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# Sea ice melting and implications on virus-host interactions and microbial community diversity in Antarctic marine ecosystems

# Fusione del ghiaccio e implicazioni su interazioni virus-ospite e diversità delle comunità microbiche negli ecosistemi marini antartici

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**Abstract**

L'Antartide è caratterizzato dalla più vasta estensione di ghiaccio marino al mondo con temperature sottozero e condizioni di vita estreme. All'interno del ghiaccio marino prosperano comunità microbiche caratterizzate da procarioti, protisti, fitoplancton e virus, in particolare nei canali che si creano nell'interfaccia ghiaccio-acqua.

L'aumento delle temperature registrate negli ultimi decenni sta accelerando sempre di più la fusione dei ghiacci, inclusi quelli Antartici, con enormi implicazioni sul funzionamento degli ecosistemi.

Tra le varie implicazioni della fusione del ghiaccio antartico, il rilascio delle diverse componenti biologiche, inclusi virus e procarioti simpagici, nell'acqua sottostante, potrebbe determinare effetti molteplici su biodiversità e funzioni degli ecosistemi marini.

Il nostro studio ha lo scopo di valutare se la fusione dei ghiacci antartici nel Mare di Ross possa alterare le interazioni virus-ospite e la composizione delle comunità microbiche nel ghiaccio e nell'acqua di mare con possibili conseguenze sulla biodiversità e il funzionamento dell'ecosistema marino antartico.

Per raggiungere questi obiettivi sono stati effettuati degli esperimenti simulando un aumento di temperatura di +2.5°C come previsto dall'IPCC per il 2100 e lo scioglimento del ghiaccio nell'acqua di mare. Sono stati valutati l'abbondanza virale e procariotica, la produzione virale, i tassi di infezione virale e la composizione tassonomica delle comunità procariotiche prima e dopo lo scioglimento dei ghiacci antartici.

I risultati indicano che le comunità microbiche e virali sono più abbondanti nel ghiaccio marino rispetto alle acque circostanti e che l'aumento della temperatura determina una diminuzione della produzione virale e dei tassi di infezione virale, in particolare nel ghiaccio (fino a 4 volte).

Inoltre, abbiamo stimato che circa la metà delle abbondanze virali e procariotiche presenti nel ghiaccio viene rilasciata nell'acqua di mare dopo la fusione e che mentre una frazione di virus simpagici di dimensioni < 0.2 μm continua ad infettare attivamente i procarioti planctonici, i virus "giganti" del ghiaccio (di dimensioni > 0.2 μm, presumibilmente virus di alghe simpagiche), non trovando ospiti specifici o condizioni ideali in acqua di mare, perdono la loro capacità infettiva.

In aggiunta, i nostri risultati mostrano che l'aumento di temperatura determina uno shift nella composizione della comunità microbica sia nell'acqua sia nel ghiaccio e che lo scioglimento dei ghiacci e il rilascio di virus simpagici hanno effetti rilevanti sulle comunità microbiche planctoniche.

In particolare, i dati di metabarcoding rivelano una diminuzione dell'abbondanza relativa per alcuni taxa, tra cui Polaribacter e Clade-Ia, guidata da virus e temperatura. Questi taxa giocano un ruolo chiave nella decomposizione della biomassa algale, per cui uno shift nella loro abbondanza relativa potrebbe avere un impatto sui cicli di remineralizzazione della materia organica negli ecosistemi antartici.

Complessivamente, considerando i risultati osservati, possiamo affermare lo scioglimento del ghiaccio marino antartico, dovuto all' aumento delle temperature, esercita un'influenza significativa sulle interazioni virus-procarioti in colonna d'acqua e sulla diversità delle comunità procariotiche. Prevedere gli effetti del riscaldamento globale su biodiversità e funzionamento degli ecosistemi polari è essenziale per identificare piani di gestione adattativa e definire decisioni informate per la mitigazione di tali effetti.

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# INTRODUCTION

*Antarctica and its sympagic community*

The study of undisturbed habitats defined as "pristine areas" is essential to create a reference frame of natural habitats without human intervention (Levin & Dayton, 2009). Far from any anthropogenic impact, Antarctica can be considered our last true wilderness serving as a unique natural laboratory for fundamental research (A. Clarke et al., 2007).

The region is dominated by the Antarctic Ice Sheet, the largest expanse of sea ice on Earth. The ice sheet extends beyond the continent during wintertime covering up to 20 x $10^6$ $km^2$ and recedes to less than 4 x $10^6$ $km^2$ extent in austral summer (December-March, Eicken, 1992a). As reported by Zwally (1983), Antarctica is characterized by strong seasonality (austral summer and winter) where the extent of Southern Ocean Sea ice follows a robust seasonal cycle of growth and decay, expanding fivefold in area from summer to winter (June-September). It reaches a minimum of 4 x $10^6$ $km^2$ during austral summer, in February, then rapidly expands equatorward at a maximum rate of 4.2 x $10^6$ $km^2$ per month from May to June, with an average rate of 2.4 x $10^6$ $km^2$ per month. The maximum extent of 20 x $10^6$ $km^2$ is reached during austral winter, in September. Subsequently, the ice edge retreats slightly from September to October, followed by a rapid retreat of 4.6 x $10^6$ $km^2$ per month from October to December. The peak retreat rate of 6.9 x $10^6$ $km^2$ per month occurs from November to December, with an average retreat rate of 3.3 x $10^6$ $km^2$ per month between September and February. The total area of the Antarctic Continent is 14 x $10^6$ $km^2$, resulting in an approximate total area of snow and ice cover in Antarctica of around 34 x $10^6$ $km^2$ at the winter maximum.

Antarctic Sea ice is a harsh environment for life (Rocchi et al., 2022a). It exhibits temperatures below zero (around -1.8°C), limited liquid water, and is characterized by the presence of brine channels with salinity exceeding 200 ‰ (Eicken et al., 2000). Brine channels form within sea ice through a process driven by the expulsion of salt (Krembs et al., 2000). Indeed, as sea ice forms, salt is expelled from the ice crystals creating a pathway for the brine to move. This process results in the formation of an intricate network of channels that run through the ice (Paterson & Laybourn-Parry, 2012). Here, microbial life including prokaryotes (bacteria and archaea), protists (e.g., nanoflagellates), phytoplankton (microalgae), and viruses can proliferate (Arrigo et al., 2010; Deming & Eric Collins, 2017; Mock & Thomas, 2005a). These organisms form the sympagic community (Brierley & Thomas, 2002) and thrive in the portion of sea ice in direct contact with underlying seawater. This peculiar sea ice layer is called *brown ice* and is the most biologically productive habitat in Antarctic Sea ice. Here, as seawater flows through the ice channels, it transports suspended or dissolved particles, nutrients, and other substances supporting the growth of sympagic algal, prokaryotic, and microeukaryotic communities within the ice channels (Krembs et al., 2000).

The sympagic algal communities can contribute up to 50% of the total primary production during the beginning of austral summer (September-November) when algal biomass accumulates in thick brown layers at the sea ice-water interface (Arrigo & Thomas, 2004a; Mangoni et al., 2009; Saggiomo et al., 2017).

The oceans surrounding Antarctica also provide an important physical component of the Antarctic region. The waters surrounding Antarctica are deep, reaching depths of 4,000 to 5,000 meters, and are a key component of the "ocean conveyor belt," a global system in which water circulates the globe based on density and currents (Convey et al., 2014). Water

masses around and below Antarctic Sea ice are among the most inhospitable habitats for life, especially during the winter months. Moreover, the high salinity hampers nutrient exchange with the underlying water column (Golden et al., 1998, 2007). However, as spring arrives, the situation shifts: rising temperatures and melting ice led to decreased salinity, allowing for water column mixing and the upwelling of nutrients from deeper waters. This, coupled with increased sunlight, results in heightened algal and microbial biomass in the water column, peaking in early summer (Garrison et al., 2003; Mundy et al., 2011).

The interaction between sea ice and the seawater around and below the ice creates a crucial habitat whose conditions significantly influence the Antarctica prokaryotic community (Eicken, 1992b; Rivkin & Putt, 1987; Spindler et al., 1990). Indeed, in these peculiar environments, thrive highly diversified and metabolically active prokaryotic communities (Bluhm et al., n.d.; J. Bowman, 2013; J. S. Bowman et al., 2012; Martin et al., 2012; Mock & Thomas, 2005b) which are involved in the biogeochemical cycles of elements playing a key role in the re-mineralization of nutrients (Maranger et al., 1994a; Yager et al., 2001) and in the transfer of the organic matter to higher trophic levels (Deming & Eric Collins, 2017). The number and the taxonomic composition of prokaryotic communities living in the sea ice and in the seawater around and below the ice can be influenced by environmental factors (temperature, availability of trophic resources, and exposure to sunlight) as well as by the interactions with other organisms, including grazers, algae, and viruses (Deming & Eric Collins, 2017; Thomas & Dieckmann, 2002a). The prokaryotic assemblages in Antarctica are dominated by members of the Proteobacteria and Bacteroidota phylum (Mock & Thomas, 2005b). Among the Proteobacteria, the Colwelliaceae family has been extensively found along the Dotson ice shelf, between the Martin Peninsula and the Bear Peninsula, and along the Antarctic coasts (Fuentes et al., 2019), while members of the families

Pseudomonadaceae and Rhodobacteraceae are often associated with algal blooms in Antarctica surface waters (Castro-Sowinski, 2019; Fuentes et al., 2019; Wagner-Döbler & Biebl, 2006). Belonging to the Bacteroidota phylum, the Flavobacteraceae were observed across various subregions of the Antarctic Ocean characterized by high phytoplanktonic production with a potential role in the remineralization of algal biomass (Abell & Bowman, 2005; J. P. Bowman et al., 2000; Moore et al., 2001). Moreover, different adaptation strategies to periods between melting and refreezing of ice have been observed in some taxa. For example, members of the family Octadecabacter and Polaromonas have been found capable of maintaining the bacterial cells at the surface probably to increase access to light and nutrients suggesting an evolutionary response to the seasonal changes in sea ice (Boetius et al., 2015a; Deming & Eric Collins, 2017). These adaptations and life strategies likely help prokaryotes to survive and remain active despite the strong seasonality that characterizes Antarctica.

### *Virus-host interactions in Antarctic Sea ice*

Marine viruses are the main agents of prokaryotic mortality (Suttle, 2007). Bacteriophage proliferation occurs predominantly through lytic or lysogenic infection. Lytic infection results in the release of viral progeny into the surrounding environment following the lysis of host cells, along with cellular debrides (Weinbauer, 2004a). As dissolved organic matter, the cellular material released by viral lyses can be a source of labile carbon for the microbial community (Danovaro et al., 2008). Temperate viruses, on the other side, can “choose” the lysogenic cycle. Temperate viruses are able to integrate their viral DNA into the host's genome and replicate alongside it, without inducing immediate cell lysis (Weinbauer, 2004a).

As main agents of microbial mortality, viruses can play a key role in shaping microbial diversity. A well-known density-dependent hypothesis, the *Kill-the-Winner* (KtW) hypothesis, states that the most abundant microbial taxa are more likely to be infected and lysed by viruses (Breitbart et al., 2018; Suttle, 2007). As suggested by the KtW theory, a high-microbe-density state is coupled with a high viral density, a high virus-to-microbe ratio (VMR) and enhanced lytic infections that kill the numerically dominant taxa. This mechanism can keep in check competitive dominants promoting the co-existence of less competitive taxa, thus enhancing microbial community biodiversity (Breitbart et al., 2018). Hence, marine viruses have a substantial influence on mortality, dynamics, diversity, and element transfer through trophic levels (Chen et al., 2021). Moreover, another process occurring in marine ecosystems is the microbial loop, a trophic pathway for carbon transfer to higher trophic levels through the consumption of dissolved organic matter by prokaryotes, which are then grazed by protists (Evans et al., 2021).

The abundance of viruses and microorganisms in Antarctic sea ice was around 3 times higher than in the surrounding surface seawater ($4.5 \pm 1.9 \times 10^6$ and $1.7 \times 10^6$ viruses $mL^{-1}$, $1.5 \pm 0.7 \times 10^6$ and $4 \times 10^5$ cells $mL^{-1}$ for viruses and prokaryotes, respectively; Rocchi et al., 2022) with the significant presence of large viruses in the ice (>110–424 nm capsid diameter; Gowing et al., 2002, 2003). In sea ice, faster microbial metabolism and higher levels of microbial biomass promoted viral proliferation (Mock & Thomas, 2005b; Gowing et al., 2002, 2003) with values ranging from $1.5x10^3$ and $8.5\ x10^3$ viruses $ml^{-1}h^{-1}$ (Paterson & Laybourn-Parry, 2012).

In sea ice, microbes with high metabolic activity have been observed (Brinkmeyer et al., 2003; Cowie et al., 2014), likely thriving on dissolved and particulate organic substances released by microalgae, such as transparent exopolymers (Riedel et al., 2006). Here,

sympagic viruses can proliferate by infecting their sympagic (prokaryotic or eukaryotic) hosts (Zhong et al., 2020). Previous evidence showed that viral activity induces lysis on prokaryotes even at temperatures below −20 °C in brines (Deming, 2009) and on protists as well (Gowing, 2003b), resulting in the release of dissolved organic carbon in sea ice environments (Boetius et al., 2015b).

As summer arrives and light duration and intensity increase, photosynthetic activity is favored, leading to the growth and accumulation of phytoplankton and other microbial components (Legendre et al., 1992) on the submerged side of the 10–20-cm-thick sea ice layer (Rocchi et al., 2022b). Early summer marks the beginning of sea ice melting, releasing the organic matter previously trapped in ice channels and the sympagic microorganisms into the seawater. An increase in organic matter and nutrient concentration in the water column leads to a surge in phytoplankton growth and grazing activity on new photosynthetic biomass and on sympagic microorganisms (prokaryotes, microalgae, and protists) which become new food sources for krill and pelagic organisms (Arrigo & Thomas, 2004a).

The lithic interaction between viruses and prokaryotes (and protists) in seawater has been extensively studied in the past (Fuhrman, 1999; Weinbauer, 2004a). After viral lyses, the release of organic material into the water column provides an important contribution to uninfected prokaryotic metabolism promoting higher prokaryotic biomass (Danovaro et al., 2011a). This process can divert the flow of organic carbon and nutrients from microbes to higher trophic levels (the so-called "*viral shunt*", Suttle et al., 2007, Chen et al., 2021).

Information on virus-induced host mortality (including prokaryotes and protists) and their role in shaping host communities in sea ice and at the interface between seawater and sea ice (Gowing, 2003b; Gowing et al., 2002) is still scarce. Temporal and spatial investigations in Antarctic sea-ice are needed to better understand the role of viruses in these extreme systems.

*Global warming and its impact on Antarctic ecosystems*

In the Fifth Report (2013), the Intergovernmental Panel on Climate Change (IPCC) stated: "*It was found the human activity's influence on the rise in atmospheric and ocean temperatures, a change in the global hydrological cycle, a decrease in snow and of ice, on the rise of the average global sea level, and on some extreme climatic phenomena* […]. *It is extremely probable that human influence has been the main cause of the warming observed since the mid-20th century*" (Evseeva et al., 2021).

Rising temperatures and changing ocean currents have resulted in accelerated ice melting, which has important implications for the carbon cycle and the stability of Antarctic ice shelves (Adusumilli et al., 2020; Hellmer et al., 2017; Rignot et al., 2013).

An average temperature increase of >0.05°C per decade has occurred across Antarctica since 1957, although not uniformly (Steig et al., 2009). While West Antarctica warmed by >0.1 °C per decade from the 1950s to the 2000s, the colder and more stable East Antarctica had been experiencing cooling until the 2000s (Clem et al., 2020).

The Southern Ocean around Antarctica has absorbed more heat than any other ocean (Bourgeois et al., 2022a), with particularly strong warming at depths below 2,000 m (Garner et al., 2022) and around the West Antarctic, which has warmed by 1 °C since 1955 (Discovering Antarctica, 2022, URL: https://discoveringantarctica.org.uk/climate-change/impacts-of-climate-change/). This is due to the presence of the wind-driven Antarctic Circumpolar Current that creates a residual overturning circulation, consisting of an upwelling of circumpolar deep waters around the Polar Front, a residual northward transport, and eventual subduction under subtropical waters (Bourgeois et al., 2022b). In particular, the upwelled water mass is cold and undersaturated with respect to anthropogenic-derived $CO_2$,

enabling it to effectively absorb significant quantities of atmospheric excess heat and anthropogenic carbon (Tjiputra et al., 2010).

According to Bourgeois (et al., 2022b), from 1850 to 2005, the area between 30°S and 55°S accounted for 27% of the worldwide uptake of anthropogenic carbon and 50% of the ocean's excess heat, despite comprising just 21% of the total global ocean area.

In particular, over the last 50 years, there has been a significant decrease in sea-ice distributions and extent in the Bellingshausen-Amundsen sector, while there has been a net increase in the Ross Sea sector (Comiso et al., 2011). These findings suggest that the duration of ice-free days on the Ross Sea continental shelf has decreased by more than 2 months over the past three decades (Stammerjohn et al., 2012).

Nonetheless, projections for future regional air temperature changes indicate a substantial warming trend in the Ross Sea sector over the next century (Bracegirdle & Stephenson, 2012). In fact, since the recorded peak in September 2007, there has been an overall, but uneven, reduction in sea ice coverage, leading to a record low in February 2017, followed by some recovery the next year (Parkinson, 2019).

Large amounts of meltwater entering the ocean reduces the salinity (Heinrich, 1988), and release organic carbon (Krembs et al., 2002) and microorganisms in the water column (Maranger et al., 1994b; Thomas & Dieckmann, 2002b).

This process can have direct or indirect impacts on the prokaryotic metabolism.

An increase in temperature and organic matter might stimulate prokaryotic metabolism leading to an increase in cellular respiration with the release of $CO_2$ (Wadham et al., 2019). Indirect impacts can be seen in the fertilization of downstream ecosystems, either through the release of nutrient-rich glacial meltwaters or through subglacial meltwater-induced upwelling of nutrient-rich marine water at tidewater glacier margins (Cape et al., 2019).

Glacially induced Ocean fertilization might lead to significant $CO_2$ drawdown by phytoplankton, thereby enhancing the biological pump (Death et al., 2014).

As viral replication and life cycle are connected to host metabolism (Zhang et al., 2020), temperature rises are expected to impact the dynamics between viruses and their hosts (Mojica & Brussaard, 2014). For example, as the growth rate of prokaryotes accelerates, the duration of the lytic cycle shortens and the burst size increases, potentially leading to higher levels of virus production (Hadas et al., 1997; Proctor et al., 1993). Moreover, higher temperatures are likely to enhance the rate of contact between viruses and hosts (Murray & Jackson, 1992), potentially leading to increased infection rates. Danovaro et al., 2011 reported that in cold water systems, higher rates of viral production were associated with warmer temperatures.

Together with organic matter and microorganisms, sympagic viruses can be released into the seawater in order to understand the impact and the potential infection rates of sympagic viruses once they are released into the water column, it is essential to monitor their abundance and virus-host dynamics in the sea ice, polar waters, and at the interface between sea ice and seawater (Ibarbalz et al., 2019; Lopez-Simon et al., 2023).

May these microorganisms released by Antarctic ice affect the dynamic and the interaction of the microbial community in the water column?

To shed light on this unanswered question, we investigated sea ice collected from the Ross Sea Ice Shelf, seawater samples from the Ross Sea, and samples at the ice-water interface to quantify and discriminate the impact of viruses on prokaryotes, microalgae, and protozoa. Moreover, to improve our understanding of virus-host dynamics in the investigated systems (sea ice, seawater, and ice-water interface) in a future climate-changing scenario, we

conducted a time-course experiment simulating an increase of temperature of +3°C according to RCP 4.5 (Representative Concentration Pathway 4.5) emission scenario.

# OBJECTIVES

The main objective of this thesis is to assess whether sea ice melting due to global warming can influence virus-host interactions in Antarctic seawater and microbial assemblage composition with potential implications on Antarctic biodiversity and food web functioning. In particular, through a time-course experiment conducted on board the Laura Bassi oceanographic vessel in two different sites of the Ross Sea, we simulated the melting of sea ice in a future scenario of temperature increase ($\Delta T + 2.5°C$) as predicted by IPPC for 2100, to assess:

- Viral and prokaryotic abundance, viral production, and infection rates before ice melting in seawater and sea ice, and after ice melting in seawater.
- Taxonomic composition of prokaryotic communities before ice melting in seawater and sea ice, and after ice melting in seawater.

# MATERIALS AND METHODS

## *Study area and sample collection*

Samples were collected in the western part of the Ross Sea (Antarctica, 72-74°S; 174-175°E) during austral summer 2022, between January and February, on board the research vessel Laura Bassi in the framework of the "GIAnt Viruses in Antarctic Ecosystems" (GIAVA) project.

In the Ross Sea (Fig.1), the bathymetry and topography, including the north-northwest oriented deep and narrow basins (such as Glomar-Challenger Basin, JOIDES Basin, and Drygalski Basin), as well as shallow banks (such as Ross Bank, Pennell Bank, and Crary Bank), were formed by the advancing ice sheet during the glacial periods (Ha et al., 2023).

Seawater samples were collected in the northern part of the JOIDES Basin (74°01.8940'S 175°06.4461'E), an NE–SW oriented basin separated into two parts by a sill (Melis & Salvi, 2009). Sea ice samples were collected in the northern part of the Drygalski Basin (72°20.1162' S 172°43.1526' E) located in the western Ross Sea which also includes the deepest area on the Ross Sea continental shelf (Ha et al., 2023). It can reach depths exceeding 1000 meters and contains water near the freezing point with a salinity greater than 34.8 (Gordon et al., 2004).

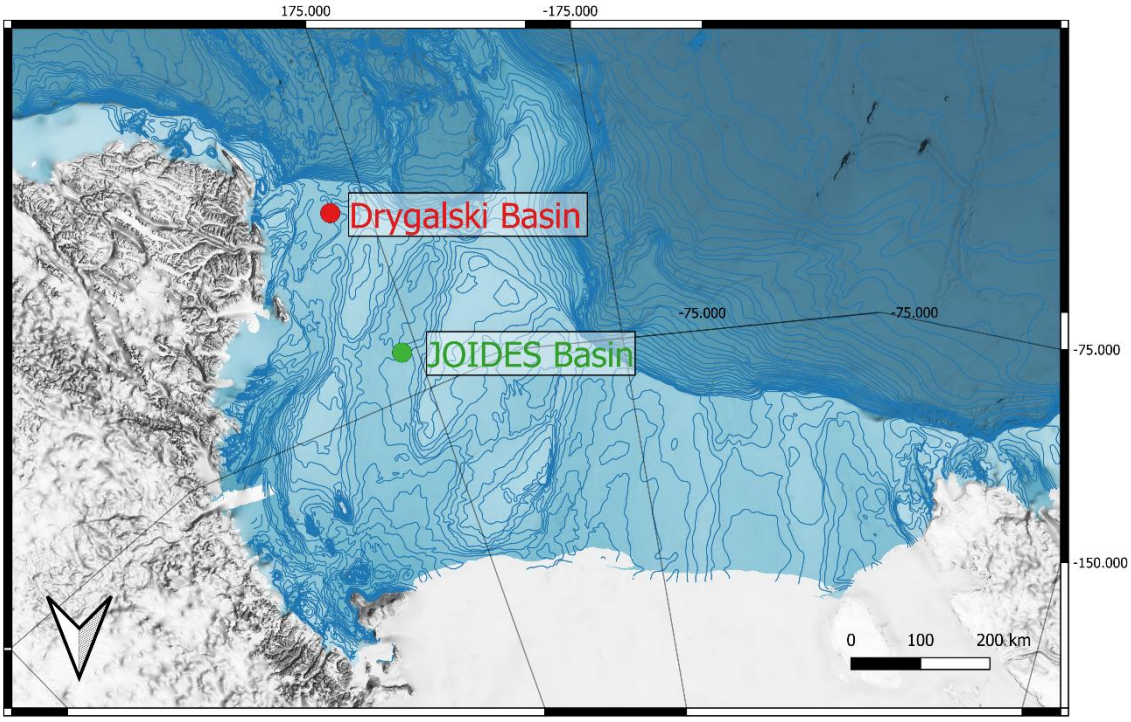


*Figure 1. Sampling area in the Ross Sea. Seawater samples were collected in the northern part of the JOIDES basin (74°01.8940'S 175°06.4461'E; in green); sea ice samples were collected in the northern part of the Drygalski Basin (72°20.1162'S 172°43.1526'E, in red).*

### *Experimental design*

To study the impact of global warming on virus-host dynamics occurring in sea ice, seawater, and at the interface between sea ice and seawater, we conducted a time-course experiment in seawater samples simulating sea ice melting with an increase in temperature of +3°C.

In detail, the experimental setup consisted of 5 treatments:

- Treatment A: 500g of sea ice;
- Treatment B: 250 ml of seawater and 250 ml of virus-free seawater (ratio 1:1);

- Treatment C: 250 ml of seawater and 250 g of ice;
- Treatment D: 250 ml of seawater and 250 ml of ice melted and filtered at 0.2 μm;
- Treatment E: 250 ml of seawater and 250 ml of ice melted and filtered at 0.02 μm.

For each treatment, three independent replicates were incubated at 2.4°C considering an increase in temperature of +2.5°C starting from an *in situ* temperature of -0.56°C for surface water. All the samples were stored at -20°C immediately after the beginning of the experiment and after 3h, 6h, 12h, and 24h.

*Prokaryotic abundance and biomass*

Total prokaryotic counts were performed using the SYBR Green I direct count procedure (Danovaro, 2009) adapting the procedure to ice samples.

Materials were sterilized in order to avoid transcontamination.

Sea ice and seawater samples were incubated for 15min with 0.02 μm pre-filtered tetrasodium pyrophosphate solution (final concentration, 5 mM), then properly diluted before filtration onto 0.2 μm pore-size Whatman Nuclepore Track - Etched Membrane polycarbonate black filters (WHA110656). Filters were then stained with 20 μl of SYBR Green I (S7563) (10000× stock) diluted 1:20 in pre-filtered TE buffer (pH 7.5), with excess stain removed 3 times using 3 ml of Milli-Q water and mounted onto microscope slides. At least 20 microscope fields and 400 cells per filter were counted randomly using epifluorescence microscopy (Zeiss Axioskop 2MOT, magnification × 1,000) under blue light (λ 498nm). For the determination of the prokaryotic biomass, prokaryotic size was converted into bio-volume following inter-calibration with Scanning Electron Microscope (SEM) based size determinations and converted into carbon content assuming 310 fgC $\mu m^{-3}$ (Danovaro, 2009), in line with previous studies (Rastelli et al., 2016).

*Viral abundance, production and viral decay*

For this procedure, we follow the protocol presented in "*Methods for the study of deep-sea sediments, their functioning and biodiversity"* by Roberto Danovaro (2009) adapting the procedure to ice samples.

Sea ice and seawater samples were incubated for 15min with 0.02 μm pre-filtered tetrasodium pyrophosphate solution (final concentration, 5 mM), centrifuged at 800 × g speed for 1 min and then properly diluted before filtration onto 0.02-μm-pore-size $Al_2O_3$ filters (Anodisc; diameter 25 mm). The filters were stained with 100 μl of SYBR Gold 2x (S11494) diluting stock solution with 0.02-μm pre-filtered TE buffer: 10 mM Tris-HCl, 1 mM EDTA). Filters were incubated in the dark for 20 minutes, rinsed three times with 3 ml of 0.02-μm pre-filtered Milli-Q water, dried under a laminar flow hood, and then mounted on glass slides with 20 μl of antifade solution (50% phosphate buffer pH 7.8, 50% glycerol, 0.5% ascorbic acid). Viral counts were obtained by EFM under blue light (λ 495nm) (Zeiss Axioskop 2MOT, magnification × 1,000) examining at least 20 fields per slide, and at least 400 viral particles per filter. Average viral production rates were determined from linear regression analyses of the increase of viral abundances over time (Dell'Anno et al., 2009), and data were normalized to the volume of sample filtered.

The percentage contribution of viral decay was calculated as the ratio between average viral decay and gross viral production where the average viral decay was determined from linear regression analyses of the decrease of viral abundance over time (6 h after the beginning of the experiment) and the gross viral production was calculated as the sum of average viral production and average viral decay (Corinaldesi et al., 2010).

*Burst size and viral-induced prokaryotic mortality (VIPM)*

Prokaryotic burst size (number of viruses released by cell lysis due to viral infection, BS) was derived from the literature using the equation: BS = 30.2+261.4x cell volume, expressed as $\mu m^3$ $cell^{-1}$; (Weinbauer & Höfle, 1998).

VIPM was calculated as follows: VIPM (% $d^{-1}$) = (AVP / BS)/ PA x 100 x 24h, where AVP is the average viral production expressed as the number of viruses produced per milliliter of seawater (or ice) per hour, BS is the burst size, PA is the prokaryotic abundance at the beginning of the experiment (Danovaro et al., 2016; Manea et al., 2019).

*Total DNA extraction*

DNA extraction was performed on sea ice and seawater samples at the beginning (T0), when the peak of viral production was reached (i.e. generally 6h after the beginning of the experiment), and at the end of the experiment (after 24 h).

Total DNA was extracted and purified using the *DNeasy PowerWater Kit* (QIAGEN) following manufacturer instructions. The melted ice and water samples (around 25ml of sample) were filtered onto 0.22 µm filters with a 47mm diameter (Advantec MFS mixed Cellulose Ester Membrane, A020H047A). Using two sets of sterile forceps, the filter membrane was inserted into a 5 ml PowerWater Bead Pro tube where 1 ml of PW1 solution was subsequently added (the solution PW1 must be warmed to 55°C for 5–10 min to dissolve precipitates before the use and it should be used while still warm). The tube was then warmed to 65° for 10 minutes and then secured horizontally to a Vortex adapter set to maximum speed for 10 minutes. After that, the tube was centrifuged at 4000 x g for 1 min.

The supernatant then was transferred to a clean 2 ml collection tube and centrifuged at 13,000 x g for 1 min. Avoiding the pellet, the supernatant was transferred to another clean 2 ml

collection tube where 200 µl of solution IRS was added. The tube was vortexed briefly to mix the solution and then incubated at 2–8°C for 5 min in ice. After the incubation, the tube was centrifuged at 13,000 x g for 1 min. Avoiding the pellet, the supernatant was then transferred to a clean 2 ml collection tube where later 650 µl of Solution PW3 (if Solution PW3 has precipitate, heat to 55°C for 5–10 min to dissolve precipitate) was added and then the tube was vortexed briefly to mix. 650 µl of supernatant were loaded onto an MB Spin Column that was centrifuged at 13,000 x g for 1 min where then the flow-through was discarded. This step was repeated until all the supernatants had been processed. The supernatant was loaded onto the MB Spin Column Filter was placed into a clean 2 ml collection tube where 650 µl of Solution PW4 was added. The tubes were centrifuged at 13,000 x g for 1 min. After discarding the flow-through, 650 µl of ethanol was added to the tube and then centrifuged at 13,000 x g for 1 min. The flow-through was again discarded and the tubes were recentrifuged at 13,000 x g for 2 min and then left opened. At the end the MB Spin Column was placed into a clean 2 ml collection tube where 50 µl of Solution EB were added to the center of the white filter membrane. The tube was then centrifuged at 13,000 x g for 1 min, the MB Spin Column was discarded, and the DNA was then ready for downstream applications and kept in a cold room at 20 degrees.

DNA quantification was carried out via readings on *Qubit* fluorometers.

### *Sequencing of 16S rRNA and bioinformatic analysis*

For the analysis of prokaryotic diversity, the 16S RNA gene was amplified using the primer set 515F-Y (5′-GTGYCAGCMGCCGCGGTAA) and 926R (5′-CCGYCAATTYMTTTRAGTTT) (Parada et al., 2016). The PCR amplification of the 16S

RNA gene conditions were as follows 95°C for 3 min; 35 cycles of 95°C for 45 s, 50°C for 45 s, and 68°C for 90s; 68°C for 5 min, and then a 4°C hold.

The amplicons were sequenced on Illumina MiSeq platform and the paired-end sequences were subsequently analyzed through the DADA2 procedure within QIIME2 (Bolyen et al., 2019) to infer biologically significant Amplicon Sequence Variants (ASVs). The reference sequences were trimmed to the region amplified by the primers and the resulting ASVs were compared against the SILVA 16S database (v138; Quast et al., 2012) or taxonomic affiliation using the VSEARCH-based inference plugin within QIIME2 (Bolyen et al., 2019).

Before the computation of alpha- and beta-diversity indices, the ASV table was filtered to taxa seen at least two times in at least 5% of samples using the phyloseq package (McMurdie & Holmes, 2013) within RStudio software (v 4.2.0).

The microeco package (Liu et al., 2021) within RStudio software (v 4.2.0) was used to remove chloroplast- and mitochondrion-affiliated sequences. The main alpha-diversity indices (i.e., ASVs Observed, Shannon index and Pielou Index) were calculated through the microbiome package (Lahti et al., 2017; RStudio software v 4.2.0). Taxonomic inference of ASVs was carried out using phyloseq package (McMurdie & Holmes, 2013; RStudio software v 4.2.0) on the rarefied table to obtain a view of the assemblage structure at the phylum and family level considering those taxa whose contributions were >0.5% to the whole assemblage and merging the remaining taxa into a single “Others” group. Unknown families were grouped at the lowest taxonomic level known.

*Statistical analyses*

Differences in the investigated variables (between controls and the treatments) were investigated through permutational analyses of variance (PERMANOVA;

PRIMER+PERMANOVA 6+ stare; M. Anderson, 2008; M. J. Anderson, 2001) with 9999 permutations considering: “Treatments” (n=6 fixed levels: Ctrl_Ice, Ctrl_SW, Tr_A, Tr_B, Tr_C, Tr_D, Tr_E). Pairwise tests were also carried out to ascertain patterns of differences among controls and treatments when the main test displayed significant results.

Analysis of variance (ADONIS2 test with 9999 permutations) was carried out within the vegan package (K. R. Clarke, 1993; Warton et al., 2012) (RStudio software v 4.2.0) using the distance matrix based on the Bray-Curtis to look for significant differences between the microbial assemblages found within controls and treatments and the three investigated times of the time-course experiment.

Differential abundance analyses were performed with the ANCOM-BC procedure (Dixon, 2003; Lin & Peddada, 2020; Nearing et al., 2022) at the genus level on the non-rarefied table to identify differentially abundant taxa between the investigated times (i.e. T0 *vs* T6h *vs* T24h).

# RESULTS

## 1. *Prokaryotic abundance and biomass*

### *1.1. Prokaryotic abundance*

Prokaryotic abundance showed values 1.79 times higher in sea ice (on average 8,25 x $10^5$ ± 9,64 x $10^4$ Cells $ml^{-1}$) than in seawater samples (on average 4.60 x $10^5$ ± 1.70 x $10^4$, PERMANOVA, $p<0.001$; Fig. 2). At the beginning of the experiment (sampling time T0), no significant differences were observed between samples incubated at *in situ* temperature and samples incubated at ΔT +2.5°C (8.06 x $10^5$ ± 2.54 x $10^4$ Cells $ml^{-1}$ and 4.01 x $10^5$ ± 3.34 x $10^5$ Cells $ml^{-1}$ for sea ice and seawater at ΔT +2.5°C, respectively). Mixing sea water and sea ice (treatment C), prokaryotic abundance displayed values lower than sea ice (PERMANOVA, $p<0.01$) and higher than seawater (PERMANOVA, $p<0.05$). Treatments D and E showed the lowest values (2.61 x$10^5$ ± 4.29 x$10^4$ and 2.51 x$10^5$ ± 1.90 x$10^4$ Cells $ml^{-1}$ for D and E, respectively).

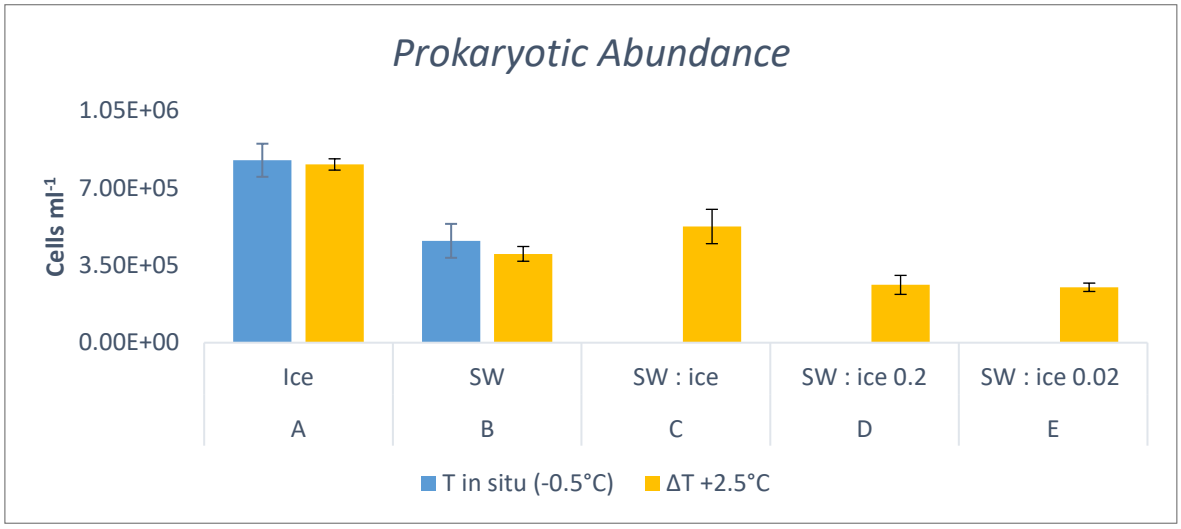


*Figure 2. Prokaryotic abundance in seawater and sea ice at in situ temperature (in blue) and sea ice, seawater, sea ice mixed with seawater, sea water mixed with 0.2 µm filtered sea ice and seawater mixed with 0.02 µm*

*filtered sea ice incubated at ΔT +2.5°C (in yellow). Prokaryotic abundance of different treatments in y-axis is expressed in cells ml$^{-1}$. Reported are average values and standard deviations.*

### *1.2. Prokaryotic biomass*

Prokaryotic biomass (Fig.3) displayed patterns similar to prokaryotic abundance, with higher values in sea ice than seawater and no significant differences between samples incubated at *in situ* temperature (8.75 x $10^{-3}$ ± 1.07 x$10^{-3}$ µgC ml$^{-1}$ for sea ice and 6.76 x $10^{-3}$ ± 1.54 x$10^{-3}$ µgC ml$^{-1}$ for seawater) and samples incubated at ΔT +2.5°C (8.81 x $10^{-3}$ ± 5.79 x$10^{-4}$ µgC ml$^{-1}$ for sea ice and 4,19 x $10^{-3}$ ± 3,74 x$10^{-4}$ µgC ml$^{-1}$ for seawater). As observed for prokaryotic abundance, treatment C showed lower values than sea ice and higher values than seawater, while treatment D and E displayed the lowest values showing no significant differences (2.71 x$10^{-3}$ ± 4.46 x$10^{-4}$ µgC ml$^{-1}$ and 2.60 x$10^{-3}$ ± 1.97 x$10^{-4}$ µgC ml$^{-1}$ for D and E, respectively).

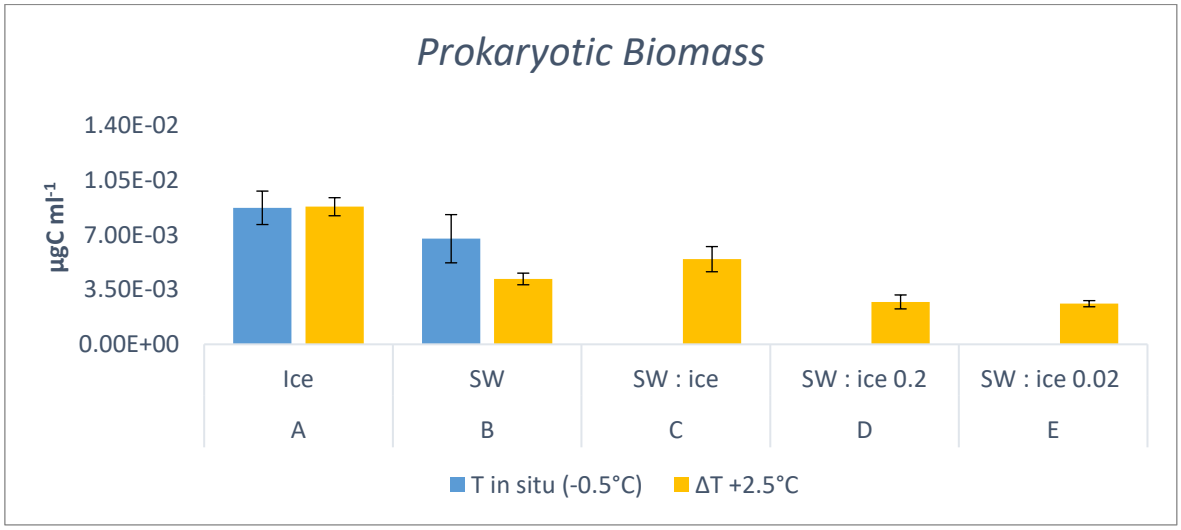


*Figure 3. Prokaryotic biomass in seawater and sea ice at in situ temperature (in blue) and sea ice, seawater, sea ice mixed with seawater, sea water mixed with 0.2 µm filtered sea ice and seawater mixed with 0.02 µm*

*filtered sea ice incubated at ΔT +2.5°C (in yellow). Prokaryotic biomass of different treatments in y-axis is expressed in µgC ml$^{-1}$. Reported are average values and standard deviations.*

## 1. Viral abundance, production and viral decay

### 1.1. Viral abundance

Viral abundance showed the highest values in sea ice (both at *in situ* temperature and at ΔT +2.5°C; on average 1.07 x10$^6$ ± 5.54 x10$^4$ viruses ml$^{-1}$; Fig.4). At sampling time T0, viral abundance didn't differ between samples at natural conditions and their respective treatments. Viruses observed in treatment C (sea ice and seawater together, 8.79 x10$^5$ ± 2.97 x10$^4$ viruses ml$^{-1}$) showed lower values compared to sea ice, but higher values than seawater. Treatments D and E showed lower values compared to treatments A, B, and C (3.65 x10$^5$ ± 5.50 x10$^3$ and 3.45 x10$^5$ ± 1.39 x10$^4$ viruses ml$^{-1}$ for treatments D and E, respectively; PERMANOVA, $p<0.05$).

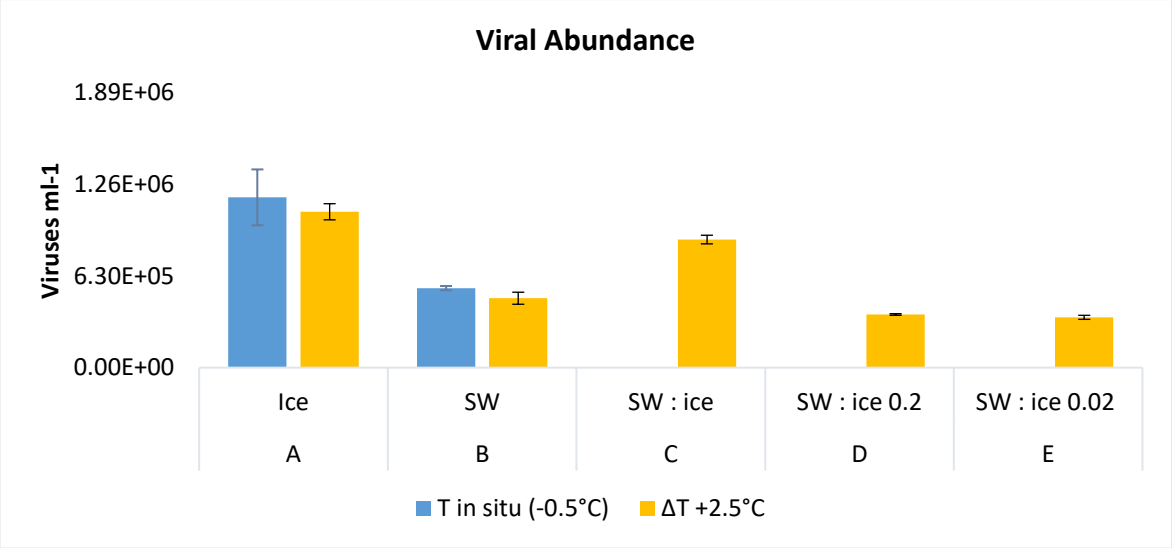


*Figure 4. Viral abundance in seawater and sea ice at in situ temperature (in blue) and sea ice, seawater, sea ice mixed with seawater, sea water mixed with 0.2 µm filtered sea ice and seawater mixed with 0.02 µm filtered*

*sea ice incubated at ΔT +2.5°C (in yellow). Viral abundance of different treatments in y-axis is expressed in viruses ml$^{-1}$. Reported are average values and standard deviations.*

### 2.2. Viral production

Viral production (Fig.5) in sea ice incubated at *in situ* temperature showed higher values than seawater at *in situ* conditions (PERMANOVA, $p<0.001$). All the samples (both sea ice and seawater) incubated at ΔT +2.5°C displayed lower values of viral production compared to samples incubated at *in situ* temperature (PERMANOVA, $p<0.001$). In detail, viral production in sea ice showed values 4 times higher at *in situ* temperature than in samples incubated at ΔT +2.5°C ($2.75 \times 10^5 \pm 1.87 \times 10^4$ and $7.00 \times 10^4 \pm 6.58 \times 10^3$ viruses ml$^{-1}$ h$^{-1}$ for sea ice at *in situ* and ΔT +2.5°C temperature, respectively; PERMANOVA, $p<0.001$), while in seawater samples viral production was 1.8 times lower at ΔT +2.5°C ($1.90 \times 10^5 \pm 1.00 \times 10^4$ and $1.07 \times 10^5 \pm 9.98 \times 10^3$ viruses ml$^{-1}$ h$^{-1}$ for sea ice at *in situ* and ΔT +2.5°C temperature, respectively; PERMANOVA, $p<0.001$). Sea ice and seawater samples showed no significant differences from treatment C; values observed in treatment E showed no significant differences with seawater incubated at higher temperature (ΔT +2.5°C).

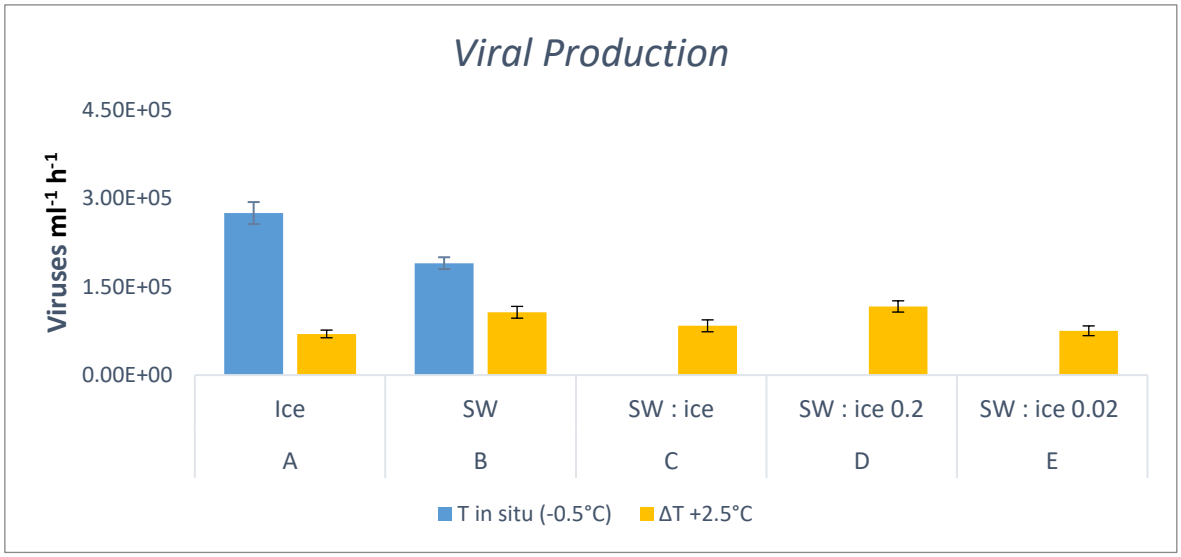


*Figure 5. Viral production in seawater and sea ice at in situ temperature (in blue) and sea ice, seawater, sea ice mixed with seawater, sea water mixed with 0.2 µm filtered sea ice and seawater mixed with 0.02 µm filtered sea ice incubated at ΔT +2.5°C (in yellow). Viral production of different treatments in y-axis is expressed in viruses $ml^{-1}$ $h^{-1}$. Reported are average values and standard deviations.*

### 2.3. *Percentage contribution of viral decay*

The percentage contribution of viral decay (Fig. 6) displayed significantly lower values in the sea ice compared to the seawater at *in situ* temperature (PERMANOVA, $p<0.01$). Significant differences were observed between sea ice incubated at ΔT +2.5°C (33.45 ± 3.85 %) and sea ice at *in situ* temperature (20.80 ± 4.26 %), as well as in seawater samples (36.22 ± 5.88 % and 23.18 ± 4.30 % at *in situ* temperature and at ΔT +2.5°C, respectively). Treatment C (30.32 ± 4.90 %) showed no significant difference with all treatments D and E.

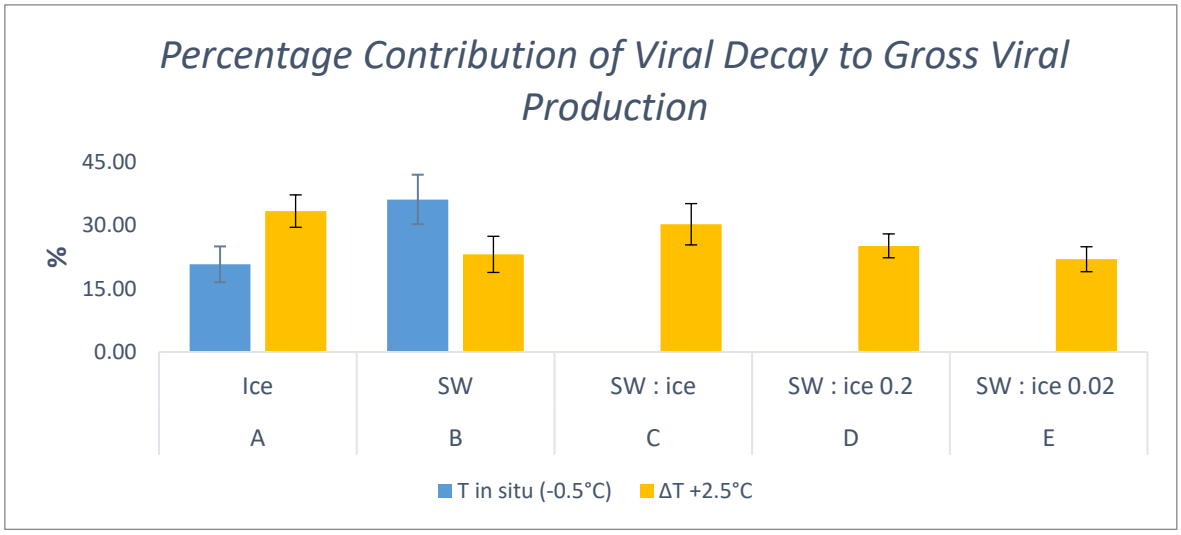


*Figure 6. Percentage contribution of viral decay to gross viral production in seawater and sea ice at in situ temperature (in blue) and sea ice, seawater, sea ice mixed with seawater, sea water mixed with 0.2 µm filtered sea ice and seawater mixed with 0.02 µm filtered sea ice incubated at ΔT +2.5°C (in yellow). Percentage contribution of viral decay of different treatments in y-axis is expressed in %. Reported are average values and standard deviations.*

### 3. *Viral-induced prokaryote mortality (VIPM)*

Virus-induced prokaryote mortality (VIPM; Fig. 7) was lower in sea ice samples than in seawater at *in situ* temperature (PERMANOVA, $p<0.05$; Fig. 4). VIPM values 3.9 and 1.5 times lower were observed in both sea ice and seawater samples incubated at ΔT +2.5°C ($3.31 \pm 0.21$ % $d^{-1}$ and $10.23 \pm 1.54$ % $d^{-1}$ for sea ice and seawater at ΔT +2.5°C, respectively) compared to *in situ* conditions ($12.81 \pm 0.92$ % $d^{-1}$ and $15.79 \pm 0.96$ % $d^{-1}$ for sea ice and seawater at *in situ* temperature, respectively; PERMANOVA, $p<0.01$).

Sea ice and seawater mixed (treatment C, $6.22 \pm 1.45$ % $d^{-1}$) significantly differed from both sea ice and seawater incubated at higher temperatures. Treatment D ($17.21 \pm 1.68$ % $d^{-1}$) did

not significantly differ from the seawater incubated at *in situ* temperature but differed significantly from treatment E (11.57 ± 2.02 % $d^{-1}$).

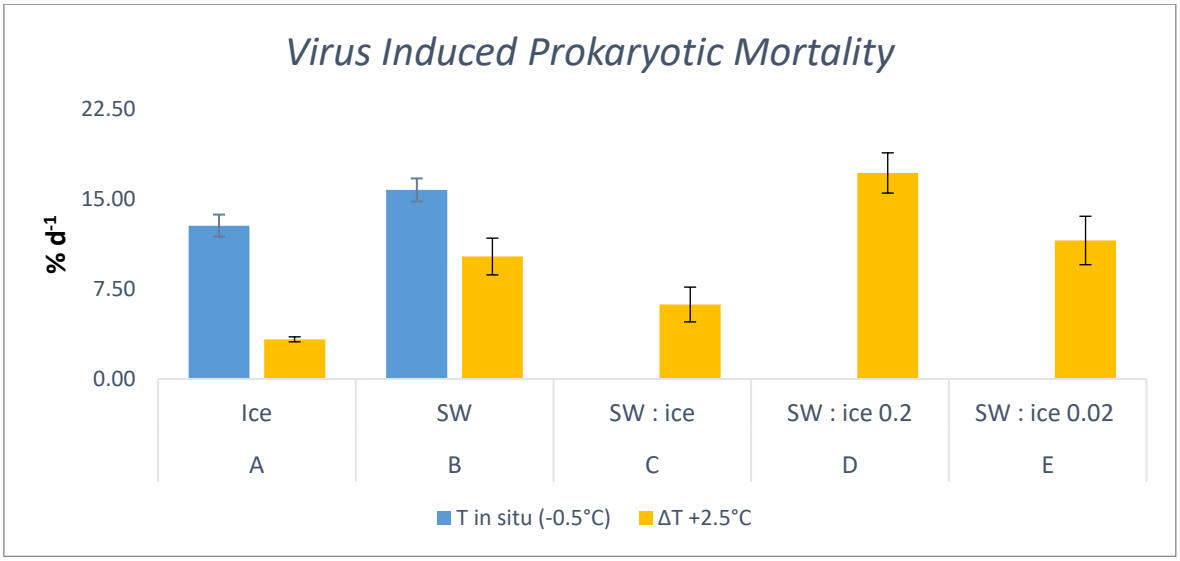


*Figure 7. Viral induced prokaryotic mortality in seawater and sea ice at in situ temperature (in blue) and sea ice, seawater, sea ice mixed with seawater, sea water mixed with 0.2 µm filtered sea ice and seawater mixed with 0.02 µm filtered sea ice incubated at ΔT +2.5°C (in yellow). Viral induced prokaryotic mortality of different treatments in y-axis is expressed in % $d^{-1}$. Reported are average values and standard deviations.*

## 4. *Prokaryotic diversity based on 16S rRNA sequencing*

After ASVs rarefaction, total ASVs were assigned to 12 phyla, 17 classes, 42 orders, 81 families, 129 genera in all the samples.

The observed ASVs and the Shannon index (calculated at the beginning of the time-course experiment) in sea ice and seawater samples incubated at *in situ* temperature displayed a similar pattern with higher values in sea ice samples (PERMANOVA, $p<0.001$, Figure 8). Comparing sea ice samples, Observed ASVs, and Shannon index displayed lower values in ice samples incubated at ΔT +2.5°C than those observed in natural conditions (PERMANOVA, $p<0.001$), while no differences were found in seawater samples in the two

temperature conditions. When sea ice and seawater were mixed together (treatment C), values 1.3 and 1.4 times higher than sea ice and seawater incubated at the same temperature were observed for the Observed ASVs and the Shannon index, respectively (PERMANOVA, $p<0.05$). Significant differences were observed between treatments C and D, as well as between treatments C and E (PERMANOVA, $p<0.05$).

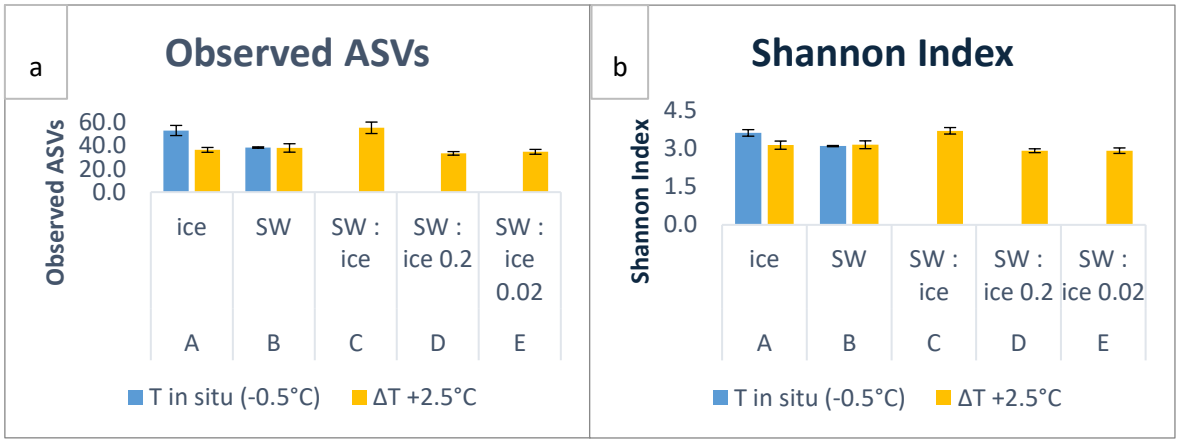


*Figure 8. α-diversity indexes in seawater and sea ice at in situ temperature (in blue) and sea ice, seawater ice mixed with seawater, sea water mixed with 0.2 µm filtered sea ice and seawater mixed with 0.02 µm filtered sea ice, each incubated at ΔT +2.5°C (in yellow). The plots show the Observed ASVs (a) and the Shannon Index (b) in the different treatments.*

At the beginning of the experiment, Proteobacteria represented the most abundant phylum in all samples contributing 49-87.6% to all prokaryotic taxa, followed by Bacteroidota (11.8-36.7%). At family level (Fig. 9), seawater samples (both unfiltered, treatment B, and filtered, treatment D and E) were dominated by members of the Clade-I group (phylum Proteobacteria) contributing on average to 22.4% in B, 26.7% in E and 20.9% in D, while the Rhodobacteraceae (phylum Proteobacteria) and Flavobacteriaceae (phylum Bacteroidota) were the most abundant taxa in sea ice samples (on average 18.8% all of prokaryotic sequences).

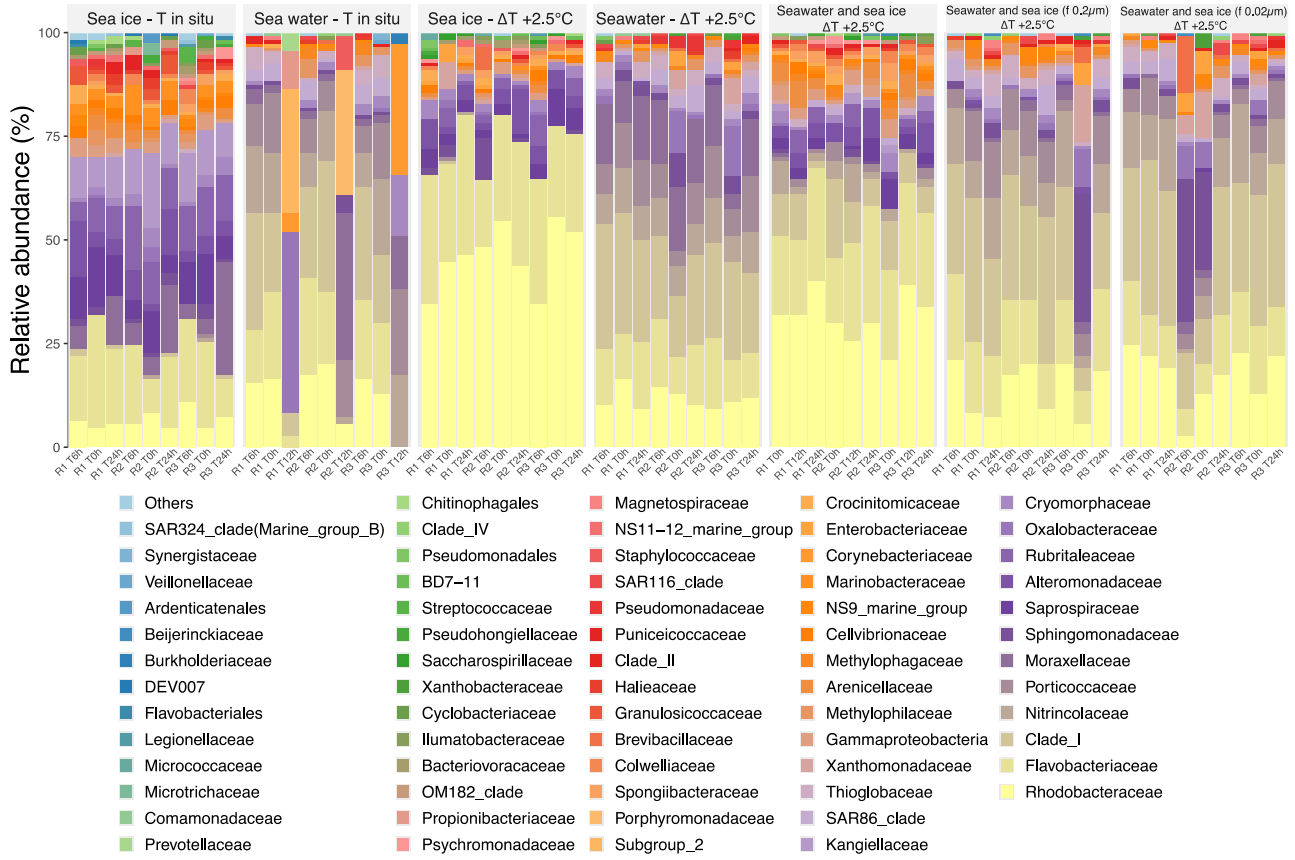


*Figure 9. Taxonomic structure of the prokaryotic assemblages. The taxonomic structure is facet into: seawater and sea ice at in situ temperature and sea ice, seawater ice mixed with seawater, sea water mixed with 0.2 μm filtered sea ice and seawater mixed with 0.02 μm filtered sea ice, each incubated at ΔT +2.5°C. Taxa are represented at family level.*

Comparing taxa changing in their relative abundance during the time-course experiment in sea ice samples incubated at *in situ* temperature (Fig. 10), we observed that members of the *Kangiellaceae* displayed a general decrease over time (from 11% at the beginning of the experiment to 9.3% after 24h; ANCOM, $p<0.05$). Among taxa showing a significant increase in sea ice samples at *in situ* temperature during the experiment, members of the *Acinetobacter* and *Polaribacter* genus changed in their relative abundance from 2.7% to 16.3% and from 3.3% to 6.3% of all prokaryotic sequences (ANCOM, $p<0.05$; Fig. 10a–10c).

Considering taxa changing in their relative abundance in sea ice samples incubated at $\Delta T$ +2.5°C (Fig. 10), *Rhodobacteraceae* and *Polaribacter* reached minimum values after 6h from the beginning of the experiment (from 24.3% and 7% to 16.7 and 4.7% for *Rhodobacteraceae* and *Polaribacter*, respectively; ANCOM, $p<0.05$), while members of the *Psychroserpens* and *Psychroflexus* reached maximum values after 6h and 24h, respectively (from 5.3% to 6% and 3.7% and 6.3% *Psychroserpens* and *Psychroflexus*, respectively ANCOM, $p<0.05$; Fig 10d-10f).

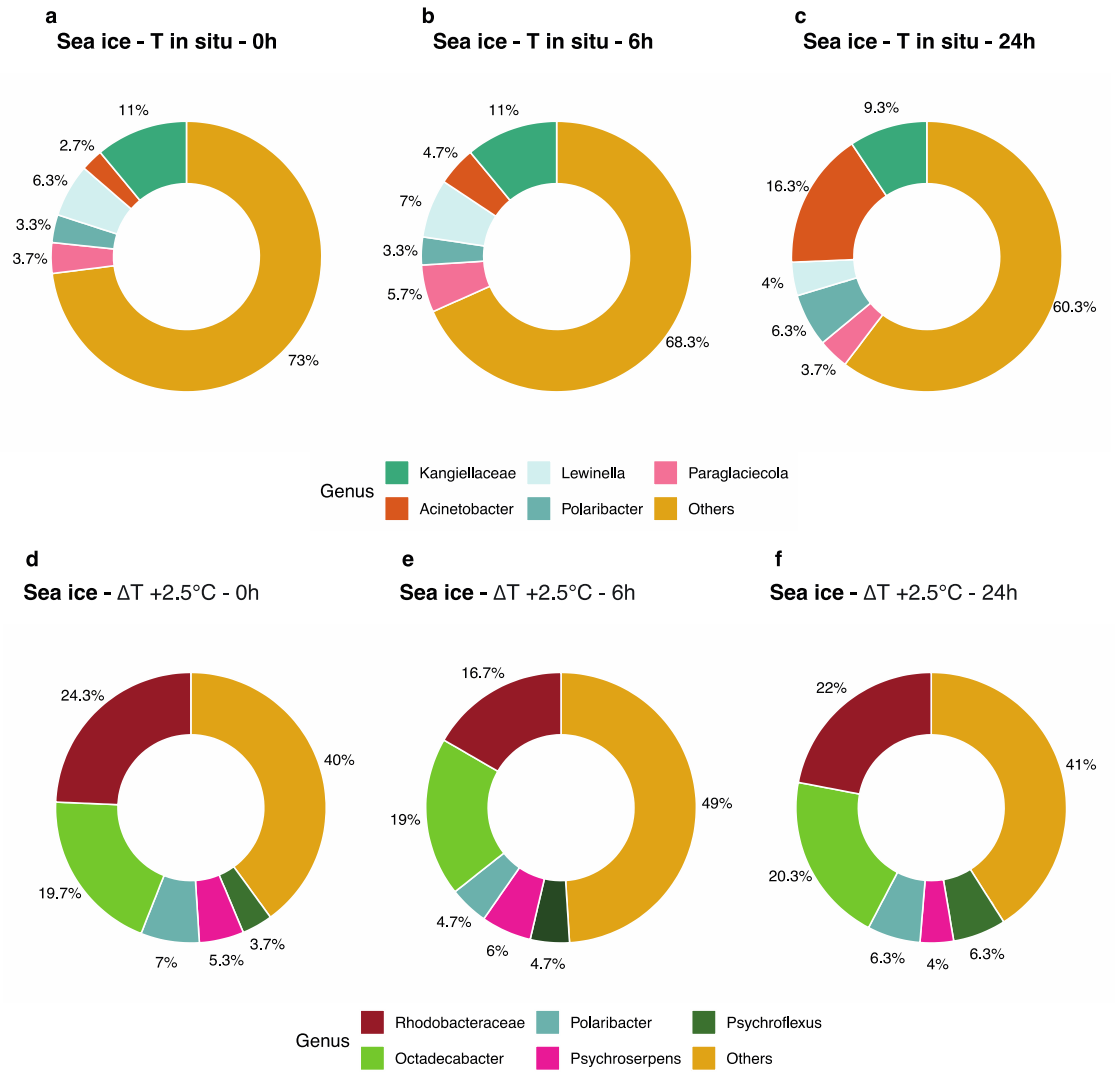


*Figure 10. Pie chart of the percentage of abundance of the five most abundant genera in sea ice samples incubated at in situ temperature (upper panel) and at ΔT +2.5°C (bottom panel) at sampling time t0h (a, d), t6h (b, e) and t24h (c, f).*

In seawater samples incubated at *in situ* temperature (Fig. 11a-11c), members belonging to the Clade_Ia and the *Nitrincolaceae* drop dramatically from 20.7% and 16% at the beginning of the experiment to 2% and 6% after 24h, respectively.

Members of the Clade_Ia and the *Nitrincolaceae* exhibited the highest relative abundance in seawater samples incubated at ΔT +2.5°C (Fig. 11d-11f) and in treatment D (Fig. 12d-12f) after 6 hours from the beginning of the experiment (ANCOM, $p<0.05$), while ASVs

associated with the SAR92_Clade reached their lowest values at 6 hours in seawater samples and the highest values at 6h in treatment D (ANCOM, $p<0.05$).

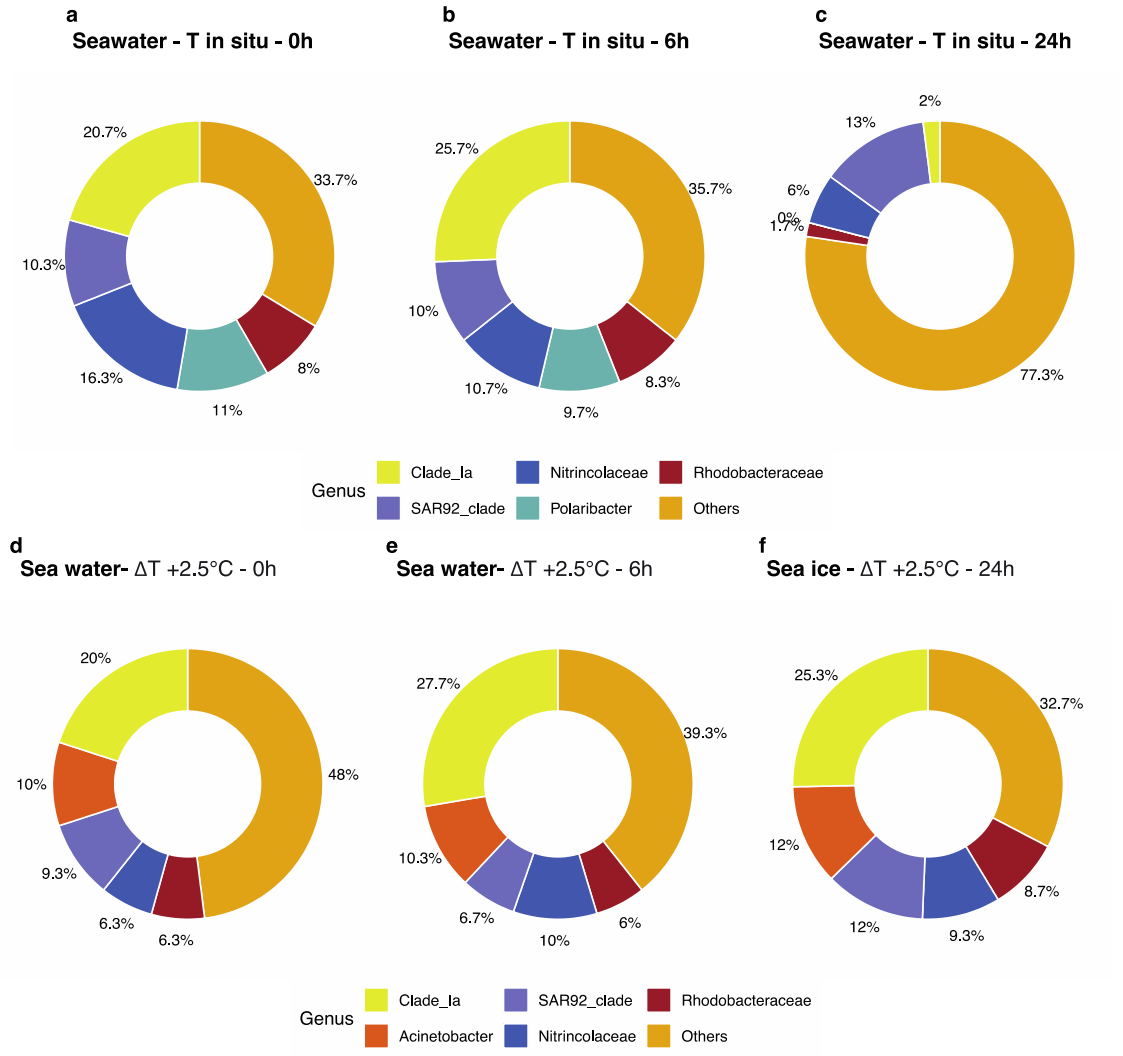


*Figure 11. Pie chart of the percentage of abundance of the five most abundant genera in seawater samples incubated at in situ temperature (upper panel) and at ΔT +2.5°C (bottom panel) at sampling time t0h (a, d), t6h (b, e) and t24h (c, f).*

When incubated sea ice and seawater together at ΔT +2.5°C (treatment C; Fig. 12a-12c), members of the *Rhodobacteraceae* group reached their maximum values of relative abundance at 6 hours, while *Nitrincolaceae* and Clade_Ia decreased over time (ANCOM, $p<0.05$). In treatment E, no significant differences were found in Clade_Ia during the

experiment, while the *Nitrincolaceae* were more abundant after 24h (ANCOM, $p<0.05$; Fig. 12g-12i).

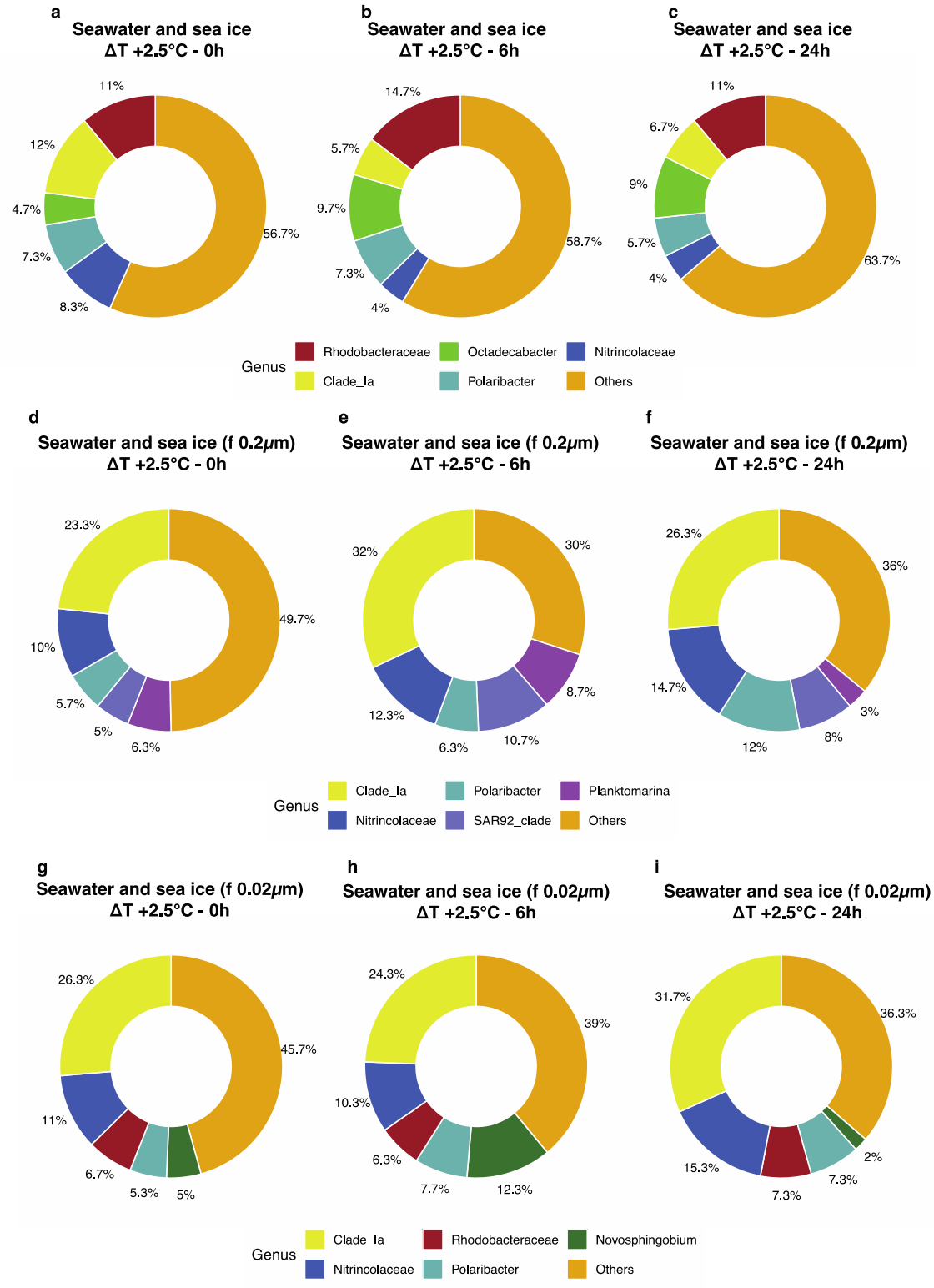


*Figure 12. Pie chart of the percentage of abundance of the five most abundant genera in seawater samples incubated at in situ temperature (upper panel) and at ΔT +2.5°C (bottom panel) at sampling time t0h (a, d), t6h (b, e) and t24h (c, f).*

Prokaryotic assemblages found in sea ice samples clustered separately from those observed in seawater samples in both *in situ* and warmer temperature conditions (PERMANOVA, $p<0.01$; Fig.13). Both sea ice and seawater samples incubated at ΔT +2.5°C displayed significant differences in prokaryotic assemblages when compared to sea ice and seawater samples incubated at *in situ* temperature (PERMANOVA, $p<0.01$). Prokaryotic assemblages observed in sea ice and seawater mixture (treatment C) showed significant differences with sea ice and seawater incubated at warmer temperature conditions (PERMANOVA, $p<0.01$). When the sea ice was filtered at 0.2 μm (treatment D) and 0.02 μm (treatment E) and mixed with seawater, no significant differences were observed in the prokaryotic assemblages between these treatments or in comparison with the seawater samples incubated at *in situ* temperature.

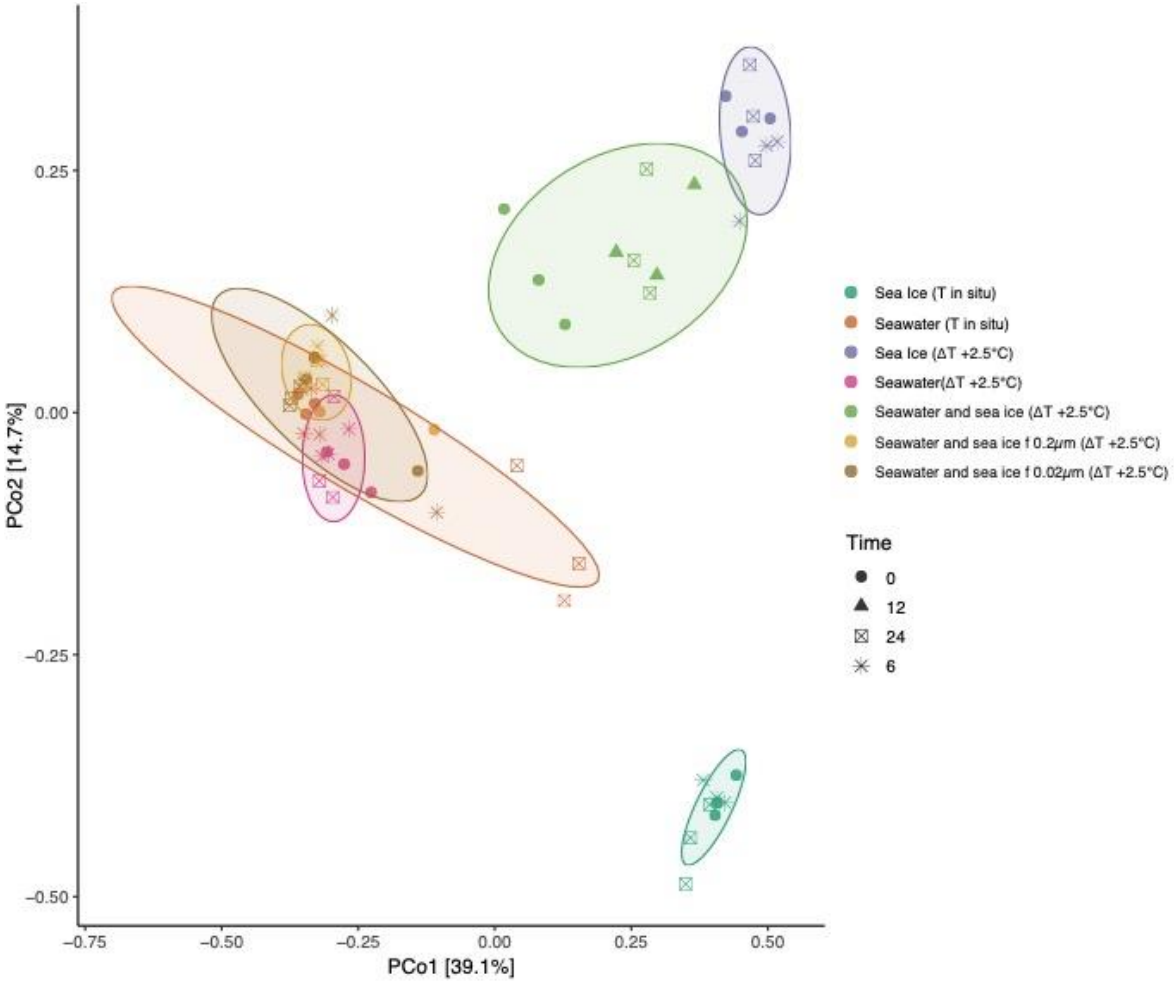


*Figure 13. PCoA plot representing inter-sample distances between seawater and sea ice at in situ temperature and sea ice, seawater ice mixed with seawater, sea water mixed with 0.2 µm filtered sea ice and seawater mixed with 0.02 µm filtered sea ice, each incubated at ΔT +2.5°C (treatment A, B, C, D and E, respectively). Inter-sample distances were calculated with Bray-Curtis distance.*

The analyses of ASVs showed that 26.9% of all ASVs were shared among sea ice and seawater incubated at natural conditions (T -0.5°C; Fig. 14a). The ASVs shared between sea ice and seawater samples were affiliated to 18 genera. ASVs affiliated to the *Acinetobacter* accounted for 17.4% of all shared ASVs, followed by *Rhodobacteraceae* and *Sulfitobacter* (Fig. 14b).

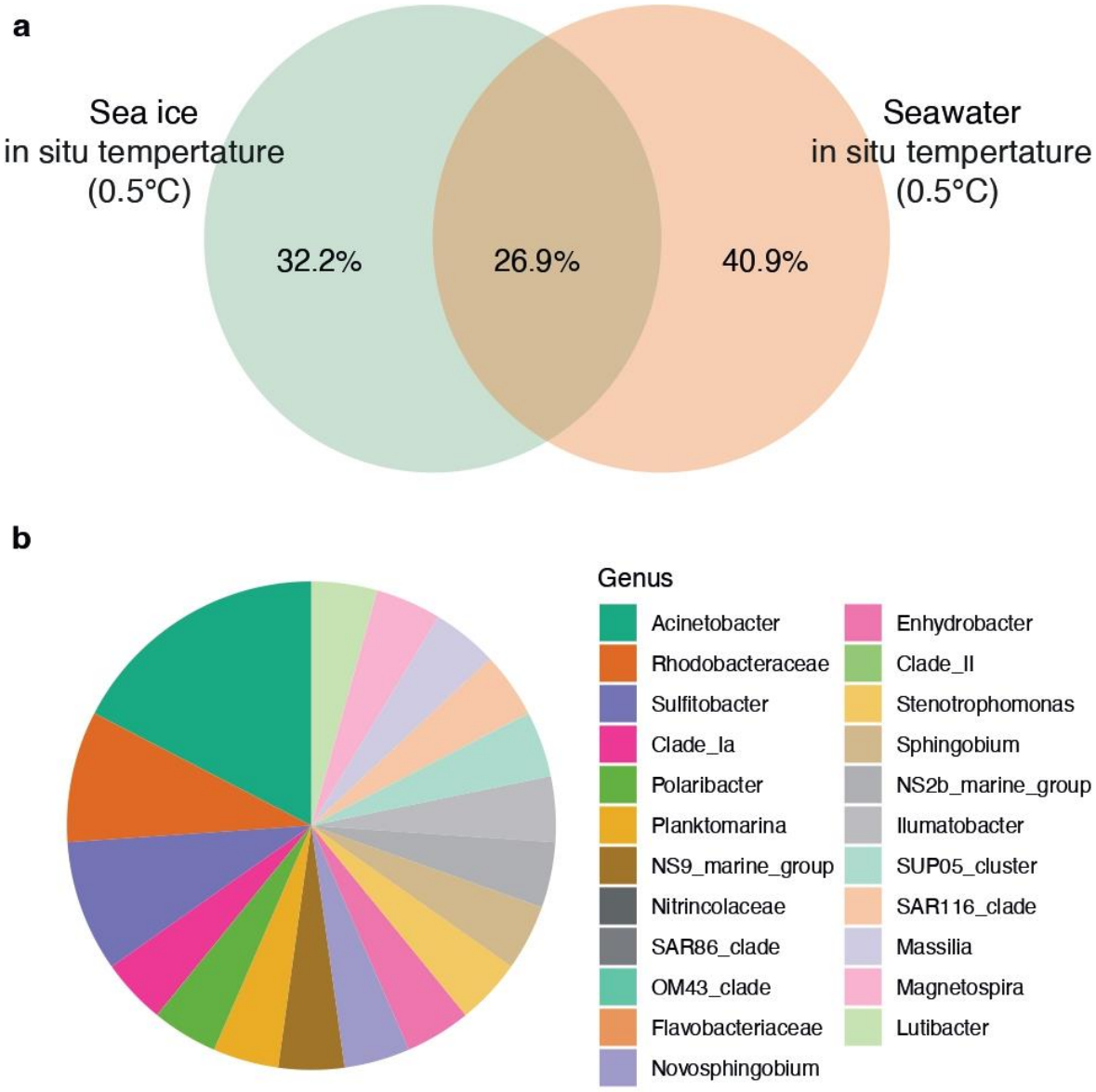


*Figure 14. ASV exclusivity analysis on the whole prokaryotic assemblage within sea ice and seawater samples incubated at in situ temperature; a) The Venn plots show the percentage of ASVs on the total number of ASVs found within sea ice and seawater samples; b) taxonomic structure of ASVs shared between sea ice and seawater samples at genus level.*

The analyses of ASVs showed that 42.3% of all ASVs were shared among sea ice and seawater incubated at ΔT +2.5°C (Fig. 15a). The ASVs shared between sea ice and seawater

samples were affiliated to 16 genera. ASVs affiliated to the *Polaribacter* accounted for the 13% of all shared ASVs, followed by *Rhodobacteraceae* and *Sulfitobacter* (Fig. 15b).

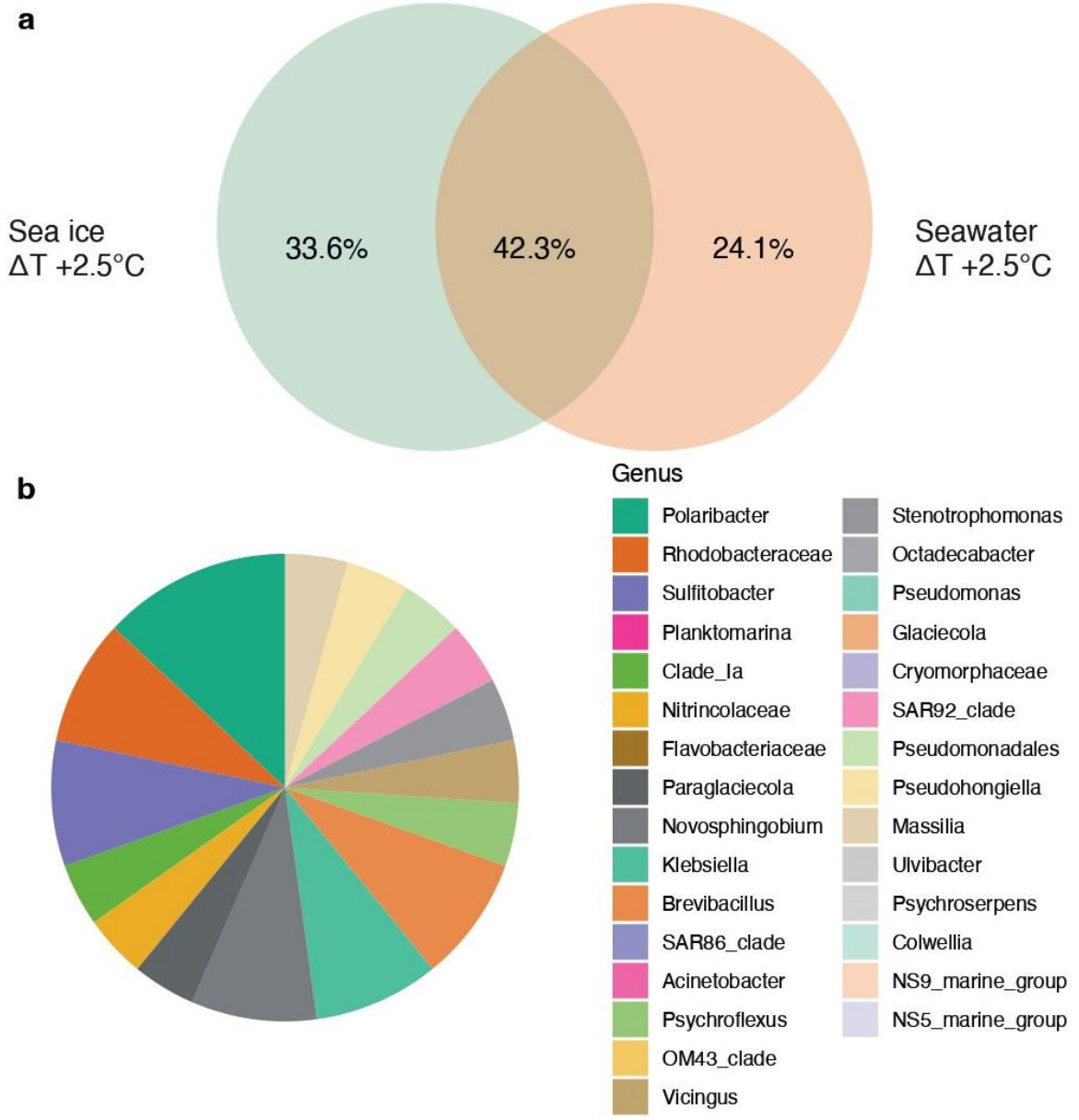


*Figure 15. ASV exclusivity analysis on the whole prokaryotic assemblage within sea ice and seawater samples incubated at ΔT +2.5°C; a) The Venn plots show the percentage of ASVs on the total number of ASVs found within sea ice and seawater samples; b) taxonomic structure of ASVs shared between sea ice and seawater samples at genus level.*

The analyses of ASVs showed that 38.3% of all ASVs were shared among sea ice, seawater and sea water mixed with sea ice incubated at ΔT +2.5°C (Fig. 16a). The ASVs shared between sea ice, seawater and sea water mixed with sea ice samples were affiliated to 16 genera. ASVs affiliated to the *Polaribacter* accounted for the 15% of all shared ASVs, followed by *Rhodobacteraceae* and *Sulfitobacter* (Fig. 16b).

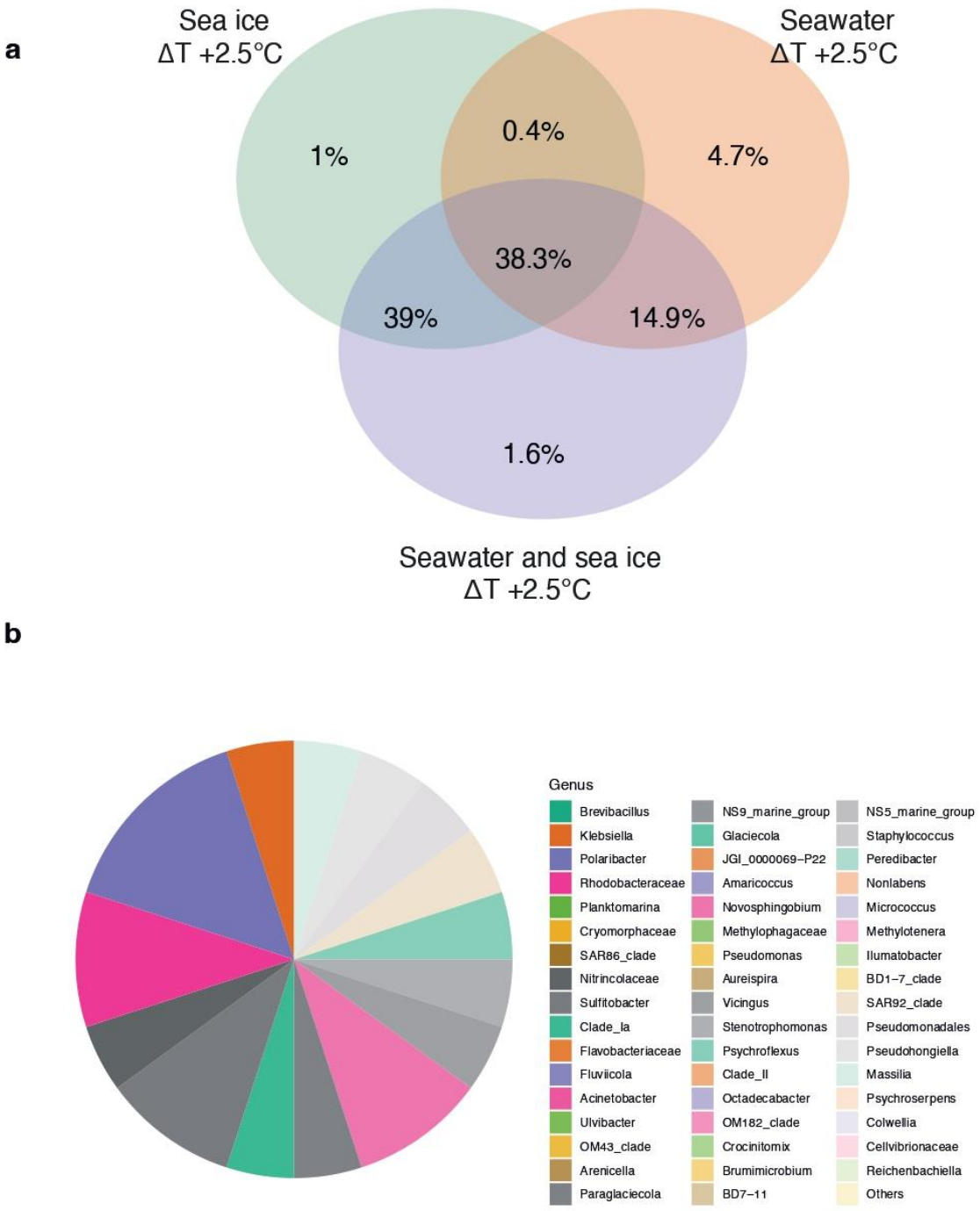


*Figure 16. ASV exclusivity analysis on the whole prokaryotic assemblage within sea ice, seawater and sea ice mixed with seawater samples incubated at ΔT +2.5°C; a) The Venn plots show the percentage of ASVs on the total number of ASVs found within sea ice and seawater samples; b) taxonomic structure of ASVs shared between sea ice and seawater samples at genus level.*

Redundancy analysis (RDA) was performed to identify whether temperature and/or viral abundance modulated the prokaryotic community structure (Figure 13). Viral abundance was the strongest driver of the community composition ($p<0.001$) followed by temperature ($p<0.001$). The direction and the length of the arrow indicate a strong positive correlation between viral abundance ($p<0.001$) in sea ice samples and sea ice and seawater mixture incubated at $\Delta T$ +2.5°C, but also a positive correlation with temperature ($p<0.001$). Sea water mixed with 0.2 μm filtered sea ice (treatment D) and sea water mixed with 0.02 μm filtered sea ice (treatment E) were negatively correlated with viral abundance ($p<0.001$). Taxa belonging to the genera *Kangiellaceae, Enhydrobacterwere* and *Lutibacter* were more relevant in sea ice samples incubated at *in situ* temperature and were positively correlated with increasing viral number, while members of the *Nitrincolaceae*, Clade-Ia and

SAR92_Clade were present in high abundance in seawater samples incubated at ΔT +2.5°C and in treatment D and E showing a negative correlation with viral.

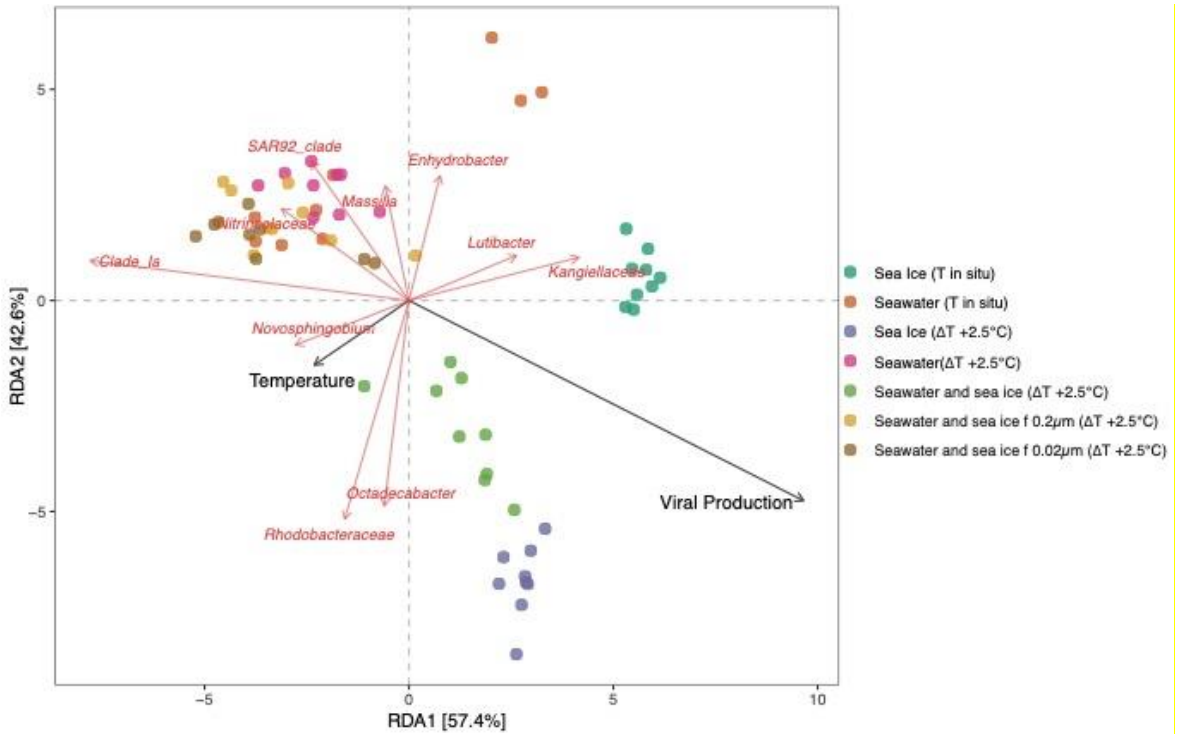


*Figure 17. Redundancy analyses (RDA) of prokaryotic communities in the investigated sea ice and seawater samples. The plot was generated using Bray-Curtis distance matrix with 9999 permutations. Each point represents prokaryotic communities found in each sample group. Vectors represent temperature and viral abundance; the direction of the arrows indicates the direction of maximum change of the variables and its length is proportional to the rate of change.*

# DISCUSSION

Sea ice provides a habitat suitable for the proliferation of microbial communities and their viruses (Deming, 2009; Deming & Eric Collins, 2017), therefore ice melting, due to climate changes, is expected to cause a release of biological components that live in sea ice, including viruses (Arrigo & Thomas, 2004b). Although Antarctic ecosystems are considered among those most impacted by climate changes (Rintoul et al., 2018), information on how microbial assemblages and virus-prokaryote dynamics could change in a global warming scenario is very limited. Nonetheless, this information is crucial to predict potential effects on biodiversity and Antarctic food-web functioning since there is evidence that the viral shunt (i.e., deviation of organic matter from higher trophic levels to the POM and DOM pools mediated by viral infections of marine organisms; (Carlson & Hansell, 2015) can have multiple implications on biogeochemical cycles and nutrient regeneration, on biodiversity patterns and global climate changes (Danovaro et al., 2011b)**.** Previous studies reported that the interaction between viruses and prokaryotes and/or protists in sea ice may determine the release of organic matter into the water column, promoting the growth of other prokaryotes (microbial loop) and enhancing grazing by higher trophic levels (microbial loop and microbial food webs) (Boras et al., 2010; Maranger et al., 2015).

Our study, conducted on the western part of the Ross Sea, aimed to investigate the effects of ice melting due to global warming on the interactions between planktonic and sympagic viruses and prokaryotes, and prokaryotic diversity. To do this, we carried out an experiment simulating ice melting due to a temperature increase of 2.5°C as predicted by IPCC for 2100 (Rasmussen et al., 2018), and we assessed the implications on prokaryotic

abundance and biomass, viral abundance, production and infection, as well as on the composition of prokaryotic assemblages in seawater.

First, we found that viral and prokaryotic abundances and prokaryote biomass were higher in sea ice samples than in seawater. This is in line with the pattern described by Rocchi et al., 2022b, which reported viral and prokaryotic abundances in Antarctic Sea ice ca. 3 times higher than in the surrounding surface seawater.

The highest microbial abundances in the Antarctic sea ice can be explained by the great availability of trophic resources for heterotrophic bacteria living in this habitat. Several studies have highlighted, indeed, the high biomass of sympagic algae (Arrigo, 2014; Arrigo et al., 1997), which produce organic matter via photosynthesis and essential nutrients for prokaryotes, thus promoting their growth. Furthermore, the resulting release of organic debris due to microalgae death enriches the nutrient pool available to prokaryotes (Arrigo, 2014; Arrigo et al., 1997).

When sea ice melted into seawater due to the increase in temperature, the abundances of viruses and prokaryotes showed intermediate values between those observed, separately, in ice and seawater at the beginning of the experiment. By removing the components with a size > 0.02 μm in the ice, we estimated that ca. half of the viruses and prokaryotes present in seawater were released by melted ice. The same result was found when only the biological components with a size > 0.2 μm were removed (treatment D; $3.65 \times 10^5 \pm 5.50 \times 10^3$ viruses $ml^{-1}$; Δ in viral abundance between C and D is 2.4 ± 0.1) suggesting that a large fraction of viruses in sea ice has a size > 0.2 μm.

The presence of dsDNA giant viruses (capsid diameter > 0.110−0.424 μm) has been observed in the sea ice of the Ross Sea (Gowing, 2003a; Gowing et al., 2002). Here, we expanded information on the quantitative contribution of sea-ice giant viruses, which could be relevant.

The high contribution of the sea-ice prokaryotes (in terms of abundances and biomasses) and viruses to the seawater due to sea ice melting, suggests that climate changes could intensify the release of this component in the marine environment.

There is evidence that viral infection rates increase as their hosts increase in number (Weinbauer, 2004b), although a host-specific interaction appears to be required (Breitbart, 2012). Therefore, if sea-ice viruses remain active after melting, they could infect marine microbes and other organisms.

Previous studies reported a 10-fold increase in viral abundance over time in the sea ice suggesting that viruses are highly active in this habitat (Maranger et al., 1994a) and high viral production rates (Bellas et al., 2013; Paterson & Laybourn-Parry, 2012). These results are justified by the high probability of contact rate between viruses and prokaryotes in the sea-ice brine inclusions (Wells et al., 2006).

In the present study, we observed that the increase in temperature decreased by 2-4 times the viral production rates in sea ice and seawater. Previous studies highlighted that temperature increases influence the interactions between viruses and prokaryotes and that viral production rates tend to be higher at higher temperatures (Danovaro et al., 2011b).

Our results expand previous information, indicating that an increase in temperature of 2.5°C for 24h (as simulated here) could affect viral-host interactions in seawater and sea ice. This was also evident in terms of viral infection rates, which decreased by 1.5-4 times in seawater and sea ice once incubated at higher temperatures compared to in situ conditions.

Following sea ice melting in seawater, we observed that viral production and infection were maintained at intermediate values between those determined, separately, in seawater and ice after the T increase. When components larger than 0.02 μm (including prokaryotes and viruses) were removed from sea ice, viral production and infection in seawater after ice

melting showed values significantly higher than those of the unfiltered samples, suggesting that a portion of sympagic viruses, especially those smaller than 0.2 μm, remain infective in seawater. At the same time, viral infection in seawater was higher when sea ice hosts larger than 0.2 μm were removed from sea ice (treatment D), suggesting a negative effect induced by sympagic host competitors on virus-prokaryote interactions.

As we observed a high contribution of giant viruses in sea ice, assumed to be represented by algal viruses (Gowing, 2003c), our findings allow us to infer that once released from the melted sea ice, algal viruses cannot find specific hosts to infect in seawater.

Sea ice and seawater samples were characterized by a different composition of the prokaryotic communities (in terms of ASVs). In seawater samples incubated at *in situ* temperature and ΔT +2.5°C (both filtered and unfiltered) no differences were observed in their prokaryotic assemblages.

In a climate-changing scenario, prokaryotic community composition displayed significant shifts between sea ice at *in situ* temperature conditions *vs* sea ice incubated at ΔT +2.5°C.

Both temperature and viral pressures on prokaryotic hosts can be considered key factors in shaping prokaryotic assemblages (Sandaa et al., 2018). Here we observed a significant correlation between prokaryotic community composition and viral production and temperature suggesting that both viruses and temperature can be responsible for the observed changes in prokaryotic assemblage composition. In particular, shifts in prokaryotic community observed in sea ice at in situ temperatures were mainly influenced by viral pressure on different taxa.

Differential abundance analyses conducted on sea ice samples at *in situ* conditions pointed out that the genus *Kangiellaceae***,** a Gamma-proteobacteria closely linked to sea-ice algae blooms during spring (Thiele et al., 2022) and one of the most abundant genera at the

beginning of our experiment, decreased over time suggesting a preferential infection of viruses on this taxon. Conversely, *Polaribacter*, found mainly associated with polar algal blooms (Thiele et al., 2022), increased over time (from 3.3% to 6.3%). Increasing temperature conditions on *Polaribacter* in sea ice samples seems to cause a decrease after 6h from the beginning of the time-course experiment. Even though information on virus-host dynamics in sea ice samples is still scarce, our data suggested that an increase in temperature might have a detrimental effect on *Polaribacter* proliferation probably due to an increase in viral infection on this taxon.

Simulating sea ice melting with an increase in temperature a release of sympagic prokaryotes and viruses is expected with possible interactions between the pelagic and sympagic compartments (Rocchi et al., 2022c). The negative effect observed on *Polaribacter* in sea ice was also observed in seawater after ice melting (treatment C), indicating that this process might determine changes in the taxonomic composition of prokaryotic communities.

On the contrary, an increase in this taxon was observed in filtered (both 0.2 μm and 0.02 μm) seawater samples incubated at ΔT +2.5°C suggesting that sympagic viruses are potentially responsible for *Polaribacter* decrease. A similar pattern can be also observed for ASVs affiliated with Clade-Ia. At the same temperature conditions, members of Clade-Ia showed a decrease in sea ice and seawater mixture after 24h. As observed for *Polaribacter*, sympagic viruses (excluded in treatment D and E) can play a crucial role in killing one of the most abundant taxa observed in seawater at the beginning of the experiment.

Overall, based on our results, we can state that sympagic viruses released by the melting of Antarctic sea ice, following an increase in temperature, exert a significant influence on prokaryotic communities in the ice itself and the surrounding seawater.

# CONCLUSIONS

Our experiment conducted in the western part of the Ross Sea expands knowledge about the effects of global warming and the resulting melting of Antarctic ice on microbial communities. Our results add valuable information to a still sparse literature, highlighting how sea ice is an ideal habitat for the proliferation of diverse microbial communities and their viruses.

We conclude that:

- The abundances and biomass of prokaryotes and viruses are higher in sea ice compared to the surrounding water;
- Approximately half of the viruses and prokaryotes found in seawater were released by melted ice;
- A significant proportion of viruses in sea ice exceed 0.2 μm in size;
- Climate change may enhance the release of sea ice prokaryotes and viruses into the marine environment;
- Increasing temperatures reduce viral production rates in both sea ice and seawater by 2-4 times;
- A 2.5°C temperature increase over 24 hours (as simulated in this study) could affect viral-host interactions in seawater and sea ice;
- The simulated temperature increase may impact viral infection rates, reducing them by 1.5 to 4 times in both seawater and sea ice;
- A portion of sympagic viruses, especially those smaller than 0.2 μm, remain infective in seawater;

- Following their release from melted sea ice, algal viruses are unable to find specific hosts to infect in seawater;
- The prokaryotic community compositions, in terms of ASVs, differ between sea ice and seawater samples;
- Both viruses and temperature changes may contribute to the observed alterations in prokaryotic assemblage composition;
- Viruses may preferentially infect the *Kangiellaceae* taxon;
- An increase in temperature might negatively impact the proliferation of *Polaribacter*;
- Sympagic viruses may play a role in the reduction of *Polaribacter* populations;
- The release of sympagic viruses from melting Antarctic sea ice, driven by rising temperatures, significantly impacts prokaryotic communities within the ice and the surrounding seawater.

# REFERENCES


Abell, G. C. J., & Bowman, J. P. (2005). Ecological and biogeographic relationships of class Flavobacteria in the Southern Ocean. *FEMS Microbiology Ecology*, *51*(2), 265–277.

Adusumilli, S., Fricker, H. A., Medley, B., Padman, L., & Siegfried, M. R. (2020). Interannual variations in meltwater input to the Southern Ocean from Antarctic ice shelves. *Nature Geoscience*, *13*(9), 616–620.

Anderson, M. (2008). PERMANOVA+ for PRIMER: guide to software and statistical methods. *Primer-E Limited.*

Anderson, M. J. (2001). A new method for non-parametric multivariate analysis of variance. *Austral Ecology*, *26*(1), 32–46.

Arrigo, K. R. (2014). Sea ice ecosystems. *Annual Review of Marine Science*, *6*, 439–467.

Arrigo, K. R., Mock, T., & Lizotte, M. P. (2010). Primary producers and sea ice. *Sea Ice*, *2*, 283–325.

Arrigo, K. R., & Thomas, D. N. (2004a). Large scale importance of sea ice biology in the Southern Ocean. *Antarctic Science*, *16*(4), 471–486.

Arrigo, K. R., & Thomas, D. N. (2004b). Large scale importance of sea ice biology in the Southern Ocean. *Antarctic Science*, *16*(4), 471–486. https://doi.org/10.1017/S0954102004002263

Arrigo, K. R., Worthen, D. L., Lizotte, M. P., Dixon, P., & Dieckmann, G. (1997). Primary production in Antarctic sea ice. *Science*, *276*(5311), 394–397.

Bellas, C. M., Anesio, A. M., Telling, J., Stibal, M., Tranter, M., & Davis, S. (2013). Viral impacts on bacterial communities in Arctic cryoconite. *Environmental Research Letters*, *8*(4). https://doi.org/10.1088/1748-9326/8/4/045021

Bluhm, B. A., Hop, H., Melnikov, I. A., Poulin, M., Vihtakari, M., Collins, R. E., Gradinger, R., Juul-Pedersen, T., & von Quillfeldt, C. (n.d.). *3.1 Sea ice biota*.

Boetius, A., Anesio, A. M., Deming, J. W., Mikucki, J. A., & Rapp, J. Z. (2015a). Microbial ecology of the cryosphere: sea ice and glacial habitats. *Nature Reviews Microbiology*, *13*(11), 677–690.

Boetius, A., Anesio, A. M., Deming, J. W., Mikucki, J. A., & Rapp, J. Z. (2015b). Microbial ecology of the cryosphere: sea ice and glacial habitats. *Nature Reviews Microbiology*, *13*(11), 677–690.

Bolyen, E., Rideout, J. R., Dillon, M. R., Bokulich, N. A., Abnet, C. C., Al-Ghalith, G. A., Alexander, H., Alm, E. J., Arumugam, M., Asnicar, F., & others. (2019). Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nature Biotechnology*, *37*(8), 852–857.

Boras, J. A., Sala, M. M., Arrieta, J. M., Sà, E. L., Felipe, J., Agustí, S., Duarte, C. M., & Vaqué, D. (2010). Effect of ice melting on bacterial carbon fluxes channelled by viruses and protists in the Arctic Ocean. *Polar Biology*, *33*(12), 1695–1707. https://doi.org/10.1007/s00300-010-0798-8

Bourgeois, T., Goris, N., Schwinger, J., & Tjiputra, J. F. (2022a). Stratification constrains future heat and carbon uptake in the Southern Ocean between 30° S and 55° S. *Nature Communications*, *13*(1), 340.

Bourgeois, T., Goris, N., Schwinger, J., & Tjiputra, J. F. (2022b). Stratification constrains future heat and carbon uptake in the Southern Ocean between 30°S and 55°S. *Nature Communications*, *13*(1). https://doi.org/10.1038/s41467-022-27979-5

Bowman, J. (2013). *Sea-ice microbial communities*.

Bowman, J. P., McCammon, S. A., Rea, S. M., & McMeekin, T. A. (2000). The microbial composition of three limnologically disparate hypersaline Antarctic lakes. *FEMS Microbiology Letters*, *183*(1), 81–88.

Bowman, J. S., Rasmussen, S., Blom, N., Deming, J. W., Rysgaard, S., & Sicheritz-Ponten, T. (2012). Microbial community structure of Arctic multiyear sea ice and surface seawater by 454 sequencing of the 16S RNA gene. *The ISME Journal*, *6*(1), 11–20.

Bracegirdle, T. J., & Stephenson, D. B. (2012). Higher precision estimates of regional polar warming by ensemble regression of climate model projections. *Climate Dynamics*, *39*, 2805–2821.

Breitbart, M. (2012). Marine viruses: Truth or dare. In *Annual Review of Marine Science* (Vol. 4, pp. 425–448). https://doi.org/10.1146/annurev-marine-120709-142805

Breitbart, M., Bonnain, C., Malki, K., & Sawaya, N. A. (2018). Phage puppet masters of the marine microbial realm. *Nature Microbiology*, *3*(7), 754–766.

Brierley, A. S., & Thomas, D. N. (2002). *Ecology of Southern Ocean pack ice*.

Brinkmeyer, R., Knittel, K., Jürgens, J., Weyland, H., Amann, R., & Helmke, E. (2003). Diversity and Structure of Bacterial Communities in Arctic versus Antarctic Pack Ice. *Applied and Environmental Microbiology*, *69*(11), 6610–6619. https://doi.org/10.1128/AEM.69.11.6610-6619.2003

Cape, M. R., Straneo, F., Beaird, N., Bundy, R. M., & Charette, M. A. (2019). Nutrient release to oceans from buoyancy-driven upwelling at Greenland tidewater glaciers. *Nature Geoscience*, *12*(1), 34–39.

Carlson, C. A., & Hansell, D. A. (2015). DOM Sources, Sinks, Reactivity, and Budgets. In *Biogeochemistry of Marine Dissolved Organic Matter: Second Edition* (pp. 65–126). Elsevier Inc. https://doi.org/10.1016/B978-0-12-405940-5.00003-0

Castro-Sowinski, S. (2019). *The ecological role of micro-organisms in the Antarctic environment*. Springer.

Chen, X., Weinbauer, M. G., Jiao, N., & Zhang, R. (2021). Revisiting marine lytic and lysogenic virus-host interactions: Kill-the-Winner and Piggyback-the-Winner. *Science Bulletin*, *66*(9). https://doi.org/10.1016/j.scib.2020.12.014ï

Clarke, A., Johnston, N. M., Murphy, E. J., & Rogers, A. D. (2007). Introduction. Antarctic ecology from genes to ecosystems: The impact of climate change and the importance of scale. In *Philosophical Transactions of the Royal Society B: Biological Sciences* (Vol. 362, Issue 1477, pp. 5–9). Royal Society. https://doi.org/10.1098/rstb.2006.1943

Clarke, K. R. (1993). Non-parametric multivariate analyses of changes in community structure. *Australian Journal of Ecology*, *18*(1), 117–143.

Clem, K. R., Fogt, R. L., Turner, J., Lintner, B. R., Marshall, G. J., Miller, J. R., & Renwick, J. A. (2020). Record warming at the South Pole during the past three decades. *Nature Climate Change*, *10*(8), 762–770.

Comiso, J. C., Kwok, R., Martin, S., & Gordon, A. L. (2011). Variability and trends in sea ice extent and ice production in the Ross Sea. *Journal of Geophysical Research: Oceans*, *116*(C4).

Convey, P., Chown, S. L., Clarke, A., Barnes, D. K. A., Bokhorst, S., Cummings, V., Ducklow, H. W., Frati, F., Green, T. G. A., Gordon, S., Griffiths, H. J., Howard-Williams, C., Huiskes, A. H. L., Laybourn-Parry, J., Lyons, W. B., McMinn, A., Morley, S. A., Peck, L. S., Quesada, A., … Wall, D. H. (2014). The spatial structure of antarctic biodiversity. In *Ecological Monographs* (Vol. 84, Issue 2, pp. 203–244). Ecological Society of America. https://doi.org/10.1890/12-2216.1

Corinaldesi, C., Dell'Anno, A., Magagnini, M., & Danovaro, R. (2010). Viral decay and viral production rates in continental-shelf and deep-sea sediments of the Mediterranean Sea. *FEMS Microbiology Ecology*, *72*(2), 208–218.

Cowie, R. O. M., Williams, G. J., Maas, E. W., Voyles, K. M., & Ryan, K. G. (2014). Antarctic sea-ice microbial communities show distinct patterns of zonation in response to algal-derived substrates. *Aquatic Microbial Ecology*, *73*(2), 123–134.

Danovaro, R. (2009). *Methods for the study of deep-sea sediments, their functioning and biodiversity*. CRC press.

Danovaro, R., Corinaldesi, C., Dell'Anno, A., Fuhrman, J. A., Middelburg, J. J., Noble, R. T., & Suttle, C. A. (2011a). Marine viruses and global climate change. In *FEMS Microbiology Reviews* (Vol. 35, Issue 6, pp. 993–1034). https://doi.org/10.1111/j.1574-6976.2010.00258.x

Danovaro, R., Corinaldesi, C., Dell'Anno, A., Fuhrman, J. A., Middelburg, J. J., Noble, R. T., & Suttle, C. A. (2011b). Marine viruses and global climate change. In *FEMS Microbiology Reviews* (Vol. 35, Issue 6, pp. 993–1034). https://doi.org/10.1111/j.1574-6976.2010.00258.x

Danovaro, R., Dell'Anno, A., Corinaldesi, C., Magagnini, M., Noble, R., Tamburini, C., & Weinbauer, M. (2008). Major viral impact on the functioning of benthic deep-sea ecosystems. *Nature*, *454*(7208), 1084–1087. https://doi.org/10.1038/nature07268

Danovaro, R., Dell'Anno, A., Corinaldesi, C., Rastelli, E., Cavicchioli, R., Krupovic, M., Noble, R. T., Nunoura, T., & Prangishvili, D. (2016). Virus-mediated archaeal hecatomb in the deep seafloor. *Science Advances*, *2*(10), e1600492.

Death, R., Wadham, J. L., Monteiro, F., Le Brocq, A. M., Tranter, M., Ridgwell, A., Dutkiewicz, S., & Raiswell, R. (2014). Antarctic ice sheet fertilises the Southern Ocean. *Biogeosciences*, *11*(10), 2635–2643.

Dell'Anno, A., Corinaldesi, C., Magagnini, M., & Danovaro, R. (2009). Determination of viral production in aquatic sediments using the dilution-based approach. *Nature Protocols*, *4*(7), 1013–1022. https://doi.org/10.1038/nprot.2009.82

Deming, J. W. (2009). Sea ice bacteria and viruses. *Sea Ice*, 247–282.

Deming, J. W., & Eric Collins, R. (2017). Sea ice as a habitat for bacteria, archaea and viruses. *Sea Ice*, 326–351.

Dixon, P. (2003). VEGAN, a package of R functions for community ecology. *Journal of Vegetation Science*, *14*(6), 927–930.

Eicken, H. (1992a). The role of sea ice in structuring Antarctic ecosystems*. In *Polar Biol* (Vol. 12). Springer-Verlag.

Eicken, H. (1992b). The role of sea ice in structuring Antarctic ecosystems*. In *Polar Biol* (Vol. 12). Springer-Verlag.

Eicken, H., Bock, C., Wittig, R., Miller, H., & Poertner, H.-O. (2000). Magnetic resonance imaging of sea-ice pore fluids: methods and thermal evolution of pore microstructure.

In *Cold Regions Science and Technology* (Vol. 31). www.elsevier.comrlocatercoldregions

Evans, C., Brandsma, J., Meredith, M. P., Thomas, D. N., Venables, H. J., Pond, D. W., & Brussaard, C. P. D. (2021). *microorganisms Shift from Carbon Flow through the Microbial Loop to the Viral Shunt in Coastal Antarctic Waters during Austral Summer*. https://doi.org/10.3390/microorganisms

Evseeva, O., Evseeva, S., & Dudarenko, T. (2021). The impact of human activity on the global warming. *E3S Web of Conferences*, *284*. https://doi.org/10.1051/e3sconf/202128411017

Fuentes, S., Arroyo, J. I., Rodr\'\iguez-Marconi, S., Masotti, I., Alarcón-Schumacher, T., Polz, M. F., Trefault, N., la Iglesia, R., & D\'\iez, B. (2019). Summer phyto-and bacterioplankton communities during low and high productivity scenarios in the Western Antarctic Peninsula. *Polar Biology*, *42*, 159–169.

Fuhrman, J. A. (1999). Marine viruses and their biogeochemical and ecological effects. *Nature*, *399*(6736), 541–548.

Garner, G., Hermans, T. H. J., Kopp, R., Slangen, A., Edwards, T., Levermann, A., Nowicki, S., Palmer, M. D., Smith, C., Fox-Kemper, B., & others. (2022). *IPCC AR6 WGI sea level projections*.

Garrison, D. L., Jeffries, M. O., Gibson, A., Coale, S. L., Neenan, D., Fritsen, C., Okolodkov, Y. B., & Gowing, M. M. (2003). Development of sea ice microbial communities during autumn ice formation in the Ross Sea. *Marine Ecology Progress Series*, *259*, 1–15.

Golden, K. M., Ackley, S. F., & Lytle, V. I. (1998). The percolation phase transition in sea ice. *Science*, *282*(5397), 2238–2241.

Golden, K. M., Eicken, H., Heaton, A. L., Miner, J., Pringle, D. J., & Zhu, J. (2007). Thermal evolution of permeability and microstructure in sea ice. *Geophysical Research Letters*, *34*(16).

Gordon, A. L., Zambianchi, E., Orsi, A., Visbeck, M., Giulivi, C. F., Whitworth, T., & Spezie, G. (2004). Energetic plumes over the western Ross Sea continental slope. *Geophysical Research Letters*, *31*(21). https://doi.org/10.1029/2004GL020785

Gowing, M. M. (2003a). Large viruses and infected microeukaryotes in Ross Sea summer pack ice habitats. *Marine Biology*, *142*, 1029–1040.

Gowing, M. M. (2003b). Large viruses and infected microeukaryotes in Ross Sea summer pack ice habitats. *Marine Biology*, *142*(5), 1029–1040. https://doi.org/10.1007/s00227-003-1015-x

Gowing, M. M. (2003c). Large viruses and infected microeukaryotes in Ross Sea summer pack ice habitats. *Marine Biology*, *142*(5), 1029–1040. https://doi.org/10.1007/s00227-003-1015-x

Gowing, M. M., Riggs, B. E., Garrison, D. L., Gibson, A. H., & Jeffries, M. O. (2002). Large viruses in Ross Sea late autumn pack ice habitats. *Marine Ecology Progress Series*, *241*, 1–11.

Ha, S., Lee, J. Il, Bak, Y. S., Yoo, K. C., & Khim, B. K. (2023). Paleoenvironmental reconstruction of glaciomarine sediments in the Drygalski Basin of the western Ross Sea since the Last Glacial Maximum. *Sedimentary Geology*, *456*. https://doi.org/10.1016/j.sedgeo.2023.106495

Hadas, H., Einav, M., Fishov, I., & Zaritsky, A. (1997). Bacteriophage T4 development depends on the physiology of its host Escherichia coli. *Microbiology*, *143*(1), 179–185.

Heinrich, H. (1988). Origin and consequences of cyclic ice rafting in the northeast Atlantic Ocean during the past 130,000 years. *Quaternary Research*, *29*(2), 142–152.

Hellmer, H. H., Kauker, F., Timmermann, R., & Hattermann, T. (2017). The fate of the southern Weddell Sea continental shelf in a warming climate. *Journal of Climate*, *30*(12), 4337–4350.

Ibarbalz, F. M., Henry, N., Brandão, M. C., Martini, S., Busseni, G., Byrne, H., Coelho, L. P., Endo, H., Gasol, J. M., Gregory, A. C., & others. (2019). Global trends in marine plankton diversity across kingdoms of life. *Cell*, *179*(5), 1084–1097.

Krembs, C., Eicken, H., Junge, K., & Deming, J. W. (2002). High concentrations of exopolymeric substances in Arctic winter sea ice: implications for the polar ocean carbon cycle and cryoprotection of diatoms. *Deep Sea Research Part I: Oceanographic Research Papers*, *49*(12), 2163–2181.

Krembs, C., Gradinger, R., & Spindler, M. (2000). Implications of brine channel geometry and surface area for the interaction of sympagic organisms in Arctic sea ice. In *Journal of Experimental Marine Biology and Ecology* (Vol. 243). www.elsevier.nl/locate/jembe

Lahti, L., Shetty, S., Blake, T., Salojarvi, J., & others. (2017). Tools for microbiome analysis in R. *Version*, *1*(5), 28.

Legendre, L., Ackley, S. F., Dieckmann, G. S., Gulliksen, B., Horner, R., Hoshiai, T., Melnikov, I. A., Reeburgh, W. S., Spindler, M., & Sullivan, C. W. (1992). Ecology of sea ice biota: 2. Global significance. *Polar Biology*, *12*, 429–444.

Levin, L. A., & Dayton, P. K. (2009). Ecological theory and continental margins: where shallow meets deep. *Trends in Ecology & Evolution*, *24*(11), 606–617.

Lin, H., & Peddada, S. Das. (2020). Analysis of compositions of microbiomes with bias correction. *Nature Communications*, *11*(1), 3514.

Liu, C., Cui, Y., Li, X., & Yao, M. (2021). microeco: an R package for data mining in microbial community ecology. *FEMS Microbiology Ecology*, *97*(2), fiaa255.

Lopez-Simon, J., Vila-Nistal, M., Rosenova, A., De Corte, D., Baltar, F., & Martinez-Garcia, M. (2023). Viruses under the Antarctic Ice Shelf are active and potentially involved in global nutrient cycles. *Nature Communications*, *14*(1). https://doi.org/10.1038/s41467-023-44028-x

Manea, E., Dell'Anno, A., Rastelli, E., Tangherlini, M., Nunoura, T., Nomaki, H., Danovaro, R., & Corinaldesi, C. (2019). Viral infections boost prokaryotic biomass production and organic C cycling in hadal trench sediments. *Frontiers in Microbiology*, *10*, 459069.

Mangoni, O., Carrada, G. C., Modigh, M., Catalano, G., & Saggiomo, V. (2009). Photoacclimation in Antarctic bottom ice algae: an experimental approach. *Polar Biology*, *32*, 325–335.

Maranger, R., Bird, D. F., & Juniper, S. K. (1994a). Viral and bacterial dynamics in Arctic sea ice during the spring algal bloom near Resolute, NWT, Canada. *Marine Ecology Progress Series*, 121–127.

Maranger, R., Bird, D. F., & Juniper, S. K. (1994b). Viral and bacterial dynamics in Arctic sea ice during the spring algal bloom near Resolute, NWT, Canada. *Marine Ecology Progress Series*, 121–127.

Maranger, R., Vaqué, D., Nguyen, D., Hébert, M.-P., & Lara, E. (2015). Pan-Arctic patterns of planktonic heterotrophic microbial abundance and processes: controlling factors and potential impacts of warming. *Progress in Oceanography*, *139*, 221–232.

Martin, A., McMinn, A., Davy, S. K., Anderson, M. J., Miller, H. C., Hall, J. A., & Ryan, K. G. (2012). Preliminary evidence for the microbial loop in Antarctic sea ice using microcosm simulations. *Antarctic Science*, *24*(6), 547–553.

McMurdie, P. J., & Holmes, S. (2013). phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. *PloS One*, *8*(4), e61217.

Melis, R., & Salvi, G. (2009). Late Quaternary foraminiferal assemblages from western Ross Sea (Antarctica) in relation to the main glacial and marine lithofacies. *Marine Micropaleontology*, *70*(1–2), 39–53. https://doi.org/10.1016/j.marmicro.2008.10.003

Mock, T., & Thomas, D. N. (2005a). Recent advances in sea-ice microbiology. In *Environmental Microbiology* (Vol. 7, Issue 5, pp. 605–619). https://doi.org/10.1111/j.1462-2920.2005.00781.x

Mock, T., & Thomas, D. N. (2005b). Recent advances in sea-ice microbiology. In *Environmental Microbiology* (Vol. 7, Issue 5, pp. 605–619). https://doi.org/10.1111/j.1462-2920.2005.00781.x

Mojica, K. D. A., & Brussaard, C. P. D. (2014). Factors affecting virus dynamics and microbial host–virus interactions in marine environments. *FEMS Microbiology Ecology*, *89*(3), 495–515.

Moore, J. K., Doney, S. C., Glover, D. M., & Fung, I. Y. (2001). Iron cycling and nutrient-limitation patterns in surface waters of the World Ocean. *Deep Sea Research Part II: Topical Studies in Oceanography*, *49*(1–3), 463–507.

Mundy, C. J., Gosselin, M., Ehn, J. K., Belzile, C., Poulin, M., Alou, E., Roy, S., Hop, H., Lessard, S., Papakyriakou, T. N., & others. (2011). Characteristics of two distinct high-light acclimated algal communities during advanced stages of sea ice melt. *Polar Biology*, *34*, 1869–1886.

Murray, A. G., & Jackson, G. A. (1992). *Viral dynamics: a model of the effects of size, shape, motion and abundance of single-celled planktonic organisms and other particles* (Vol. 89).

Nearing, J. T., Douglas, G. M., Hayes, M. G., MacDonald, J., Desai, D. K., Allward, N., Jones, C. M. A., Wright, R. J., Dhanani, A. S., Comeau, A. M., & others. (2022). Microbiome differential abundance methods produce different results across 38 datasets. *Nature Communications*, *13*(1), 342.

Parada, A. E., Needham, D. M., & Fuhrman, J. A. (2016). Every base matters: Assessing small subunit rRNA primers for marine microbiomes with mock communities, time series and global field samples. *Environmental Microbiology*, *18*(5), 1403–1414. https://doi.org/10.1111/1462-2920.13023

Parkinson, C. L. (2019). A 40-y record reveals gradual Antarctic sea ice increases followed by decreases at rates far exceeding the rates seen in the Arctic. *Proceedings of the National Academy of Sciences of the United States of America*, *116*(29), 14414–14423. https://doi.org/10.1073/pnas.1906556116

Paterson, H., & Laybourn-Parry, J. (2012). Antarctic sea ice viral dynamics over an annual cycle. *Polar Biology*, *35*(4), 491–497. https://doi.org/10.1007/s00300-011-1093-z

Proctor, L. M., Okubo, A., & Fuhrman, J. A. (1993). Calibrating estimates of phage-induced mortality in marine bacteria: ultrastructural studies of marine bacteriophage development from one-step growth experiments. *Microbial Ecology*, *25*, 161–182.

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., & Glöckner, F. O. (2012). The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Research*, *41*(D1), D590–D596.

Rasmussen, D. J., Bittermann, K., Buchanan, M. K., Kulp, S., Strauss, B. H., Kopp, R. E., & Oppenheimer, M. (2018). Extreme sea level implications of 1.5 °c, 2.0 °c, and 2.5 °c temperature stabilization targets in the 21st and 22nd centuries. *Environmental Research Letters*, *13*(3). https://doi.org/10.1088/1748-9326/aaac87

Rastelli, E., Dell'Anno, A., Corinaldesi, C., Middelboe, M., Noble, R. T., & Danovaro, R. (2016). Quantification of viral and prokaryotic production rates in benthic ecosystems: A methods comparison. *Frontiers in Microbiology*, *7*, 1501.

Riedel, A., Michel, C., & Gosselin, M. (2006). Seasonal study of sea-ice exopolymeric substances on the Mackenzie shelf: implications for transport of sea-ice bacteria and algae. *Aquatic Microbial Ecology*, *45*(2), 195–206.

Rignot, E., Jacobs, S., Mouginot, J., & Scheuchl, B. (2013). Ice-shelf melting around Antarctica. *Science*, *341*(6143), 266–270.

Rintoul, S. R., Chown, S. L., DeConto, R. M., England, M. H., Fricker, H. A., Masson-Delmotte, V., Naish, T. R., Siegert, M. J., & Xavier, J. C. (2018). Choosing the future of Antarctica. In *Nature* (Vol. 558, Issue 7709, pp. 233–241). Nature Publishing Group. https://doi.org/10.1038/s41586-018-0173-4

Rivkin, R. B., & Putt, M. (1987). PHOTOSYNTHESIS AND CELL DIVISION BY ANTARCTIC MICROALGAE: COMPARISON OF BENTHIC, PLANKTONIC AND ICE ALGAE 1. *Journal of Phycology*, *23*, 223–229.

Rocchi, A., Sotomayor-Garcia, A., Cabrera-Brufau, M., Berdalet, E., Dall'Osto, M., & Vaqué, D. (2022a). Abundance and activity of sympagic viruses near the Western Antarctic Peninsula. *Polar Biology*, *45*(8), 1363–1378. https://doi.org/10.1007/s00300-022-03073-w

Rocchi, A., Sotomayor-Garcia, A., Cabrera-Brufau, M., Berdalet, E., Dall'Osto, M., & Vaqué, D. (2022b). Abundance and activity of sympagic viruses near the Western Antarctic Peninsula. *Polar Biology*, *45*(8), 1363–1378. https://doi.org/10.1007/s00300-022-03073-w

Rocchi, A., Sotomayor-Garcia, A., Cabrera-Brufau, M., Berdalet, E., Dall'Osto, M., & Vaqué, D. (2022c). Abundance and activity of sympagic viruses near the Western Antarctic Peninsula. *Polar Biology*, *45*(8), 1363–1378. https://doi.org/10.1007/s00300-022-03073-w

Saggiomo, M., Poulin, M., Mangoni, O., Lazzara, L., De Stefano, M., Sarno, D., & Zingone, A. (2017). Spring-time dynamics of diatom communities in landfast and

underlying platelet ice in Terra Nova Bay, Ross Sea, Antarctica. *Journal of Marine Systems*, *166*, 26–36.

Sandaa, R. A., Storesund, J. E., Olesin, E., Paulsen, M. L., Larsen, A., Bratbak, G., & Ray, J. L. (2018). Seasonality drives microbial community structure, shaping both eukaryotic and prokaryotic host–viral relationships in an arctic marine ecosystem. *Viruses*, *10*(12). https://doi.org/10.3390/v10120715

Spindler, M., Dieckmann, G. S., & Lange, M. A. (1990). Seasonal and geographic variations in sea ice community structure of the Weddell Sea, Antarctica. In *Antarctic ecosystems: Ecological change and conservation* (pp. 129–135). Springer.

Stammerjohn, S., Massom, R., Rind, D., & Martinson, D. (2012). Regions of rapid sea ice change: An inter-hemispheric seasonal comparison. *Geophysical Research Letters*, *39*(6).

Steig, E. J., Schneider, D. P., Rutherford, S. D., Mann, M. E., Comiso, J. C., & Shindell, D. T. (2009). Warming of the Antarctic ice-sheet surface since the 1957 International Geophysical Year. *Nature*, *457*(7228), 459–462.

Suttle, C. A. (2007). Marine viruses—major players in the global ecosystem. *Nature Reviews Microbiology*, *5*(10), 801–812.

Thiele, S., Storesund, J. E., Fernández-Méndez, M., Assmy, P., & Øvreås, L. (2022). A Winter-to-Summer Transition of Bacterial and Archaeal Communities in Arctic Sea Ice. *Microorganisms*, *10*(8). https://doi.org/10.3390/microorganisms10081618

Thomas, D. N., & Dieckmann, G. S. (2002a). *Antarctic Sea Ice--a Habitat for Extremophiles*. https://www.science.org

Thomas, D. N., & Dieckmann, G. S. (2002b). *Antarctic Sea Ice--a Habitat for Extremophiles*. https://www.science.org

Tjiputra, J. F., Assmann, K., & Heinze, C. (2010). Anthropogenic carbon dynamics in the changing ocean. *Ocean Science*, *6*(3), 605–614.

Wadham, J. L., Hawkings, J. R., Tarasov, L., Gregoire, L. J., Spencer, R. G. M., Gutjahr, M., Ridgwell, A., & Kohfeld, K. E. (2019). Ice sheets matter for the global carbon cycle. In *Nature Communications* (Vol. 10, Issue 1). Nature Publishing Group. https://doi.org/10.1038/s41467-019-11394-4

Wagner-Döbler, I., & Biebl, H. (2006). Environmental biology of the marine Roseobacter lineage. *Annu. Rev. Microbiol.*, *60*, 255–280.

Warton, D. I., Wright, S. T., & Wang, Y. (2012). Distance-based multivariate analyses confound location and dispersion effects. *Methods in Ecology and Evolution*, *3*(1), 89–101.

Weinbauer, M. G. (2004a). Ecology of prokaryotic viruses. In *FEMS Microbiology Reviews* (Vol. 28, Issue 2, pp. 127–181). https://doi.org/10.1016/j.femsre.2003.08.001

Weinbauer, M. G. (2004b). Ecology of prokaryotic viruses. In *FEMS Microbiology Reviews* (Vol. 28, Issue 2, pp. 127–181). https://doi.org/10.1016/j.femsre.2003.08.001

Weinbauer, M. G., & Höfle, M. G. (1998). Significance of viral lysis and flagellate grazing as factors controlling bacterioplankton production in a eutrophic lake. *Applied and Environmental Microbiology*, *64*(2), 431–438.

Wells, E., Deming, J. W., Wells, L. E., & Deming, J. W. (2006). Communication Viruses in winter sea iceL Brief report Modelled and measured dynamics of viruses in Arctic winter sea-ice brines. *Environmental Microbiology*, *8*(6), 1115–1121. https://doi.org/10.1111/j.1462-2920.2005.00984.x

Yager, P. L., Connelly, T. L., Mortazavi, B., Wommack, K. E., Bano, N., Bauer, J. E., Opsahl, S., & Hollibaugh, J. T. (2001). Dynamic bacterial and viral response to an algal bloom at subzero temperatures. *Limnology and Oceanography*, *46*(4), 790–801.

Zhang, R., Weinbauer, M. G., & Peduzzi, P. (2020). Aquatic viruses and climate change. *Current Issues in Molecular Biology*, *41*, 357–380. https://doi.org/10.21775/cimb.041.357

Zhong, Z.-P., Rapp, J. Z., Wainaina, J. M., Solonenko, N. E., Maughan, H., Carpenter, S. D., Cooper, Z. S., Jang, H. Bin, Bolduc, B., Deming, J. W., & others. (2020). Viral ecogenomics of arctic cryopeg brine and sea ice. *Msystems*, *5*(3), 10–1128.

Zwally, H. J. (1983). *Antarctic sea ice, 1973-1976: Satellite passive-microwave observations* (Vol. 459). Scientific and Technical Information Branch, National Aeronautics and Space~….