 1990; Wommack and Colwell, 2000; Suttle, 2005; 2007). The numbers of viruses range between 10^5 and 10^8 ml^{-1} in bulk Arctic and Antarctic sea ice from spring to autumn (Gowing et al., 2002). The lowest values have been observed during the winter in Antarctic bulk ice (Paterson and Laybourn-Parry, 2012), whereas the highest numbers of viruses occur during freezing or spring algal mass growth (Maranger, Bird and Juniper, 1994; Deming and Collins, 2017). Hence, as the potential hosts increase, viruses do, what explain the significant relationship with bacterial abundance, activity and chlorophyll-a (chl-a) concentrations (Maranger, Bird and Juniper, 1994; Gowing et al., 2004). In aquatic environments, a typical virus-to-bacteria ratio is 10:1 (Maranger and Bird, 1995). Bacterial and viral density dictates their contact rate, which is one of the key controls in virus–host interactions. The semi-enclosed environment of brine channels may increase this contact rate, especially during winter, when the brine channels are narrower and even more concentrated (Wells and Deming, 2006). In spring the sun light goes through the sea ice (1-2m thick) enhancing the development of phytoplankton growth (i.e. diatoms, phototrophic nanoflagellates) (Figure 2), mainly in the bottom layer of the sea ice that is in contact with the water column, very rich in inorganic nutrients (Behrenfeld et al., 2006; 2014; 2016; 2017).

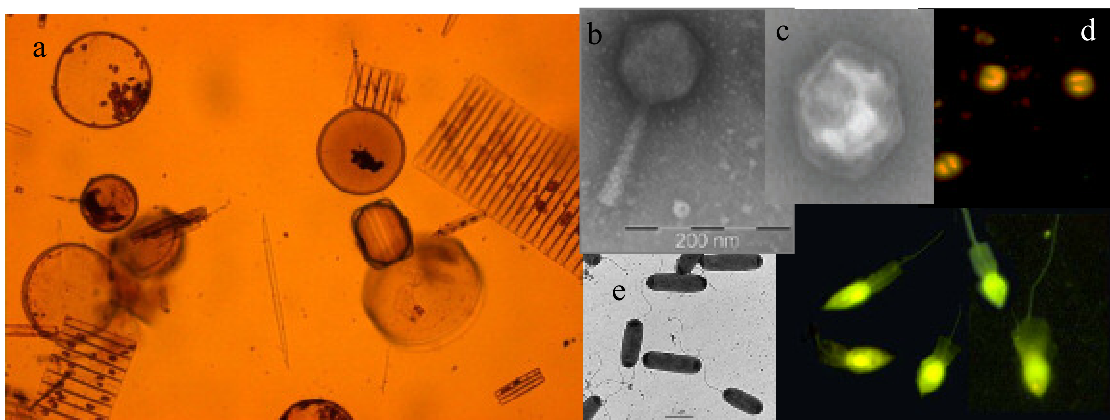

Figure 2. Pictures of (a) diatoms, (b, c) virus, (d) nanoflagellates and (e) bacteria, all belonging to the sea ice.

Lysis

Subsequently, an increase in microbial food web activity occurs within these ice channels (Evans et al., 2009; Lindell et al., 2005; Hurwitz et al., 2013; Béja et al., 2001; Wells and Goldberg, 1992; Guidi et al., 2016). Along summer, the sea ice is in part melted and broken, liberating a high proportion of these microorganisms (Figure 2) to the water column. In there, their activity will be enhanced even more, and will contribute to carbon fluxes, as particulate organic carbon (POC) to higher trophic levels through grazing, or to be returned as dissolved organic matter (DOM) to the water column by virus mediated lysis (Suttle, 2005; Wilhelm and Suttle, 1999; Fuhrman, 1999; Suttle, 2007; Danovaro et al., 2008; Brussaard, 2004; Brussaard, 2008; O'Dowd et al., 2015) (Figure 3).

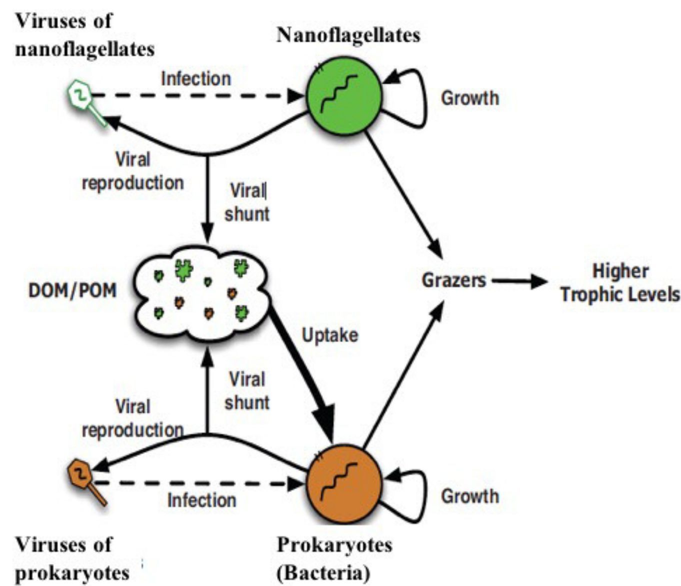

Figure 3. The lysis of microbes by viruses releases cellular material into the environment. Some of this cellular material can be utilized by microbes for subsequent metabolic processes. Here, we note that available dissolved organic material (DOM) and particulate organic material (POM) is utilized primarily by prokaryotes. (DOM: dissolved organic material; POM: particulate organic material) modified from (Weitz and Wilhelm, 2012).

Polar interaction ocean-atmosphere

The atmosphere of planet Earth protects life by absorbing ultraviolet solar radiations and by warming its surface through heat retention. In the atmosphere that overlays the 71% of the Earth's surface that is covered by oceans, marine aerosols contribute substantially to the global aerosol budget and have a large impact on the planetary albedo and climate (Reddington et al., 2017). However, aerosols remain the least understood and constrained aspect of the climate system (Boucher et al., 2013). Aerosol concentration, size distribution, chemical composition and physico-chemical behaviour play a crucial role in governing radiation transfer. Aerosols both scatter and absorb incident solar radiation and

affect cloud microphysics. The effect of aerosols on cloud properties is recognized in the IPCC Assessment Report (IPCC 2017) as the largest single contributor to uncertainty in predicting climate change. However, aerosol sources and processes, including critical climate feedback mechanisms, are still not fully characterized, especially in pristine environments (Carslaw et al., 2013).

Clouds

Marine aerosols could affect cloud formation, thereby influencing sunlight irradiation and precipitation. However, the extent to which and the manner in which viruses influence climate remains uncertain (Rosenfeld et al., 2019). Clouds are a fundamental part of the terrestrial energy budget, and any process that can cause systematic changes in cloud microphysics is of scientific interest. To form a cloud droplet, water vapor needs to condense to aerosols acting as cloud condensation nuclei (CCN) of sizes of at least 50-100 nm (Seinfeld et al., 2016), with changes in the number of CCN influencing cloud microphysics (Twomey et al., 1987; Pierce and Adams, 2009; Svensmark et al., 2017).

Aerosol

Marine airborne particles are produced from both primary and secondary processes (Figure 4). Primary aerosol production occurs from the interaction between wind and the ocean surface, resulting in the mechanical production of sea-spray droplets which can comprise both organic (POA, primary organic marine aerosol, both DOC Dissolved Organic Carbon and POC Particulate Organic Carbon, including microgels) and inorganic matter (NaCl) (O'Dowd and de Leeuw, 2007). Two main categories of microgels are TEP (Transparent Exopolymer Particles) - mainly polysaccharides-like gels and CSP (Coomassie Stainable Particles) - mainly protein-like gels. Microgels can accumulate in the ocean surface and once emitted, they can affect the number of organic aerosols in the atmosphere, hence affecting our climate. This is because water vapor in the atmosphere condenses on aerosol, strongly regulating cloud formation.

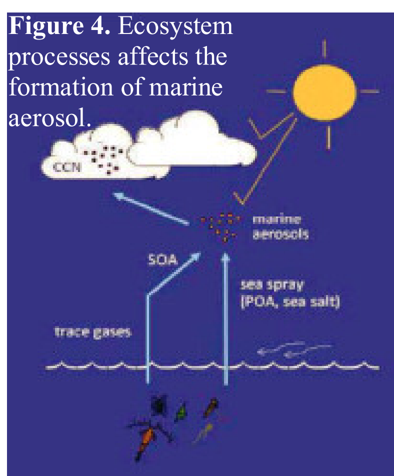

Figure 4. Ecosystem processes affects the formation of marine aerosol.

By contrast, Secondary organic marine aerosols (SOA) derive from gas-to-particle conversion processes which involve biogenically emitted precursors. This is the case of the dimethyl sulfide (DMS gas) release to the atmosphere through SSA, which is transformed

in sulphate aerosol particles. Specifically, some phytoplankton taxa as dinoflagellates (Figure 2), cryptophytes and haptophytes are great producers of dymethylsulfopropionate (DMSP) which is released to the water column after zooplankton grazing or viral lysis (Turner et al., 1988; Keller et al., 1989; Kettle et al., 1999; Belviso et al., 1990; Matrai and Keller, 1994; Hill et al., 1998; Laroche et al., 1999; Kiene et al., 2000, Archer et al., 2001). This DMSP is transformed to dimethyl sulphite (DMS) through phytoplanktonic and/or bacteria enzymatic processes (Laroche et al., 1999; Zhuang et al., 2011; Wolfe and Steinke, 1996; Hill et al., 1998). Finally, the DMS is emitted to the atmosphere by aerosolization and its derivate sulphate promotes cloud condensation (Charlson et al., 1987, Decesari et al., 2011; Brooks and Thornton, 2018).

Focusing on primary marine sea spray

Atmospheric aerosols have long been known to alter climate by scattering incoming solar radiation and acting as seeds for cloud formation, these processes have implications in the chemistry of the Earth's environment and climate. Sea spray aerosol (SSA) is produced at the ocean surface and thus emitted over nearly three-quarters of our planet and it can be then lofted into the upper troposphere, where it contributes to the global radiation budget. Sea spray aerosol (SSA) particles (i.e. salt, virus, bacteria) are generated by breaking waves and bursting of whitecap foam bubbles (Blanchard et al., 1957; Lewis et al., 2011) and constitute one of the most abundant aerosol particle types in the atmosphere.

Sources of organic primary marine aerosols

The organic part of primary sea spray remains unknown. Previous studies have suggested that the organic fraction of SSA in the marine boundary layer increases during periods of high biological activity in the ocean. For this reason, environmental studies correlated aerosol production with chlorophyll-a bloom (O’Dowd et al., 2004, Lee et al. 2015)

The "Chlorophyll-a lag" issue

However, Rinaldi et al. (2013) suggested that chlorophyll-a is only partially suitable for predicting organic enrichment. A temporal lag between water chlorophyll-a concentration and atmospheric organic matter enrichment in aerosol was observed, suggesting that biological processes in oceanic surface waters should be considered when modeling the production of primary marine organic aerosol (Rinaldi et al., 2013). Indeed, recent results suggest that marine microbiology, in particular viruses, may play a role in controlling the

chemical composition and impacts of marine sea spray aerosol (O'Dowd et al., 2015; Schiffer et al., 2018). To further understand this, we carried out some experiments with aerosol marine chamber. Therefore, what we did was studying virus in controlled chamber experiments to link virus role (marine microbiology) with aerosol particles (biogeochemistry of the atmosphere).

AIM OF THE CURRENT WORK

Under the multidisciplinary PI-ICE (Polar atmosphere-ice-ocean Interactions: Impact on Climate and Ecology) project, during the period 2018-2020 a polar field study was carried out in the Western Antarctic peninsula lead by the ICM-CSIC. Such project - PI-ICE aimed to directly identify atmospheric aerosols emitted in the polar regions, their biological origin and their impact on marine clouds formation.

Here, the aim is to test if the lysis of prokaryotes (bacteria) and eukaryotes (heterotrophic and phototrophic nanoflagellates) - produced by viruses in melted sea ice - affects the production of primary organic marine aerosols. To achieve this goal, we carried out melted sea ice - atmosphere experiments in a controlled marine aerosol chamber.

Within the PI-ICE experiments, the work of the candidate of this master consisted in calculating the mortality of viruses affecting the populations of prokaryotes and nanoflagellates over time.

Specifically, from an experimental approach, this master's degree thesis, aims to:

- test how viruses interact and affect the populations of prokaryotes and small protists (heterotrophic and phototrophic nanoflagellates) over time, **by measuring the viral production and mortality over these populations.**
- evaluate whether the viral lysis of prokaryotes and protists due to virus infection release organic compounds, which could be involved in marine aerosol formation that could give rise to the creation of cloud condensation nuclei.

In addition, we use chemical data (DOC, DMSP and TEPs) measured over the experiments, by other members of the PI-ICE team, to establish relationships between this chemical data and the released DOC by the lysed prokaryotes and protists cells. Here, we

want to detect if changes in released DOC, due to the viral activity, contribute to the pool of these organic compounds, and if that could be reflected in the formation of aerosol compounds.

2 MATERIALS AND METHODS

2.1 EXPERIMENTAL DESIGN

2.1.1 Area of study and sampling strategy (water and sea ice)

The PI-ICE field expedition visited different geographic locations alongside the upper part of the Western Antarctic Peninsula (WAP, Figure 5). The field study was divided into two parts, the first one onboard the RV BIO- Hesperides, from 21/01/2019 to 5/02/2019, and the second one in the Antarctic Spanish BAE Juan Carlos I (BAE), in Livingstone Island, from 6/02/2019 to 19/03/2019. For the present study, during the first part, four different ice samples were collected from a robber boat in the Weddell Sea (station SI9, Figure 5d), stored in 60 L clean plastic boxes and maintained frozen in a controlled temperature chamber, first in the BIO-Hesperides and after in the BAE. Sea ice was choosing according of different brown-green colours, indicating that ice is inhabited by rich biological assemblages. In the second part, mainly focused on air-sea aerosol chamber experiments, we collected 200 litres of coastal seawater in Livingston Island (station SW15, Figure 5c).

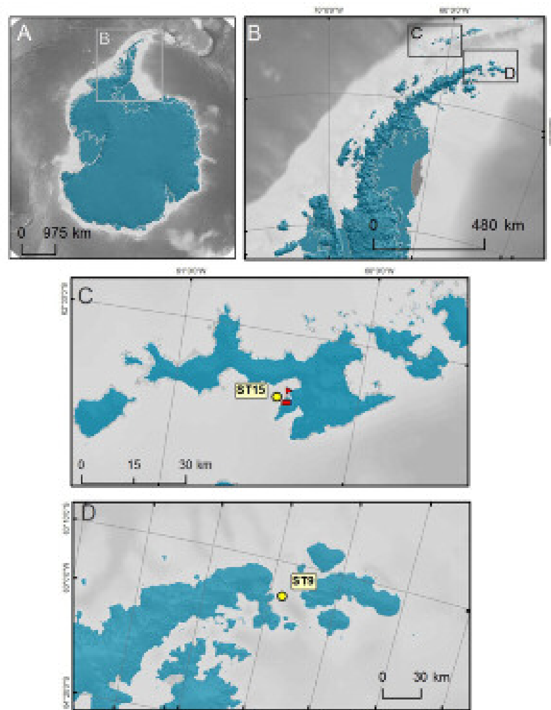

Figure 5. (a) Map of Antarctica and the Southern Ocean with the area of study. (b) Zoom of the Antarctic Peninsula. (c) Livingston Island zoom with the station SW15 where the PI-ICE group sampled water and (d) Weddell sea where ice was taken in the SI9.

The bulk water (200L) was filtered in situ through 200µm mesh size and collected in a three-times rinsed Teflon-coated container to avoid plastic contamination. Temperature and salinity were measured directly and continuously from the boat with a calibrated thermometer via the flow-through thermosalinograph SBE 21 SeaCAT. Once in the lab of

the BAE, the sample was divided in two parts: 1) for analyses of several chemical and chlorophyll concentration or just stored frozen for further analyses once back in the laboratory at the Institute of Marine Sciences (ICM-CSIC) in Barcelona; and 2) for the Sea Ice experiments by filtering the rest of the sample through 0.2 µm for melting the sea ice.

2.2 EXPERIMENTS IN BASE (BAE JCI)

2.2.1 MARINE AEROSOL CHAMBER

We conducted four sea ice experiments in a bubble-bursting aerosol generation 70 L stainless steel tank, sealed with a methacrylate (transparent) lid (Figure 6a). Bubble bursting was created with water jets coming from the top by recirculating the seawater with a peristaltic pump. Air was blown into the chamber (with a clear air compressor) and the physico-chemical characteristics of the particles coming out of the chamber were determined in different chemical and physical aerosol instruments (Figure 6b).

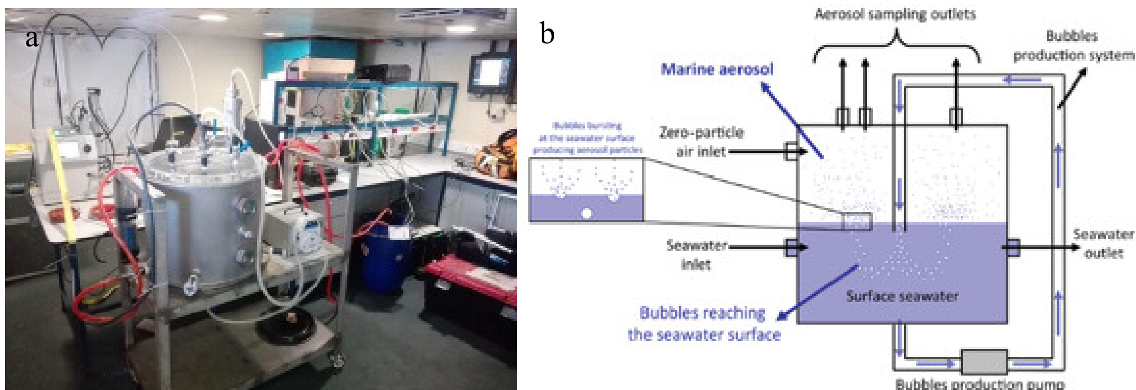

Figure 6. (a) Aerosol real chamber with temperature control thermostat at the ICM-CSIC and (b) scheme of the aerosol chamber taken from (Rastelli et al., 2017).

First, 30 kg of sea ice were melted through 24 hours with 30 L of the 0.2 µm-filtered seawater SW15 (free of microorganisms except viruses), in clean containers. Sea ice samples were used for these experiments. Sea ice was melted following the common procedure, 75% of sea ice from station SI9 and 25% of water from station SW15 to facilitate the melting without compromising the biology of the cells. Second, the chamber was filled up to 60L with the melted sea ice. Each one of the experiments lasted for 72 hours and were maintained at constant light (ambient of the lab) and temperature of about 1°C, by recirculating cold water through the external surface wall of the chamber (Figure 6a).

2.2.2 EXPERIMENTS OF LYSIS AND MARINE AEROSOL CHAMBER

During each experiment, the melted sea ice was under to two different phases: still and bubbling repeated twice, in total of 4 experiment periods (Figure 7). The first bubbling period (24h) was done to assess the melted sea-ice biogeochemical characteristics before and after a bubble-bursting natural event (BB and AB, Before Bubbling and After Bubbling, Figure 7). The second bubbling period (24-48h) was done to evaluate the effect of introducing a stress on the natural conditions by the addition into the tank of a marine viral concentrate of about 20%-50% of the initial viral concentration, what we call the viral attack (VA, AVA, Viral Attack, After Viral Attack, Figure 7). This addition of viral concentrate was left to incubate 24h (still period) and then another bubbling period of 24h was applied (Figure 7). Finally, in order to study air-sea-ice interactions, we collected primary aerosol generated in the controlled aerosol marine chamber.

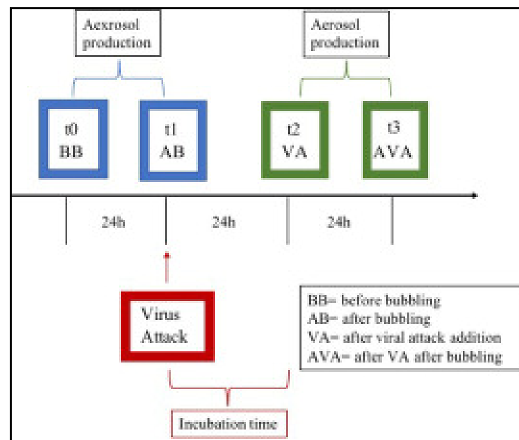

Figure 7. Scheme of the tank used for conducting experiments with ice samples. The water was recirculated with 4 different times, two bubbling phases were made to simulate the conditions of a rough sea and the addition of the virus concentrate. Actions and sampling were carried out on each of the 4 experiments.

Sampling was carried out for different chemical and microbiological variables in each still and bubbling phase of the experiments. A total of 4 different experiments were run (EXP 1, EXP 2, EXP 3, EXP 4). Furthermore, viral mortality measurements were conducted after the first bubbling period, before viral attack (addition of viral concentrate), and after 24h the viral attack, before the second bubbling. Samples for many of the variables were taken (organic and inorganic nutrients, viral abundance, prokaryotes etc…), stored conveniently, and analysed and processed in the Institute of Marine Sciences (ICM) in Barcelona.

For a better understanding and development of the work, pictures of prokaryote abundance have been taken with a computer attached to the epifluorescence microscope (1250x, Olympus RX 61) at the ICM-CSIC.

2.2.3 MORTALITY EXPERIMENT SETUP

To perform measurements of microbial mortality by viruses, only two samples were collected from the melted sea ice in the bubbling chamber. One, after 24 h bubbling before viral concentrate addition (control, or natural conditions), and two, 24 h after viral addition (viral attack VA, or extreme conditions) (Figure 7).

Virus mediated mortality on prokaryotes

2.2.3.1a *Viral production and prokaryotic mortality*

Viral production (VP, Virus mL^{-1} d^{-1}) and prokaryotic losses due to viruses (rate of lysed cells, RLC) were measured using the virus reduction approach (Weinbauer et al., 2002, Wilhelm et al., 2002). This method distinguishes between the production of lytic (VPL), and lysogenic phages (VPLyso) by inducing lysis with mitomycin C.

To perform the VP measurements, one litre of melted sea-ice water was filtered through an 0.8-µm–pore size polycarbonate filter (Nuclepore) and then concentrated by a spiral-wound cartridge (0.22-µm pore size, VIVAFlow 200), obtaining 40 mL of prokaryote concentrate, and keep the 0.2 µm filtered melted sea-ice water. Virus-free sea-ice melted water was collected by filtering 0.5 litre of sea-ice melted water prokaryotes free using a cartridge of 30 kDa molecular mass cut-off (VIVAFlow 200). A mixture of virus-free water (160 mL) and prokaryote concentrate (40 mL) was prepared and distributed into six sterile 50-mL falcon plastic tubes. Three of the tubes were kept as controls to measure viral lytic production (VPL), while mitomycin C (Sigma) was added to the other three tubes as the inducing agent of the lytic cycle in prophages (1 µg ml^{-1} final concentration) (Figure 8).

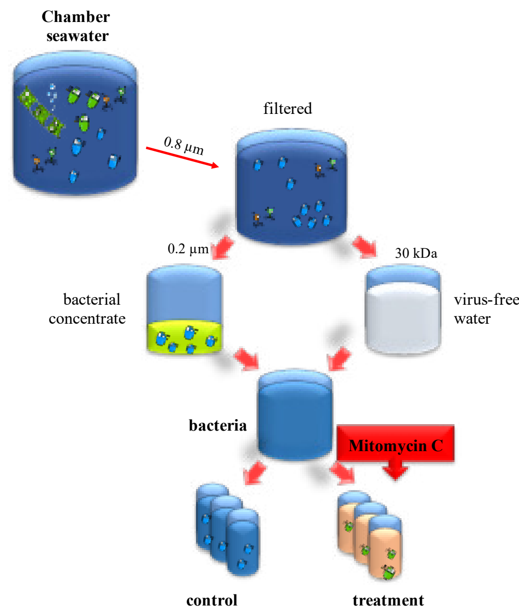

Figure 8. Scheme of the dilutions to achieve the mortality experiment for prokaryotes.

The tubes were incubated in a thermostatic chamber simulating in situ temperature, in the dark for 12-24 h. Samples for viral and prokaryotic abundances were collected at time zero and every 4 h of incubation, fixed with glutaraldehyde (0.5% final concentration) and stored. Viruses and prokaryotes from viral production incubations, and rate of lyses cells were counted by flow cytometry in the ICM-CSIC laboratory (as described above). Calculations of viral lytic and lysogenic production were made according to Weinbauer et al. (2003). As part of the prokaryotes is lost during the prokaryotic concentration process, the VPL and VPLyso were multiplied by the prokaryote correction factor (from 1.6 to 10 in our study) which enable to calculate the VP (lytic and lysogenic) in relation to the ambient prokaryotic abundance. This factor was obtained dividing the abundance of prokaryotes in the aerosol chamber by the abundance of prokaryote after tangential concentration (Winget et al., 2005).

VP= total Δ Viruses / t; Total viral production (viruses mL^{-1} d^{-1}) (Mit C treatment) (Figure 9)

VPL= Δ Viruses control/ t; Viral lytic production (viruses mL^{-1} d^{-1}) (Control)

VPLyso= VP-VPLytic

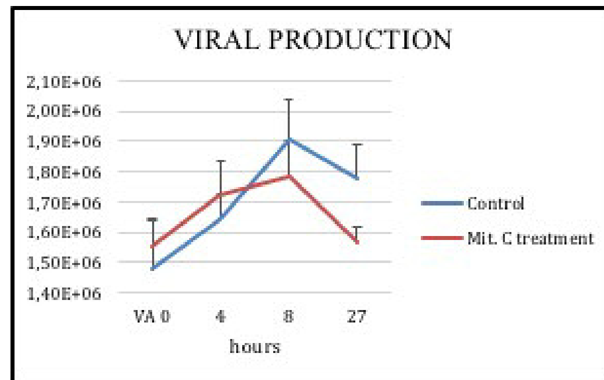

Figure 9. Example of the evolution of viral production, the increase of viruses over time, both lysis and lysogeny.

2.2.3.1b *Rate of lysed prokaryote cells*

The virus mediated prokaryotic mortality was assessed as the rate of lysed cells mL^{-1} d^{-1} (RLC), which is obtained dividing the VPL by the burst size (BS), as described in Guixa-Boixereu, (1997). The BS is the number of viruses released by a prokaryotic lysed cell, and was estimated from VP measurements, as in Middelboe and Lyck (2002), Wells and Deming (2006) and Boras et al. (2009). This is obtained dividing the increase of viral abundances by the decrease of prokaryote abundance during the first 4 hours of incubations (Jiang and Paul, 1996 and Boras et al., 2009), obtaining a value around 24 viruses $prokaryote^{-1}$, which is according to Wommack and Colwell (2000). Increase of viral abundance during short time intervals (4 h) in VP measurements was divided by a decrease of prokaryote abundance in the same period of time. We assumed that the prokaryotes production and viral decay in this time interval were negligible.

Virus mediated mortality on nanoflagellates

2.2.3.2a *Viral production and nanoflagellate mortality*

A similar procedure was performed for the heterotrophic and phototrophic nanoflagellate (HNF and PNF) mortality measurements. The two samples before and after the viral attack were collected from the tank in order to get cell concentrates of HNF and PNF nanoflagellates. For that 1 L of sample was concentrated using 2 L filtration system (Nalgene) on a 0.8 µm policarbonate filter to eliminate prokaryotic cells and viruses and keep the concentrate of nanoflagellates above the filter. The concentrate of nanoflagellates, was rinsed twice, with 250 mL of virus free sea-ice melted seawater to remove as much as possible viruses and prokaryotes from the concentrate. During the process the 0.8 µm filter was changed several times, to avoid the clogging of the filter, and maintain the cells in suspension. Once we got 100 mL of concentrate, this was diluted with 1 L melted sea ice

virus-free water, that is obtained as before, using a cartridge of 30 kDa molecular mass cut-off (VIVAFlow 200). Next, 300 mL of the mixture was distributed in into three sterile 500 mL plastic flasks and incubated for 48 h at ambient light and temperature (0-1°C). Subsamples of 15 mL were collected every 8-12 hours (Figure 10).

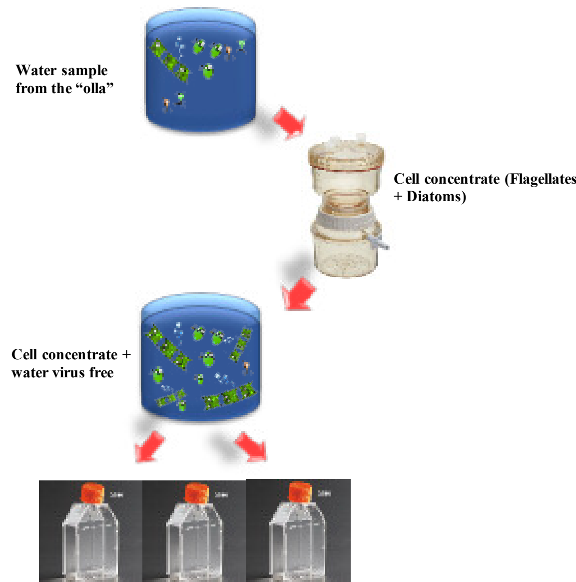

Figure 10. Scheme of the dilutions to achieve the mortality experiment for nanoflagellates.

In this case we only measured the Viral Lytic Production (VLP), that was multiplied by a correction factor: number of PNF+HNF mL^{-1} in the tank at the time we took the sample and divided by PNF+HNF mL^{-1} at time 0 after the cell concentration. In this case we only considered the increase over time of the V3 and V4 fractions, that presumably correspond to viruses infecting eukaryotic cells.

2.2.3.2.b *Rate of lysed PNF and HNF cells*

In order to achieve the rate of lysed nanoflagellates cells (HNF+NPF; cells lysed $ml^{-1}d^{-1}$, RLC), the viral lytic production was divided by the BS. Since, we cannot distinguish the rate for each lysed type of nanoflagellate separately, we considered the average of BS between HNF (70 vir/HNF, Massana et al., 2007) and PNF (263 vir/PNF, Maat et al., 2017), taking into account, the proportion of the abundances of each type of nanoflagellate.

2.2.3.3 *Calculation of released carbon*

We also wanted to detect the concentrations of carbon in prokaryote and eukaryote cells before and after the addition of the viral attack in order to have an idea of the carbon released after cell lysis. The rate of released carbon (ng C L^{-1}) from lysed prokaryotes was

obtained multiplying the rate of lysed prokaryote (cell $mL^{-1}d^{-1}$) by the corresponding cell C/cell Volume factor described in Gundersen et al. (2002), which is: fg C $cell^{-1}$ = 108.8* $(V)^{0.898}$.

We estimated the rate of released C (ng C L^{-1}) from lysed nanoflagellates by multiplying the rate of RLC (cell $mL^{-1}d^{-1}$) by the corresponding cell C/cell V factor that in the case of protists is: pgC $cell^{-1}$= 0.216* $(vol)^{0.939}$ (Menden-Deuer and Lessard, 2000).

3 MEASUREMENTS

3.1 MICROBIOLOGICAL VARIABLES

3.1.1 Abundances of viruses and prokaryotes

For both viruses and prokaryotes, 2 mL of melted sea ice samples from the tank were taken and fixed with glutaraldehyde (0.5 % final conc.), maintained for 15 min in the dark at 4°C, flash frozen in liquid nitrogen and stored at -80°C, until their analyses. For viruses, once in the Institute of Marine Science (ICM-CSIC) laboratory, these sub-samples were thawed at 80°C and stained with SYBR Green I (Molecular Probes, Invitrogen), before counting in a FACScalibur flowcytometry (FACSCalibur, Becton & Dickinson) following Brussaard (2004), and Boras et al. (2009). To achieve reliable counts, we should acquire an optimal range of 80-800 of events min^{-1}. For that we have to select the best dilution that fits that range for each sample, making parallel dilutions of 10x or 20x times the original sample. These dilutions were carried out with an aliquot of the sample mixing it with the sterile buffer TE (10mM Tris-hydroxymethyl aminomethane, Roche Diagnostics; 1mM ethylenediamine tetracetic acid, Sigma-Aldrich) up to 500µL. This buffer is used to avoid electronic coincidence; any other diluent has had a negative effect on virus reading in the flow cytometer (Brussaard, 2004). For each session, a blank was prepared, composed entirely of the TE buffer. Then, we dyed the samples with 5µL of a solution of SYBR® green I (10,000x concentrated in DMSO; Invitrogen, Molecular Probes) which remained in a thermal bath at 80ºC for 10 minutes, which favoured the entry of dye through capsid and DNA staining. After leaving them to rest for 5 minutes in the dark we proceeded to its analysis. Each sample was treated for 1 minute in the flow cytometer and Milli-Q (ultra-pure deionized water with an electrical resistivity of 18.2 MΩcm-1) was used as preservation fluid between samples. With the use of Milli-Q a better signal is observed, necessary when working on the lower threshold of measurement of the flow cytometer

(Brussaard, 2010). Also, each diluted sample was weighed before and after was circulated through the flow cytometer to obtain the flow rate (volume per minute) that was on average 60 μL min^{-1}. This, together with the events min^{-1}, and dilution factors, is used for obtaining the final viral abundance mL^{-1}. A similar procedure was taken for the prokaryote count. In this case, dilutions were not performed unless strictly necessary, that is, when the appropriate reading threshold of the flow cytometer was exceeded. The samples were prepared by directly extracting an aliquot portion of 400μL of water and adding 4μL of SYBR® green I. In this case, each sample was treated for 2 minutes in the flow cytometer and likewise, Milli-Q was used as preservation fluid between sample and sample (30 μL min^{-1}). The program used to work with the FCM technique was the Cell Quest Pro. Prior to the measurement of each sample, it was necessary to configure the parameters for separate analysis of viruses and prokaryotes. For viruses, depending on their fluorescent signal, viruses were classified as low, medium (V1 and V2) or high (V3 and V4) fluorescence that correspond to their content in DNA. Presumably, fractions of V1 and V2 are mainly attributed to bacteriophages, and V3 and V4 to viruses of eukaryotes (Evans et al., 2009). Particularly, the V4 fraction corresponded to haptophyceae (i.e. phaeocystis sp.) viruses.

3.1.2 Abundances of heterotrophic and phototrophic nanoflagellates

To determine the abundance of heterotrophic (HNF) and phototrophs (PNF) nanoflagellates melted sea ice samples of 15 to 20mL were taken, fixed with glutaraldehyde (1% final concentration), stained with DAPI (4,6-diamidino 2-phenylindole) for 5 min (conc. final 1 μg mL^{-1}, Sieracki et al., 1985) and filtered through previously blackened 0.6μm (pore diameter) polycarbonate filters to avoid background fluorescence. The filters were mounted on a slide, placed on top of a drop of immersion oil (low fluorescence, Olympus). Then another drop of oil was added on top of the filter and covered with a cover slide. Slides were frozen at -20° C until observation and counting by epifluorescence microscopy (1250x, Olympus RX 61) at the ICM-CSIC. Two types of nanoflagellates were established: phototrophic and heterotrophic. In the epifluorescence microscope, phototrophic nanoflagellates (PNF) can be distinguished for showing red fluorescence due to the photosynthetic pigments contained in the chloroplasts when blue light (excitation 550nm and emission at 590nm) struck the sample, while the heterotrophic nanoflagellates showed green-yellow fluorescence. To be sure that what we were seeing was a cell and not an artefact, filters were observed under ultraviolet radiation (excitation

400nm and emission 450 nm), so that the nucleus of the cell fluoresced in blue, due to staining with DAPI. In addition, we considered four cell size categories: 2µm, 2-5µm, 5-10µm, 10-20µm. For each filter 3 transects of 5mm were made and each nanoflagellate was counted, differentiating them by size and type.

3.2 OTHER MEASUREMENTS

Chlorophyll a concentration and chemical variables

The following variables were determined by other members of the PI-ICE team.

3.2.1 *Chlorophyll-a concentration*

Samples (150 ml of melted sea ice) for the estimation of chlorophyll-*a* (Chl-a) concentration were collected on glass fibre filters (GF/F) and extracted in acetone (90% V/V) for 24 hours in the dark at 4ºC. All samples were kept frozen at -80°C in the BAE fridge. Concentrations were determined by fluorimetry with a calibrated Turner Designs fluorometer at the BAE following the method developed by Holm-Hansen et al. (1965).

3.2.2 *Dissolved organic matter concentration*

We sampled (two subsamples of) 30 mL of melted sea ice for dissolved organic carbon (DOC) quantification and DOM optical characterization, which were filtered through pre-combusted Whatmann GF/F filters. The filtrates were collected in acid-clean 30 mL polycarbonate bottles and stored at -20ºC for DOC measurements. DOC will be measured with a Shimadzu TOC-V CSH organic carbon analyser at the Nutrient analysis service of the ICM (CSIC).

3.2.3 *Transparent exopolysaccharides concentration and Dymethylsulfopropionate*

Samples of 150 mL were collected in each time of the experiments for transparent exopolysaccharides (TEPs) concentrations. Three subsamples were filtered at low, constant vacuum (150 mm of Hg) using polycarbonate filters (0.4-µm pore-size). We measured following the same procedure of (Zamanillo et al., 2019).

Considering DMSP, to 30 mL sea ice melted samples, two pellets of NaOH were added for hydrolysis to DMS for at least 24 h at room temperature in the dark. DMSP concentrations were calculated by subtraction of the endogenous DMS. DMS calibration was performed with DMS solutions generated by dissolution and hydrolysis of solid DMSP in high purity water.

4 RESULTS: MICROBIAL MEDIATED MORTALITY BY VIRAL LYSIS

Prokaryotes and nanoflagellates mortalities were measured in the four sea-ice melted experiments, in the two treatments: before (control) and after viral attack.

4.1 Prokaryote mortality mediated by viral lysis

4.1.1 *Viral production and prokaryotic mortality*

In the four sea-ice melted experiments in the tank (control and viral attack), we have detected viral lytic production (VPL), while viral lysogenic production (VPLyso), was estimated in all control samples, and at both treatments in EXP4. The VPL, varied between 9.84 x 10^4 and 7.88 x 10^5 viruses mL^{-1} day^{-1} in the control, and between 2.16 x 10^5 and 1.94 x 10^6 viruses mL^{-1} day^{-1} after the addition of the viral concentrate (Figure 11), and VPLyso varied between 4.77 x 10^4 and 8.70 x 10^5 in the control, and reached 7.74 x10^4 in EXP4 in the viral attack treatment (Table 1).

Table 1. VPLyso values along the four experiments.

Measurements	**VPLyso** (mL^{-1} d^{-1})	**LysoBac** (mL^{-1} d^{-1})	**% LysoBac** (d^{-1})
SeaIce 1	4.77E+04	1.99E+03	1.16
SeaIce 1 VA	ns	ns	ns
SeaIce 2	8.70E+05	3.63E+04	2.45
SeaIce 2 VA	ns	ns	ns
SeaIce 3	2.02E+05	8.40E+03	0.28
SeaIce 3 VA	ns	ns	ns
SeaIce 4	7.75E+04	3.23E+03	0.23
SeaIce 4 VA	7.44E+04	3.10E+03	0.37

4.1.2 *Rate of lysed prokaryotes and released prokaryote carbon*

Once we got viral lytic production, this was divided by the burst size (viruses released for prokaryotes, in our case was 24) to obtain the rate of lysed prokaryotes (cells ml^{-1} day^{-1}), which varied from 3.95 x 10^3 to 3.28 x 10^4 in the control, and after viral attack, it increases from 8.99 x 10^3 and 1.00 x 10^5 (Figure 11).

Applying the corresponding factor of carbon content per prokaryotic cell volume (fg C $cell^{-1}$ = 108.8* $(V)^{\wedge 0.898}$), we estimated the dissolved organic carbon released from lysed

prokaryote in each experiment and treatment. The lowest values were from the control, while the highest values were found after viral attack oscillating from 37.44 ng $L^{-1}d^{-1}$ in the first experiment (EXP1) to 948.52 ng $L^{-1}d^{-1}$in the third (EXP3) after the viral attack (Figure 11). Our study suggests that there is an increase in the number of produced viruses over time thanks to the lysis of prokaryotes; but mainly after viral attack in EXP3 and EXP4 (Figure 11). The same pattern is maintained by the lysed prokaryotes and the released prokaryote carbon (Figure 11).

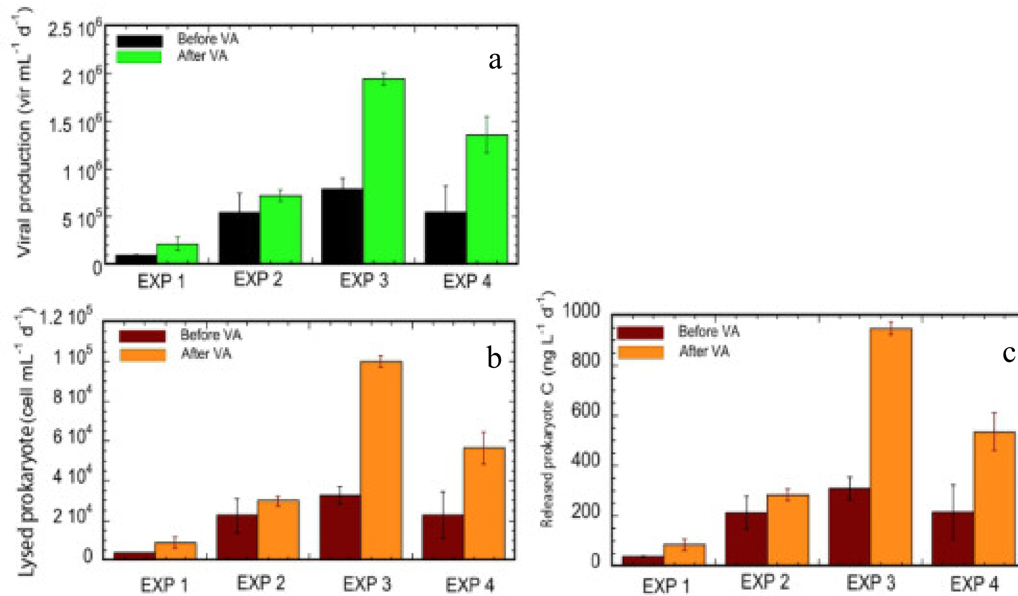

Figure 11. (a)Viral Lytic production, (b) rate of lysed prokaryotes and (c) released prokaryote carbon from lysed cells, in the four experiments, before and after viral attack.

4.2 Nanoflagellates mortality mediated by viral lysis

As for prokaryotic mortality, we have, first measured viral production due to nanoflagellates (heterotrophic and phototrophic) infection in each treatment (control and after the viral attack) of the four ice melted experiments.

4.2.1 *Viral production and nanoflagellates mortality*

Here, we only have measured viral lytic production (VPL). Lysogeny was not assayed, because it is not clear how this type of infection could be detected.

Viral lytic production (VPL) varied between 6.00 x 10^2 and 4.59 x 10^4 viruses mL^{-1} day^{-1} in the control, and between 3.98 x 10^3 and 8.74 x 10^4 viruses mL^{-1} day^{-1} after the addition of the viral concentrate (Figure 12). The highest values for VPL were obtained in EXP3 and EXP4, similarly what happen with VPL for prokaryotes.

4.2.2 *Rate of lysed nanoflagellate cells and released nanoflagellate carbon*

Dividing the VPL by the burst size (BS) which oscillates between 44 and 153 we got the rate of lysed nanoflagellates (cells mL^{-1} day^{-1}), that ranged between 6.85 and 9.88 x 10^2 cells mL^{-1} day^{-1}in the control, and in the viral attack treatment increases from 7.41 x 10^1 and 2.41 x 10^3 cells mL^{-1} day^{-1}. The highest values were registered in EXP4 (either in the control as in the viral attack treatment) (Figure 12).

Concerning to the released carbon along the four measurements, there are many differences in the rate of released C, and they follow the same trend as for the rate of lysed nanoflagellates. Values ranged from 1.78 x 10^1 ng $L^{-1}d^{-1}$ in the control of EXP2 to 8.28 x 10^3 ng $L^{-1}d^{-1}$ in EXP4 after the viral attack.

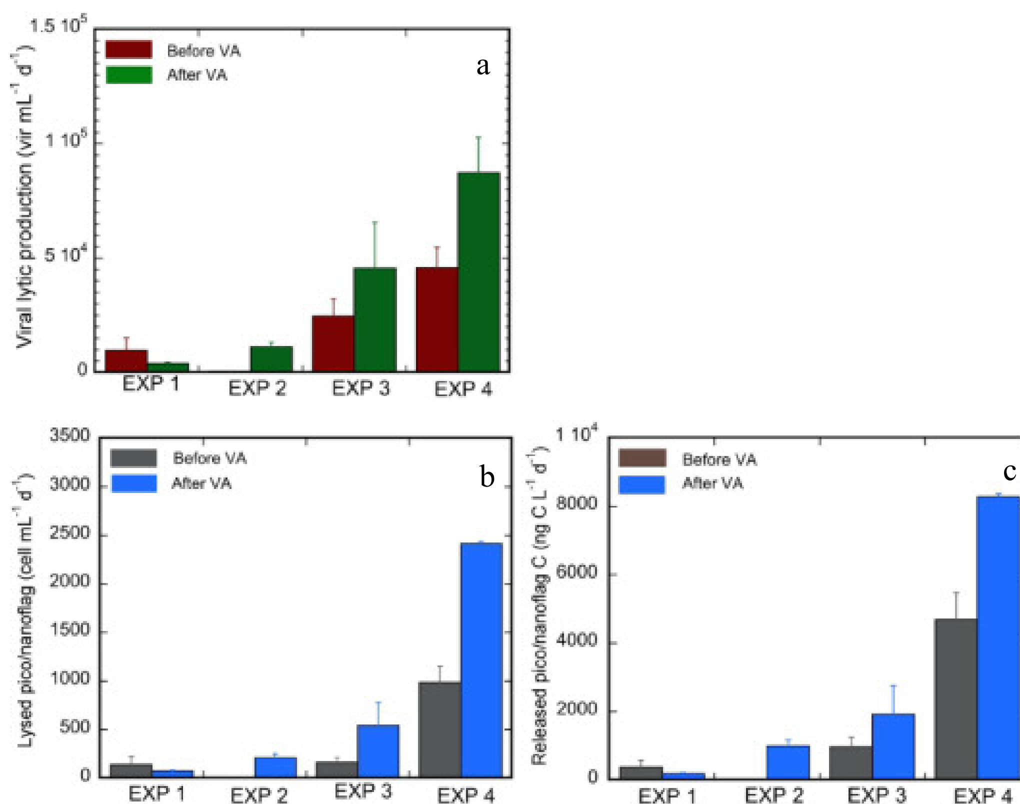

Figure 12. (a) Viral lytic production, (b) rate of lysed nanoflagellate cells and (c) rate of released dissolved organic carbon, in the four experiments, before and after viral attack.

In summary, mortality on microbial communities (prokaryotes and nanoflagellates) indicates that there is an increase in viral lytic production over time due to their infection and lysis of the hosts communities, mainly in the viral attack treatment, and this was more evident in the EXP3 and EXP4. The same pattern is maintained by the lysed microbial cells and the released carbon.

4.3 Contribution of released carbon from both prokaryotes and nanoflagellates

During mortality experiments, before and after the addition of viral concentrate, we detected the different concentrations of carbon released from prokaryotes and eukaryotes. Carbon released after viral lysis from prokaryotes and nanoflagellates contributes differently among the sea-ice melted experiments (Figure 13). Percentage of prokaryote to nanoflagellate carbon release varied from 21% to 33% in Exp3, showing that the contribution of dissolved carbon released by nanoflagellates is considerably higher than that of prokaryotes. In addition, the Exp3 and Exp4 showed the highest mortality and released of carbon, mainly after viral attack. The lowest concentration value belongs to the natural condition, 0.5×10^3 ng L^{-1} d^{-1} and the highest belongs to Exp4, in the viral attack treatment, 9.0×10^3 ng L^{-1} d^{-1}, where, 96% of the contribution is attributed to nanoflagellates (Figure 13).

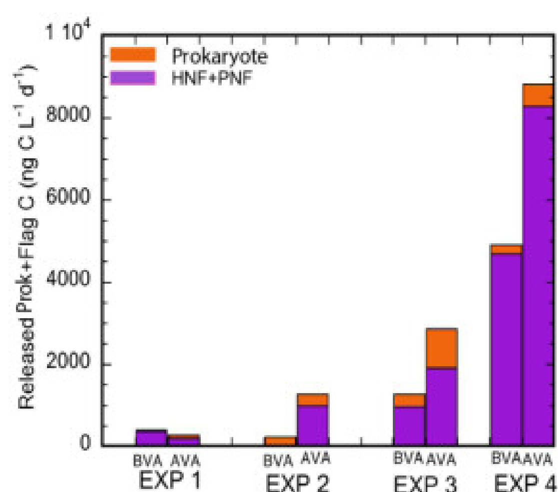

Figure 13. Contribution of released carbon due to viral lysis in the four experiments before (BVA) and after viral attack (AVA).

5 COMPLEMENTARY RESULTS FROM PI-ICE

In this section we present data collected and analysed by other colleagues. Whilst, I personally produced the data presented in section 4 (results), in this section I present briefly other data for explaining the sea ice marine aerosol chamber experiments (see methods 2.2). These data presented in this section 5 will be then discussed - along with my data of section 4 - in the final section 6 (discussion). [appendix*]

5.1 Biological parameters (complementary data)

In the tank, microbial abundances were measured in each four melted sea ice experiments, subjected to all treatments. Viral abundances of the total fractions averaged of $1.79 \times 10^6 \pm 1.01 \times 10^6$ cell ml^{-1} in EXP1, $6.43 \times 10^6 \pm 2.31 \times 10^6$ cell ml^{-1} in EXP2, in the EXP3 averaged of $1.11 \times 10^7 \pm 1.54 \times 10^6$ cell ml^{-1} and of $4.81 \times 10^6 \pm 4.89 \times 10^5$ cell ml^{-1} in EXP4 (Figure 14). The V1 and V2 fractions were the most abundant in all of them with an average of $2.86 \times 10^6 \pm 1.86 \times 10^6$ cell mL^{-1}, they correspond mostly to bacteriophages, instead, the V3 and V4 fractions correspond to nanoflagellate viruses with an average of $2.43 \times 10^5 \pm 1.95 \times 10^5$ cell mL^{-1}. There is a little increase of the V4 fraction mainly in the third experiment. In general, there is an increment of the all virus fractions after the addition of the virus concentrate, as expected (Figure 14). Abundance of prokaryote was much more variable along each experiment with the highest average value in EXP3: $2.53 \times 10^6 \pm 3.08 \times 10^5$ cell mL^{-1}. In three to four experiments they decrease in number directly after the "viral attack", which could be due to viral lysis. The heterotrophic nanoflagellates (from $1.19 \times 10^2 \pm 3.76 \times 10^1$ cell ml^{-1} in the EXP1 to $3.12 \times 10^3 \pm 9.72 \times 10^2$ cell mL^{-1} in the EXP4) were more abundant than the phototrophic ones (from $1.13 \times 10^2 \pm 1.33 \times 10^2$ cell mL^{-1} in the EXP1 to $4.40 \times 10^2 \pm 7.71 \times 10^1$ cell mL^{-1} in the EXP3). Summarizing we observed lower microbial abundances between the two first experiments (1 and 2) and the two last ones (3 and 4). Interestingly, we also detected differences between the sea ice ecosystems 3 and 4. The third one was characterized by high viral (average of $1.11 \times 10^7 \pm 1.54 \times 10^6$ cell mL^{-1}) and prokaryote (average of $2.53 \times 10^6 \pm 3.08 \times 10^5$ cell mL^{-1}) abundances and relatively low nanoflagellates abundance (average of $9.42 \times 10^2 \pm 2.88 \times 10^2$ cell mL^{-1}); instead, the fourth one showed higher nanoflagellates abundance (average of $3.40 \times 10^3 \pm 9.65 \times 10^2$ cell mL^{-1}) and the lower microbial abundances (viruses average of $4.81 \times 10^6 \pm 4.89 \times 10^5$ and prokaryote average of $1.11 \times 10^6 \pm 2.75 \times 10^5$, cell mL^{-1}) (Figure 14).

Finally, chlorophyll a concentration, reached the highest value at time cero of each experiment, after melting the sea ice and filling the tank. It followed a similar trend that the big diatoms that inhabiting the sea ice (data not shown), decreasing dramatically after 24 h bubbling. We thought that diatoms were subjected to sedimentation when they were put in the tank, due to this fact chlorophyll decays. In the first experiment chlorophyll concentration passed from 14.84 ng L^{-1} to 1.05 ng L^{-1}, the same pattern was maintained in all the measurements (Figure 14).

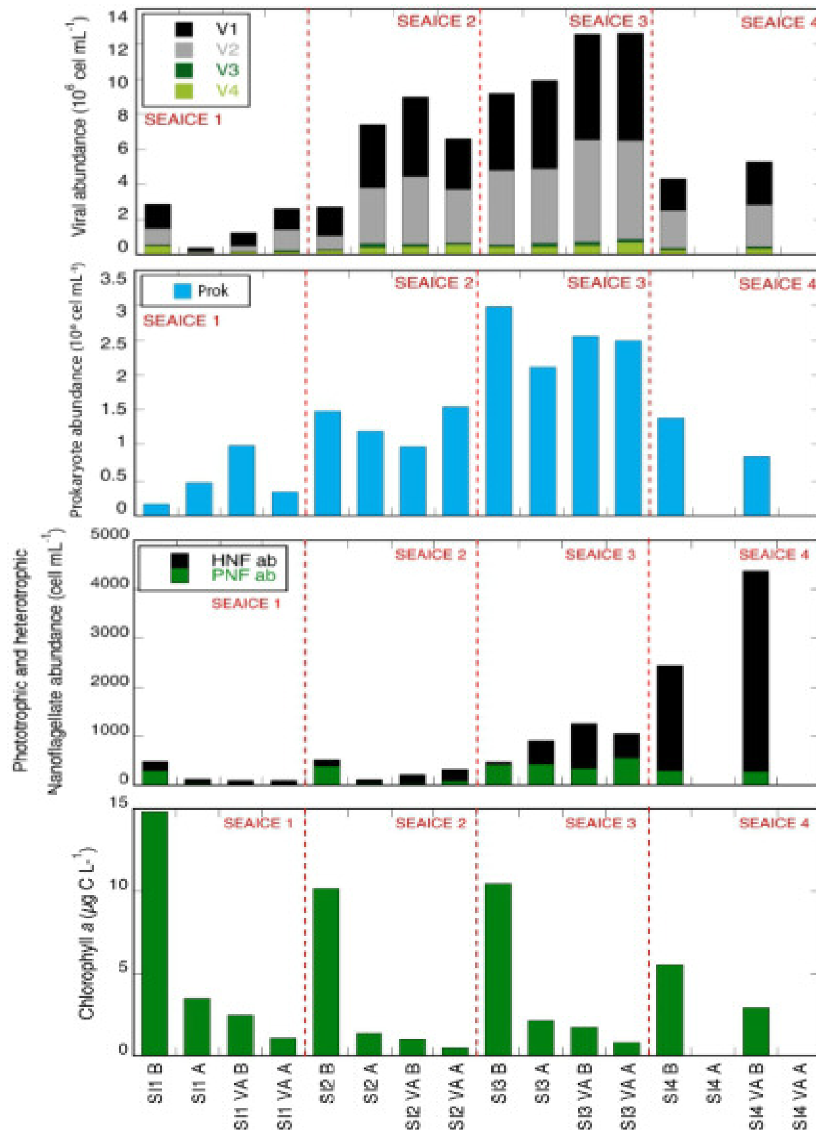

Figure 14. Viral abundance, prokaryote abundance and nanoflagellates abundance and chlorophyll-*a* concentrations along the four chamber experiments (SI B: sea ice before bubbling; SI A: after 24h of bubbling; SI VA B: after 24h after the addition of the viral attack; SI VA A: after the viral attack, after the second bubbling time).

5.2 Polar marine biogeochemistry (complementary data)

Prokaryotes and small eukaryotic cells, due to their lysis due to viral infection, among other biological and chemical processes are involved in the production of organic matter, in particular dissolved organic carbon (DOC), dimethyl sulphopropionate (DMSP) and transparent exopolysaccharides (TEP) (Figure 15 and 16). During the four experiments, the bulk of DOC and TEP follow a similar trend, their concentrations increased in the experiments after the addition of the viral concentrate. DOC concentration varies with a mean of 2.58 x 10^2 µM, in the third measurement it passes from 3.04 x 10^2 µM to 5.3 x 10^2 µM. We could appreciate the same trend with regard to TEP concentrations with an

average of 1.88 x 10^2 µg L^{-1} (Figure 16). DMSP concentration achieved always the highest value at time cero of the experiment following the chlorophyll concentration.

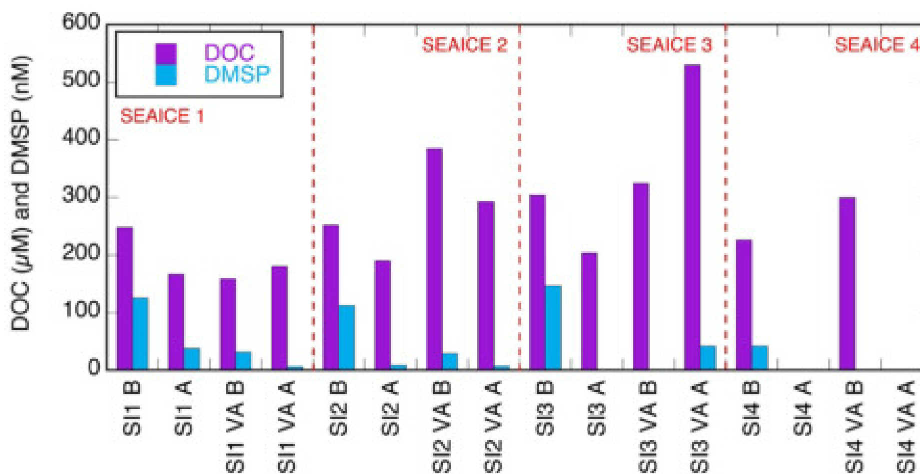

Figure 15. DOC and DMSP concentrations during the all four chamber experiments.

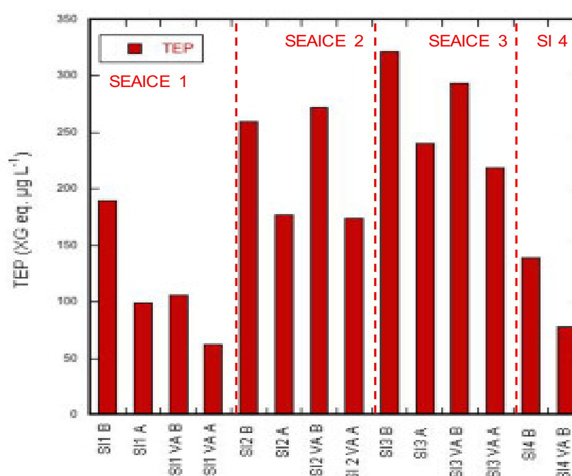

Figure 16. TEP concentrations during the all four chamber experiments.

6 DISCUSSION

In the present section we verified our hypotheses about the impact of viruses on prokaryote and nanoflagellate communities in Antarctic sea ice and providing new information on mortality rates of these microorganisms, and their possible contribution in the formation of organic compounds, which could be transformed in aerosol particles.

In a nutshell, we take the results of my thesis described in the previous section 4 and we discuss them with additional data taken from other colleagues at ICM-CSIC (section 5).

It is an important part of the multidisciplinary PI-ICE (Polar atmosphere-ice-ocean Interactions: Impact on Climate and Ecology) project. PI-ICE general objective is to directly identify the atmospheric aerosols emitted in polar regions, their biological origin

and their impact, with a particular emphasis on the biogenic cycle of nitrogen in the ice-water-atmosphere interface. The specific aims were (1) design and construction of a portable chamber to generate marine aerosols and to study the role of atmosphere-ice-ocean interactions in aerosol formation in polar areas; (2) determine and qualitatively characterise aerosol emissions in polar regions by laboratory experiments with surface waters directly affected by ice melting; (3) obtain real data from natural aerosols on a fixed platform (Juan Carlos I Antarctic Base) to enable the characterization of a wide range of primary and secondary aerosols, including newly formed particles and aerosolized biogenic toxins.

Marine viruses constitute a major ecological and evolutionary driving force in the marine ecosystems (Mann et al., 2003; Suttle, 2007). They control microbial abundance and community structure (Fuhrman, 1999). In addition, viruses exert a profound effect on food web interactions and affect global geochemical cycles (Evans et al., 2009; Lindell et al., 2005; Hurwitz et al., 2013; Béja et al., 2001). Marine viruses are agents of microbial (prokaryotes, eukaryotes) mortality that have biogeochemical and ecological significance (Fuhrman, 1999). Viral lysis converts prokaryotes and nanoflagellates to progeny viruses and particulate and dissolved organic matter, channelling matter and energy away from higher trophic levels and generating substrates for heterotrophic prokaryotic producers (Suttle, 2005). In our study all the mortality measurements, both of prokaryotes and nanoflagellates result in increments in viral production, lysed prokaryotes or nanoflagellates and released carbon. In these measurements, mortality rates of prokaryotes and eukaryotes due to viral activity achieved the maximum values in the “stressed” condition. Concerning our experimental design, we should take into account that we have no replicate of each experiment so we can’t figure out if the increment in the mortality after the addition of the viral attack really depends on the latter or maybe just because there is no bubbling phase. But we address the cause of the rise in mortality is the addition of the viral concentrate.

6.1 Comparison between mortality rates (results 4) and other chemical variables (results 5.2)

Our results clearly indicated that viral activity depends on the initial microbial community from the sea ice, which is enhanced after viral concentrate addition. Also, we expect that the rate of released dissolved organic carbon from the lysed microorganisms should be related with the concentration of different compounds produced during each experiment.

We observed that the rate of DOC released by lysed prokaryotes was significantly related ($r=0.705$, $p<0.05$) to DOC concentration (Figure 17); as well as with TEP concentration ($r=0.756$, $p<0.05$) (Figure 17). This means that increasing the concentration of released carbon, the concentrations of DOC or TEP increased too.

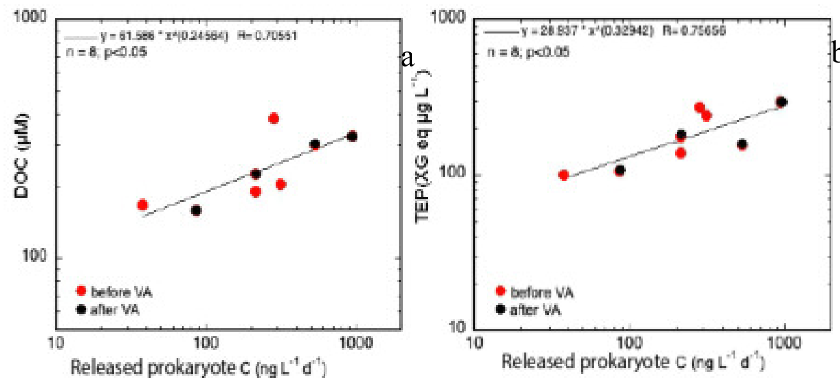

Figure 17. Positive relationships between Released prokaryote carbon and (a) DOC and (b) TEP.

Regarding DMSP concentration, although few data could be considered, it was significantly related with viral lytic production ($r=0.84$, $p<0.05$), rate of lysed nanoflagellates ($r=0.83$, $p<0.05$), and rate of released carbon ($r=0.83$, $p<0.05$) (Figure 18).

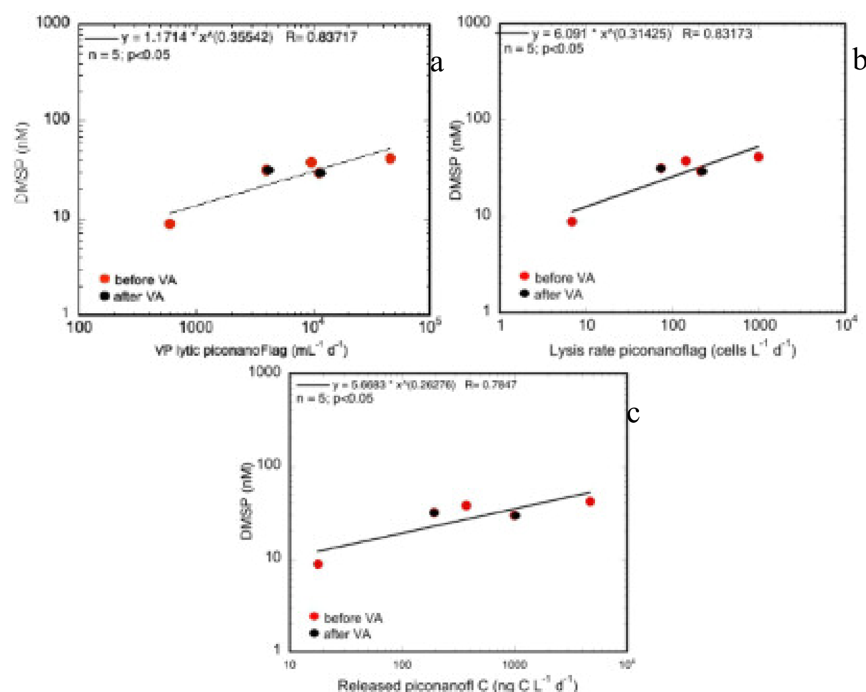

Figure 18. Positive correlations between (a) viral production, (b) lysed nanoflagellates rate and (c) released nanoflagellates carbon and DMSP concentrations.

These results suggested that viral activity contributed in the formation of these compounds (DOC, TEPS, and DMSP). It is likely that the increase of TEP is due to prokaryotes lysis,

whilst the increases of DMPS is related to nanoflagellates lysis, as briefly summarized below.

6.2 Lysis of prokaryotes

After viral infection on prokaryotes we detected lysogeny in all control experimental samples, and in both treatments in the fourth experiment. This type of viral cycle, where the virus is inserted in the host genome (prophage), was described by Paul and Jiang (1996), and was related to the trophy of the system (poor biomass and activity). This is not the case of these sea-ice melted waters with high nutrient concentration. However, lysogeny was also detected in Antarctic waters (Brum et al., 2016; Vaqué et al., 2017) the lytic production was always higher, and in Arctic waters influenced by polar melted waters (Boras et al., 2010). After viral addition in three of the fourth experiments, lysogeny was not detected anymore. Today, there is still a debate of what trigger one type or other of infection (Knowles et al., 2016), which does not only depend on the trophy of the system, but in the fitness of the hosts (Thingstad et al., 1993). Our results contribute highly to this knowledge in a system were viral activity is still poorly studied. From our results, we identified strong and positive relationships between released bacterial carbon together with DOC and TEP concentrations. Transparent gelatinous or TEP compounds are excreted by microorganisms as physical protection from radiation and drying (Wurl and Holmes, 2008); depending on their characteristics and environmental conditions, they can also rise in the water column by directly influencing aerosol formation (Azetsu-Scott and Passow, 2004). Such hypothesis could explain how microbiology is involved in aerosol formation. Prokaryotes and viruses would be implicated in the release of different types of organic compounds, part of which will contribute into the formation of cloud condensation nuclei.

6.3 Lysis of nanoflagellates

Since the early 90', marine viruses have been considered to play a possible role in the CLAW hypothesis (Charlson, Lovelock, Andreae and Warre, the four authors that launched that hypothesis, Charlson et al., 1987); this states a biological climate regulation where marine microorganisms, could contribute to the formation of aerosol particles, which could act as cloud condensation nuclei in the marine-atmospheric boundary layer. One of the most important and studied component responsible in that process is the dimethylsulphide (DMS, Malin et al., 1992, 1994, 1998; Wilson et al., 1998; Hill et al., 1998; Liss et al., 1993; Bratbak et al., 1995), which comes from the dimethylsulfoniopropionate (DMSP; Vairavamurthy et al., 1985) and is produced by

different groups of phytoplankton as haptophytes, cryptomonads, small dinoflagellates (Turner et al., 1988; Keller et al., 1989; Kettle et al., 1999). The DMSP, after the effect of enzymatic activity, will be transformed in DMS, that with the sea spray will pass to the atmosphere as DMS gas. With the lysed rates measurements, we found a positive and strong relationship (Figure 18) between released nanoflagellates carbon and DMSP concentration, supporting literature findings. Part of these nanoflagellates were phototrophic, mainly phaeocystis (haptophytes). So, our results agree with the findings of other authors.

6.4 Aerosol generation

As we described earlier, viruses play an important role in both global and small-scale biogeochemical cycling, influencing community structure and phytoplankton bloom termination (Suttle et al., 2005; 2007). Given that the vast majority of the biomass in oceans comprises microorganisms, it is expected that viruses and other prokaryotic and eukaryotic microbes will play important roles in biogeochemical processes. Viral infections in marine plankton can be lysogenic (viral genomes integrated into the genomes of their hosts) or lytic (causing host cell bursting), frequently followed by host death. Thus, lytic infections represent an important source of mortality of marine plankton.
Figure 19 summarizes some of the data described in Section 4 and 5, including: Chl-a, heterotrophic (HNF) and phototrophs (PNF) nanoflagellates, Bacteria and Virus abundances, POC and DOC, contribution of released carbon due to viral lysis in the four experiments before (BVA) and after viral attack (AVA), and aerosol particles produced from the marine aerosol chamber (Figure 6, aerosol data were measured by means of Scanning Mobility Particle Sizer, particle size range 8-600nm, further details are beyond the scope of this master).

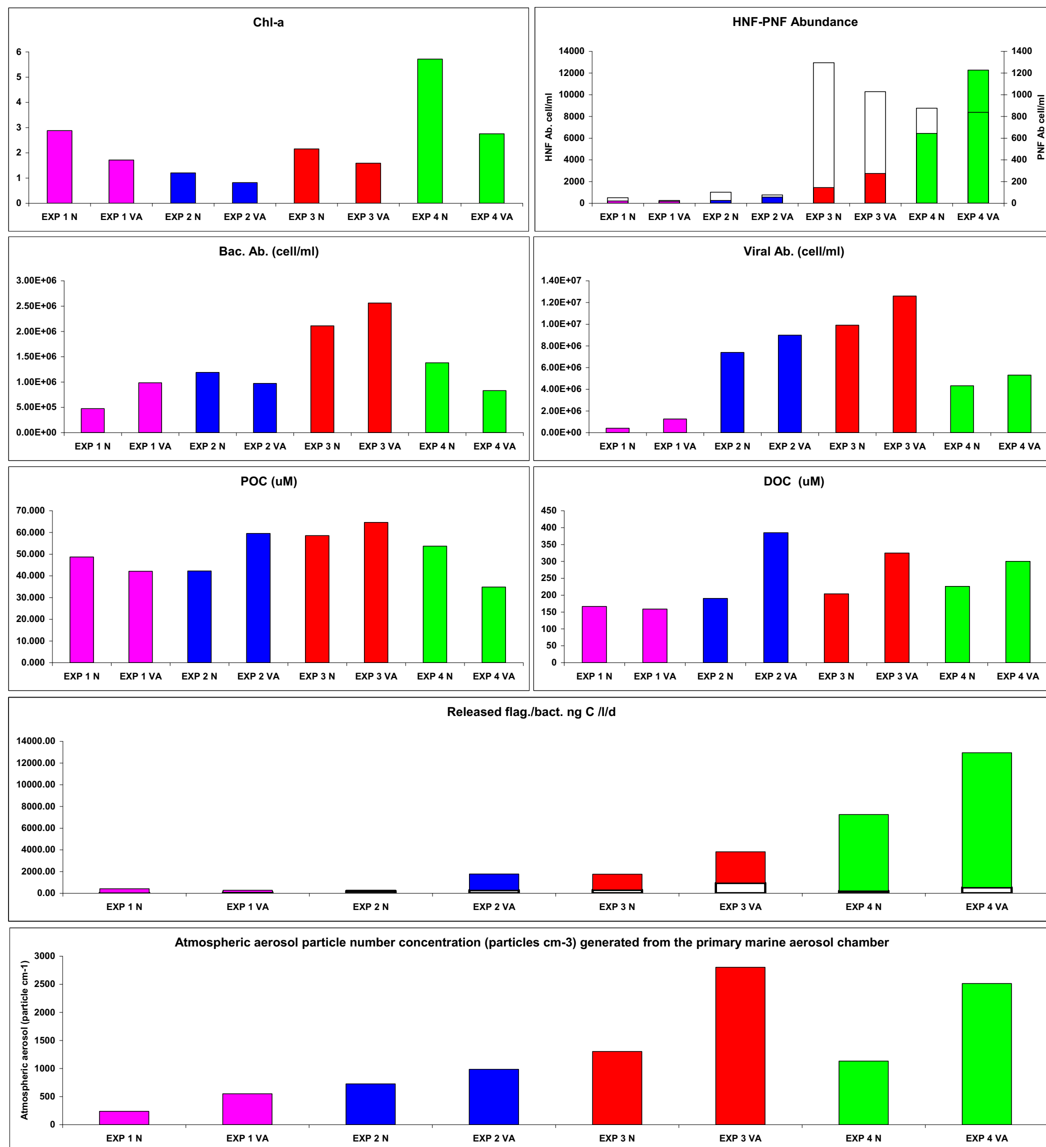

Figure 19. Particle number concentrations during the four sea ice experiments before (N Normal) and after viral attack (after Viral Attack, VA).

Briefly, four different scenarios can be summarised in Figure 19:

- EXP1 and EXP2 are characterized by low values of Chl-a and low values of HNF-PNF abundances - also the released carbon from the viral attack seem moderate. Aerosols coming out from the aerosol chamber by bubbling the EXP1 and EXP2 waters also do not seem high, and generally the picture is not clear - suggesting these two experiments of low mortality values are reflected in low aerosol production
- EXP3 and EXP4 have a large number of HNF and PNF abundances. Whilst EXP3 also have a large number of bacteria, EXP4 is more characterised by high PHF abundances and low bacteria ones. The mortality results of EXP3 and EXP4 are

quite high, reflected in high aerosol production coming out from the chamber. Whilst EXP3 has some contribution from released fresh Carbon from Bacteria (prokaryotes), most of the EXP4 is linked with lysis from nanoflagellates. It is interesting to note how nanoflagellates increases in number, also at the expenses of bacteria concentration in EXP4.

7 CONCLUSIONS

Viruses are very abundant in the sea ice and have an important impact on daily bacterial and phytoplankton infection and mortality rates. They have great influence in many biogeochemical cycles, including nutrient cycling, particle-size distribution and sinking rates as well as being responsible for bacterial and algal biodiversity and species distributions. What is clear is that after viral infection, phytoplankton (nanoflagellates) and bacterial (prokaryotes) cells release organic compounds to the water column. DOM, during waves movements, can pass from sea surface to the atmosphere as aerosol particles. During the last years, the aerosol is known as a source of clouds and have an outsized effect on the planet's climate but with big uncertainty of its role towards climate change.

Here, we aimed to address how viruses interact and affect the populations of prokaryotes (bacteria) and small protists (heterotrophic and phototrophic nanoflagellates) over time and whether viral lysis relate with the concentration of organic compounds from Antarctic ice to the water column and atmospheric aerosol production. We performed different chamber experiments (before and after the addition of viral concentrate) with melted sea ice in order to study mortality of nanoflagellates and prokaryotes due to viral infection. In addition, we did other experiments (4 times, 2 bubbling phases and the VA) with a marine controlled chamber to study the link between released organic matter and aerosol particles formation.

Viral mortality rates on prokaryotes and eukaryotes in the four melted sea-ice experiments showed significant relationships with DOC, TEP and DMSP compounds, suggesting that microbial processes could contribute in the production of these metabolites. In addition, the production of aerosol particles from the tank follows the same trend than the rates of lysed cells. Then, we could argue that these substances could pass to the atmosphere and promote the formation of condensation clouds nuclei.

Specifically, we observed that:

- Viral abundance in each experiment was always higher after viral attack than before and reached the highest value in Experiment 3.
- Viral production appeared in all experiments and before and after viral attack, while lysogeny only was detectable in the control samples.
- Rate of lysed prokaryotes and the organic carbon released from these lysed cells achieved higher values after viral attack, and the highest values were achieved in Experiments 3 and 4.
- Rate of lysed eukaryotes and the organic carbon released from these eukaryotic cells also reaches higher values after viral attack, and the highest values were observed in the Experiment 4.
- The contribution of released carbon from all microorganisms was higher for eukaryotic cells.
- Finally, the similar trend of the viral mortality rates together with the abundances of particles found in the collector of the tank during the four experiments, indicate that these microbial processes are participating in the aerosol formation. The highest particle number concentrations belong to the Experiments 3 and 4.

All these findings should motivate future studies to focus on identifying individual molecules and microbes that contribute to marine aerosol types, the mechanisms that govern their biochemical production during phytoplankton blooms, and the transfer of these molecules and microbes into sea spray aerosol.

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This research is part of Polar CSIC Activities

***In a nutshell, the results of this thesis can be summarised as follow**

(1) **Lab work developed in the study by the candidate**

Samples process

a-Prokaryote mortality measurements

Counts of viruses and prokaryotes by flowcytometry:

Viral production: 192 sub-samples of viral abundance

Rate of lysed prokaryotes and burst size: 192 sub-samples of prokaryote abundance

b-Protist mortality measurements

Counts of viruses by flowcytometry and protists (heterotrophic and phototrophic) by epifluorescence microscopy:

Viral production: 72 sub-samples of viral abundance

Rate of lysed protists and BS: 72 subsamples for heterotrophic and 72 for phototrophic nanoflagellates

(2) **Data analyses**

Calculation of viral production (lytic and lysogenic), rate of lysed cells, and use of cell/carbon conversion factors from the literature

(3) **Elaboration of the Master thesis**

1- Recompilation of the literature of the topic to understand the objective of the study and test the hypothesis derived from the plausible contribution of viral activity in the aerosol formation

2- Understand the experimental approach

3- Use of the obtained results and the complementary data (provided by other members of the PI-ICE team) to elaborate the final Master document.