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Université Libre de Bruxelles
Faculté des Sciences / Ecole Interfacultaire de Bioingénieurs
Ecologie des Systèmes Aquatiques

Interactions between the microbial network and the organic matter in the Southern Ocean: impacts on the biological carbon pump

Isabelle Dumont

Thèse de doctorat
présentée en vue de l'obtention du grade de
Docteur en Sciences Agronomiques et Ingénierie Biologique

Mai 2009

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Résumé

L'océan Antarctique (± 20% de la surface totale des océans) est un endroit essentiel pour la régulation du climat de notre planète grâce à sa capacité d'absorber le dioxyde de carbone (CO_2) atmosphérique par des mécanismes physico-chimique et biologique. La pompe biologique à carbone est un processus majeur de fixation de CO_2 par les organismes autotrophes à la surface de l'océan et de transfert de carbone organique vers le fond de l'océan. Ce processus est influencé par l'importance de la production primaire ainsi que par l'intensité de la reminéralisation de la matière organique dans la colonne d'eau. Ainsi, le cycle annuel de la glace via sa production/reminéralisation *in situ* mais aussi via l'ensemencement de l'océan avec des microorganismes et des nutriments organiques et inorganiques (en particulier le fer) a un impact sur le cycle du carbone dans l'Océan Antarctique, notamment en favorisant l'initiation d'efflorescences phytoplanctoniques dans la zone marginale de glace.

Plus précisément, nous avons étudié les interactions entre le réseau microbien (algues, bactéries et protozoaires) et la matière organique dans le but d'évaluer leurs impacts potentiels sur la pompe biologique de carbone dans l'Océan Austral. Deux écosystèmes différents ont été étudiés : la glace de mer et le milieu océanique grâce à des échantillons prélevés lors des campagnes de glace ARISE, ISPOL et SIMBA et lors de la campagne océanographique SAZ-Sense, couvrant une période allant de la fin de l'hiver à l'été.

La glace de mer est un environnement très particulier dans lequel les microorganismes planctoniques se trouvent piégés lors de la formation de la banquise et dans lesquels ils subissent des conditions extrêmes de température et de salinité, notamment. Les banquises en océan ouvert étudiées (0,3 à 1,2 m d'épaisseur, températures de -8.9°C à -0.4°C, volumes relatifs de saumure de 2.9 à 28.2% et salinités de saumures entre 10 et jusque >100) étaient composées de glace columnaire et granulaire. Les algues de glace étaient principalement des diatomées mais des flagellés autotrophes (tels que des dinoflagellés ou *Phaeocystis* sp.) ont été typiquement observés dans les couches de glace de surface. Les biomasses algales maximales se trouvaient généralement dans la couche de glace de fond sauf à SIMBA où les maxima se trouvaient en surface, probablement en raison de l'épaisseur des couches de neige et de glace, limitant la lumière disponible dans la colonne de glace. Au début du printemps, la croissance algale était contrôlée par l'espace disponible (càd le volume des saumures) tandis qu'au printemps/été, la disponibilité en nutriments majeurs a pu la contrôler. A toutes les saisons, des concentrations élevées en matière organique (MO) dissoute et particulaire on été mesurées dans la glace de mer par rapport à l'océan. Des monomères dissous (sucres et acides aminés) étaient accumulés dans la glace, surtout en hiver. Au printemps et été, les polysaccharides dissous dominaient le réservoir de sucres. La MO était présente sous forme de TEP qui par leurs propriétés de gel modifie l'habitat interne de la glace. Ce biofilm retient les nutriments et gêne le mouvement des microorganismes. La composition et la distribution de la MO dans la glace étaient en partie reliées aux algues de glace. De plus, la thermodynamique de la couverture de glace peut contrôler la distribution des microorganismes et de la MO, comme observé lors de la fonte de la glace à ISPOL et lors du refroidissement de la banquise à SIMBA. La distribution des bactéries n'est pas corrélée avec celle des algues et de la MO dans la glace. En effet, la consommation de la MO par les bactéries semble être limitée non pas par la nature chimique des substrats mais par un facteur extérieur affectant le métabolisme bactérien tel que la température, la salinité ou une toxine. Le dysfonctionnement de la boucle microbienne menant à l'accumulation de la MO dans la glace a donc été mis en évidence dans nos échantillons.

De plus, le biofilm formé par les TEP est aussi impliquée dans l'attachement des cellules et autres composés aux parois des canaux de saumure et donc dans la séquence de largage lors de la fonte. Cette séquence semble propice au développement d'efflorescences phytoplanctoniques dans la zone marginale de glace. Les microorganismes originaires de la glace (surtout ceux de taille < 10 µm) semblent capables de croître dans la colonne d'eau et l'apport en nutriments organiques et inorganiques apparaît favorable à la croissance des microorganismes pélagiques.

Enfin, l'influence des activités hétérotrophes sur l'export de carbone et l'efficacité de la pompe biologique à carbone a été évaluée dans la couche de surface (0-100 m) et mésopélagique (100-700 m) de l'océan. Au contraire de la glace, les biomasses et activités bactériennes suivaient les distributions de la chlorophyll *a* et de la MO. Elles diminuent fortement en dessous de 100-200 m, néanmoins les valeurs intégrées sur la hauteur de la colonne d'eau indiquent que la reminéralisation de la MO par les bactéries dans la zone mésopélagique est loin d'être négligeable, spécialement dans une région dominée par les diatomées.

Abstract

The Southern Ocean (ca. 20% of the world ocean surface) is a key place for the regulation of Earth climate thanks to its capacity to absorb atmospheric carbon dioxide (CO_2) by physico-chemical and biological mechanisms. The biological carbon pump is a major pathway of absorption of CO_2 through which the CO_2 incorporated into autotrophic microorganisms in surface waters is transferred to deep waters. This process is influenced by the extent of the primary production and by the intensity of the remineralization of organic matter along the water column. So, the annual cycle of sea ice, through its *in situ* production and remineralization processes but also, through the release of microorganisms, organic and inorganic nutrients (in particular iron) into the ocean has an impact on the carbon cycle of the Southern Ocean, notably by promoting the initiation of phytoplanktonic blooms at time of ice melting.

The present work focussed on the distribution of organic matter (OM) and its interactions with the microbial network (algae, bacteria and protozoa) in sea ice and ocean, with a special attention to the factors which regulate the biological carbon pump of the Southern Ocean. This thesis gathers data collected from a) late winter to summer in the Western Pacific sector, Western Weddell Sea and Bellingshausen Sea during three sea ice cruises ARISE, ISPOL-drifting station and SIMBA-drifting station and b) summer in the Sub-Antarctic and Polar Front Zone during the oceanographic cruise SAZ-Sense.

The sea ice covers were typical of first-year pack ice with thickness ranging between 0.3 and 1.2 m, and composed of granular and columnar ice. Sea ice temperature ranging between -8.9°C and -0.4°C, brines volume ranging between 2.9 to 28.2% and brines salinity from 10 to >100 were observed. These extreme physico-chemical factors experienced by the microorganisms trapped into the semi-solid sea ice matrix therefore constitute an extreme change as compared to the open ocean. Sea ice algae were mainly composed of diatoms but autotrophic flagellates (such as dinoflagellates or *Phaeocystis* sp.) were also typically found in surface ice layers. Maximal algal biomass was usually observed in the bottom ice layers except during SIMBA where the maxima was localised in the top ice layers likely because of the snow and ice thickness which limit the light available in the ice cover. During early spring, the algal growth was controlled by the space availability (i.e. brine volume) while in spring/summer (ISPOL, SIMBA) the major nutrients availability inside sea ice may have controlled algal growth. At all seasons, high concentrations of dissolved and particulate organic matter were measured in sea ice as compared to the water column. Dissolved monomers (saccharides and amino acids) were accumulated in sea ice, in particular in winter. During spring and summer, polysaccharides constitute the main fraction of the dissolved saccharides pool. High concentrations of transparent exopolymeric particles (TEP), mainly constituted with saccharides, were present and their gel properties greatly influence the internal habitat of sea ice, by retaining the nutrients and by preventing the protozoa grazing pressure, inducing therefore an algal accumulation. The composition as well as the vertical distribution of OM in sea ice was linked to sea ice algae. Besides, the distribution of microorganisms and organic compounds in the sea ice was also greatly influenced by the thermodynamics of the sea ice cover, as evidenced during a melting period for ISPOL and during a flood-freeze cycle for SIMBA. The bacteria distribution in the sea ice was not correlated with those of algae and organic matter. Indeed, the utilization of the accumulated organic matter by bacteria seemed to be limited by an external factor such as temperature, salinity or toxins rather than by the nature of the organic substrates, which are partly composed of labile monomeric saccharides. Thus the disconnection of the microbial loop leading th OM accumulation was highlighted in sea ice.

In addition the biofilm formed by TEP was also involved in the retention of cells and other compounds (DOM, POM, and inorganic nutrients such as phosphate and iron) to the brine channels walls and thus in the timing of release of ice constituents when ice melts. The sequence of release in marginal ice zone, as studied in a microcosm experiments realized in controlled and trace-metal clean conditions, was likely favourable to the development of blooms in the marginal ice zone. Moreover microorganisms derived from sea ice (mainly <10 µm) seems able to thrive and grow in the water column as also the supply of organic nutrients and Fe seems to benefit to the pelagic microbial community.

Finally, the influence of the remineralization of organic matter by heterotrophic bacterioplankton on carbon export and biological carbon pump efficiency was investigated in the epipelagic (0-100 m) and mesopelagic (100-700 m) zones during the summer in the sub-Antarctic and Polar Front zones (SAZ and PFZ) of the Australian sector (Southern Ocean). Opposite to sea ice, bacterial biomass and activities followed Chl *a* and organic matter distributions. Bacterial abundance, biomass and activities drastically decreased below depths of 100-200 m. Nevertheless, depth-integrated rates through the thickness of the different water masses showed that the mesopelagic contribution of bacteria represents a non-negligible fraction, in particular in a diatom-dominated system.

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List of abbreviations

CO_2:	carbon dioxide
SO:	Southern Ocean
C:	carbon
PP:	primary production
OM:	organic matter
DOC:	dissolved organic carbon
POC:	particulate organic carbon
EPS:	exopolymeric substances
TEP:	transparent exopolymeric particles
d-TCHO:	dissolved total saccharides
d-MCHO:	dissolved monosaccharides
d-PCHO:	dissolved polysaccharides
p-TCHO:	particulate saccharides
DFAA:	dissolved free amino acids
SAZ:	Sub-Antarctic Zone
PFZ:	Polar Front Zone
Chl *a*:	chlorophyll *a*
BGE:	bacterial growth efficiency
BP:	bacterial production
EPA:	ectoproteolytic activity
BB:	bacterial biomass

1. Global warming and Southern Ocean

Since last decades, more and more efforts to explain and broadcast global warming issues to a large audience have been made by scientists and policy makers. This approach has been rewarded by the Nobel Peace Prize given to the Intergovernmental Panel on Climate Change (IPCC) and Al Gore in 2007. In an extreme view, climate change could even be felt as a sort of "fashion" or marketing tool (a process known as "greenwashing", with e.g. publicity for low pollution vehicles). However nowadays the global warming is incontestable; Earth's global temperature has increased by 0.74°C over the last 100 years (1906–2005) (Figure I.1, IPCC report 2007). Since the industrial revolution (late 18th-early 19th), the atmospheric concentration of greenhouse gases has significantly increased: atmospheric concentration of the most important greenhouse gas, carbon dioxide (CO_2) reaching 379 ppm in 2005 is now far higher than the natural range over the last 650 000 years (180 to 300 ppm) (Figure I.1, IPCC report 2007). The consumption of fossil energies, the modification of land use or the cement industries are some examples of the modern sources of anthropogenic CO_2 emission. It is very likely (> 90% certainty) that the global warming of Earth is caused by the human activities (IPCC report 2007).

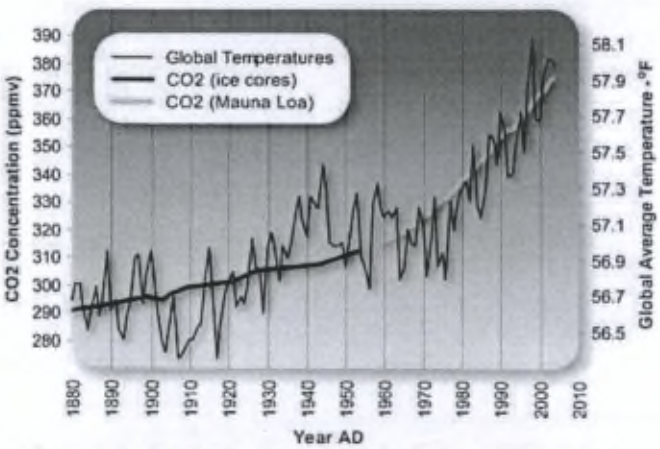

Figure I.1: Global average temperature and carbon dioxide concentration, 1880-2004. (Source: www.whrc.org/resources/online_publications/warming_earth/scientific_evidence.htm)

When addressing climate issues, discussing the role of the ocean is straightforward. The oceans, which cover ca. 70% of the surface of our planet, influence the Earth's climate by absorbing and storing heat and gases. At a millennium scale, oceans control and regulate the atmospheric concentration of CO_2 (Falkowski 2002). The ocean constitutes a major reservoir of carbon of 38,000 Gt C (Figure I.2), mainly in the dissolved inorganic form. About 680-700 Gt C is under the dissolved organic form and represents the second largest pool of oceanic carbon (Hansell 2003). Thus dissolved organic matter in the ocean contains roughly the same amount of carbon as is present in the atmosphere in the form of CO_2 (Figure I.2, Hansell and Carlson 2001, Nagata 2008).

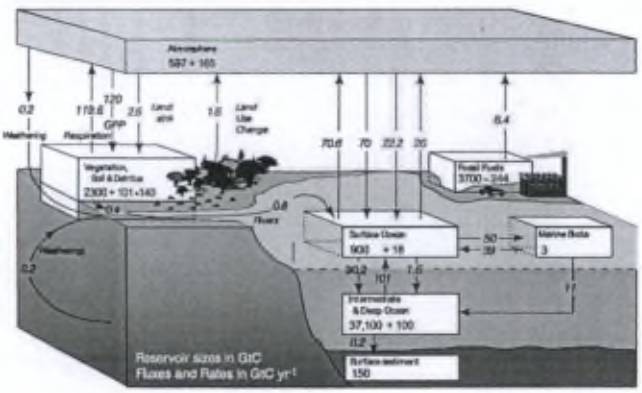

Figure I.2: Carbon cycle (Source: IPCC report 2007)

The Southern Ocean (SO) representing ca. 20% of the World Ocean surface is suggested to be a key place for the regulation of Earth's climate (Bopp et al. 2001, Takahashi et al. 2002) The SO extent is defined by the water masses around the Antarctic continent and is limited in the north by a quasi-stationary front called Polar Front or Antarctic Convergence (Figure I.3). The Polar Front limits the boundary between cold and warm surface sub-Antarctic waters and its position varies between latitudes of 48°S and 61°S.

Figure I.3: Southern Ocean extent defined by the Antarctic Convergence. (*Credits and source:* Philippe Rekacewicz. http://maps.grida.no/go/graphic/the_antarctic_convergence)

The SO is also characterized by a highly fluctuating seasonal sea ice cover (Figure I.4). Depending on its thermodynamical stage, the sea ice cover may or not constitute a physical barrier to air-ocean gas exchanges (Delille et al. 2007). The surface of the sea ice which covers the Antarctic Ocean varies between 3.8 x 10^6 km² (in February) and 19 x 10^6 km² (in September) (Zwally et al. 1983). During winter, the surface of the SO covered by sea ice reaches up to 55% of its superficies. This also means that the major fraction (90%) of the sea ice is annual and melts each year. Two types of ice have been defined, depending on their localisation. "Fast ice" is the ice formed near the coast and which extents up to 35 km seawards (Lange et al. 1989, Jeffries et al. 1993). In opposition, "Pack ice" refers to floating consolidated sea ice that is either freely floating or has been blocked by land-attached ice while drifting. Pack ice is commonly associated to sea ice formed in the open ocean.

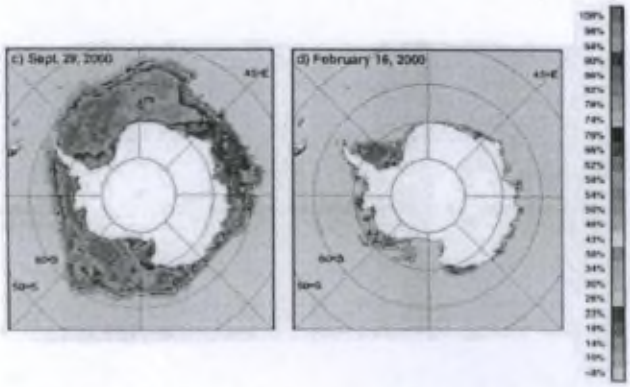

Figure I.4: Sea ice extent around Antarctica in early autumn (September) and early spring (February) (Comiso 2003)

The SO hosts the largest of the world's ocean currents: the Antarctic Circumpolar Current (ACC). The ACC connects together the Indian, Atlantic and Pacific Ocean basins (Figure I.5) and has thus a powerful influence on the Earth's climate. Then, the SO is an important actor of the global thermohaline circulation. The thermohaline circulation is an 'overturning' circulation in which warm water flows poleward near the surface and is subsequently converted into cold water that sinks and flows equatorward in the interior (Toggweiler 1994). The cooling of surface waters, the sea ice formation during winter and subsequent rejection of sea salts constitute a major drive for the world's ocean circulation.

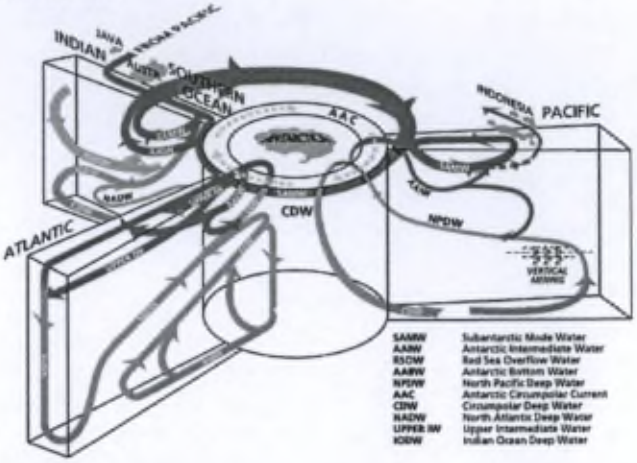

Figure I.5: water currents around Antarctica. Rintoul et al. (2001).

The significant role of the SO in the air-ocean exchanges of CO_2, and Earth's climate, happens on the one hand by physical mechanisms and on the other hand by biological mechanisms. Indeed where deep water formation occurs, the cooling of surface waters increases their capacity to dissolve CO_2 (the dissolution of CO_2 in seawater indeed increases when temperature decreases). These cold and thus denser water masses sink and bring CO_2 with them, separating it from contact with the atmosphere. Because of the time scales of the overall oceanic circulation, these waters will be separated from contact from the atmosphere for laps of time of thousand of years (Chisholm 2000). This mechanism is refereed to as the ***physico-chemical pump*** (Figure I.6 right).

The second CO_2 absorption mechanism involves autotrophic organisms. In presence of light and inorganic nutrients, the CO_2 dissolved in surface waters, in equilibrium with atmospheric CO_2, is transformed into organic molecules by the photosynthesis process (Figure I.6 left).

$$CO_2 + 2\,H_2O + \text{photons} \rightarrow (CH_2O)_n + H_2O + O_2$$

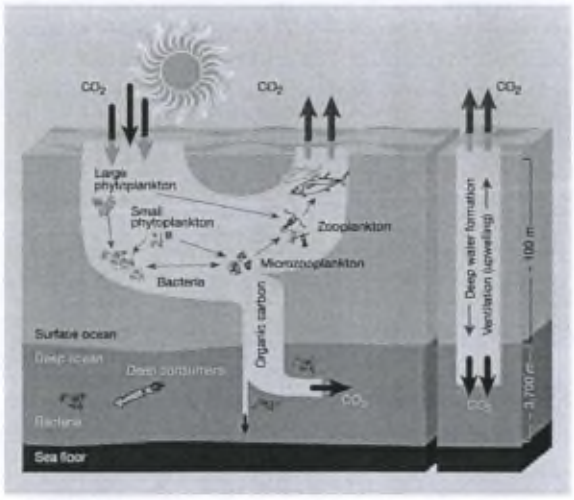

Figure I.6: Physico-chemical (right) and biological (left) carbon pumps (Chisholm 2000).

A part of this organic carbon is transferred to herbivorous and carnivorous organisms forming the food web. Another fraction leaves the surface ocean and sinks to the deep ocean as particulate or dissolved organic carbon. This export of particulate organic carbon occurs via sedimentation of aggregates (marine snow) or faecal pellets. Dissolved organic carbon, when accumulated in the surface layer, can be exported by deep convection. This downward transport of particulate and dissolved organic carbon is called the ***biological carbon pump***. This mechanism is further developed in the second section of the introduction part.

The present rapid warming of the Earth's atmosphere might have several consequences such as increase of the sea level, melting of ice, slowdown or shutdown of the oceanic circulation, precipitation changes, ocean acidification or changes in ecosystems structure (IPCC report 2007). While the decrease of the ice extent in Arctic is already observed (Comiso 2003), the situation in Antarctic is less clear (Goosse et al. 2009). Firstly the expected reduction of sea ice extent will have a positive feedback effect on climate change through the greater absorption of heat by newly exposed seawater. Secondly, it would also affect the oceanic circulation through the modification of the formation of dense water masses from sea ice formation and consequent brine rejection. Thirdly, as sea ice is a habitat for microorganisms especially algae, the modification of the primary productivity in sea ice may influence the carbon cycle through a) its seeding role for phytoplankton bloom in the marginal ice zone and b) its importance as food source for under-ice zooplankton and krill during winter. The shortage of krill, a key species of the Antarctic, would in turn in account for the decline in the numbers of higher trophic levels (penguins, whales, seals or seabirds).

2. Biological carbon pump in the Southern Ocean

The biological carbon pump is the transfer of photosynthetically fixed organic carbon from surface to the deep ocean (see section 1 and Figure I.6). Its efficiency is determined by the fraction of carbon finally buried in the seafloor. It depends on the magnitude of primary production, the structure of the algal community and the related food web but also on the importance and location in the water column of the bacterial organic matter degradation (Ducklow et al. 2001a). At the difference of the degradation of organic matter occurring throughout the water column, primary production is restricted to the upper ocean where there is light enough to support photosynthesis. The biological carbon pump of the SO is also likely influenced by sea ice as developed here after.

2.1. Primary production in the Southern Ocean

2.1.1. An HNLC open ocean

As mentioned above, primary production (PP) is the basis of the biological C pump. The PP and algal growth is determined by physical factors such as light availability and temperature but also by nutrients availability. The Southern Ocean has been defined as a "high nutrient low chlorophyll" (HNLC) region, which means a region where phytoplanktonic biomass remain low despite the presence of high levels of major nutrients in surface waters year-round (Chisholm and Morel 1991, Banse and English 1997). At first glance, this situation seemed abnormal because in most of the ecosystems the major inorganic nutrients are totally consumed by the algal uptake at the end of the growth period. Then minor nutrients mainly iron have been reported as limiting element of PP in this remote ocean (Martin and Fitzwater 1988, de Baar et al. 1995). In the SO however light and iron have been shown as co-limiting factors of PP (Lancelot et al. 1993, de Baar et al. 2005).

2.1.2. Structure of the microbial community: Microbial network vs. linear trophic chain

Nutrients supply (in particular iron) and light levels have an effect on phytoplanktonic growth and species composition. In low iron (and light) conditions (Figure I.7, upper panel), the phytoplanktonic community is dominated by small cells (<10 µm, nanophytoplankton). These small phytoplanktonic cells can be rapidly grazed by microprotozooplankton. In such a trophic network, no bloom occurs even if the nanophytoplankton is not really limited itself because prey and grazer are ubiquist and have the same turnover rate. This ***microbial*** trophic

network leads to the retention of C and nutrients in the surface waters thanks to a constant and rapid recycling, and thus limits the carbon export.

By contrast, in high iron and sufficient light conditions (Figure I.7, lower panel), large phytoplanktonic cells (such as diatoms) can develop. The large diatoms are grazed by meso- and meta-zooplankton. Because of their respective generation times, bloom events are possible. Such a high algal concentration may favour on the one hand, sinking and aggregates formation by collision and on the other hand, viral lysis releasing particulate (POC) and dissolved (DOC) organic carbon. All these may be exported to the deep ocean. Faecal pellets also contribute to the downward flux. Still this ***linear*** trophic chain is spatially and temporally ephemeral in Antarctic (Boyd 2002).

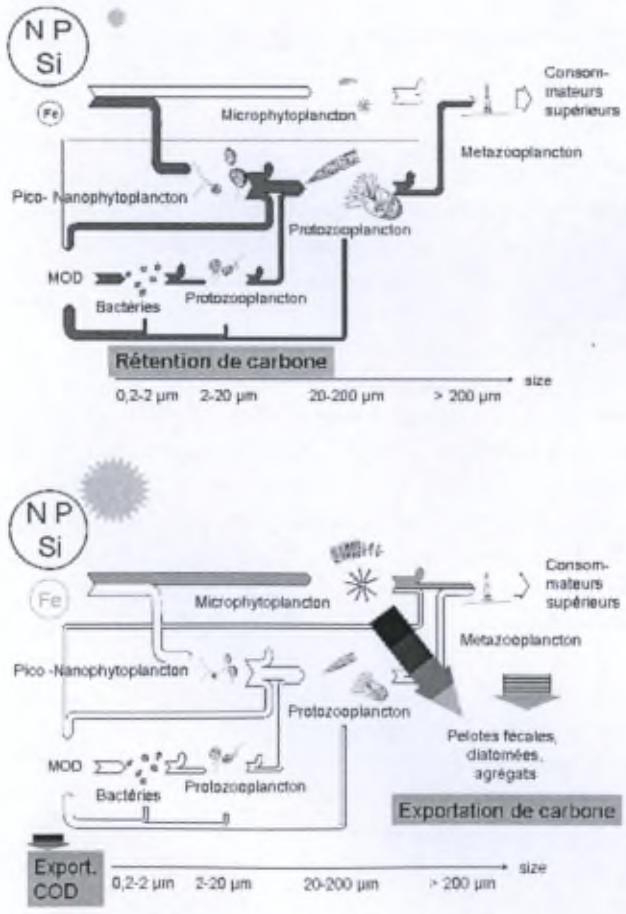

Figure 7: Microbial network and linear trophic chain

2.2. Role of remineralization of organic matter

As organic matter (OM) sinks into the deep ocean, its carbon content is remineralized back to CO_2 by heterotrophic microorganisms (see also 4.4.). The deeper the remineralization will take place, the longer the regenerated CO_2 will be stored away from the atmosphere. Heterotrophic bacteria, as the primary consumers of organic matter, control its remineralization (Azam et al. 1983, Kirchman 1990). The full understanding of the bacterial remineralization of the OM during its downward transfer is thus essential to evaluate its impact on the biological carbon pump. Especially the microbial processes taking place in the mesopelagic or twilight zone of the ocean (~100-1000 m) need to be better documented (Dehairs et al. 2008).

Indeed it is estimated that more than 90% of the organic carbon annually exported from global surface waters (~10 Gt C/yr) is remineralized in mesopelagic waters (Boyd and Trull 2007). The mesopelagic zone is considered as the depth layer where most of the changes in sinking organic matter occur (Aristegui et al 2005). A steep decrease of POC content with depth has been observed (see e.g. Martin et al. 1987, Buesseler et al. 2007), but also a depth-depending change in the biochemical composition of the sinking organic material (Sheridan et al. 2002, Lee et al. 2004). Delivery of OM to the twilight zone occurs via sinking particles (dead organisms), marine snow, faecal pellets, migrant zooplankton or vertical diffusion or mixing (Turner 2002). In the mesopelagic zone, even if heterotrophic activities are generally lower than in surface, the integrated activities over the much thicker layer covered by mesopelagic waters might represent a non-negligible fraction of the whole water column (del Giorgio and Duarte 2002). Still the bacterial processes occurring at depth may differ from surface ones with e.g. difference in bacterial communities (Herndl et al. 2005, Tamburini et al. 2009).

2.3. Role of sea ice

Sea ice influences nutrients and carbon cycles by providing a habitat for a high diversity of micro-organisms which develop intense activities.

Sea ice primary production is highly variable but in average annual PP ranges between 0.3 and 34 g C m^{-2} yr^{-1} (Arrigo 2003). Total annual primary production of Antarctic sea ice assemblages has been estimated to range between 63 and 70 Tg C yr^{-1} which corresponds to about 5% of the estimated total PP of the seasonal ice zone (Lizotte 2001). According to Arrigo et al. (2008), sea ice PP contributes largely (10 to 28%) to total ice-covered region production. Still the sea ice PP only represents a small fraction (1%) of the total of all open ocean regions even if the PP in the marginal ice zone

Table I.1: Surface, Primary Production (PP), daily PP and annual PP are given for each of these provinces, along with their contribution to the PP of all Southern Ocean. The four ecological provinces of the Southern Ocean were defined by Arrigo et al. (2008) on basis of sea ice coverage and bathymetry, which include the pelagic, the marginal ice zone (MIZ), the continental shelf and the part of the MIZ on the continental shelf (MIZ-shelf).

From Arrigo et al. (2008)	Surface	PP	%	Daily PP	Annual PP
	10^6 km^2	$Tg\ C\ yr^{-1}$		$mg\ C\ m^{-2}\ d^{-1}$	$g\ C\ m^{-2}\ yr^{-1}$
All Southern Ocean	34.14 ± 0.23	1949 ± 70		156	57.0
• **Pelagic**	32.0 ± 0.19	1729 ± 61	~ 90	148	54.0
• **MIZ**	1.51 ± 0.07	86.7 ± 12.6	4.5	158	57.5
• **Continental shelf**	0.39 ± 0.04	66.1 ± 12.2	~ 3.5	460	109
• **MIZ-shelf**	0.25 ± 0.01	27.3 ± 4.67	1.4	303	67.8
Sea ice		63-70*			0.3-34**

* Lizotte (2001), ** Arrigo (2003)

(MIZ) is the second most important contributor to the annual PP in the Southern Ocean (Table I.1, Arrigo et al. 2008). Indeed phytoplankton blooms in the MIZ have been recognized as a major site of primary production in the Southern Ocean (Lizotte 2001), likely because of the formation of a shallow (favorable light) and stable surface mixed layer due to freshening after ice melt (e.g. Smith and Nelson 1986) and the seeding of populations of algae released from sea ice (e.g. Garrison et al. 1987). In addition release of iron from melting ice may be a factor responsible for the occurrence of algal blooms in the MIZ (Sedwick and DiTullio 1997, Lancelot et al. submitted).

In fact, the relative importance of sea ice PP as compared to the total Southern Ocean PP is not the only important factor but rather, the timing and the geographical location of the ice-based contribution are critical in determining the contribution of sea ice for primary production of the Southern Ocean (Lizotte 2001).

3. Sea ice

3.1. Incorporation of OM, nutrients and micro-organisms during sea ice formation

The microorganisms (algae, bacteria and protozoa), nutrients and organic matter present in the water column at the time of ice formation can be incorporated into the ice matrix in formation (e.g. Krell et al. 2005). They can be physically concentrated by the mechanisms of sea ice consolidation (Garrison et al. 1983, 1989, Giannelli et al. 2001), involving harvesting or scavenging of cells by frazil ice crystals rising through the water column (Weeks and Ackley 1982) or by the increase of available crystal substrate in the skeletal layer of the columnar ice or in the platelet ice layer (Dieckmann et al. 1986). Wave induced pumping also contributes to enrichment (Weissenberger et al. 1992, Weissenberger and Grossmann 1998).

The formation of sea ice results of the cooling of the seawater in contact with the atmosphere. The processes by which the ice forms will determine its texture (granular *vs* columnar). When seawater cools down the freezing point (-1.8°C for a salinity ca. 34 ‰), crystals of ice (of 2-4 mm) start to appear (Figure I.8). This stage is called "frazil ice". These crystals, less dense than seawater, rise in the water column and accumulate at the surface of the ocean. They form then "grease ice". The ice continues to grow and under still meteorological conditions, a matrix of 1 to 10 cm of granular ice called "nilas" will form. Under turbulent conditions, winds and waves compressed frazil crystals into larger units (from 30 cm to some meters of diameter), of granular texture, called "pancake ice". After this stage (nilas or pancake), ice continues to grow but the process is completely different. Some water molecules, not in contact with air anymore, freeze at the bottom of the existing ice bloc. The crystals formed by this process are long and orientated and constitute the columnar ice.

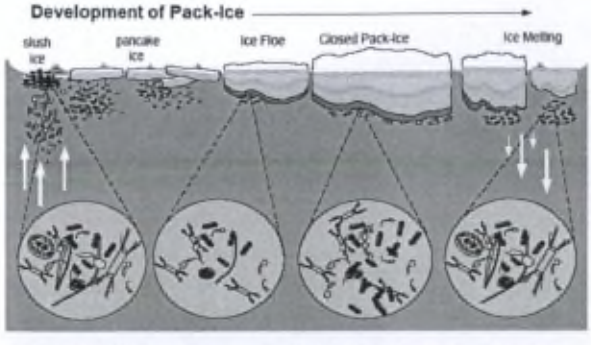

Figure I.8: Schematic representation of pack ice development where sea ice formation and growth are coupled to the colonization by microbial communities. Source: Belem (2002), modified from Ackley and Sullivan (1994).

When snow falls occur, a relatively important layer of snow may accumulate above the ice sheet some days after its formation. Because of the weight of the snow,

the ice sheet can move down and allow seawater to infiltrate into the snow. "Snow ice" can be formed when this snow full of water refreeze and create a solid ice layer at the top of the ice unit.

During the ice growth, salts and "impurities" (i.e. nutrients, microorganisms and organic matter) are rejected out of the ice crystal matrix into an interstitial liquid phase called brine. Depending on the ice sheet temperature and salinity, isolated pockets, tubes or interconnected channels of brine can be observed (Golden et al. 1998). The brine liquid phase is the place where sea ice microorganisms really live, being attached to the surface of the brine channels or pockets.

3.2. Life inside sea ice

3.2.1. Physico-chemical environment

Sea ice is a particularly variable environment, extreme and ephemeral for microorganisms (Brierley and Thomas 2002). During their incorporation into the ice, the microbial communities undergo major changes in their chemical and physical environment (temperature, salinity, light, pH, nutrients...) as compared to the water column. These conditions further continuously change during the consolidation and ultimately during the melting of sea ice. Due to the confinement of microorganisms into isolated microenvironments, chemical conditions such as nutrient concentrations, toxic compounds accumulation or limitation of gas exchanges may strongly influence microbial activities. Also, significantly higher concentrations of organic matter in sea ice as compared to seawater which are likely present as transparent exopolymeric particles modify the viscosity of the ecosystem (Thomas and Papadimitriou 2003, Krembs and Deming 2008, further details in section 5).

3.2.2. Locations of microbial communities

Throughout the ice cover, high gradients of temperature, salinity, brine volume and light are typically observed (Figure I.9).

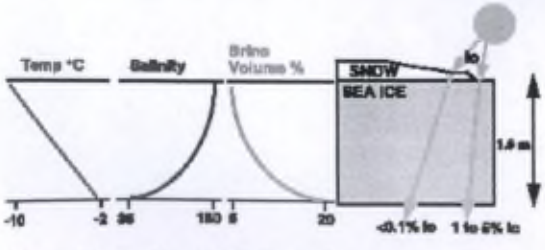

Figure I.9: Profiles of irradiance, temperature, salinity and brine volume in an ice sheet. Thomas and Dieckmann (2002).

The growth of microorganisms in sea ice is controlled by ice formation processes (Eicken 1992) and physical and chemical environmental factors such as light conditions, salinity, temperature, ice texture, nutrient availability and trophic interactions (Garrison 1991, Palmisano and Garrison 1993, Ackley and Sullivan 1994, Lizotte 2001). Structure of the brine channels and surfaces are also key parameters (Krembs et al. 2000, 2001).

The vertical distribution of microbial communities is highly variable (Horner 1985, Horner et al. 1988, 1992). Different assemblages are typical of fast and pack ice (Arrigo 2003). In pack ice, the main communities are found in surface, interior and bottom communities (Figure I.10). Each of those meets different environmental conditions, as detailed in Ackley and Sullivan (1994).

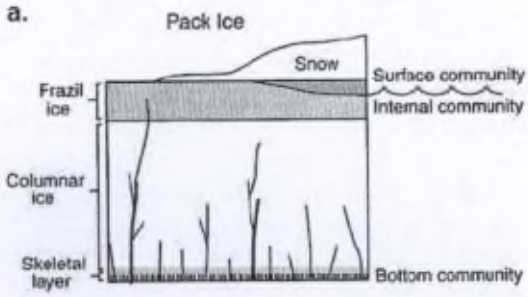

Figure I.10: Schematic illustration of pack ice showing the major physical features and locations of microbial habitats (from Arrigo and Thomas 2004)

The surface community, originating from seawater infiltration, benefit from optimal light conditions while nutrient depletion can occur. Yet some flooding and infiltration events could bring nutrients and allow high algal growth (Arrigo et al. 1995, Thomas et al. 1998).

The interior community results from the incorporation of microorganisms by frazil ice or from autumn bloom. Their habitat (brine channels and pockets) has extreme conditions of temperature and salinity. Generally restricted nutrient supply occurs as less exchange with seawater is possible.

The bottom community develops in the lower part of the congelation ice. It receives low light levels while it experiences relatively stable temperature and salinity conditions. Nutrients replenishment is possible from the seawater beneath.

3.2.3. Composition of microbial communities

The sympagic (ice-associated, Horner et al. 1992) community includes bacteria, viruses, unicellular algae,

fungi, worms and crustaceans. A high diversity of microorganisms belonging to the eukaryotes, prokaryotes and *Archaea* has been observed in sea ice (Lizotte 2003). Diatoms are the most reported autotrophic protists but other autotrophic microorganisms such as ciliates, dinoflagellates or prymnesiophytes (e.g. *Phaeocystis sp.*) also inhabit sea ice. Heterotrophic microorganisms include bacteria, heterotrophic protists and *Archaea*. The initial incorporation of microorganisms reflects the algal community composition in the water column at time of freezing (Garrison and Buck 1985). Bacteria are incorporated when attached to algal cells (Weissenberger and Grossmann 1998). Then environmental conditions potentially exert a selection on the microorganisms which could lead to a succession of species in function of the adaptation capability of each microorganism (Lizotte 2003) and trophic interactions.

3.3. Melting of sea ice

Sea ice melting releases into the seawater low salinity water coming from the crystal matrix. Under favourable meteorological conditions (i.e. low wind and waves), this low-salinity, low-density water will form a shallow mixed layer (<40 m, Smith and Nelson 1986). This physical process creates favourable light conditions for phytoplankton growth.

In addition sea ice melting releases microorganisms, organic matter and nutrients (e.g. iron) into the surface ocean layer. Sea ice algae are potentially a seeding population for ice-edge blooms as suggested by similarities between ice and pelagic communities (Leventer 2003). Still once in the water column, algae could also directly sediment (Riebesell et al. 1991) or be grazed by copepods (Fransz 1988) and krill (Marschall 1988). Release of iron from melting ice may be a factor responsible for the occurrence of algal blooms in the MIZ: Sedwick and DiTullio (1997) observed that the input of iron along the ice-receding edge could increase iron concentration and trigger the phytoplanktonic production. Similarly, the supply of organic substrates by sea ice melting could support bacterial growth in surface waters (Kähler et al. 1997, Giesenhagen et al. 1999). Complexation between iron and organic matter, like more than 90% of dissolved iron in ocean surface water (Wu and Luther 1995, Boyé et al. 2001) and the one suggested in sea ice (Schoemann et al. 2008), could also influence the bioavailability of iron (Maranger and Pullin 2003, Hutchins et al. 1999, Maldonado et al. 2005) and keeps iron in solution, avoiding loss by precipitation and adsorption onto particles.

The fate of the sea ice components would depend on the mixed layer depth, on the timing and rate at which ice compounds are released into the pelagic system, but also on the kind of compounds (e.g. high or low aggregation capacity) and on the interactions with the pelagic fauna. Nevertheless these multiple interlaced processes are determining the efficiency of the biological carbon pump of the Southern Ocean.

4. Organic matter

4.1. Definitions and biogeochemical significance

The organic matter is a matter composed of molecules containing carbon. Historically, the term "organic" means that this kind of compound has to be synthesized in living organisms and opposes to inorganic compounds such as minerals.

The organic matter is composed of a) the biomass of living microorganisms and b) the "detrital", nonliving compounds which are derived from dead organisms or are actively produced (e.g. remnants of dead cells, excretion products...) but which do not constitute the living biomass itself. Generally the nonliving compounds are mainly in the dissolved fraction.

The organic matter, in particular the dissolved organic matter (DOM) is an essential component of the marine food web and carbon cycle (Pomeroy et al. 2007). DOM is central to the concept of "microbial loop" which is the pathway where DOM taken up by bacteria is converted into living POM and in turn grazed upon by protists (Figure I.11, Azam et al. 1983). This pathway is considered as major carbon flux in marine ecosystem that supports higher trophic levels and drives nutrient regeneration in the upper ocean; one-half of oceanic primary production on average is channelled into the microbial loop (Azam 1998).

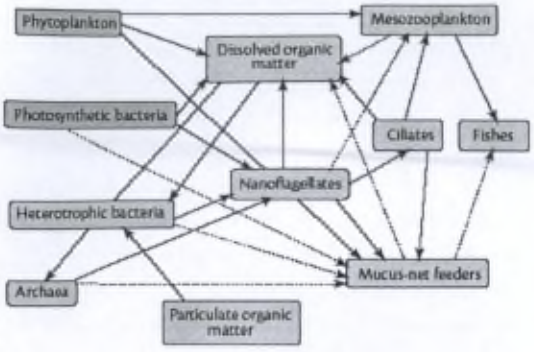

Figure I.11: Simplified diagram of the ocean's food web showing the dominant roles of the microbial loop. The major fluxes of carbon and energy are delineated by continuous lines; fluxes usually of lesser magnitude are delineated by broken lines. Other than the meso-zooplankton (including mucus-net feeders) and fishes (all right boxes), the boxes represent organisms that are a part of the microbial loop (green = photosynthetic and yellow = heterotrophic). Pomeroy et al. (2007)

4.2. Characterization of organic matter

Organic matter in seawater is a complex mixture of organic compounds with diverse physical structures, chemical compositions and reactivity (Nagata 2008).

4.2.1. Size distribution

In oceanography, organic matter is usually classified into particulate and dissolved organic matter (POM and DOM). This separation is purely operational, depending on the filter porosity (between 0.2 and 0.7 µm). The organic matter is in fact a continuum made of molecules of increasing sizes from small molecules to macromolecules, colloids, small and large particles (Figure I.12, Verdugo et al. 2004). Transformations between organic pools also occur along the size continuum of organic matter.

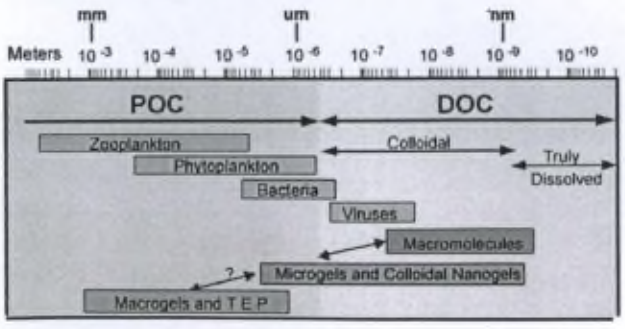

Figure I.12: Size continuum of organic matter and marine gels. Verdugo et al. (2004)

Dissolved organic matter (DOM) is the dominant fraction (97%, Benner 2002) of total oceanic carbon. The low molecular weight (LMW) fraction (less than 1 kDa) is the major size fraction of DOM throughout the whole water column in the ocean (Ogawa and Tanoue 2003, Figure I.13b). Most old DOM in the deep ocean is LMW-DOM (75–80% of the bulk DOC). This observation suggests that the HMW-DOM (> 1 kDa) is relatively reactive while the LMW-DOM is the major form of refractory DOM in the ocean (Ogawa and Tanoue 2003). This is in agreement with the size-reactivity continuum hypothesis of Amon and Benner (1996) which proposed that lability of DOM decreases with decreasing molecular size. In fact two distinctive LMW-DOM components and different processes occurring at different time scales have to be considered: a fast cycling and labile LMW-DOM pool (such as dissolved free amino acids and free sugars) and a slow cycling, refractory LMW-DOM pool produced by diagenetic processes (Nagata 2008).

4.2.2. Biochemical composition

Most of the oceanic dissolved organic matter (> 80%) is still uncharacterized at the molecular level (Benner 2002, Ogawa and Tanoue 2003, Figure I.13c). However, saccharides are the most abundantly identified component of oceanic DOM and typically account for 10-25% of DOC (Pakulski and Benner 1994, Bhosle et al. 1998, Borsheim et al. 1999). Amino acids, making also part of the identified fraction of the DOM, account for 1-3% of the DOC in surface waters (Benner 2002). Dissolved total saccharides (d-TCHO) exist as free monomers (MCHO) and as combined sugars, such as polysaccharides (PCHO). Similarly, in sea ice, saccharides have already been reported as a major component of the DOM pool (Herborg et al. 2001). Amon et al. (2001) reported a large contribution of neutral sugars and amino acids in Arctic ice, which are freshly produced and are of algal origin.

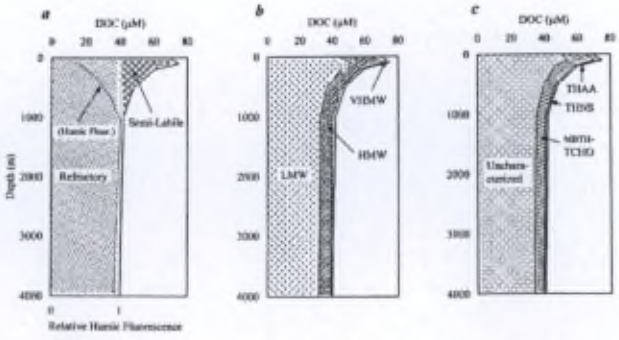

Figure I.13: Typical vertical distributions of DOC in the ocean according to **a**: conceptual classification of biological reactivity, **b**: size distribution and **c**: chemical composition. See Ogawa and Tanoue (2003) and text for details.

In surface oceans (up to ~100 m), the composition of about 80% of the particulate organic matter (POM) is known (Wakeham et al. 1997, Figure I.14). POM is

Table I.2: Characteristics of Labile, Semi-Labile and Refractory DOC in the Oceans. From Nagata (2008).

Pool	Concentration (μM)	Turnover time	Function	Chemical Identity
Labile	<1	<hours-days	Fueling bacterial production	Includes dissolved free amino acids, free sugars, and labile protein
Semi-Labile	10-30	Months-years	Export	Unknown
Refractory	40	>1000 years	Storage	Unknown

mainly composed of proteins, polysaccharides and lipids as algae (Bertilsson and Jones 2003). By contrast, below ~100 m, the fraction of POM characterized decreases up to about only 20% deep oceans (Figure I.14).

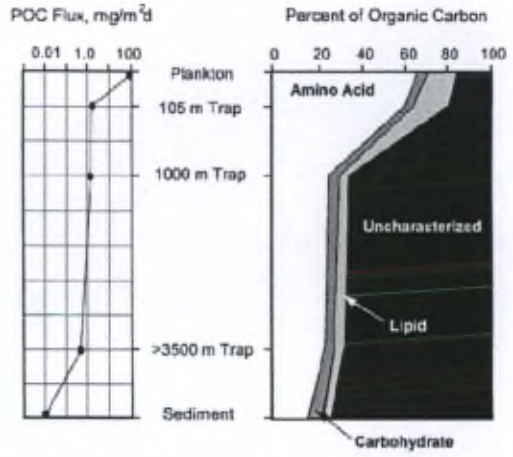

Figure I.14: Typical vertical distribution of POC in the ocean. Wakeham et al. (1997), Hedges et al. (2000).

At the interface between DOM and POM, a biogeochemically important class of compounds are the transparent exopolymeric particles (TEP). TEP are formed from dissolved precursors, extracellular polymeric substances (EPS) produced by algae and bacteria. TEP consists mostly of polysaccharides, negatively charged and are very sticky particles that exhibit the characteristics of gels (Passow 2002). Other substances, including proteins or trace elements (e.g. iron) may be enclosed in or adsorbed on to TEP (Passow 2002). The size of TEP and precursors ranges from colloidal (1 kDa) to hundreds of micrometres (Figure I.12, Passow 2002). TEP play major roles in attachment of cells to surface, aggregation, sedimentation, organic carbon mineralization, microbial loop and cycling of dissolved metals (Bhaskar and Bhosle 2006). Studies in Antarctic and Arctic sea ice have also highlighted the importance of TEP (Krembs and Engel 2001, Krembs et al. 2002, Meiners et al. 2004) in sea ice.

4.2.3. Reactivity

The DOM pool can also be divided in three fractions based on lability (or degradability), i.e. in function of the turnover rate of the organic matter (Figure I.13a, Table I.2, Carlson 2002, Nagata 2008). Labile material constitutes a small percentage of the DOC pool. It is composed of dissolved monosaccharides (d-MCHO) and dissolved free amino acids (DFAA) meaning that they are easily taken up by bacteria and found at nanomolar concentrations in the open ocean (Table I.2, Carlson 2002). An important feature of the semi-labile DOC is that it is exportable and thus its consumption can occur in a location different from where it is produced (Nagata 2008).

4.3. Sources and transformations of OM

In aquatic systems, the first important source of OM is due to algae which use solar energy to transform inorganic carbon into reduced C compounds (Bertillson and Jones 2003). Upon cell death, the photosynthetically fixed organic substances are released into the surrounding water, either directly as dissolved compounds or as particulate detritus that can act as a secondary source of DOM (Azam and Cho 1987). Other mechanisms of production and transformation of the organic matter include (Figure I.15, Nagata 2000):

- extracellular release by microorganisms (DOM, EPS)
- grazer mediated release and excretion
- release via cell lysis (by viral, bacterial, physical or osmotic mechanisms)
- solubilization of particles (Cho and Azam 1988)
- bacterial transformations and release
- abiotical formation DOC => gel => POC (Chin et al. 1998)

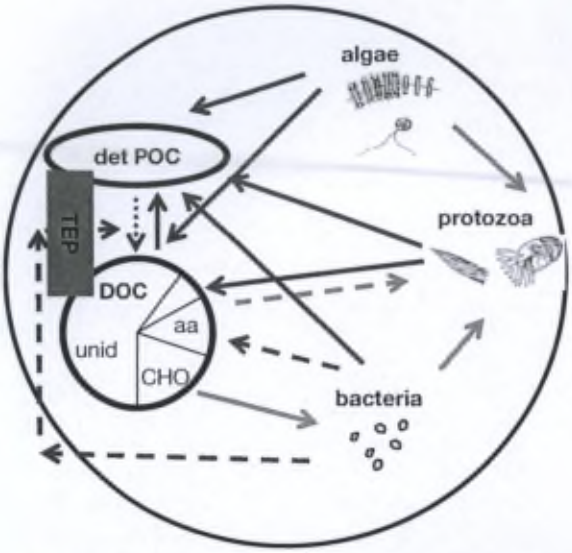

Figure I.15: Pathways of production, transformations and consumption of OM

It should be noted that in response to stress conditions such as the extreme environmental conditions of sea ice (e.g. temperature, salinity…) or at the end of an algal bloom the secretion and excretion of the following compounds could be enhanced:

- ice-active substances (IAS; Raymond 2000, Janech et al. 2006) i.e. proteins that have an affinity for ice crystals
- dimethylsulphoniopropionate (DMSP)
- extracellular polymeric substances (EPS) (Brierley and Thomas 2002)

4.4. Bacterial mineralization of organic matter

Mineralization is the degradation process of the organic matter, in which the matter is converted from an organic substance to inorganic substances, thereby becoming mineralized.

The consumption of organic matter by heterotrophic organisms is mainly due to heterotrophic bacteria. Still the uptake DOC by protozoa can also be observed (Marchant and Scott 1993, Sherr and Sherr 1988). Especially in sea ice, it seems that some algae are mixotroph (combining autotrophy and heterotrophy) to maintain under stress conditions (Palmisano and Garrison 1993, Brierley and Thomas 2002).

The main processes involved in the bacterial degradation of OM have been described by Billen and Servais (1989) and are synthesized in the Figure I.16.

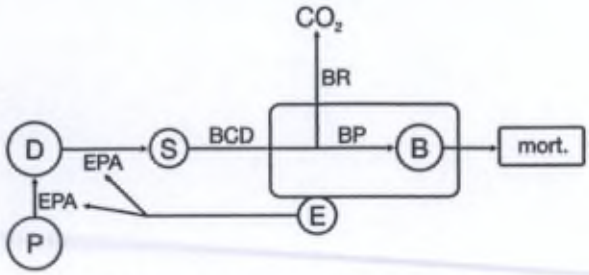

Figure I.16: Model of bacterial degradation of organic matter. Billen and Servais (1989). See text for details.

In this conceptual model the OM pool is divided into 2 pools: dissolved (D) and particulate (P). Inside each of these pools, distinction is made between: rapidly biodegradable, slowly biodegradable and refractory (turnover rates from days to millennia, see 4.2.3). Basically, bacteria are able to directly consume only small substrates (S). More complex molecules have to be hydrolysed by bacterial ectoenzymes (E) prior to be taken up by bacteria (B). After their uptake, substrates are metabolized by the bacterial cell, i.e. anabolized, with production of biomass (BP) and catabolised, through respiration (BR) and excretion. The ratio between biomass production (BP) and organic matter consumed (BCD) define the growth efficiency (BGE).

The consumption of organic matter by bacteria depends on intrinsic factors linked to the organic matter itself and on extrinsic factors linked to the bacterial metabolism. The nature of the organic matter is related to its chemical characteristics such as molecular weight, nutrient content, saccharides, amino acids, lipids content. It is determined by the source of OM, the physiological state of the producer, the species and the diagenetic state of the matter (Amon et al. 2001). Factors regulating bacterial metabolism shape in turn the degradation of OM. These factors are the temperature, pressure, nutrients availability, phylogenetic composition and physiological state of the bacterial assemblages. In sea ice, bacterial activity and viability is likely limited by low temperature (Delille 1992, Nedwell 1999, Pomeroy and Wiebe 2001), high salinity, or inhibitory compounds such as acrylic acid resulting from the breakdown of DMSP (Brierley and Thomas 2002). In the mesopelagic zone, the effect of pressure upon bacteria seems important to take into account (Tamburini et al. 2003, 2009).

5. Interactions between OM and micro-organisms in the Southern Ocean

In the Southern Ocean, the average concentration of DOC is < 60 µM C (Ogawa and Tanoue 2003) and POC is < 10 µM C (Knox 2006). Dissolved organic carbon (DOC) and particulate organic carbon (POC) background concentrations (i.e. concentration in deep ocean) are respectively 42 µM C and 3 µM C (Carlson et al. 2000). By contrast, in sea ice, extremely high levels of organic matter have been recorded, which are up to several orders of magnitude higher than those in seawater (Thomas et al. 1998, 2001a, Herborg et al. 2001). This organic matter is either allochtonous i.e. trapped during the ice formation (Giannelli et al. 2001) or autochtonous i.e. produced within the sea ice via *in situ* biological activities. The enrichment of organic matter in sea ice results from the following processes, certainly co-acting:

- physical concentration by mechanisms of sea ice formation (see 3.1)
- *in situ* production, by microorganism (see 4.3). High biomasses of microorganisms can indeed occur in sea ice. In particular, in the autumn and in the late winter, favourable conditions such as light and nutrient availability can be encountered in the sea ice as compared to the water column, leading to a large accumulation of biomass.
- exclusion of large heterotrophs from the sea ice matrix because of the narrow brine channels and therefore limitation the consumption of the high biomass
- limitation of bacterial activities and/or low substrate quality (Thingstad et al. 1997) potentially hamper the use of the available organic matter pool.

The accumulation of OM in sea ice clearly results from a disconnection from the production and consumption processes (Thomas and Papadimitriou 2003). As shown in Figure I.15, the interactions between the OM and the microbial community are multiple. These OM production and consumption mechanisms and the relationships between microorganisms are greatly influenced by the physico-chemical properties of the environment where microorganisms live. Indeed micro-organisms produce OM for different reasons such as in response to environmental stress: e.g. temperature, salinity or nutrients (Nagata 2000). In some conditions, like in the sea ice or at the end of a bloom, the production of EPS by algae is enhanced (Passow 2002).

Above all, the presence of this OM could in turn modify the environment and has consequences on the microorganisms. The presence of OM around micro-organisms may modify or shape their habitat and have direct or indirect consequences on the microbial network. For instance, the EPS/TEP produced within the sea ice to protect the cell against temperature or salinity damage, modifies the internal sea ice habitat by the formation of a 'glue-like' environment (Krembs and Deming 2008). The consequences of such a gelatinous structure may serve or deserve microorganisms. On the one hand it could create a favourable microenvironment around the cell which concentrate nutrient or act as a protection against predators. On the other hand, this microenvironment could sequester toxic compounds and limit gas transfer or reduce microorganisms' movement. As a consequence, trophic relationships between microorganisms can be greatly modified in this particular habitat, with the alteration of grazing activities by the reduction of the movement of grazers (Decho 1990). This could spatially disconnect the microbial loop. Furthermore, the EPS released at the end of a phytoplanktonic bloom favour the aggregation of cells, and thus their export out of the surface layer. As such compounds (acidic polysaccharides) likely create chemicals links with iron the nature of the OM, its metal sequestration capacity and its aggregation potential will influence the distribution and availability of iron for microorganisms.

6. Objectives

The production in autumn-winter of sea ice and its melting in spring-summer constitute one of the main characteristics of the Southern Ocean. This ice cover greatly modifies not only the ocean light environment and the air-sea exchange but also the carbon cycling and the efficiency of the biological pump. Indeed by sequestring nutrients (including iron), detrital organic matter and microorganisms, ice formation creates a new physico-chemical habitat for microorganisms modifying their growth conditions and trophic interactions. Their release in the water column at the time of ice melting also impacts on the pelagic system, providing some food for organisms using ice as a refuge and some nutrients and seeds for phytoplankton blooms enhancement in the marginal ice zone. However, part of the release material is directly exported below the surface layer and contributes to the local biological pump. The magnitude and depth location of bacterial remineralization of the exported organic matter determines the efficiency of the biological carbon pump.

As part of a general question on the contribution of sea ice to the Antarctic biological pump, the general objective of this thesis aims to improve our knowledge of organic matter in the ocean and sea ice realms of the Southern Ocean and its interactions with the microbial network. This was approached by addressing the four following questions:

- **How are organic matter and microbial communities distributed in pack ice at the end of winter?**
- **How do organic matter and microbial communities evolve during spring/summer, before melting?**
- **How does the sea ice melting impact on the ocean surface production and carbon export?**
- **What is the fate of exported organic matter during transfer to the deep ocean and how does it depend on the surface production?**

To address these questions, we studied the sea ice microbial network in relation with the physico-chemical characteristics of the ice habitat, at a seasonal scale and in different Antarctic locations, with the objective of determining the factors governing the distribution of microorganisms (algae, bacteria, protozoa) in the pack ice. In parallel we determined the quantity and the type of organic matter present in the sea ice in order to understand the dynamics of the organic pool and to decipher the biological processes governing both production and consumption.

The fate of sea ice material (organic matter, iron) and microorganisms when released in the surface ocean at the time of ice melting was studied under different laboratory-controlled melting experiments.

Finally, we studied the fate of the exported material was approached by investigating the composition of organic matter in relation with the distribution of bacteria biomass and activity in the water column of an ice free part of the Southern Ocean. The link between organic matter surface production and bacterial degradation was approached based on the comparison between an iron-replete and iron-deplete derived carbon export.

My thesis work funded by FRIA (Fonds pour la Recherche en Industries Agro-alimentaires) was conducted in the scope of two Belgian multidisciplinary projects, BELCANTO and SIBCLIM. The goal of BELCANTO (**BEL**gian research on **C**arbon uptake in the **ANT**arctic Ocean) was to assess and understand the present-day functioning of the CO_2 biological pump in the Southern Ocean and to predict its evolution in response to scenarios of increasing atmospheric CO_2. The main objective of SIBCLIM (**S**ea **I**ce **B**iogeochemistry in a **CLIM**ate change perspective) was to study, understand and quantify the physical and biogeochemical processes associated with the sea ice biota that govern the emissions of marine gases of climatic significance. It aims to ultimately upgrade the existing ecological models (e.g. take into consideration the sea ice dynamics in Antarctic ecosystem modelling).

7. Outline

After a section describing the **Materials and Methods** used in this work, the <u>first part</u> of the manuscript deals with the sea ice microbial network and organic matter and its controlling mechanisms. The **first chapter** describes the microbial community composition in sea ice during the winter/spring (ARISE). The **second and third chapters** show the distribution and the characterization of organic matter in pack ice respectively a) during the transition winter/spring/early summer (ARISE and ISPOL) and b) during early spring and a flood-freeze cycle (SIMBA). The **fourth chapter** discusses about the impacts of sea ice melting on the pelagic ecosystem.

The <u>second part</u> of the manuscript (**chapter 5**) focuses on the pelagic realm and concerns the bacterial remineralization of the organic matter during summer in a region which is not directly influenced by sea ice (SAZ-Sense).

Finally, the results presented in the different chapters are discussed together and a general conclusion is given. Some leads for future work are also proposed.

Materials and Methods

1. Study sites

1.1. ARISE

The "ARISE in the East" (Antarctic Remote Ice Sensing Experiment) research cruise onboard the RV *Aurora Australis* (AAV0103) took place in the Australian sector (*63-66°S, 109-118°E*) of the Southern Ocean (Figure M.1) between the 1st and 27th October 2003 (end of winter-early spring). The ARISE cruise was a spatio-temporal study with the repetition of one-day-long ice stations at different locations of a relatively limited spatial domain. Sea ice, brines and seawater samples analysed in this study were collected at stations IV, V, VII, IX, XII and XIII.

1.2. ISPOL

The ISPOL (Ice Station POLarstern) cruise onboard the RV *Polarstern* took place in the Western Weddell Sea (*68°S, 55°W*) near the Antarctic Peninsula (Figure M.1) in November - December 2004 (spring-summer). During the ISPOL cruise, the RV *Polarstern* was anchored to a large pack ice floe (few km in size) in order to follow the temporal variations of the physical and biological atmospheric-ice-ocean processes at a drifting station during the transition from austral spring to summer (Hellmer et al. 2008). Sea ice, brines and seawater samples were collected on seven occasions, between the 29.11.04 and 30.12.04, at regular intervals (usually every five days).

1.3. SIMBA

The SIMBA (Sea Ice Mass Balance in Antarctica) drifting station experiment was carried out onboard the RV *N.B. Palmer* between the 1st and 23rd October 2007 in the Bellingshausen Sea (*69-71°S, 90-95°E*) (Figure M.1). Sampling was conducted at two contrasted locations of the pack ice floe, namely the "Brussels" and "Liège" stations. These 2 sites were chosen for their contrasted ice and snow conditions: mostly thin columnar ice and thin snow cover at Brussels site *vs.* mostly thick granular ice and thick snow cover at Liège site. Each station was visited at 5-days regularly spaced intervals, in the course of nearly 4 weeks. Sea ice, brines and seawater samples were collected between Julian Days 274 and 294 at Brussels station and between Julian Days 276 and 296 at Liège station.

1.4. SAZ-Sense

The Sub-Antarctic Zone SENSitivity to Environmental Change (SAZ-Sense) project was conducted onboard the R.V. *Aurora Australis* (AAV0307) from 17th January to 20th February 2007 (mid-summer) in the Sub-Antarctic Zone (SAZ) and Polar Front Zone (PFZ) of the Australian sector of the Southern Ocean (Figure M.1). Water stations of short (12 h stay, 6 "transect" stations) and long (5 days stay, 3 "process" stations) duration were sampled. The sites sampled during this study can be grouped into three main zones: a) the SAZ west (135-145°E) of Tasmania (Stns SS2 and P1; W-SAZ); b) the PFZ (Stn P2) and c) the SAZ east (150-160°E; E-SAZ) of Tasmania (Stns SS9, SS10, SS12, P3, SS21 and SS24).

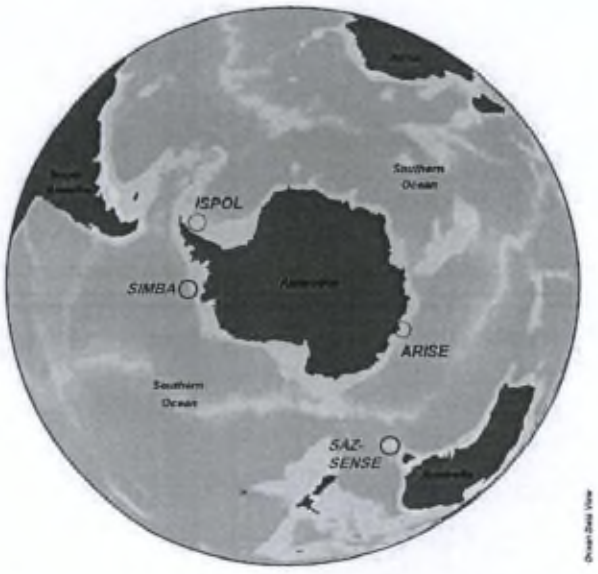

Figure M.1: Localisation of the 4 cruises studied in the present work.

2. Sampling

During sea ice cruises, 3 types of samples were collected: sea ice, brines and seawaters (Figure M.2).

A clean sampling site (20 m x 20 m, 70 x 30 m, 100 x 60 m) located 1 km away from the ship was delimited in order to conduct clean sampling. Within the 'clean area', smaller adjacent 5 x 5 m (10 x 10 m) units were chosen for each sampling day (Tison et al. 2008). Precautions to avoid organic matter contaminations, which were also meant to be trace metal clean (Lannuzel et al. 2006, 2008) were used: clean room garments (Tyvek overall, overshoes and polyethylene gloves) over warm clothes of operators were worn on site and a special electropolished stainless-steel corer (14 cm diameter) was used to collect the ice cores. The different ice cores (dedicated to different analysis) were retrieved about 20 cm apart from each other. Cores were immediately stored at -30°C. Brine collection was made by the 'sackhole' technique, i.e. by gravity drainage of brines into a hole drilled into the sea ice cover (Thomas and Dieckmann 2003). Brines (from 2 depths meant to correspond to a temperature <-5°C and >-5°C) as well as under-ice seawater (0 m, -1 m and -30 m) were collected using a portable peristaltic pump (Cole-Parmer, Masterflex E/P) and acid cleaned tubing. Brines and seawater samples were transferred into acid-washed bottles abundantly rinsed with the collected samples until further processing. For microbial analysis, the full length of the ice cores was then cut into 4 (ARISE) or 6 (ISPOL and SIMBA) subsections, each 6 to 10 cm thick (Figure M.2). Surface and bottom layers of an ice core were always sampled while the other ice sections were chosen on visual observation of ice discoloration. The ice pieces were transferred into acid washed polyethylene containers and further treated as described below for the analysis of inorganic nutrients, chlorophyll *a* (chl *a*) and microscopic investigations. For dissolved organic carbon (DOC) and particulate organic carbon (POC) determination, sea ice sections were transferred into glass beakers pre-combusted at 450°C for 4 h.

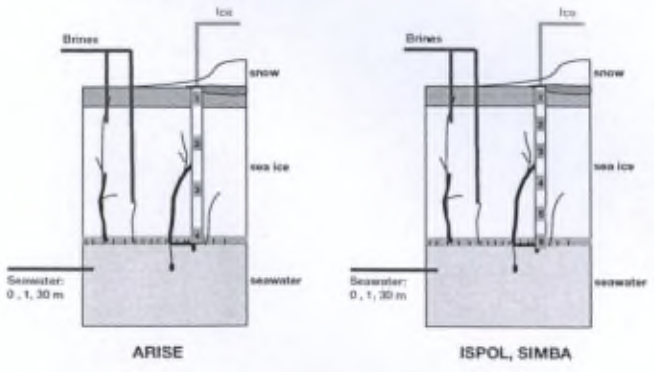

Figure M.2: Sampling strategy during the sea ice cruises, for ARISE on the left and ISPOL and SIMBA on the right.

In case of the oceanographic cruise, seawater samples were collected using a CTD cast system, mounted on a rosette sampler equipped with 24 12L-Niskin bottles. Seawater was collected from 0 to 700 m at between 7 and 15 sampling depths. Sub-sampling was carefully performed to avoid organic contamination. Seawater was collected into acid cleaned polycarbonate bottles and immediately treated.

3. Melting experiments

Two kinds of melting experiments were realized during this work. The first one was realized onboard during the ISPOL cruise while the second one was realized in the laboratory.

3.1. Direct melting

The set-up of this microcosm experiment was realized according to the procedure of Mathot et al. (1991) and Giesenhagen et al. (1999) in order to simulate the melting of sea ice. The experiment was conducted in controlled and iron-clean conditions. Sub-sampling was conducted taking precautions to avoid contamination; bottles have been soaked in HCl 6 N and abundantly rinsed with ultra-high purity water (UHP). This experiment was reproduced twice, using sea ice (bottom ice) and seawater (-30 m), sampled on the 09.12.04 (series a) and on the 25.12.04 (series b). Seawater was pre-filtered using a 0.2 µm membrane cartridge (Sartobran sterile capsule). A piece of 5 cm of bottom ice was thawed with filtered "clean" seawater (1:4, v:v) at 4°C in an acid clean recipient. One litre of homogenized melted ice was filtered under trace metal conditions on 0.2 µm polycarbonate filter. This direct melting experiment consisted of four different additions of seawater and melted sea ice (Table M.1), followed in parallel in 20 L microcosms.

Table M.1: Set-up of the direct microcosms experiment, series a (09.12.04) and b (25.12.04)

Microcosm #	Type of seawater	Type of melted ice added	Comments
M1	unfiltered	none	Pelagic biota evolution without sea ice
M2	unfiltered	unfiltered	"Natural" simulation
M3	filtered (0.2 µm)	unfiltered	Ice biota evolution in seawater
M4	unfiltered	filtered (0.2µm)	Impact of dissolved constituents from ice (dFe and DOM) on pelagic biota

The first microcosm, M1, contained only seawater and served as a control (i.e. simulate the evolution of the planktonic network). The second, M2, was a mix between ice and seawater (1:100, v:v) and simulated "natural" conditions (i.e. the melting of the inferior 10 cm of bottom ice in a water layer of 10 m). The third, M3, aimed to study

the fate of sympagic organisms without interactions due to planktonic organisms; sea ice was added to filtered seawater. Finally, the fourth microcosm, M4, contained seawater amended with filtered melted sea ice, in order to simulate a seeding of dissolved components such as DOM, and total dissolvable iron from the ice to the pelagic system. The microcosms were incubated at -1°C and under a light intensity of 45 µE m^{-2} s^{-1}. They were sampled over time (ca. 10 days) in order to follow the evolution of DOC, POC, and microorganisms (algae, bacteria and protozoa). Major inorganic nutrients and total dissolvable iron were also measured at initial time.

3.2. Sequential melting

In order to study the temporal sequence of release of ice components (microorganisms, OM and Fe), an experiment in controlled and trace-metal clean conditions was realized in laboratory. The conceived device (Figure M.3) enabled to realistically simulate the physical melting of the sea ice, i.e. in triggering melting due to increase of air temperature without increasing seawater temperature. Seawater (13.5 L, collected during the CLIVAR SR3 cruise in the Antarctic Pacific sector (*135-150°E*) in December 2001, [dFe] = 1.05 nM) was placed in a high density polyethylene container, at 2°C, under constant mixing and refrigerated thanks to a tube filled with ethanol in order to maintain seawater temperature around -1.9°C, under a light intensity of 45 µE m^{-2} s^{-1}. A bottom ice piece of 20 cm high (diameter = 14 cm) coming from an ice core sampled on the 04.12.04 during ISPOL was then put to melt in this system. All pieces in contact with sea ice were cleaned to work in trace metal clean conditions. The seawater was sampled with a peristaltic pump (Masterflex) by a tube placed at the bottom of the container. Salinity, temperature, total dissolvable iron, DOC and POC were followed until complete melting of the ice piece (ca. 5 days).

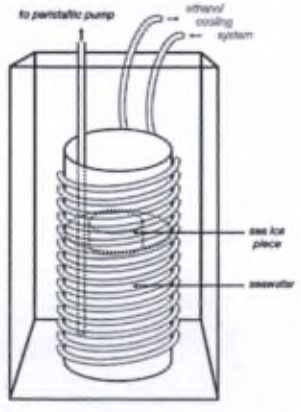

Figure M.3: Schematic view of the melting device used in the sequential melting experiment

For each sub-sampling time (t), a second seawater sample (noted t*) was taken and let in the incubator until the next sub-sampling time (t+1) in order to discriminate between the contribution of ice melting only ($C_{t+1} - C_t$) and the evolution of the compounds in the seawater between the 2 sub-sampling times ($C_t - C_t$). For each parameter, fluxes from sea ice have been estimated by $F_{t+1} = (C_{t+1} - C_t)/ t$, with C, the concentration of the compound and t, the time elapsed between t+1 and t.

4. Physico-chemical parameters

4.1. Ice texture, salinity and temperature

The ice structure was determined by thin section analysis and photographs taken under polarized light. Based on ice crystal size and orientation, two stratigraphic units were distinguished: granular ice and columnar ice. High-resolution analyses of the $\delta^{18}O_{ice}$ allowed further discrimination between snow ice and frazil ice within the granular facies (Jeffries et al. 1989, Eicken 1998). Ice temperature was measured on site using a calibrated probe (TESTO 720) inserted every 5 or 10 cm along the freshly sampled core. Bulk ice salinity was determined by conductivity using WP-84-TPS meter. The brine volume fraction in the sea ice (V_b/V = brine volume/bulk sea ice volume ratio) was calculated on the basis of temperature and salinity values following the equations of Cox and Weeks (1983) and Leppäranta and Manninen (1988), revisited in Eicken (2003).

4.2. Nutrients

Ice sections were melted in the dark at 4°C and then filtered together with "sackhole" brine and seawater samples through 0.4 µm polycarbonate filters. The filtrates and the microcosms subsamples were determined for inorganic nutrients (NO_3^-/NO_2^-, NH_4^+, PO_4^{3-} and $Si(OH)_4$) by colorimetry following the methods described in Grasshoff et al. (1983) for ARISE and Papadimitriou et al. (2007) for ISPOL. To avoid matrix effect, standards used for calibration were prepared in artificial seawater solutions with salinities similar to those of the samples analyzed. Brine volume-normalized concentrations have been calculated by dividing the bulk ice concentration by the brine volume.

For the sequential melting experiment, samples for Fe analysis were kept in polyethylene bottles and acidified at pH 1.8 with ultrapure HNO_3 (Ultrex, JT Baker). Total dissolvable Fe (unfiltered) and dissolved Fe (filtered on 0.2 µm Nuclepore polycarbonate fiters) samples from the "direct" melting experiments were measured according to Lannuzel et al. (2006) by flow injection analysis. Unfiltered Fe samples from the sequential experiment were analyzed according to de Jong et al. (2008) by isotopic dilution combined with multiple collector inductively coupled plasma mass spectrometry (ID-MC-ICP-MS) using nitrilotriacetic acid chelating resin for pre-concentration and matrix separation.

5. Organic Matter

All the material and glassware used for organic carbon sampling and measurements were either made of glass cleaned by ashing (4 h at 450°C), or washed with chromic-sulphuric acid (Merck), or of Teflon cleaned by 10% HCl soaking and rinsed with ultra high purity water (UHP) (18.2M Millipore milli-Q system) obtained from a water purification system equipped with a UV-lamp and organic cartridge (Milli-Q Element, Millipore).

5.1. Melting of ice samples for organic carbon

A preliminary experience was realized in order to test the influence of the melting protocol on the organic matter (OM) concentration measured in the melted samples. Two handlings were possible to apply to the sea ice samples: a) directly melted "as is" or b) with addition of filtered seawater in order to reduce osmotic shock. As observed by Garrison and Buck (1986), fragile microorganisms such as ciliates may be disrupted during their passage from salinity to another and then released their internal content. Such a process could lead to the overestimation of the dissolved fraction. However Thomas et al. (1998) reported that for diatom dominated samples, there is no need to dilute samples.

Two sea ice pieces sampled at Mc Murdo in 2003 were chosen for their contrasted low and high biomass levels. A sea ice piece was divided in 2 equal parts under organic-clean methods. The first piece was melted "as is" while sterile artificial seawater was added to the other piece (1:4, v:v). Subsamples of each melted samples were taken to measure concentration of dissolved organic carbon and dissolved saccharides. Results suggested that no major lysis phenomenon occurred during this experiment. The direct melting protocol was thus applied to OM samples hereafter

Thus, sea ice sections dedicated to organic matter analysis were thawed in precombusted glass beaker at 4°C in the dark. For POC and DOC, ice sections were melted onboard for the 3 sea ice cruises. Melted sub-samples were then processed as explained below. For qualitative analyses of organic matter (saccharides, amino acids and TEP), sub-samples were also collected from onboard melting in case of the SIMBA cruise but for previous ones (ARISE and ISPOL), samples were taken from a retrieved twin ice core, melted in the laboratory. All handling was performed in a class 100 laminar flow hood. Brines and seawaters samples were treated directly as explained hereafter.

5.2. Dissolved and particulate organic carbon

Particulate organic carbon (POC) was collected on pre-combusted (450°C, 4h) Whatman GF/F filters and stored at -20°C until analysis. After drying at 60°C, POC was analyzed with a Fisons NA-1500 elemental analyzer following carbonate removal from the filters by HCl fumes overnight. Filtered samples for dissolved organic carbon (DOC) were stored in pre-combusted (450°C, 4h) 20-ml glass ampoules with addition of 25 µl H_3PO_4 (concentration 50 %), which were sealed to avoid contact with air. Samples were kept in the dark at 4°C until analysis. The DOC was measured by high temperature catalytic oxidation (HTCO; procedure of Sugimura and Suzuki 1988) with a Shimadzu TOC-5000 analyzer for ARISE samples and a Dohrmann Apollo 9000 analyzer for ISPOL and SIMBA samples. Carbon concentration was determined using a five-point calibration curve performed with standards prepared by diluting a stock solution of potassium phthalate in Ultra pure water (Milli-Q Element system, Millipore). Each value corresponds to the average of at least five injections. Samples were measured in duplicate and the relative standard deviation never exceeded 2%. The accuracy of our DOC measurements was tested by analyzing reference materials provided by the Hansell laboratory (University of Miami). We obtained an average concentration of 45.1 ± 0.7 µM C (n=10) for deep-ocean reference material (Sargasso Sea Deep water, 2600 m) and 1.4 ± 0.7 µM C (n=10) for low-carbon reference water. Our values are within the nominal values provided by the reference laboratory (44.0 ± 1.5 µM C and 2.0 ± 1.5 µM C, respectively).

5.3. Dissolved and particulate saccharides

Melted samples were filtered through pre-combusted GF/F filters (Whatman, 450°C, 4 h). Filters and filtrates were stored frozen (-20°C) until analysis. Total particulate saccharides (p-TCHO) and total dissolved saccharides (d-TCHO) were determined following the colorimetric TPTZ (2,4,6-tripyridyl-*s*-triazine) method of Myklestad et al. (1997). The whole procedure was carried out in darkness because the reagents are light-sensitive (van Oijen et al. 2003). d-TCHO includes mono- (d-MCHO) and poly-saccharides (d-PCHO) which are respectively the concentration before and after hydrolysis (d-TCHO = d-MCHO + d-PCHO). Dissolved and particulate samples were hydrolysed with HCl 0.1 N at 100°C, during 20h (Burney and Sieburth 1977). Calibration curves were obtained with D(+)-glucose and the values of saccharides were expressed as glucose equivalent. They were converted into carbon concentration using a conversion of 6 mol of C per mole of glucose, since each glucose molecule contains 6 carbon atoms. The coefficient of variation (CV, standard deviation/mean) of all triplicate measurements was below 5% and the detection limit was 1.0 µM C. Three blanks per batch of samples were always treated and analyzed in the same way as the samples and subtracted from the concentration of the samples.

5.4. Dissolved Free Amino Acids (DFAA)

Filtered (Whatman GF/F, 450°C, 4 h) sub-samples were stored frozen (-20°C) until analysis. Dissolved free amino acids (DFAA) were measured following the fluorometric method of Parsons et al. (1984). The coefficient of variation (CV, standard deviation/mean) of triplicate measurements

was below 5%. A calibration curve was made with glycine. The detection limit was 0.1 µM glycine. Concentrations were expressed in µM C by using a conversion of 2 mol of C per mol of glycine. Similarly, the concentrations were converted into µM N using 1 mol of N per mol of glycine.

5.5. Transparent Exopolymeric Particles (TEP)

For transparent exopolymeric particles (TEP), unfiltered samples were poisoned with formaldehyde (2% final conc.) to avoid bacterial development and kept at 4°C until further processing. TEP were determined according to the spectrophotometric method of Passow and Alldredge (1995). Briefly, 10-15 ml subsamples were filtered onto 0.4 µm polycarbonate filters (Nuclepore) using a vacuum pressure <150 mm Hg. TEP were stained with Alcian Blue solution (0.02% Alcian Blue, pH 2.5). Filters were then extracted with 6 ml of 80% H_2SO_4 for 2 h in the dark with constant stirring. Absorbance was measured against a distilled water blank at 787 nm. The calibration was made using Xanthan Gum solution. Results are expressed as Xanthan Gum weight equivalent per liter (XAGeq l^{-1}). All TEP determinations were performed in triplicate.

6. Microorganisms

For the determination of Chl *a* and microorganisms, ice core sections were melted in the dark at 4°C in seawater prefiltered through 0.2 µm polycarbonate filters (1:4, v:v), to avoid rupture of microorganisms cells due to osmotic shocks during sample melting (Garrison and Buck 1986).

6.1. Chlorophyll a

Samples were filtered onto Whatman GF/F filters for ARISE, ISPOL and SAZ-Sense. During SIMBA and also for the ISPOL microcosm experiments, samples were filtered onto 10 and 0.8 µm Nuclepore filters. Chl *a* were extracted in 90% v:v acetone in the dark at 4°C for 24h, and quantified fluorometrically according to Yentsch and Menzel (1963).

6.2. Abundance and biomass of bacteria, algae and protozoa

Subsamples were fixed with lugol for inverted light microscopy and with formaldehyde for epifluorescence microscopy and kept at 4°C.

Algae and protozoa were enumerated by inverted light microscopy (100 x magnification and 320 x magnification) according to the method of Utermöhl (1958) and by epifluorescence microscopy (400 x magnification) after DAPI staining (Porter and Feig 1980). Autotrophic species were distinguished from heterotrophs by the red autofluorescence of Chl *a* observed under blue light excitation. A minimum of 100 organisms was counted per taxonomic group. Microscopic size measurements were converted to cell volumes, according to a set of geometric correspondences (Hillebrand et al. 1999). Algae and protozoan biomass was calculated from the abundance and the specific carbon biomass (carbon per cell) estimated from the relationships established by Menden-Deuer and Lessard (2000).

Bacteria were enumerated by epifluorescence after DAPI staining (Porter and Feig 1980). A minimum of 1000 cells was counted in at least 10 different fields at 1000x magnification. A relative standard deviation of 15% (n=20) was estimated on the bacterial abundance determination. Bacterial biovolumes were determined by image analysis (Lucia 4.6 software) and calculated by treating rods and cocci as cylinders and spheres, respectively (Watson et al. 1977). They were converted to carbon biomass by using the relation established from data measured by Simon and Azam (1989): $C = 92\ V^{0.598}$ where C is the carbon per cell (fg C $cell^{-1}$) and V is the biovolume (μm^3). For SAZ-Sense, a carbon conversion factor 12 fg C $cell^{-1}$ (Fukuda et al. 1998) was applied.

Note that the following sections 6.3 to 6.6 only concern the SAZ-Sense cruise.

6.3. Bacterial community structure

Bacterioplankton community structure was examined by FISH (fluorescence *in situ* hybridization) using probe EUB338 for *Eubacteria*, ALF1b for the α-subclass of the *Proteobacteria*, BET42a for the β-subclass of the *Proteobacteria*, GAM42a for the γ-subclass of the *Proteobacteria*, CF319a for the *Cytophaga-Flavobacter* group, ARCH915 for *Archaea* and a negative control probe for nonspecific probe binding (Table M.2). Bacterioplankton samples were prepared for FISH using a modification of the method described by Glöckner et al. (1996). Seawater samples were fixed with formaldehyde (2% final concentration). After fixation during 12-24 h, bacteria were stained with DAPI and filtered onto a 0.2-µm-pore-size polycarbonate membrane (Poretics), rinsed with 0.2-µm-pore-size-filtered seawater and stored at -20°C. A piece of the filter was placed on a Parafilm-covered glass slide, overlaid with 30 µl of hybridization solution containing 2 ng/µl of Cy3-labeled oligonucleotide probe, and incubated in a sealed container for 24 h at 42°C. The hybridization solution contains 0.9 M NaCl, 20 mM Tris-HCl (pH 7.4), 0.01% sodium dodecyl sulfate, 50 mM EDTA and varying formamide concentrations determined to achieve specificity for the targeted group of bacteria (Table M.2). After hybridization, the sample was washed during 15 min at 38°C in a solution containing 20 mM Tris-HCl (pH 7.4), 10 mM EDTA, 0.01% sodium dodecyl sulfate and appropriate concentrations of NaCl i.e. 102 mM for EUB338, BET42a and GAM42a, 440 mM for ALF1b, 308 mM for ARCH915 and 80 mM for CF319a. Stained cells were determined by image analysis (Lucia 4.6 software) from epifluorescence observations of 10 fields using a Leica DMRXA microscope fitted with Cy3 and DAPI filters and equipped with a Nikon DXM 1200 camera.

Table M.2: Oligonucleotide probes used in this study. %FA = percentage of formamide in the hybridization buffer. 1: Amann et al. (1990), 2: Manz et al. (1992), 3: Manz et al. (1996), 4: Stahl and Amann (1991)

Probe	Specificity	Probe sequence (5'–3')	Target site 16S or 23S rRNA position (nucleotide)	% FA	Refs.
EUB338	*Eubacteria*	GCTGCCTCCCGTAGGAGT	16S (338–355)	30	1
ALF1b	alpha-*proteobacteria*	CGTTCGYTCTGAGCCAG	16S (19-35)	40	2
BET42a	beta-*proteobacteria*	GCCTTCCCACTTCGTTT	23S (1027–1043)	30	2
GAM42a	gamma-*proteobacteria*	GCCTTCCCACATCGTTT	23S (1027–1043)	30	2
CF319a	*Cytophaga-Flavobacterium*	TGGTCCGTGTCTCAGTAC	16S (319–336)	30	3
ARCH915	*Archaea*	GTGCTCCCCCGCCAATTCCT	16S (915–934)	25	4

6.4. Bacterial viability

The proportion of bacterial non-viable cells was estimated from Sytox-positive cells (Roth et al. 1997). A stock solution of Sytox Green stain (Molecular probes) was prepared in dimethylsulfoxide to a final concentration of 5 mM. The cells were stained with 1 µM SYTOX Green (final conc.) for at least 10 min at room temperature in the dark, as proposed on the product information sheet. The Sytox-positive cells were determined by epifluorescence microscopy (see above).

6.5. Bacterial enzymatic activity

Ectoproteolytic activity (EPA) was determined fluorimetrically (Kontron SFM 25 fluorimetre) as the maximum hydrolysis rate of model substrate for leucine-aminopeptidase (L-leucine-7-amino-4-methyl-coumarin) added at saturating concentration (40 mM). Wavelengths for excitation and emission were 360 and 445 nm. Fluorescence in the samples was measured as a function of time at *in situ* temperature in the dark. Increase of fluorescent units with time was converted into activity from a standard curve prepared with the end product of the reaction, 7-amino-4-methylcoumarin (Sigma). The relative standard deviation was 12% (n = 20).

6.6. Bacterial Production and BGE estimations

Bacterial production (BP or more precisely referring to heterotrophic prokaryotic production) was determined by incorporation of ^{3}H-labelled thymidine into macromolecules precipitated by cold trichloroacetic acid (TCA) according to Fuhrman and Azam (1982). Three 20 ml (for depths <200 m) and three 40 ml (for depths >200 m) aliquots of water sample were dispensed into plastic vials. All samples received methyl-[^{3}H]-thymidine (specific activity: 74.5 Ci mmol^{-1}; Amersham), to a final concentration of 10 nM, by addition of 100 or 200 µl ^{3}H-thymidine. One of these subsamples served as a blank (t0) and the two others were incubated for 4 h (<200 m) and 8 h (>200 m) at *in situ* temperature. The thymidine incorporation was stopped by precipitating the samples with trichloroacetic acid (TCA, 5% final concentration). The samples were filtered through 0.22 µm pore size cellulose acetate filters (Sartorius) and rinsed 5 times with 2 ml 5% ice cold TCA. The filters were immediately stored at -20°C. Back to the home laboratory, they were placed in scintillation cocktail (EcoLume, MP Biomedicals), and the radioactivity incorporated in the cold TCA precipitable material was measured by a Packard Tricarb 1900TR Liquid Scintillation Analyzer. Corrections for quenching were made by transformed spectral indices of external standards and stored quench curves. Radiotracer incorporation (nmol l^{-1} h^{-1}) was converted into bacterial production (cell l^{-1} d^{-1}) using the published thymidine conversion factor (TCF) of 8.6 x 10^{17} cell per mol ^{3}H incorporated estimated by Ducklow et al. (1999). Biomass production rate was obtained by use of the CCF of 12 fg C cell^{-1} (Fukuda et al. 1998).

The measurements of BP at discrete depths throughout the water column can be used to estimate depth integrated bacterial respiration (BR) and bacterial carbon demand (BCD) from the following relations: a) BR= [(BP/BGE) – BP] and b) BCD = BP + BR = BP/BGE. To calculate this, we rely on the use of a thymidine conversion factor (TCF) and a bacterial growth efficiency factor (BGE). The values of each of these conversion factors (CFs) found in literature are rather variable and consequently greatly influence the estimation of the carbon fluxes. We tested, and discuss in Chapter 5, the impact of three different ranges of TCFs and BGEs. We applied a constant carbon conversion factor (CCF) of 12 fg C cell^{-1} and used a low (TCF = 0.5 x 10^{18} cells mol^{-1} and BGE = 0.38), a middle (TCF = 0.86 x 10^{18} cells mol^{-1} and BGE = 0.15), and a high estimate (TCF = 2.0 x 10^{18} cells mol^{-1} and BGE = 0.09) to calculate bacterial respiration and bacterial carbon demand. The value of 0.15 for BGE is the median of 239 oceanic studies (del Giorgio and Cole 2000) and the lower and higher ones are the extreme values found by Carlson et al. (1999). The choice of the TCF is made after Ducklow et al. (1999) and Ducklow (2000). In addition, we also use the model of del Giorgio and Cole (1998) to estimate BGE from BP (in µg C l^{-1} h^{-1}) at each depth, following the relation: BGE = (0.037 + (0.65 x BP)) / (1.8 + BP).

The sea ice habitat

Microbial network, organic matter and controlling mechanisms

Foreword

This first section is dedicated to sea ice habitat and presents the results concerning the microbial network, the organic matter, their controlling mechanisms and the impact of the release of these compounds during sea ice melting.

Chapter 1 presents the distribution of microorganisms (algae, bacteria and protozoa) in first year pack ice and their controlling mechanisms during the *Aurora Australis* cruise ARISE held in October-November 2003 in the Australian sector of the Southern Ocean.

Chapter 2 looks at the distribution of dissolved and particulate organic matter in first year pack ice a) from the *Aurora Australis* cruise ARISE held in October-November 2003 in the Australian sector of the Southern Ocean and b) from the *RV Polarstern* cruise ISPOL in November-December 2004 in the Weddell Sea.

Chapter 3 presents similar results about the distribution of organic matter in sea ice from the SIMBA cruise onboard the RV *NB Palmer* in October 2007 in the Bellingshausen Sea.

Finally **Chapter 4** deals with the impact of sea ice melting on the pelagic ecosystem. It presents results from laboratory experiments realized during the ISPOL cruise and from the distribution of the microbial community in sea ice during the ISPOL cruise in November-December 2004 in the Western Weddell Sea.

***Chapter 1**: Biogeochemistry and microbial community composition in sea ice and underlying seawater off East Antarctica during early spring*
Becquevort S., I. Dumont, J-.L. Tison, D. Lannuzel, M-L Sauvée, L. Chou, V. Schoemann
Polar Biology, in press, *DOI 10.1007/s00300-009-0589-2*

***Chapter 2**: Distribution and characterization of dissolved and particulate organic matter in Antarctic pack ice*
Dumont I., V. Schoemann D. Lannuzel, L. Chou, J-.L. Tison, Becquevort S.
Polar Biology, in press, *DOI 10.1007/s00300-008-0577-y*

***Chapter 3**: Distribution and chemical characterization of organic matterin the Antarctic pack ice zone, Bellingshausen Sea.*
Dumont I., F. Masson, V. Schoemann, J-L. Tison and S. Becquevort. Draft with co-authors.

***Chapter 4**:* Impacts of Antarctic pack ice melting on planktonic microbial communities in the Western Weddell Sea
Dumont I., Schoemann V., Lannuzel D., de Jong J., Delille B., Tison J-L., Becquevort S. Draft with co-authors.

Chapter 1

Biogeochemistry and microbial community composition in sea ice and underlying seawater off East Antarctica during early spring

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ORIGINAL PAPER

Biogeochemistry and microbial community composition in sea ice and underlying seawater off East Antarctica during early spring

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Abstract Pack ice, brines and seawaters were sampled in October 2003 in the East Antarctic sector to investigate the structure of the microbial communities (algae, bacteria and protozoa) in relation to the associated physico-chemical conditions (ice structure, temperature, salinity, inorganic nutrients, chlorophyll *a* and organic matter). Ice cover ranged between 0.3 and 0.8 m, composed of granular and columnar ice. The brine volume fractions sharply increased above −4°C in the bottom ice, coinciding with an important increase of algal biomass (up to 3.9 mg C l^{-1}), suggesting a control of the algae growth by the space availability at that period of time. Large accumulation of NH_4^+ and PO_4^{3-} was observed in the bottom ice. The high pool of organic matter, especially of transparent exopolymeric particles, likely led to nutrients retention and limitation of the protozoa grazing pressure, inducing therefore an algal accumulation. In contrast, the heterotrophs dominated in the underlying seawaters.

Keywords Sea ice · Brine volume fraction · Nutrients · Organic carbon · Sympagic organisms

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Introduction

The seasonal occurrence of sea ice is an important feature in the Southern Ocean, with sea ice extending from 4×10^6 km^2 in summer to 20×10^6 km^2 in winter (Zwally et al. 1983; Comiso 2003). Sea ice plays a key role in the Earth Climate system, by governing the deep water formation, in addition to heat and gas exchanges between the lower atmosphere and sea surface (Dieckmann and Hellmer 2003). From a biological point of view, sea ice is a rich environment for microbial communities (algae, bacteria, protozoa) and for a wide variety of life forms of vital importance for the whole Antarctic ecosystem (Lizotte 2003). The "frozen ocean" provides extreme and highly variable habitats both spatially and temporally. Microorganisms are indeed subject to contrasted chemical and physical constraints (e.g. temperature, salinity, space, light, pH, nutrients), which vary in space (horizontally and vertically) and time (ice formation and melting, Eicken 1992; Thomas and Dieckmann 2002). On a micro-scale, the biomass is essentially confined to the brine pockets and channels (Eicken 2003), representing less than 10% of the sea ice volume in the winter–spring period. Microorganisms within the sea ice are physically constrained in such a way that many of the biological and chemical interactions are likely to be comparable to those reported for aquatic biofilm studies (Mock and Thomas 2005).

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The distribution, concentration and taxonomic composition of sympagic communities are related, on one hand, to the abundance and species composition of the planktonic microorganisms present in the water column at the time and location of sea ice formation and, on the other hand, to sea ice properties such as age and texture (Spindler et al. 1990). The structuring of the sea ice communities starts initially by the incorporation of pelagic organisms upon sea ice formation. Most of the cells are passively incorporated in the ice, being harvested during frazil ice formation and/or by the percolation pumping mechanism (Shen and Ackermann 1990). Cells with the capability to adhere to the ice matrix might also be introduced into the bottom skeletal layer of columnar/congelation ice later in the season, as water underneath the ice cover is continually exchanged due to tidal currents and the thermohaline circulation (Lizotte 2003).

As the season progresses, the evolution of the sympagic communities depends on two major physical factors: light and temperature. First, light conditions in the ice environment depend on seasonal changes in solar radiation, ice thickness and snow cover, which can lead to reflection and attenuation of incident irradiance (Arrigo 2003). Then temperature controls volume as well as salinity of the brines. The microorganisms living within the ice therefore not only have to cope with low temperatures but also to withstand high osmotic pressures and low space availability. Moreover, due to temperature variations, the size and the degree of connection between brine pockets and channels change with time (Perovich and Gow 1996). The sea ice porosity is consequently affected and the exchanges between the atmosphere, the sea ice and the underlying water are altered. For instance, sea ice can be considered as permeable and brines can move through the solid when the brine volume fraction represents 5% of the total ice volume for a temperature of −5°C and a bulk ice salinity of 5 (Golden et al. 1998).

The reduction of permeability also has an important impact on the structure of the microbial community (Granskog et al. 2003). Sea ice thermodynamics may constrain the migration of organisms and alter trophic relationships e.g. by preventing predation by large organisms. The permeability stage of ice cover may also control the growth of autotrophs by limiting the supply of nutrients from the underlying water, as well as restraining transports of contaminants and heat.

Finally, biological processes taking place in sea ice may potentially regulate the pelagic ecosystem. During winter, the organic matter accumulated in the sea ice may represent an important food source for large consumers such as krill (Garrison 1991). When the ice melts, the material incorporated within the ice matrix can be ultimately released into the water column. The organisms released from decaying sea ice may settle rapidly as aggregates (Riebesell et al. 1991) and feed the benthic trophic web, being quickly grazed by zooplankton and incorporated into fast settling pellets (Scharek et al. 1994), or act as an inoculum for the planktonic blooms (Garrison et al. 1987). Moreover, nutrients such as iron (Fe) and dissolved organic matter (DOM) sequestrated in the sea ice in winter time can be released in the under ice water and in turn stimulate the planktonic microorganisms growth in the high nutrient low chlorophyll (HNLC) Southern Ocean (Lannuzel et al. 2007; Dumont et al. 2009). These processes may influence the carbon biological pump, e.g. by increasing the carbon export from surface to the deep waters.

The objectives of the present study were to investigate the relationships between physical, chemical and biological properties in the sea ice environment. We present results on the microbial community (abundance and biomass of algae, bacteria and protozoa) in the sea ice and in the underlying seawater (0, −1 and −30 m depths) in relation to physical (ice structure, salinity, temperature and brine volume fraction) and chemical (inorganic nutrients, dissolved and particulate organic carbon and transparent exopolymeric particles) parameters at the end of winter–early spring season in the pack ice zone of the western Pacific Ocean sector.

Materials and methods

Sampling and study area

Samples were collected during the research cruise AA-03-V1 "Arise in the East" aboard the Australian RV Aurora Australis, between 1 and 27 October 2003 in the area 63–66°S/109–118°E (Table 1). Sea ice, brines and under-ice water samples were collected under trace metal clean conditions (Lannuzel et al. 2006).

At each station, a set of closely spaced ice cores (maximum 20 cm between each consecutive core, on a homogeneous level ice floe) were collected with an electropolished stainless steel ice corer (14 cm internal diameter). While one core was wrapped in plastic bags and kept frozen (−28°C) for later analysis of ice texture and salinity, ice cores dedicated to biological and chemical parameters were stored in plastic bags at −28°C in the dark until further processing. Within 24 h after sampling, these cores were cut into four sections of 6 cm height. These 6-cm sections were sub-sampled from the full length of the core, chosen so that a top, two intermediate and a basal sections were included from every core. The sections were transferred into acid washed polyethylene containers and further treated as described below for the analysis of inorganic nutrients [NO_3^-/NO_2^-, NH_4^+, PO_4^{3-}, $Si(OH)_4$], chlorophyll *a* (Chl *a*), and microscopic investigations. For dissolved organic carbon (DOC), particulate organic carbon (POC) and transparent exopolymeric particles (TEP)

Table 1 Physical (texture: G = granular, C = columnar; temperature; salinity and brine volume fraction) and chemical [brine volume-normalized concentrations of $Si(OH)_4$, NO_3^-/NO_2^-, PO_4^{3-} and NH_4^+] characteristics along with chlorophyll *a* (Chl *a*) and transparent exopolymeric particles (TEP) concentrations in sea ice core sections

	Depth (cm)	Snow (cm)	Texture	Temperature (°C)	Salinity	Brine volume (%)	$Si(OH)_4$ (μM)	NO_3^-/NO_2^- (μM)	PO_4^{3-} (μM)	NH_4^+ (μM)	Chl *a* (μg l^{-1})	TEP-C[b] (mg l^{-1})
Station IV	0–6	2	G	−8.9	8.6	4.7	270.0	111.7	7.15	5.14	0.22	0.42
1 October 2003	20–26		C	−5.9	3.6	2.9	153.2	34.7	0.14	19.20	0.14	0.25
64°37.7′S	36–42		C	−3.7	4.4	5.8	21.6	17.2	0.74	29.23	0.58	0.06
117°44.5′E	42–48[a]		C	−3.0	6.0	13.1	61.8	109.9	79.44	65.70	19.25	2.02
Station V	7–13	40–50	G	−5.2	6.6	6.9	140.0	26.6	4.74	11.66	3.92	0.44
7 October 2003	40–46		C	−3.5	3.1	3.4	80.9	29.5	0.50	29.23	1.00	0.02
64°34.0′S	69–75		G/C	−1.9	2.3	6.3	20.7	15.8	0.35	15.56	1.96	0.04
116°37.8′E	75–81[a]		C	−1.8	3.3	8.9	72.2	49.3	46.54	19.97	27.98	0.94
Station VII	11–17	5–10	G	−6.2	9.1	6.9	169.8	41.8	0.71	6.70	0.36	ND
9 October 2003	32–38		C	−4.7	4.5	3.4	191.0	22.4	2.88	25.07	2.10	ND
64°38.0′S	56–62		C	−3.2	3.8	6.3	26.0	12.3	0.59	8.19	2.16	ND
116°40.7′E	62–68[a]		C	−2.7	7.3	8.9	105.4	109.2	85.86	72.10	34.26	ND
Station IX	6–12	40–50	G	−4.3	5.2	6.4	140.1	15.6	1.47	16.48	1.22	0.24
11 October 2003	20–26		C	−3.6	4.7	6.6	49.2	15.3	0.52	8.76	0.62	0.15
64°24.1′S	39–45		C	−2.8	4.9	8.4	26.8	12.0	1.09	6.59	0.93	0.37
115°17.5′E	45–51[a]		C	−2.4	6.1	16.3	62.5	34.6	24.76	11.52	12.98	0.78
Station XII	0–6	6	G	−6.7	7.7	4.7	299.6	131.8	12.66	9.94	0.16	0.18
14 October 2003	24–30		C	−4.7	5.2	6.8	111.6	91.1	1.19	12.37	0.28	0.04
63°56.2′S	40–46		G/C	−3.4	5.9	11.8	23.9	8.5	2.07	9.10	0.73	0.13
114°19.4′E	60–66[a]		C	−1.9	4.1	12.0	48.6	8.3	20.61	11.86	14.67	0.36
Station XIII	0–6	4	G/C	−7.9	9.0	5.8	342.9	113.5	6.00	17.61	0.04	ND
20 October 2003	8–14		G/C	−6.1	5.3	4.3	274.9	89.8	2.02	5.59	0.22	ND
65°16.1′S	14–20		G/C	−5.1	4.7	3.5	254.4	89.8	1.01	35.39	0.39	ND
109°27.8′E	20–26[a]		G/C	−4.0	6.2	6.2	176.7	151.9	15.38	13.11	9.10	ND

[a] Bottom sections

[b] Data from Dumont et al. (2009) converted to carbon equivalents according to Engel and Passow (2001)

ND not determined

determination, sea ice sections were transferred into glass beakers precombusted at 450°C for 4 h.

In addition, access holes "sack holes" were drilled into the sea ice cover at two depths (ice levels above or below −5°C, a first-order proxy of the permeability threshold, Golden et al. 1998) to allow the gravity driven brine collection. Brines and under-ice seawater (0, −1 and −30 m) were collected using a portable peristaltic pump (Cole-Parmer, Masterflex E/P) and acid cleaned tubing. Seawater and brines samples were transferred into acid washed bottles successively rinsed with ultra high purity water (UHP) (18.2 MΩ Millipore milli-Q system) and the collected samples.

Ice structure, temperature and salinity

The ice structure was determined by thin section analysis and photographs taken under polarized light. Based on ice crystal size and orientation, two stratigraphic units were distinguished: granular ice and columnar ice. High-resolution analyses of the $\delta^{18}O_{ice}$ allowed further discrimination between snow ice and frazil ice within the granular facies (Jeffries et al. 1989; Eicken 1998). Ice temperature was measured on site using a calibrated probe (TESTO 720) inserted every 5 or 10 cm along the freshly sampled core. Bulk ice salinity was determined by conductivity using WP-84-TPS meter. The brine volume fraction in the sea ice ($\frac{V_b}{V}$ = brine volume/bulk sea ice volume ratio) was calculated on the basis of temperature and salinity values following the equations of Cox and Weeks (1983) and Lepparänta and Manninen (1988), revisited in Eicken (2003).

Nutrients

Ice sections were melted in the dark at 4°C and filtered in the same way as the "sack holes" brine and seawater samples through 0.4-µm polycarbonate filters. Inorganic nutrients [NO_3^-/NO_2^-, NH_4^+, PO_4^{3-} and $Si(OH)_4$] were analyzed in the filtrates by colorimetry following the methods described in Grasshoff et al. (1983). To avoid matrix effect, standards used for calibration were prepared in artificial seawater solutions with salinities similar to those of the samples analyzed.

Chlorophyll *a*

For the determination of Chl *a*, ice core sections were melted in the dark at 4°C in seawater prefiltered through 0.2-µm polycarbonate filters (1:4 volume ratio), to avoid rupture of the sympagic microorganisms cells due to osmotic shocks during sample melting (Garrison and Buck 1986). Subsamples were filtered onto Whatman GF/F filters and Chl *a* was extracted in 90% v:v acetone in the dark at 4°C for 24 h, and quantified fluorometrically according to Yentsch and Menzel (1963).

Organic matter

For the determination of organic matter, sea ice sections were melted at 4°C in the dark in precombusted (450°C, 4 h) glass beakers. This process took no longer than 24 h. In samples dominated by diatoms, this melting process has been shown as not to be problematic with respect to the release of intracellular content (Thomas et al. 1998). Particulate organic carbon (POC) was collected on precombusted (450°C, 4 h) Whatman GF/F filters, stored at −20°C until analysis. After drying the filters at 60°C, POC was analyzed with a Fisons NA-1500 elemental analyzer following carbonate removal from the filters by HCl fumes overnight. Filtered samples for DOC were stored in precombusted (450°C, 4 h) 20-ml glass ampoules with 25 µl H_3PO_4 (concentration 50%), which were sealed to avoid contact with air. Samples were kept in the dark at 4°C until analysis. The DOC was measured by high temperature catalytic oxidation (HTCO; procedure of Sugimura and Suzuki 1988) with a Shimadzu TOC-5000 analyzer. Triplicate TEP subsamples were determined according to the spectrophotometric method of Passow and Alldredge (1995). Briefly, 10–15 ml subsamples were filtered onto 0.4 µm pore size polycarbonate filters (Nuclepore) using a vacuum pressure <150 mm Hg. TEP were stained with Alcian Blue solution (0.02% Alcian Blue, pH 2.5). Filters were then extracted with 6 ml of 80% H_2SO_4 for 2 h, in the dark with constant stirring. Absorbance was measured against a distilled water blank at 787 nm. TEP concentrations were recorded as µg gum Xanthan equivalents and converted into carbon equivalents according to Engel and Passow (2001).

Bacteria, algae and protozoa abundance and biomass

Ice core sections sampled for the determination of the microorganism abundance and biomass were melted according to the same procedure as for the Chl *a* analysis described above.

Algae and protozoa were enumerated by inverted light microscopy (100× magnification and 320× magnification) according to the method of Utermölh (1958) and by epifluorescence microscopy (400× magnification) after DAPI staining (Porter and Feig 1980). Autotrophic species were distinguished from heterotrophs by the red autofluorescence of Chl *a* observed under blue light excitation. A minimum of 100 organisms was counted per taxonomic group. Microscopic size measurements were converted to cell volumes, according to a set of geometric correspondences (Hillebrand et al. 1999). Algae and protozoan biomass was calculated from the abundance and the specific carbon

biomass (carbon per cell) estimated from the relationships established by Menden-Deuer and Lessard (2000).

Bacteria were enumerated by epifluorescence after DAPI staining (Porter and Feig 1980). A minimum of 1,000 cells was counted in at least 10 different fields at 1,000× magnification. A relative standard deviation of 15% ($n = 20$ filters from the same sample) was estimated on the bacterial abundance determination. Bacterial biovolumes were determined by image analysis (Lucia 4.6 software) and calculated by treating rods and cocci as cylinders and spheres, respectively (Watson et al. 1977). They were converted to carbon biomass by using the relation established from data measured by Simon and Azam (1989): $C = 92\ V^{0.598}$ where C is the carbon per cell (fg C cell^{-1}) and V is the biovolume (μm^3).

Brine concentration estimation

The actual habitat of sea ice organisms, the brine channel system, is very difficult to sample. The most widely applied method to collect brines by drainage into "sack holes" cored in the ice (Thomas and Papadimitriou 2003) was used in the present study (see "Sampling and study area"). However, brines samples collected by sack hole sampling do not really sample the particulate phase which is mostly retained in the ice matrix and will be underestimated in the sampled brines. Moreover, the brines collected in sack holes are collected from an undefined area of sea ice (Thomas and Papadimitriou 2003). We confronted hereafter measured brine concentration with brine concentration estimated (C_b^{est}) on the basis of the ratio of the depth-integrated calculated brine volume (V_b^{int}) to depth-integrated bulk sea ice concentrations ($C_{\text{ice}}^{\text{int}}$):

$$C_b^{\text{est}} = \frac{C_{\text{ice}}^{\text{int}}}{V_b^{\text{int}}} \times 100$$

The values were depth-integrated from the top of ice core to the brine collection depths.

Results

Physical environment

Detailed results of the ice texture, temperature, salinity and brine volume of sea ice cores collected during the "Arise in the East" Antarctic cruise during September–October 2003 are presented elsewhere (Tison et al. 2005; Lannuzel et al. 2007). The main physical characteristics of the sea ice sections considered in the present paper are summarized in Table 1. The six sampled stations were located within the seasonal marginal ice zone. Sea ice thickness ranged between 0.3 and 0.8 m. The top of the core was characterized by a layer of granular ice originating from frazil ice growth during the early stages of ice formation and/or snow ice formation due to seawater infiltration in the snow cover. Granular ice accounted for between 15 and 51% of the total ice thickness. The lowermost layer of the ice presented a columnar structure formed through congelation growth at the base of the ice sheet. At stations XII and XIII, the cores presented more complex textural sequences with some bands of granular ice observed along the sea ice core, suggesting rafting had occurred (Lannuzel et al. 2007).

Ice temperature varied between −8.9 and −1.8°C (Table 1). Minimum temperatures were observed in the upper ice section of the cores. At station IV, more than 50% of the ice cover was below −5°C, while most of the ice cover was above −5°C at stations V and IX. Intermediate temperature regimes were observed at the other stations (VII, XII and XIII). This considerable inter-station variability of the temperature profiles clearly results from contrasted surface snow thicknesses (2–50 cm, Table 1). For most of the stations, salinity values showed the typical C-shaped salinity profile, characteristic of first-year ice in winter–spring period (e.g. Weeks and Ackley 1986). Bulk ice salinities ranged between 2.3 and 9.1, whereas brine salinities varied between 61.5 and 103.5. Sea ice brine volumes ranged between 2.9 and 16.3%. Maximum values were observed in the bottom sections (median 10.5%). In the upper layers, brine volumes were <5% at stations IV and XII, and >5%, the permeability threshold for the other stations. Station IX displayed generally higher porosity, with brine volumes being >5% along the whole core.

Chemical environment

Nutrient concentrations measured in the sea ice and water column are gathered in Tables 1 and 2, respectively. In sea ice, brine volume-normalized $Si(OH)_4$ concentrations ranged from 20.7 to 342.9 μM, with values measured in the upper layers (median 219.9 μM) higher than the intermediate and bottom layers (median 65.1 and 67.4 μM, respectively). Bulk ice silicate values were highly correlated with salinity values (Pearson correlation: $r = 0.97$, $P < 0.001$, $n = 51$; Fig. 1a). Brine volume-normalized NO_3^-/NO_2^- concentrations in the ice varied between 8.3 and 151.9 μM. Like silicate, nitrate (and nitrite) concentrations followed relatively well the theoretical dilution lines (TDL), except in some brines and intermediate sea ice sections, where several nitrate (and nitrite) values obtained were below the TDL (Fig. 1b). For PO_4^{3-}, the brine volume-normalized concentrations in the bottom layers (median 35.7 μM) were up to 45 times greater than surface seawater levels. This enrichment is illustrated in Fig. 1c, where bulk ice concentrations clearly lie above the seawater dilution curve. In contrast, the PO_4^{3-} concentrations measured

directly in the brines were remarkably low in comparison to expected values from the TDL. Likewise, NH_4^+ concentration analyzed in the brine samples were low (Fig. 1d) but concentrations in the ice were always above the TDL.

Dissolved organic carbon concentrations ranged between 0.85 and 8.22 mg C l^{-1} (median 1.49 mg C l^{-1}) in the bulk sea ice samples (Fig. 2). Maximum concentrations were observed in the bottom layers (median 4.37 mg C l^{-1}). Concentrations in brine samples (median 1.94 mg C l^{-1}, range 1.52–5.03 mg C l^{-1}; Table 3) were in the same range as those measured in the bulk ice samples (Fig. 2).

Table 2 Chemical and biological characteristics of the seawaters, $n = 17$

	Range	Median
Nutrients		
$Si(OH)_4$ (μM)	49.6–67.4	62.1
NO_3^-/NO_2^- (μM)	18.8–29.9	29.1
PO_4^{3-} (μM)	1.6–2.1	1.96
NH_4^+ (μM)	<0.056–0.4	<0.056
Chlorophyll *a* (μg l^{-1})	0.02–0.10	0.03
Organic carbon		
Dissolved (mg l^{-1})	0.52–3.26	1.08
Particulate (mg l^{-1})	0.02–0.11	0.06
TEP-C (mg l^{-1})	0.10–0.64	0.31
Organism biomass		
Algae (μg C l^{-1})	<0.01–1.59	0.13
Bacteria (μg C l^{-1})	0.61–5.15	2.08
Protozoa (μg C l^{-1})	<0.01–2.32	0.08

Concentrations measured in brines were therefore lower (13 times in average) than the brine concentrations estimated from bulk ice DOC and calculated brine volumes (Table 3). In the underlying seawater (Table 2), DOC concentrations (median 1.08 mg C l^{-1}, range 0.52–3.26 mg C l^{-1}) were lower than in the sea ice. Estimated TEP-C ranged from 0.02 to 2.02 mg C l^{-1} (median 0.25 mg C l^{-1}) in bulk sea ice samples (Table 1) with maximum values in the bottom layers (median 0.86 mg C l^{-1}). Measured brines concentrations were very low (median 0.12 mg C l^{-1}) and two order of magnitude lower than estimated brines concentrations (Table 3). In the underlying waters, the TEP concentrations remained high, between 0.10 and 0.64 mg C l^{-1} (Table 2).

POC and microorganisms distribution

Particulate organic carbon concentrations ranged between 0.35 and 4.78 mg C l^{-1} in bulk sea ice samples (Fig. 2). Maximum values were always measured in the bottom layers (median 3.41 mg C l^{-1}), while lower values, but still high as compared to seawater, were observed in the intermediate layers (median 0.66 mg C l^{-1}). Concentrations measured in the brine samples were much lower (57 times, in average) than the expected estimated values based on bulk ice POC and calculated brine volumes (Table 3). The POC concentrations measured in the underlying seawater (Table 2) ranged between 0.02 and 0.11 mg C l^{-1} (median 0.06 mg C l^{-1}). Measured total POC contains a biotic component (the biomass of microorganisms estimated by microscopy) and a detrital component ($POC_{detrital} = POC_{total} - POC_{biotic}$).

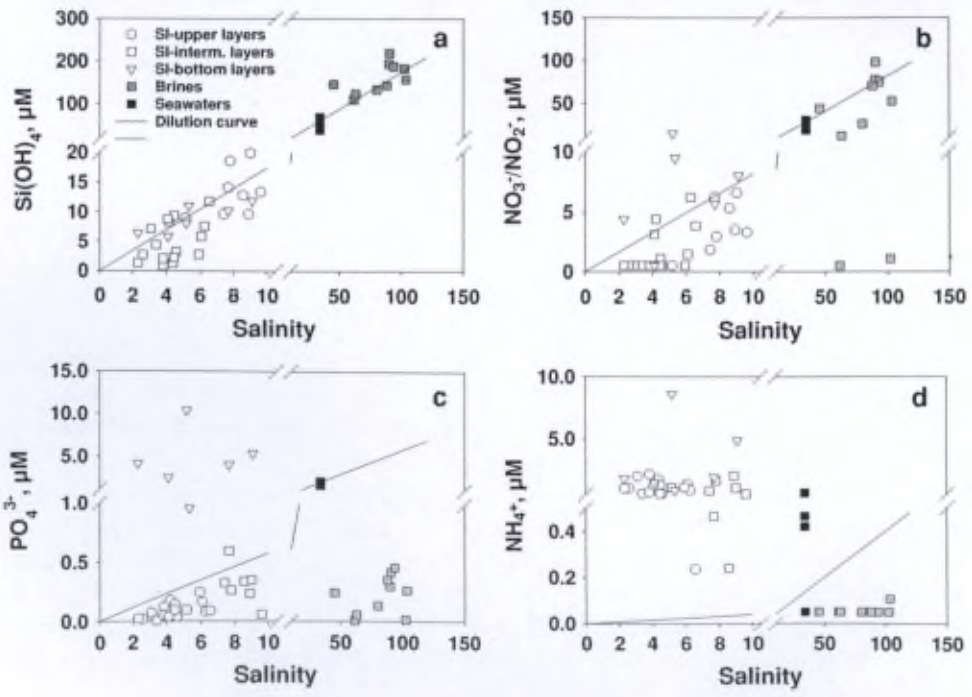

Fig. 1 Nutrients: **a** Silicate, **b** nitrate/nitrite, **c** phosphate and **d** ammonium, as a function of salinity in melted cores (bulk ice concentration), brine and seawater samples. The theoretical dilution line (TDL) was established from the nutrient concentration in under-ice water (−30 m) of the same station

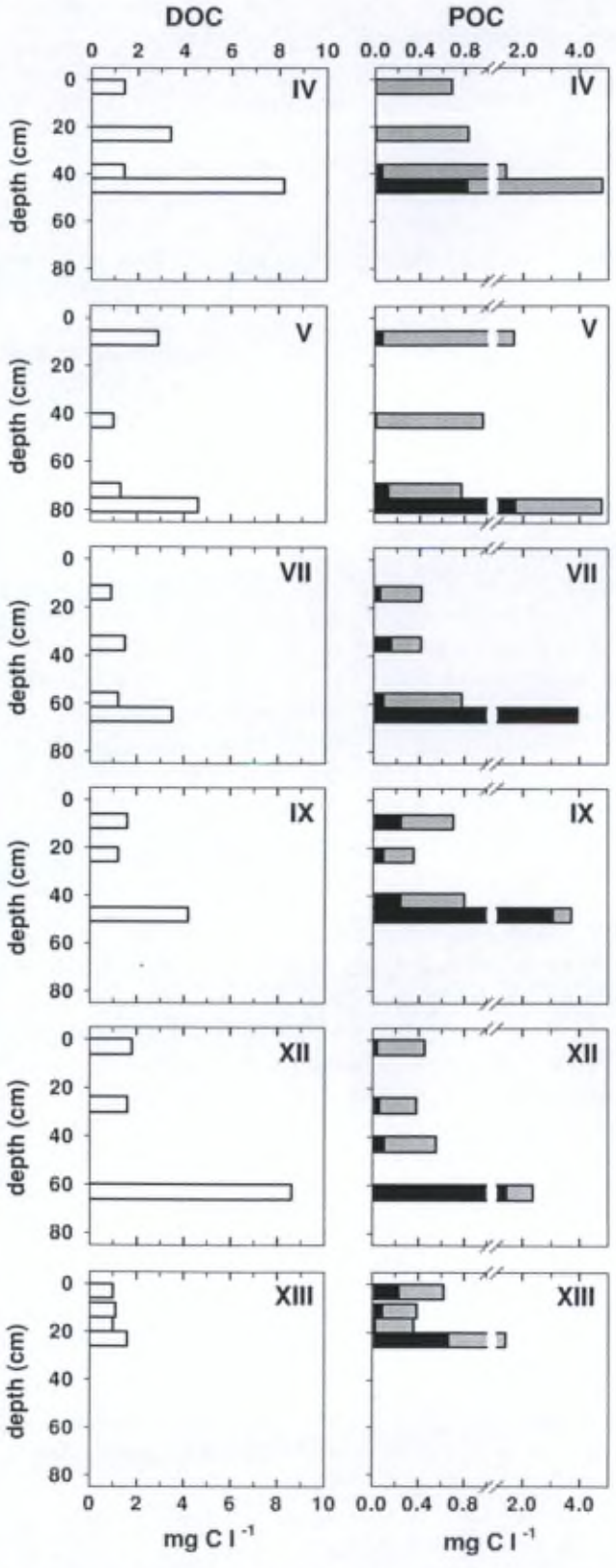

Fig. 2 Vertical distribution of organic carbon (dissolved and particulate) in the four sea ice sections. Particulate including biotic (*in black*) and detrital (*in gray*) organic carbon

Table 3 Dissolved organic carbon (DOC), particulate organic carbon (POC), transparent exopolymeric particles carbon (TEP-C), bacteria, algae and protozoa biomasses in the brine samples

	Measured values	Estimated values
DOC (mg l^{-1})	1.94	25.4
$n = 8$	1.52–5.03	10.9–68.9
POC (mg l^{-1})	0.18	10.2
$n = 9$	0.09–0.29	5.3–26.3
TEP-C (mg l^{-1})	0.12	12.3
$n = 8$	0.09–0.55	6.8–66.7
Bacteria (μg l^{-1})	9.75	484.8
$n = 9$	1.52–74.69	108.5–708.2
Algae (μg l^{-1})	8.99	551.1
$n = 9$	1.81–39.35	117.7–3076.7
Protozoa (μg l^{-1})	1.04	127.7
$n = 9$	0.02–6.54	68.3–1771.9

Measured (collected by sack hole) and estimated values based on bulk ice concentration and calculated brine volume (see "Materials and methods"). Median and range are given; n = number of samples

The latter is not directly associated to living organisms but results from previous biological activities. However, it is worthwhile to note that the biomass estimation by microscopy is not a very precise measurement of the microorganism standing crop. The conversion from the abundance of the microorganisms to carbon involves regression analyses and conversion factors that are subject to errors. If detritus content is high as compared to the biotic fraction (which can be observed in the microscopy samples), the carbon content of detritus can be estimated by difference without being strongly affected by the error on the microscopic estimate. The detrital POC pool generally dominated the POC in sea ice (Fig. 2; range 61–99%, median 81%), except in the bottom layer where the biotic pool could represent as much as 100% of the total POC. In seawater, POC was mainly detrital (Table 2; 87–99% of total POC, median 9%).

The biotic POC in the bottom layer was largely dominated by algal C biomass (Table 4). In sea ice, algal biomass ranged from 0.8 to 3,906 μg C l^{-1}, with values higher than 310 μg C l^{-1} in bottom ice (Fig. 3). Maximum concentrations of Chl *a* were recorded in bottom section as well (Table 1) and a sharp increase of algal biomass is observed from brine volume fraction higher than 8% (Fig. 5). In underlying seawater, Chl *a* concentrations were below 0.10 μg l^{-1} (Table 2). Similarly to POC, algal biomass in brine samples was lower than the estimated algal biomass based on bulk ice algal biomass and calculated brine volume (Table 3). The algal biomass in the bulk ice was largely dominated (median 82% of total algae biomass) by large algae (ESD > 20 μm) whereas the proportion of small algae was higher in the brine samples

Table 4 Contribution (%) of algae, bacteria and protozoa biomass in total biotic carbon; ratio of heterotroph to autotroph biomass (HB/AB ratio) in the sea ice samples, brines and seawater samples

	Algae (%)	Bacteria (%)	Protozoa (%)	HB/AB ratio
Upper layer	48	28	22	1.07
$n = 6$	7–85	9–73	<1–41	0.18–12.67
Intermediate layers	58	30	4	0.73
$n = 12$	46–92	7–52	<1–41	0.85–1.23
Bottom layer	97	2	<1	0.03
$n = 6$	47–99	1–4	<1–50	0.01–1.15
Brines	9	83	9	10.13
$n = 8$	2–39	32–97	<1–47	1.53–54.11
Seawaters	8	82	11	8.50
$n = 17$	<1–31	46–100	<1–46	2.21–525.53

Median and range are given, n = number of samples

(median 53% of total algae biomass, $n = 24$). In seawater, the algal biomass was also dominated by <20 μm cells with some exceptions (median 73% of total algal biomass, $n = 17$).

The autotrophic composition in sea ice was highly variable and showed a patchy distribution of biomass, reflecting the heterogeneity of the ice environment (Fig. 4). Autotrophic dinoflagellates could represent most of the autotrophic biomass in the upper layer, whereas diatoms (pennates and centrics) largely dominated in the other ice layers. Pennates generally dominated the diatomaceous biomass, with long chains of *Fragilariopsis* sp. only observed in the bottom ice. Centrics (mainly *Corethron* sp. and *Eucampia* sp.) were occasionally observed. In bottom ice, large autotrophic ciliates >10 μm were abundant at station IX, representing 58% of the autotrophic biomass (Fig. 4). Autotrophic nanoflagellates biomass was not significant in the ice assemblage. In the water column below, the autotrophic biomass was on average three orders of magnitude lower than in the sea ice and the autotrophic nanoflagellates represented a large contribution (median 36% of algal biomass, $n = 17$) of the pelagic autotrophic biomass.

Bacterial biomass ranged between 2.1 and 108.0 μg C l^{-1} in sea ice, with maximum values recorded at the maximum of algal carbon in the bottom layers (Fig. 3; median 26.0 μg C l^{-1}). However, in this ice section, their contribution to total C biomass stayed below 4% (Table 4). In the upper and intermediate layers, bacterial biomass represented from >9% up to 73% and from 7% up to 52%, respectively of the total biomass. In the brines and seawater samples, their contribution reached 83 and 81% (median values) respectively (Table 4). As estimated for algae, only a small fraction of bacteria were sampled in the brines (Table 3) as compared to the expected values estimated from bulk ice samples.

Protozoan biomass ranged between 0.7 and 331.0 μg C l^{-1} (Fig. 3). As for algae and bacteria, the maximum of biomass was reported in the bottom layer. Again, the biomass distribution among each group of protozoa was remarkably variable (Fig. 4). Dinoflagellates and ciliates generally dominated the ice protozoa biomass. The equivalent spherical diameter (ESD) ranged between 2 and 51 μm (median 11 μm, $n = 550$) and between 11 and 39 μm (median 17 μm, $n = 120$) for dinoflagellates and ciliates, respectively. In seawater, flagellates (including nano- and dino-flagellates) with ESD $\leq$ 10 μm dominated (range 2–10; median 4 μm, $n = 400$). In sea ice, the protozoa contribution to biotic carbon ranged between <1 and 50% (Table 4). In the brines and seawaters samples, their contribution varied between <1–47% and <1–46%, respectively.

The ratio of heterotroph (i.e. bacteria and protozoa) to autotroph biomass (HB/AB ratio) ranged between 0.01 and 12.7 in the sea ice (Table 4), with very low values in the bottom layer (median 0.03, $n = 6$). On the opposite, in the underlying seawater, the heterotrophs largely dominated with HB/AB varying between 2.21 and 525.53 (median 8.50, $n = 17$).

Discussion

The sampling period of the ARISE cruise (October) corresponds to the austral late winter–early spring. Values of salinities in the ice cores collected were similar to those previously observed in Antarctic sea ice for end-of-winter conditions (Clarke and Ackley 1984). Higher bulk ice salinity values were found in top layers of the ice cores, indicating that no significant brine drainage had already taken place from the top, as it is often the case later in the season (Tison et al. 2008). The collected ice cores consisted of granular and columnar ice textures, which is characteristic of first-year Antarctic pack ice. Sea ice growth processes are key mechanisms for the incorporation and distribution of microorganisms (Horner et al. 1992; Weissenberger and Grossmann, 1998; Lizotte 2003). Granular ice is associated with dynamic turbulent conditions and forms usually at high growth rates, while columnar ice forms more slowly (Eicken 2003). A rapid growth involves the concentration of cells, by nucleation of frazil ice crystal or by scavenging of cells as frazil crystals float up through the water column (Garrison et al. 1989; Horner et al. 1992). In contrast, columnar ice formation has tendency to reject algal cells in the initial growth period (Palmisano and Garrison 1993; Weissenberger and Grossmann 1998). Consequently, sympagic communities found

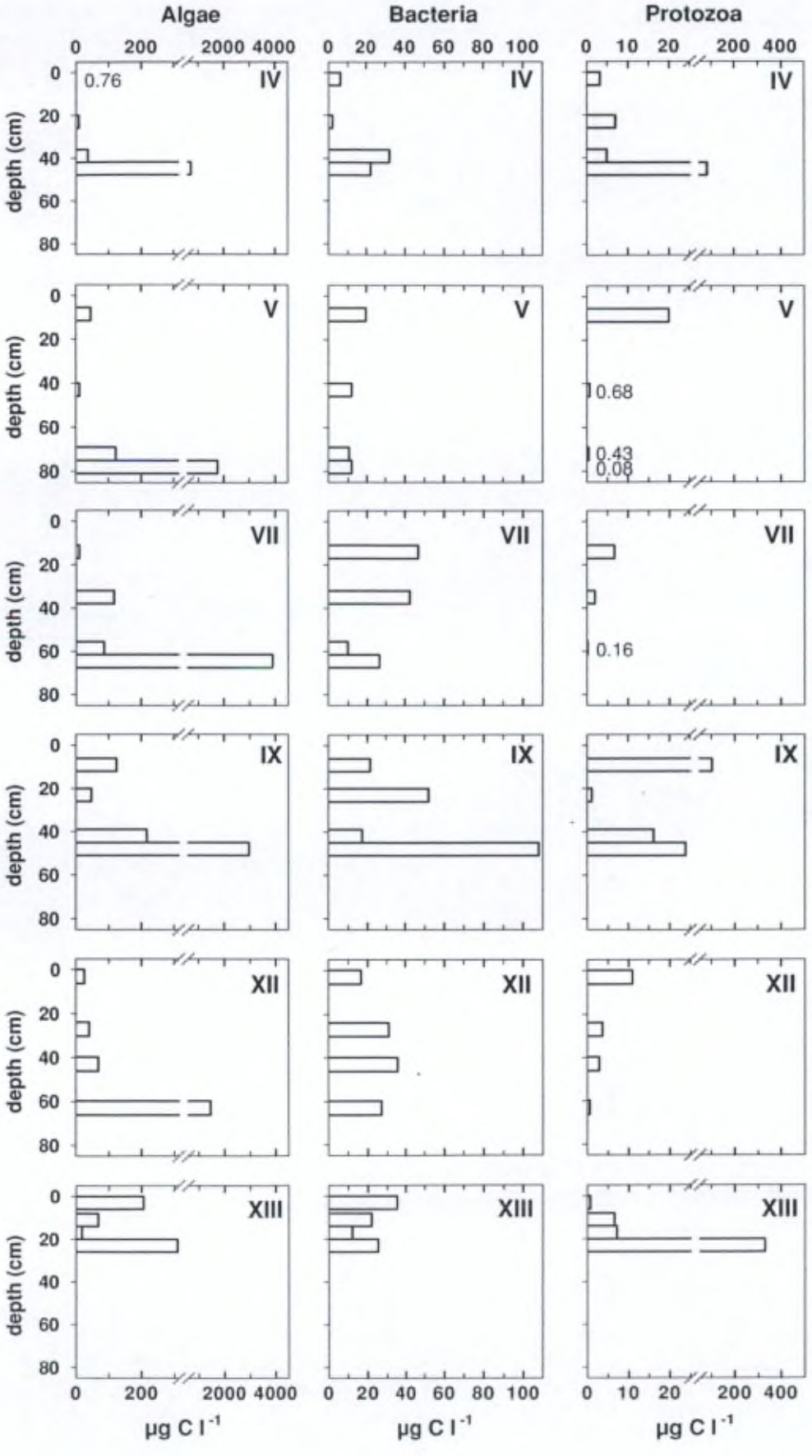

Fig. 3 Vertical distribution of sympagic microorganisms (algae, bacteria and protozoa) in the four sea ice sections

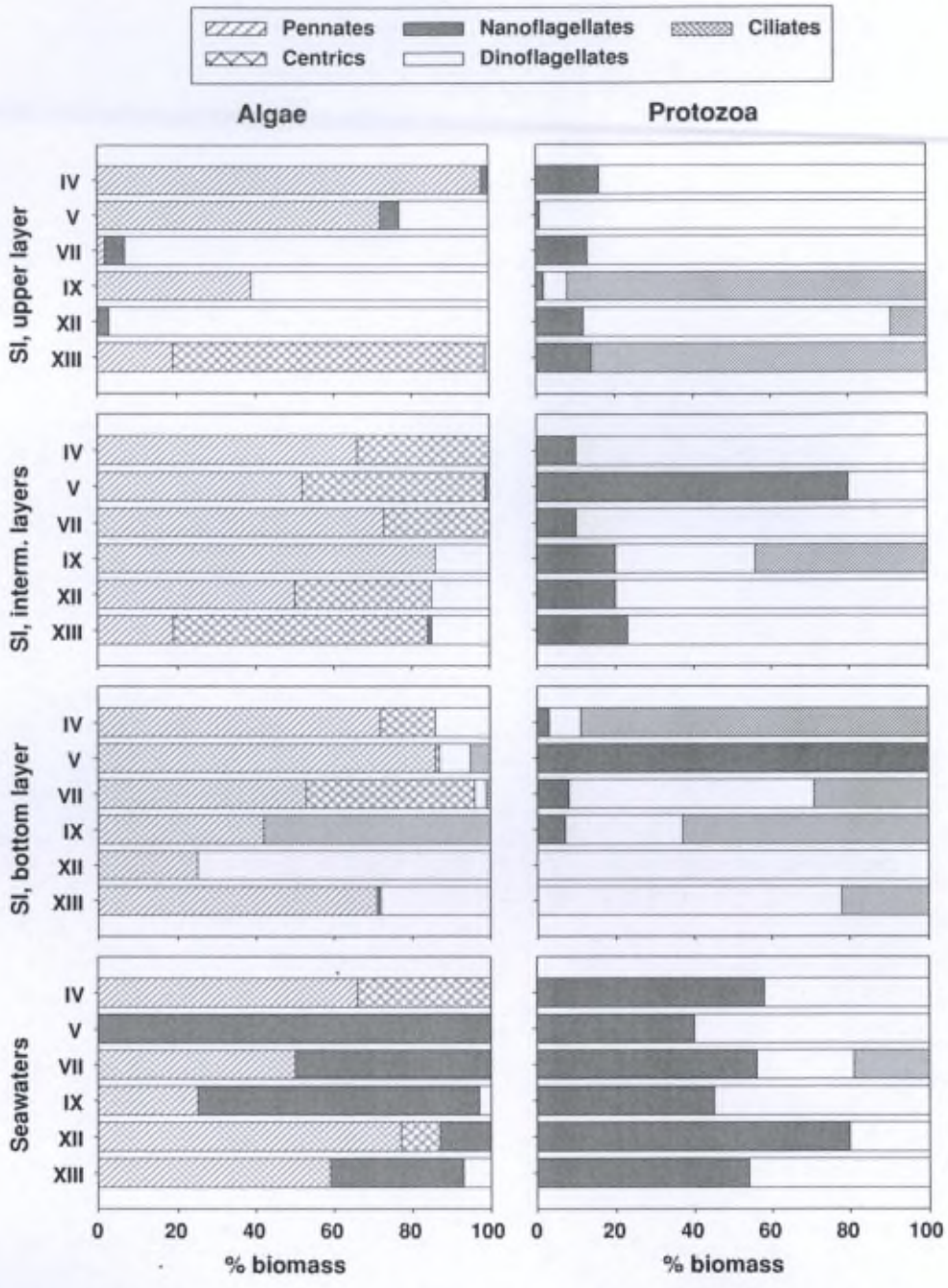

Fig. 4 Distribution of major algae and protozoa taxonomic groups in the sea ice [*upper*, *intermediate* (average values) and *bottom layers*] and underlying seawaters

in granular ice could be composed of organisms from the previous autumn, such as dinoflagellates forming cysts (Garrison and Close 1993). Nevertheless, contrasts in the initial concentration between various textural units were probably damped as time goes by, from autumn to the winter–spring (Eicken et al. 1991). In the late winter, the slowly growing bottom skeletal ice layer of the columnar ice provides a unique habitat where large accumulations of organisms, in particular algae, are observed (Garrison and Mathot 1996). In our samples, no clear relationship between biological occurrence and textural type (granular and columnar ice) was observed.

Generally, abiotic factors, i.e. light, temperature, salinity, nutrients are considered to control ice algal growth and explain spatial and temporal distribution of sea ice organisms (Arrigo and Sullivan 1994). Temperature, which controls the brine salinity and the brine volume fraction, structures the sea ice ecosystem (Eicken 1992). The brine volume fractions reported in this study were relatively stable (between 3 and 8%) but increased sharply, when a threshold temperature of −4°C was reached in the columnar ice and mainly in the bottom section. The brine volumes and more specifically the internal surfaces of brine channels and pockets could be a key factor in controlling

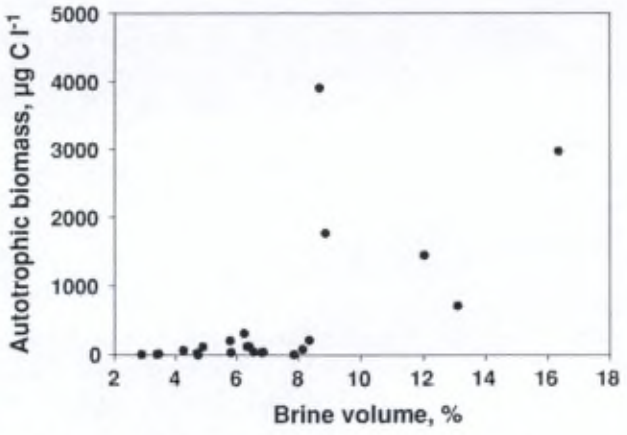

Fig. 5 Autotrophic biomass as a function of brine volume fraction in the pack ice

the accumulation and colonization of microorganisms in the sea ice (Krembs et al. 2000). As reported by Perovich and Gow (1996), for brine volumes of 3–8%, the mean surface area of the brine pockets remains essentially constant. As the ice further warms, the mean area gradually increases as the brine volume increases from 8 to 20%, and then sharply from 20 to 40%, due to the connection of pockets by the brine channels. In our study, a sharp increase of algal biomass is indeed observed from brine volume fraction higher than 8%, suggesting a control by the brine volume fraction on the algae accumulation at the beginning of the productive season in the sea ice (Fig. 5). For brine volumes ranging from 3 to 8%, the autotrophic biomass remained relatively constant, but above the limit of 8%, which roughly corresponds to −4°C, autotrophic organisms can accumulate (Fig. 5). During tank experiments, it has been shown that diatom biomass could accumulate when ice temperatures increased from −4 to −3°C (Krembs et al. 2001).

Both the ice–water exchanges and the environmental space available for sympagic organisms are driven by the brine volume. The percolation theory for columnar ice shows that, below a critical brine volume of 5%, which corresponds to a temperature and salinity of −5°C and 5 respectively, sea ice becomes impermeable, thus limiting the circulation of brines. This phenomenon is of prime importance for ice algal growth which becomes rapidly macro-nutrient limited (Arrigo 2003). When the ice is impermeable, nutrients found in the sea ice internal habitat mainly originate from their initial entrapment during ice formation or from recycling. As the ice starts to warm up, gravity brine drainage allows the transport of nutrients from the underlying water across the ice–water interface via convection mechanisms. Alternatively, nutrients replenishment can also occur when flooding introduces seawater within the surface layers. Nutrient concentrations in the sea ice cores will therefore result from a subtle balance between physical processes (permeability and brine drainage/convection) and/or biological activity (uptake and remineralization) depending on the temporal thermodynamic stage of the ice cover. Parameters governed by physical mechanisms should vary with salinity, because the latter depends on temperature and brine drainage/convection processes only. In order to reveal a potential biological control on nutrient concentrations in the ice cover as compared to those in the water column, theoretical dilution lines (TDL) have been plotted for each nutrient (Fig. 1). The estimated TDL can only be considered as a rough guide to processes involved in concentrating nutrients from seawater into brines. Since we do not have any data on nutrients concentrations in the water column when the sea ice was formed, we assume that the initial nutrient concentration was similar to the one measured in the seawater at −30 m. We also have to assume that no nutrient fractionation occurs during ice growth and during further desalination processes, which is a plausible approximation given the fact that molecular diffusion (with specific diffusion coefficients) contributes partly to these processes. Results obtained by Meese (1989) for cations that are not involved in biotic processes seem to indicate that this approximation is valid. Briefly, a negative deviation of TDL is associated to the biological uptake while a positive deviation indicates remineralization processes. Our results show that silicate concentrations are centered on the dilution curve and nitrates, although roughly increasing with salinity, already show some signs of depletion, with most of the ice samples slightly below the TDL and some of the brine samples clearly exhibiting strong deficits. Other studies showed that nitrate and silicate concentrations in the winter sea ice were centered on TDL, indicating that little nutrient uptake had taken place prior to the sampling time (Dieckmann et al. 1991; Arrigo et al. 1993). As the season progresses, nutrient concentrations fell increasingly below TDL presumably due to algae uptake (Arrigo et al. 1993; Papadimitriou et al. 2007). Although phosphate and ammonia are not correlated with salinity, a large accumulation of these two nutrients in the bottom section is observed. The very high correlation between PO_4^{3-} and NH_4^+ ($r^2 = 0.938$, $P < 0.001$, $n = 22$) suggests that their accumulation could result from similar processes. High accumulation of PO_4^{3-} and NH_4^+ are also observed in other sea ice studies (Dieckmann et al. 1991; Arrigo et al. 1995; Thomas et al. 1998). The remineralization of the observed large accumulated organic pools in the ice as well as the direct release of algal osmolytes due to cell lysis (Cota et al. 1990; Lizotte 2003; Thomas and Papadimitriou 2003) could lead to these very high concentrations.

In early spring, the major nutrients concentrations in the microenvironment where the algae are living, i.e. the brine

volume-normalized nutrients concentrations, are very high (Table 1, this study) as well as for micronutrients like iron (Lannuzel et al. 2007). The concentrations are at least 5 (range 5–88) times higher for Si, 18 (range 18–165) times for N and 122 (range 122–1,570) times for DFe above the average values of the half-saturation constant [Ks(Si) = 3.9 ± 5.0 μM, Ks(N) = 1.6 ± 1.9 μM, Kμ(Fe) = 0.35 ± 0.44 nM] characterizing diatoms nutrient uptake in oceanic waters (Sarthou et al. 2005). The PO_4^{3-} availability could however be sometimes limiting in the intermediate layers where its concentration reached values lower than diatoms estimated half-saturation constant [Ks(P) = 0.24 ± 0.29 μM]. Nevertheless, the PO_4^{3-} brine concentrations in the upper and bottom sea ice samples were at least six times above the value of the half-saturation constant. These results suggest that the algae growth was not limited by nutrients availability at the beginning of the productive season. The growth of the sympagic organisms at low temperature can however be limited by reduced affinity for substrates such as for nitrate and silicate (Thomas and Papadimitriou 2003). For instance, the half-saturation constant for the uptake of silicic acid is known to be high for diatoms from the Southern Ocean as compared to other region (Ks > 60 μM, Sommer 1986; Nelson et al. 2001).

Diatoms which dominated the algae community in sea ice are nevertheless relatively flexible in their intracellular Si content, and near-maximum cell division rates can be sustained even when ambient concentrations of silicic acid limit Si uptake by diminishing the cellular Si content (Martin-Jézéquel et al. 2000). Hence Si uptake rates may appear to be limited by ambient silicic acid concentrations, but this does not necessarily imply limitation of growth.

During our investigation, high concentration of TEP have been observed in sea ice samples, in agreement with the range of values reported for Antarctic pack ice (Meiners et al. 2004) and in Arctic fast and pack ice (Krembs and Engel 2001; Krembs et al. 2002; Meiners et al. 2003; Riedel et al. 2006, 2007a). Pennate diatoms have been reported to secrete high TEP concentrations for both adhesion and mobility by gliding (Wetherbee et al. 1998). The ability to move vertically should be an advantage in the sea ice environment in order to gain access to better light and nutrients conditions. Bacteria are also capable of producing TEP (Simon et al. 2002), but in a lower amount as compared to algae (Meiners et al. 2004; Riedel et al. 2006). Previous studies on sea ice have reported high numbers of bacteria attached to algal cells (Grossmann and Gleitz 1993; Grossmann 1994; Grossmann and Dieckmann 1994) and algal derived TEP (Meiners et al. 2004). During the present cruise, TEP were fairly well correlated with Chl *a* (Dumont et al. 2009). The high measured concentrations of TEP likely have the potential to anchor the cells to the ice surface and retain ice components into this viscous phase. The difference between values measured in brine samples (sack holes) and values estimated from bulk ice values and brine volume could result from the attachment or entrapment of the microorganisms in the ice matrix. A large fraction of microorganisms would therefore not be sampled by the drainage collection into "sack holes".

Also, dissolved organic carbon seems to have been retained on the brine pockets and channels walls in sea ice, as suggested by the large difference between concentration measured in the "sack holes" samples and estimated brines concentrations based on bulk ice DOC concentrations and brine volume fractions. These results corroborate the view of Krembs et al. (2000) and Mock and Thomas (2005) who consider that the sea ice internal habitat resembles that of a biofilm adhering to the brine channel walls, with an overlying flowing liquid phase i.e. the brine fraction. Biofilms can be defined as microorganisms attached to a surface and embedded in an extracellular gel-like matrix of polymeric substances (Fischer 2003). TEP are clearly an integral part of the structural organization of the biofilm. TEP have also been shown to adsorb dissolved organic compounds from the bulk fluid (Davey and O'Toole 2000). DOC concentrations were within the range previously observed in the sea ice (Thomas et al. 1995; Krembs et al. 2002), with very high values in the bottom layers (Meiners et al. 2008; this study). These DOC concentrations reached values close to those of POC in the bottom ice. Sea ice DOC/POC ratio ranged from 1:1 to 4:1, whereas seawater ratio typically varied from 5:1 to 61:1. These measured DOC/POC ratio in the sea ice environment are very low in comparison to traditional oceanic water value of 15:1 (Millero 1996; Kepkay 2000). An explanation for that might be the abiotic transformation of DOC into POC when a DOC threshold is reached, maintaining a constant DOC/POC ratio. In Antarctic summer ice floe, DOC concentrations up to five times lower than those of POC have even been reported (Kattner et al. 2004). POM and/or TEP formation from DOM can indeed be described using coagulation dynamics (Chin et al. 1998; Mari and Burd 1998). Low DOC/POC ratio are typically observed in biofilms and aggregates (Giani et al. 2005), due to high biotic DOC consumption and remineralization or transformation through coagulation process into TEP and/or POC (Passow 2000). Moreover, when reaching salinities as high as those measured in the brine pockets (in this study >61), a substantial increase of cations such as Na^+, Ca^{2+}, Mg^{2+} and Fe^{3+} may enhance floc formation and aggregation of suspended dissolved organic carbon (Decho 2000).

In addition to the accumulation of DOC and TEP, high inorganic nutrients such as PO_4^{3-} and NH_4^+ concentrations were measured. A proportion of the inorganic nutrients produced by the remineralization of organic matter might not instantly turned over but remains in the

biofilm, which acts as a reservoir and buffers the direct effects of major nutrients depletions. For instance, Fe (oxy)hydroxides associated with DOM may bind PO_4^{3-} (Maranger and Pullin 2003). Such an association could have promoted the accumulation of DOM-Fe–P complex in the sea ice, as suggested by the parallel accumulation of TEP, DOC, Fe and PO_4^{3-} in the sea ice bottom layers. This could allow the maintenance of an eutrophicated environment (sea ice environment) in a HNLC region such as the Southern Ocean. The median heterotrophic/autotrophic (HB/AB) ratio measured in our sea ice samples, <0.73 (median, $n = 24$), is characteristic of eutrophic environments (Gasol et al. 1997) where autotrophic biomass is largely composed of micro-sized (>20 μm) diatoms, while bacteria and protozoa biomass stays much more modest, as observed in this study. When the season progresses, later in spring and summer, sea ice samples can present lower HB/AB ratios (Garrison et al. 1986; Mathot et al. 1992), as it is observed for bottom ice samples in the present study (Fig. 6).

The gel structure characterizing the brine system could also modify typical trophic relationships observed in marine ecosystems. Several authors have found a positive relationship between bacterial and algal biomass/production in sea ice during spring/summer (Grossi et al. 1984; Kottmeier et al. 1987; Kottmeier and Sullivan 1990; Stewart and Fritsen 2004). However, as it is the case in the present study, Kottmeier et al. (1987) found no significant relationship between algae and bacteria in late winter sea ice. Maximum bacterial biomass was observed at maximum algal biomass, but the relative contribution to total C biomass was very low (Table 4). On the contrary, their contribution was relatively higher at low level of total microorganism biomass. Although we observed very high TEP and DOC concentrations, bacterial biomass remained relatively low and there was no relationship with these potential substrates (Pearson correlation: $r = 0.130$, $P > 0.10$, $n = 16$ for bacteria vs. TEP; $r = 0.125$, $P > 0.10$, $n = 26$ for bacteria vs. DOC). It has been proposed that sea ice can be an environment where the development of a microbial loop is often hampered, leading to the accumulation of POM and DOM (Lizotte 2003). As observed previously by Meiners et al. (2004), the integrated sea ice TEP in our study represented 687–12,343% of the integrated bacterial biomass, with very high percentage at the most winter station (station IV as defined in Lannuzel et al. 2007). Sea ice TEP seems nevertheless potentially to serve as a carbon source for sympagic bacteria, as suggested by a coupling between TEP and NH_4^+ regeneration (end-product of bacterial remineralization; Riedel et al. 2007a). Confirming this, a very high correlation between TEP and NH_4^+ concentrations (Pearson correlation: $r = 0.89$, $P < 0.001$, $n = 14$) is also observed in the present study. However, as observed in annual sea ice in the Ross Sea, rapid turnover of nitrogen-organic compounds is not always associated to high bacterial growth (Guglielmo et al. 2000).

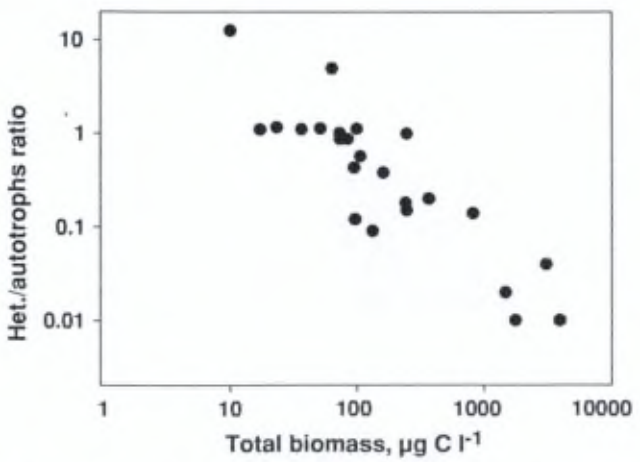

Fig. 6 Heterotrophic to autotrophic biomass ratio as a function of total microorganism (algae, bacteria and protozoa) biomass in the pack ice zone

Despite the fact that bacteria are exposed to very high substrate concentrations, protozoa grazing might control their biomass accumulation. Protozoa can indeed consume bacteria as well as small-sized algae. Our current understanding of sea ice grazing activity is however limited by the lack of appropriate investigation methods (Lizotte 2003). Given these limitations, sea ice feeding relationships could be analyzed from the absence/presence of prey and grazers and numerical response curves. Ciliates and dinoflagellates, as observed in this study, dominated the protozoa biomass in sea ice (Garrison and Mathot 1996). The particle size spectrum ingested by ciliates appears to be determined by their cell size (Fenchel 1987), a ratio between predator and prey size of 1:10 is usually observed. In our studies, ciliates with ESD < 40 μm dominated and those ciliates could consequently only graze on cells smaller than 4 μm i.e. bacteria and flagellates. However, Scott et al. (2001) reported a wide range of food particles biomass from femto- to nano-sized cells for an Antarctic sea ice ciliate (*Pseudocohnilembus* sp., ESD < 12 μm). Dinoflagellates, dominating the protozoa biomass in the sea ice, have been shown to feed on particles that approach or exceed their own sizes (Gaines and Elbrächter 1987). In the Antarctic marginal ice zone, the size range of autotrophic prey and predators overlaps. The ESD of dinoflagellates enumerated in our samples is around 11 μm (median). Those dinoflagellates are therefore likely to consume nano-sized particles. In Antarctic seawaters protozoa biomass linearly increased with the availability of potential food such as bacteria and small algae (<20 μm, Becquevort et al. 1992; Becquevort 1997). In the present study, protozoa biomass significantly

correlated with the food biomass (Pearson correlation: $r = 0.620$, $P < 0.001$, $n = 22$) but increased only when a food threshold was reached (Fig. 7). There would be a range of food concentrations over which ingestion cannot offset maintenance metabolism, so that consumer growth rate would be ≤ 0. If mobility needed for the capture of food represents a substantial energy cost, it would seem advantageous to limit predation activity when food concentration is too low to provide a compensating energy return (Taylor 1978). The presence of high TEP concentration altering the physical conditions by setting a gel, could change grazer mobility (Joubert et al. 2006), and then regulate the prey–grazer interactions. Accordingly, Riedel et al. (2007b) found a negative correlation between TEP concentration and experimentally derived ingestion rates of bacterivores in Arctic sea ice.

During seasonal melting, the accumulated components will be released from the sea ice to the seawater and some of them may serve as inoculum. Aggregated ice algae might be quickly grazed by krill (Scharek et al. 1994) or rapidly settle (Riebesell et al. 1991). The released algal cells would then never have the chance to serve as an inoculum for open water blooms. In the present study, diatoms dominated in the ice environment, whereas flagellates dominated in the water column. The similarity between sea ice and water column algal assemblages is indeed not demonstrated in all studies (Leventer 2003). The planktonic organisms could have the opportunity to grow thanks to the release of Fe and OM concentrated in the sea ice. In the underlying seawater, the DFe (Lannuzel et al. 2007), DOC and TEP concentrations were lower than in the sea ice; but were still relatively high for Antarctic seawaters (for DFe: 0.05–0.3 nM, de Baar and de Jong 2001; for DOC: <0.72 mg C l^{-1}, Ogawa and Tanoue 2003). The OM release seems actively consumed by bacteria which largely dominated biotic C biomass (Table 3). DOM released during ice melting is indeed known to be rapidly used by bacteria (Kähler et al. 1997; Giesenhagen et al. 1999). Consequently, extremely high values of HB/AB ratios were reported. These ratios reflected the community structure dominating in sea ice underlying waters at the end of winter (Garrison and Mathot 1996).

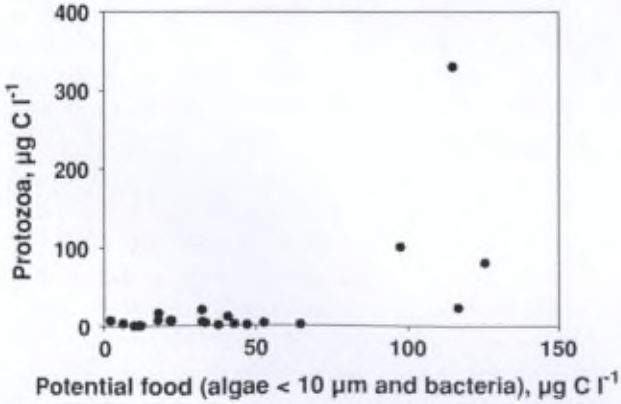

Fig. 7 Relationship between protozoa biomass and potential food, i.e. algal <20 µm and bacterial biomass

Conclusion

Sea ice algal growth at the beginning of the productive season was not limited by nutrients, but rather by available brine space and surface. A large fraction of microorganisms embedded in TEP, were retained to the brine channel/pockets, forming a gel "biofilm". It constitutes specific microenvironments for microorganisms (algae, bacteria and protozoa) where the high retention of nutrients and the low presence of their grazers allowed a large accumulation of algae. The increased autotrophic biomass was largely dominated by micro-sized diatoms. Despite large substrate concentration, the observed bacteria and protozoa biomass increase was much more modest. The accumulated OM could then be released in the seawater at the time of ice melting, stimulating the planktonic microbial loop.

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Chapter 2

Distribution and characterization of dissolved and particulate organic matter in Antarctic pack ice

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ORIGINAL PAPER

Distribution and characterization of dissolved and particulate organic matter in Antarctic pack ice

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Abstract Distribution and composition of organic matter were investigated in Antarctic pack ice in early spring and summer. Accumulation of organic compounds was observed with dissolved organic carbon (DOC) and particulate organic carbon (POC) reaching 717 and 470 μM C, respectively and transparent exopolymeric particles (TEP) up to 3,071 μg Xanthan gum equivalent l^{-1}. POC and TEP seemed to be influenced mainly by algae. Particulate saccharides accounted for 0.2–24.1% (mean, 7.8%) of POC. Dissolved total saccharides represented 0.4–29.6% (mean, 9.7%) of DOC, while dissolved free amino acids (DFAA) accounted for only 1% of DOC. Concentrations of TEP were positively correlated with those of saccharides. Monosaccharides (d-MCHO) dominated during winter–early spring, whereas dissolved polysaccharides did in spring–summer. DFAA were strongly correlated with d-MCHO, suggesting a similar pathway of production. The accumulation of monomers in winter is thought to result from limitation of bacterial activities rather than from the nature of the substrates.

Keywords Sea ice · Organic carbon · Monosaccharides · Polysaccharides · Amino acids · TEP

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Introduction

Antarctic sea ice is a very dynamic ecosystem with an extent fluctuating seasonally between 4 and 20 million km^2 (Zwally et al. 1983). Such a vast annual formation and subsequent melting of sea ice have huge implications for the whole Southern Ocean, and at a larger scale for global climate (Brierley and Thomas 2002), by way of both physical and biological processes. Sea ice provides a habitat for microorganisms like algae, bacteria and protozoa, which live in the system of brine channels and pockets inside the sea ice cover. Extremely high levels of organic matter have been recorded in sea ice, which are up to several orders of magnitude greater than those in seawater (Thomas et al. 1998, 2001a; Herborg et al. 2001). This organic matter is either allochthonous, i.e. trapped during the ice formation (Giannelli et al. 2001) or autochthonous, i.e. produced within the sea ice via in situ biological activities.

Mechanisms of organic matter production include: extracellular release by microorganisms, grazer mediated release and excretion, release via cell lysis (by viral, bacterial, physical or osmotic mechanisms), solubilisation of particles and bacterial transformations and release (Nagata 2000). Furthermore, in response to the extreme environmental conditions encountered in sea ice, such as high salinity or

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low temperature, secretion and excretion by sea ice microorganisms of specific compounds like ice-active substances (Raymond 2000; Janech et al. 2006), dimethylsulphoniopropionate (DMSP) or extracellular polymeric substances (EPS) are enhanced (Brierley and Thomas 2002). Transformations between dissolved and particulate organic pools also occur: dissolved organic matter can physically aggregate in minutes to hours to form more polymerized molecules (Chin et al. 1998) or conversely, particles can be transformed into dissolved form via, e.g. the enzymatic activity of heterotrophic bacteria (Cho and Azam 1988).

The consumption of organic matter by heterotrophic organisms is mainly due to bacteria but the uptake of dissolved organic carbon (DOC) by protists, algae and *Archaea* is sometimes observed (Sherr and Sherr 1988; Marchant and Scott 1993; Palmisano and Garrison 1993; Brierley and Thomas 2002). The consumption of organic matter by bacteria depends on the nature of the substrate and on the composition and physiological state of bacterial assemblages. Bacterial activity (e.g. enzymatic activities) and viability may be limited by low temperature (Delille 1992; Nedwell 1999; Pomeroy and Wiebe 2001), high salinity, or antibiotic effects of compounds such as dimethylsulfide (DMS) and acrylic acid resulting from the breakdown of DMSP (Thomas and Papadimitriou 2003).

The observed accumulation of organic matter clearly reveals an uncoupling between these production and consumption processes (Carlson 2002; Thomas and Papadimitriou 2003). A major consequence of this high pool of organic matter in the ice and in particular of these EPS is a profound modification of the habitat where microorganisms live. Indeed, gel-like particles such as transparent exopolymeric particles (TEP), formed biotically (Passow 2002) or abiotically (Chin et al. 1998) from EPS, have been found in particularly high concentrations in the sea ice ecosystem (Krembs and Engel 2001; Krembs et al. 2002; Meiners et al. 2004). The viscous nature of TEP yield to the brine channels system special characteristics, notably for the retention of nutrients, organisms' attachment, movement, space availability or protection against predators (Decho 1990; Passow 2002). Then the classic microbial loop can be modified and further lead to that large accumulation of organic matter.

Improving our knowledge of the quantity and the type of organic matter present in the sea ice is crucial to understand the dynamics of the organic pool and to decipher the biological processes governing both production and consumption of organic matter in sea ice. In addition, through the seasonal decay of the ice cover, the sea ice microbial community and organic matter may affect many processes in the water column such as the initiation of blooms, the solubility and bioavailability of iron (Geider 1999) and the efficiency of the biological carbon pump.

In this paper, we investigate the distribution and concentration of the dissolved and particulate fraction of organic matter along the size continuum in Antarctic pack ice sampled during the ARISE (spring 2003) and ISPOL (summer 2004) cruises. In order to better tackle the processes involved in the removal and production of the organic matter, not only are total particulate and dissolved concentrations described, but also the distribution of saccharides, amino acid and transparent exopolymeric particles.

Materials and methods

Sampling

Sea ice and under-ice seawater samples were collected during two Antarctic cruises in the pack ice zone: (a) the "ARISE IN THE EAST" (Antarctic Remote Ice Sensing Experiment) research cruise onboard the RV *Aurora Australis* in the Australian sector (64–65°S, 112–119°E) of the Southern Ocean in September–November 2003 and (b) the ISPOL (Ice Station POLarstern) cruise onboard the RV *Polarstern* in the Western Weddell Sea, Antarctic Peninsula (68°S, 55°W) in November 2004–January 2005 (Fig. 1). The ARISE cruise was a spatio-temporal study with the repetition of 1-day-long ice stations at different locations of a relatively limited spatial domain during the early spring (Fig. 1). During the ISPOL cruise, the RV *Polarstern* was anchored to a large ice floe (few km in size) in order to follow the temporal variations of the physical and biological atmospheric–ice–ocean processes at a drifting station during the transition from austral spring to summer (Hellmer et al. 2008). Sea ice samples analysed in this study have been collected, during ARISE at stations IV, V, IX and XII and at the ISPOL station on the 4/12, 9/12, 14/12, 19/12 and 30/12 (Fig. 1).

The ice covers sampled during these two cruises were typical of first year sea ice, grown in calm conditions, dominated by columnar/congelation ice with a thin surface layer (5–10 cm) of granular ice crystals (frazil and/or snow ice). The total ice thickness ranged from 0.6 to 0.9 m. The ARISE stations have been categorized into winter (cold)- and spring (warm)-type stations, taking into account their respective salinity, temperature and brine volume profiles and related impact on the ice permeability (see classification in Lannuzel et al. 2007). Having undergone a similar genesis, stations IV (winter type) and IX (spring type) can be more particularly compared to each other (Lannuzel et al. 2007). The ice characteristics during the ISPOL cruise were representative of the late spring–early summer transition. Detailed ice physical properties are presented in Lannuzel et al. (2007) for ARISE and in Lannuzel et al. (2008) and Tison et al. (2008) for ISPOL. On the basis of this

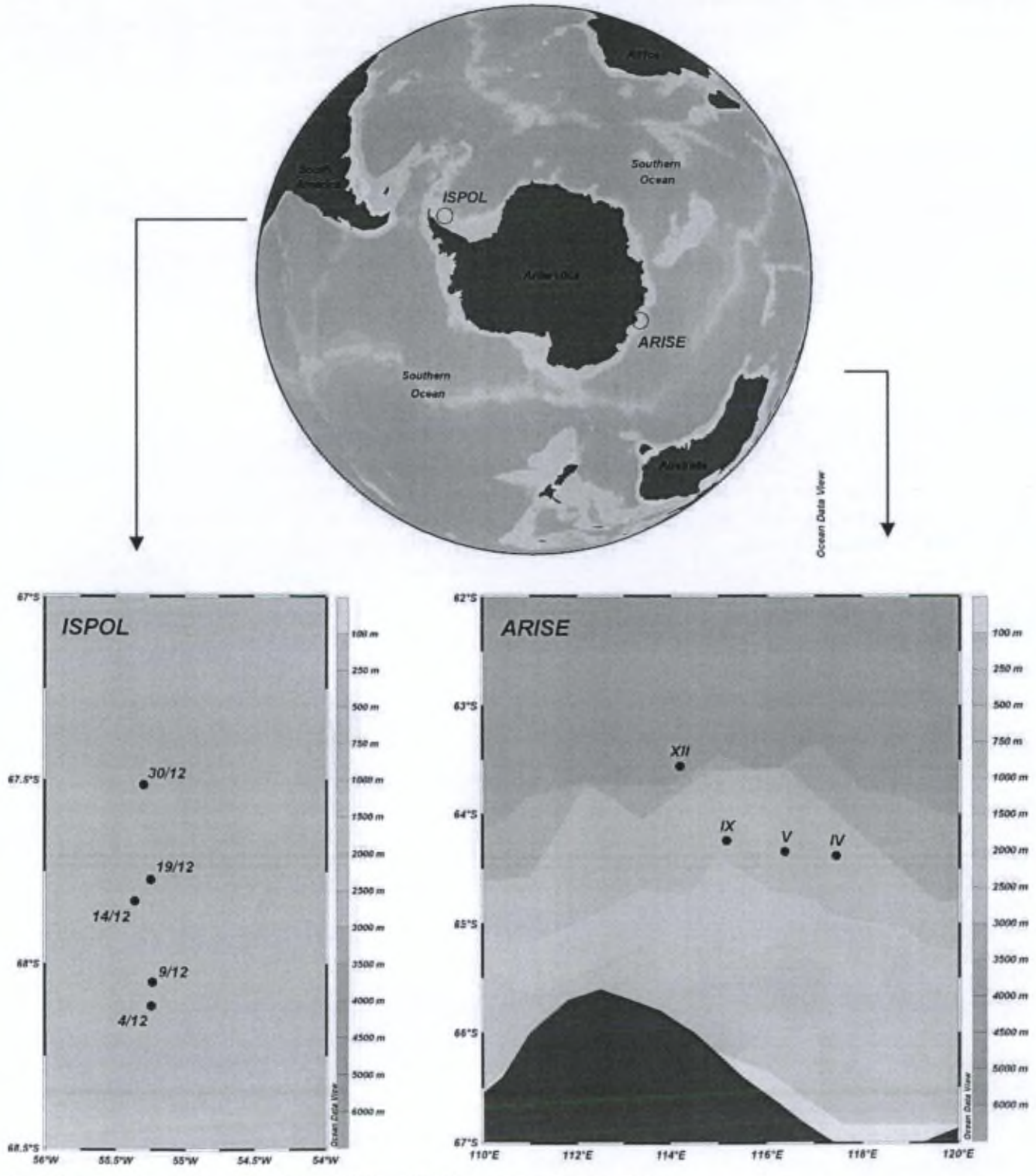

Fig. 1 Locations of sampling stations during ARISE and ISPOL expeditions (Schlitzer 2008)

broad classification and textural similarity, results from the two cruises will be compared hereafter on a temporal scale.

Sampling was conducted at sites of 20 × 20 m, located 1 km away from the ship, using precautions to avoid organic matter contaminations, which were also meant to be trace metal clean (Lannuzel et al. 2006, 2007, 2008). Clean room garments (Tyvek overall, overshoes and polyethylene gloves) over warm clothes of operators were worn on site and a special electropolished stainless-steel corer, of 14-cm diameter, was used to collect the ice cores. The ice

cores were stored at −28°C until further processing. Under-ice seawater (0, −1 and −30 m) was collected thanks to a portable peristaltic pump (Cole-Palmer, Masterflex E/P) and acid clean tubing. Seawater samples were transferred into acid washed bottles abundantly rinsed with collected samples.

Onboard ship, ice cores used for organic carbon quantification (DOC and POC) were cut into 4 (ARISE) or 6 (ISPOL) sections of 6-cm height. These 6-cm sections were subsamples from the full length of the core, chosen on basis of the ice texture and visual observation of ice algae. A "twin" ice core (maximum 20 cm apart from the previous one) dedicated to the analysis of TEP, saccharides and amino acids was kept at −28°C and brought back to the home laboratory. This ice core was then cut into 6-cm thick ice sections, at the same depths as for the organic carbon quantification.

Organic matter sample processing and analysis

All the material and glassware used for organic carbon sampling and measurements were either made of glass cleaned by ashing (4 h at 450°C), or washed with chromic-sulphuric acid (Merck), or made of Teflon cleaned by 10% HCl soaking and rinsed with ultra high purity water (UHP; 18.2 MΩ cm) obtained from a water purification system equipped with a UV-lamp and organic cartridge (Milli-Q Element, Millipore).

For POC and DOC, ice sections were melted onboard at 4°C in the dark, during less than 24 h. Melted samples were filtered through precombusted GF/F filters (Whatman, 450°C, 4 h). Samples for POC were kept frozen (−20°C). DOC samples were acidified with H_3PO_4 (0.05% final concentration) and stored in precombusted glass vials with Teflon septum in the dark at 4°C. Samples were analysed back in the home laboratories. POC was determined with a NA-2000 Fisons Instrument elemental analyzer (detection limit, 0.8 µmol C). DOC was measured by high-temperature catalytic oxidation (HTCO; procedure of Sugimura and Suzuki 1988) using a Shimadzu TOC-5000 analyzer for ARISE samples and a Dohrmann Apollo 9000 analyzer for ISPOL samples. Carbon concentration was determined using a five-point calibration curve performed with standards prepared by diluting a stock solution of potassium phthalate in UHP water (Milli-Q Element system, Millipore). Each value corresponds to the average of at least five injections. Samples were measured in duplicate and the relative standard deviation never exceeded 2%. The accuracy of our DOC measurements was tested by analysing reference materials provided by the Hansell laboratory (University of Miami). We obtained an average concentration of 45.1 ± 0.7 µM C (n = 10) for deep-ocean reference material (Sargasso Sea Deep water, 2,600 m) and 1.4 ± 0.7 µM C (n = 10) for low-carbon reference water. Our values are within the nominal values provided by the Hansell laboratory (44.0 ± 1.5 and 2.0 ± 1.5 µM C, respectively). Under-ice seawater DOC and POC concentrations were determined using the same methodology.

For qualitative analyses of organic matter (saccharides, amino acids and TEP), ice sections were thawed in precombusted glass beaker at 4°C in the dark. All handling was performed in a class 100 laminar flow hood. For TEP, unfiltered samples were poisoned with formaldehyde (2% final concentration) and kept at 4°C until further processing. Melted samples were filtered through precombusted GF/F filters (Whatman, 450°C, 4 h). Filters and filtrates were stored frozen (−20°C) until analysis. Total particulate saccharides (p-TCHO) and total dissolved saccharides (d-TCHO) were determined following the colorimetric TPTZ (2,4,6-tripyridyl-*s*-triazine) method of Myklestad et al. (1997), modified by Hung et al. (2001). The whole procedure was carried out in the dark because the reagents are light-sensitive (van Oijen et al. 2003). d-TCHO includes mono- (d-MCHO) and polysaccharides (d-PCHO) which are respectively the concentration before and after hydrolysis (d-TCHO = d-MCHO + d-PCHO). Dissolved and particulate samples were hydrolysed with HCl 0.1 N at 100°C, during 20 h (Burney and Sieburth 1977). Calibration curves were obtained with D(+)-glucose and the values of saccharides were expressed as glucose equivalent. They were converted into carbon concentration using a conversion of 6 mol of C per mole of glucose. The coefficient of variation (CV, standard deviation/mean) of all triplicate measurements was below 5% and the detection limit was 1.0 µM C. Three blanks per analytical session were always treated and analysed in the same way as the samples and subtracted from the concentration of the samples.

Dissolved free amino acids (DFAA) were measured following the fluorometric method of Parsons et al. (1984). The coefficient of variation (CV, standard deviation/mean) of triplicate measurements was below 5%. A calibration curve was made with glycine. The detection limit was 0.1 µM glycine. Concentrations were expressed in µM C by using a conversion of 2 mol of C per mol of glycine. Similarly, the concentrations were converted into µM N using 1 mol of N per mol of glycine.

Transparent exopolymeric particles were determined according to the spectrophotometric method of Passow and Alldredge (1995). Briefly, 10–15 ml subsamples were filtered onto 0.4-µm pore size polycarbonate filters (Nuclepore) using a vacuum pressure <150 mm Hg. TEP were stained with Alcian Blue solution (0.02% Alcian Blue, pH 2.5). Filters were then extracted with 6 ml of 80% H_2SO_4 for 2 h, in the dark with constant stirring. Absorbance was measured against a distilled water blank at 787 nm. The calibration was made using Xanthan Gum solution. Results are expressed as

Xanthan Gum weight equivalent per litre (XAG l^{-1}). All TEP determinations were performed in triplicate.

Algal and bacterial communities

For microscopic observations of algae and quantification of chlorophyll *a* (chl *a*), ice core sections were melted in the dark at 4°C in 0.2-µm-prefiltered seawater (1:4, V:V; Garrison and Buck 1986). Algae were enumerated by inverted light microscopy (100× and 320× magnifications) according to Utermölh (1958) and by epifluorescence microscopy (400× magnification) after DAPI staining (Porter and Feig 1980). Autotrophic biomasses were obtained using geometric shapes (Hillebrand et al. 1999) and specific carbon biomass estimations from Menden-Deuer and Lessard (2000). Subsamples for chl *a* were filtered onto Whatman GF/F filters and then extracted in 90% (V:V) acetone in the dark at 4°C overnight. Chl *a* was quantified fluorometrically according to Yentsch and Menzel (1963). For bacterial counts, ice core sections were melted in the dark at 4°C in 0.2 µm-prefiltered seawater (1:4, V:V) with addition of formaldehyde (2% final conc.). Bacteria were enumerated by epifluorescence microscopy after DAPI staining (Porter and Feig 1980). Their biovolumes were estimated according to Watson et al. (1977) and converted into biomass after Simon and Azam (1989).

Results

Algal and bacterial communities in sea ice

During both cruises, minimum chl *a* concentrations were found in internal layers while maximum values were observed at the bottom of the ice cores (Table 1). Chl *a* ranged between 0.14 and 27.98 µg l^{-1} during ARISE and between 0.16 and 28.41 µg l^{-1} during ISPOL. The dominant algal species present in sea ice are presented in Table 2. Typically, different species were found in the surface and in the bottom layers, especially for ISPOL. The surface autotrophic community was dominated by dinoflagellates during ARISE, whereas it was dominated by the Haptophyte *Phaeocystis* sp. during ISPOL. Some pennate diatoms (*Fragilariopsis* sp.) were also observed in the surface layer. In the bottom layer, algal biomass was mainly composed of pennate diatoms. *Fragilariopsis* sp., *Cylindrotheca* sp., *Amphiprora* sp. and *Nitzschia* sp. constituted the larger fraction of the diatom biomass. The bottom layer of station IX had the particularity to be the only place where autotrophic ciliates accounted for more than 50% of the algal biomass (Table 2). In the internal layers, pennate and centric diatoms were the dominant species in ARISE samples, whereas pennates and *Phaeocystis* sp. were mainly present in ISPOL internal layers. The bacteria were more randomly distributed along the ice core with maximum biomass sometimes not found in the bottom layer (Table 1). Bacterial biomasses ranged from 2.1 to 108.0 µg C l^{-1} during ARISE and from 1.6 to 19.3 µg C l^{-1} during ISPOL.

Distribution of particulate organic matter in sea ice

Particulate organic carbon concentrations ranged from 29 to 399 µM C during ARISE and from 14 to 470 µM C during ISPOL (Table 1). Maximum values were always recorded in the bottom layer of the ice core for each station, whereas minimum values were observed in internal layers. Particulate saccharides (p-TCHO) represented from 0.2 to 24.1% of the POC for all samples. In general, the minimum contribution of p-TCHO to POC (% p-TCHO/POC) was observed in the internal layers, whereas higher contribution was found in the surface and bottom layers (Fig. 2). Particulate saccharides concentrations ranged from below the detection limit to 38 µM C and from below the detection limit to 52 µM C, respectively for ARISE and ISPOL (Table 1). Highest values were also reached in the bottom layer. In this layer, if we compared the two ARISE stations with similar genesis, the particulate saccharides values were about 3 times higher at station IV (winter type) than at station IX (spring type). At the ice–water interface, during ISPOL, POC and p-TCHO concentrations increased slightly between 4/12 and 14/12, which then decreased to reach comparatively very low values on 30/12 (Fig. 2).

Distribution of transparent exopolymeric particles (TEP) in sea ice

Transparent exopolymeric particles concentrations (expressed in Xanthan Gum equivalent) ranged from 20 to 2703 µg XAG l^{-1} for ARISE sea ice samples and from 3 to 3,071 µg XAG l^{-1} for ISPOL sea ice samples (Table 1). Distributions of TEP along the core were similar to those of other organic components (Fig. 3): maximum concentrations of TEP were always encountered in the bottom layers and minimal concentrations were observed in the internal part of the ice core. In the bottom layer for ARISE, TEP concentrations at station IV were twice as high as those measured at station IX. TEP increased slightly at the beginning of the ISPOL observation period and then decreased drastically on the 19/12 and 30/12 at the ice–water interface (Fig. 3).

Distribution of dissolved organic matter in sea ice

The dissolved fraction was the main pool of the organic matter, representing 77 ± 16% of the total carbon pool

Table 1 Concentrations of chlorophyll *a* (chl *a*, μg l^{-1}), particulate organic carbon (POC, μM C), particulate saccharides (p-TCHO, μM C), dissolved organic carbon (DOC, μM C), dissolved monosaccharides (d-MCHO, μM C), dissolved total saccharides (d-TCHO, μM C), dissolved free amino acids (DFAA, μM C), transparent exopolymeric particles (TEP, μg XAG l^{-1}) and bacterial biomass (μg C l^{-1}) for ARISE and ISPOL expeditions

	Depth (cm)	chl *a* (μg l^{-1})	POC μM C	p-TCHO (μM C)	DOC (μM C)	d-MCHO (μM C)	d-TCHO (μM C)	DFAA (μM C)	TEP (μg XAG l^{-1})	BB (μg C l^{-1})[a]
ARISE Stn IV	0–6	0.22	57	2.0	119	3.0	3.0	0.3	556	6.2
01-oct-03	20–26	0.14	69	1.1	286	1.3	1.3	0.2	337	2.1
64°37.7′S	36–42	0.58	116	1.7	119	2.2	4.3	0.1	82	32.2
117°44.5′E	42–48	19.25	398	29.2	685	93.4	123.6	29.8	2,703	22.4
ARISE Stn V	7–10	3.93	142	2.7	244	8.1	14.9	2.0	590	19.7
07-oct-03	40–46	1.00	80	2.6	80	7.3	14.7	1.2	20	11.9
64°34.0′S	69–75	1.96	65	3.6	104	4.6	8.8	1.9	57	10.5
116°37.8′E	75–81	27.98	399	38.5	381	43.2	51.6	16.1	1,258	11.9
ARISE Stn IX	6–12	1.22	59	7.5	131	15.7	28.2	1.5	322	21.4
11-oct-03	20–26	0.62	29	2.4	101	5.1	10.0	0.5	199	52.0
64°24.1′S	39–45	0.93	67	0.7	ND	6.5	12.2	1.5	499	17.2
115°17.5′E	45–51	12.99	308	9.9	347	11.5	17.6	6.0	1,041	108.0
ARISE Stn XII	0–6	0.16	38	9.1	153	6.9	9.7	1.7	237	16.5
14-oct-03	24–30	0.28	32	2.3	ND	5.2	9.7	2.2	50	30.9
63°56.2′S	40–46	0.73	46	4.5	128	9.9	9.9	<DL	171	35.8
114°19.4′E	60–66	14.68	197	17.0	717	32.9	36.3	6.3	479	27.1
ISPOL 4/12	3–9	0.44	47	4.7	ND	9.2	22.0	0.9	52	9.8
	9–15	0.44	36	5.1	ND	6.2	7.0	1.0	125	9.3
68°11.5′S	40–46	0.45	14	0.5	667	<DL	10.5	0.2	38	7.5
55°24.4′W	60–66	0.16	20	0.7	388	<DL	4.8	0.1	6	6.5
	78–84	3.44	62	2.6	342	2.9	21.7	0.9	3	4.4
	84–90	26.47	264	37.8	701	38.9	116.1	11.4	2,087	11.0
ISPOL 9/12	3–9	0.97	49	9.7	204	14.3	33.4	0.6	68	6.0
	9–15	2.22	75	3.9	472	7.0	25.9	0.8	28	6.5
68°05.1′S	40–46	0.40	23	1.5	259	2.7	9.3	0.2	52	1.8
55°23.7′W	60–66	0.25	16	1.3	434	1.2	14.2	0.3	102	1.8
	78–84	2.56	95	7.2	138	4.7	22.9	1.2	262	2.6
	84–90	21.48	396	48.0	471	34.8	139.2	12.3	3,071	6.3
ISPOL 14/12	0–9	0.69	60	8.9	570	2.8	22.3	1.0	184	8.4
	9–15	1.23	64	2.2	346	<DL	21.5	0.4	166	19.3
67°83.0′S	40–46	0.27	27	1.1	374	<DL	3.3	0.1	127	1.9
55°36.3′W	60–66	0.30	38	<DL	321	<DL	4.1	0.2	117	2.5
	74–80	2.19	64	3.7	442	4.2	20.7	0.5	137	2.0
	80–86	24.23	470	52.1	476	23.6	88.0	13.8	2,668	2.9
ISPOL 19/12	4–10	0.78	24	1.1	302	4.4	17.7	<DL	178	17.6
	10–16	1.06	26	2.5	297	5.6	20.4	0.7	74	5.6
67°77.2′S	40–46	0.20	15	11.8	301	13.8	59.8	1.2	195	1.8
55°24.9′W	60–66	0.65	19	4.9	201	9.0	22.7	0.8	112	2.5
	77–83	4.20	52	5.4	265	8.9	28.7	1.9	180	4.8
	83–89	28.41	172	33.8	353	29.5	103.0	7.8	1,442	8.6
ISPOL 30/12	3–9	1.69	30	2.6	438	6.8	35.2	0.4	72	1.6
	9–16	0.97	29	3.2	457	3.9	44.6	0.8	303	5.2
67°51.4′S	40–46	0.59	30.3	1.3	230	<DL	5.1	1.0	84	12.7
55°29.7′W	59–65	0.95	ND	ND	106	1.0	7.4	0.5	86	3.5
	74–80	3.62	44.5	1.4	106	0.5	15.2	0.6	37	2.5
	80–86	24.77	167.3	3.6	208	16.3	38.6	6.7	385	3.3

[a] BB data for ARISE coming from Becquevort et al. (in revision)

ND not determined, *<DL* below detection limit

Table 2 Dominating taxa, % of estimated algal carbon biomass, in the surface, interior and bottom assemblages for ARISE and ISPOL expeditions

Stations	Algal community composition		
	Surface	Internal	Bottom
ARISE Stn IV	Pennate diatoms (98%) Nanoflagellates (2%)	Pennate diatoms (66%) Centric diatoms (34%)	Pennate diatoms (72%) Centric diatoms (14%) Dinoflagellates (14%)
ARISE Stn V	Pennate diatoms (72%) Dinoflagellates (23%) Nanoflagellates (5%)	Pennate diatoms (52%) Centric diatoms (47%) Nanoflagellates (1%)	Pennate diatoms (86%) Dinoflagellates (8%) Ciliates (5%) Centric diatoms (1%)
ARISE Stn IX	Dinoflagellates (61%) Pennate diatoms (39%)	Pennate diatoms (86%) Dinoflagellates (14%)	Ciliates (58%) Pennate diatoms (42%)
ARISE Stn XII	Dinoflagellates (97%) Nanoflagellates (3%)	Pennate diatoms (50%) Centric diatoms (35%) Dinoflagellates (15%)	Dinoflagellates (75%) Pennate diatoms (25%)
ISPOL 4/12	*Phaeocystis* sp. (89%) Pennate diatoms (10%)	*Phaeocystis* sp. (40%) Nanoflagellates (32%) Pennate diatoms (27%)	Pennate diatoms (99%) Dinoflagellates (1%)
ISPOL 9/12	*Phaeocystis* sp. (77%) Pennate diatoms (17%) Nanoflagellates (5%)	Pennate diatoms (41%) *Phaeocystis* sp. (36%) Nanoflagellates (19%)	Pennate diatoms (99%)
ISPOL 14/12	*Phaeocystis* sp. (73%) Pennate diatoms (26%) Nanoflagellates (1%)	Pennate diatoms (41%) *Phaeocystis* sp. (39%) Nanoflagellates (19%)	Pennate diatoms (99%)
ISPOL 19/12	*Phaeocystis* sp. (71%) Pennate diatoms (28%)	Pennate diatoms (56%) *Phaeocystis* sp. (26%) Nanoflagellates (18%)	Pennate diatoms (100%)
ISPOL 30/12	*Phaeocystis* sp. (78%) Pennate diatoms (22%) Dinoflagellates (1%)	Pennate diatoms (66%) Nanoflagellates (18%) *Phaeocystis* sp. (14%)	Pennate diatoms (98%) Dinoflagellates (1%)

Major pennate diatoms observed in the surface layers were *Fragilariopsis* sp., *Cylindrotheca* sp. and in bottom layers were *Cylindrotheca* sp., *Nitzschia* sp., *Amphiprora* sp., *Fragilariopsis* sp

(TOC = DOC + POC). DOC concentrations ranged from 80 to 717 μM C during ARISE and from 106 to 701 μM C during ISPOL (Table 1). Like for its particulate counterpart, highest concentrations were generally observed in the bottom layer of the ice core. Dissolved saccharides (d-TCHO) and amino acids (DFAA) followed the same trend along the core, with highest values almost always found in the layer at the interface between sea ice and seawater (Fig. 4, 5). Total dissolved saccharides (d-TCHO) accounted for 0.4–29.6% of the dissolved organic pool for all samples. The range of d-TCHO concentrations was similar for the two campaigns and comprised between 1.3 and 124 μM C during ARISE and between 3.3 and 139 μM C during ISPOL (Table 1). However, in the ARISE samples, monosaccharides were the main form of d-TCHO (on average 69 ± 20%) all along the cores, whereas in the ISPOL cores, polysaccharides were dominant (on average 78 ± 17%). DFAA concentrations ranged from 0.1 to 29.8 μM C for the ARISE samples and from 0.1 to 13.8 μM C for ISPOL ones. DFAA accounted for only 1.0 ± 1.2% of the DOC for all samples. The contribution of dissolved saccharides to DOC (%d-TCHO/DOC) and that of dissolved amino acid (%DFFA/DOC) were generally lower in the interior of the ice (Fig. 4, 5). Comparing the results obtained at ARISE stations, higher concentrations of d-TCHO and DFAA were observed in the bottom layer of station IV (winter type) compared to station IX (spring type) (Fig. 4, 5). Monosaccharides reached about 100 μM C in this layer at station IV (Table 1). For the ISPOL samples, the minimum concentration in the bottom layer was reached on the 30/12.

Concentrations of organic matter in the water column

In the seawater underlying the sea ice, POC concentrations averaged 5.6 ± 2.5 μM C (range, 2.3–9.6 μM C) during ARISE and 4.1 ± 3.1 μM C (range, 2.0–10.8 μM C) during ISPOL. DOC concentrations were 106 ± 76 μM C (range, 44–272 μM C) during ARISE and a concentration of 90 μM C was observed on the 25/12 during ISPOL (no other data available).

Discussion

Properties of organic matter in the sea ice environment

High levels of dissolved and particulate organic matter have been observed in Antarctic sea ice (Tables 3, 4) as well as in Arctic or Baltic sea ice (Thomas et al. 1995;

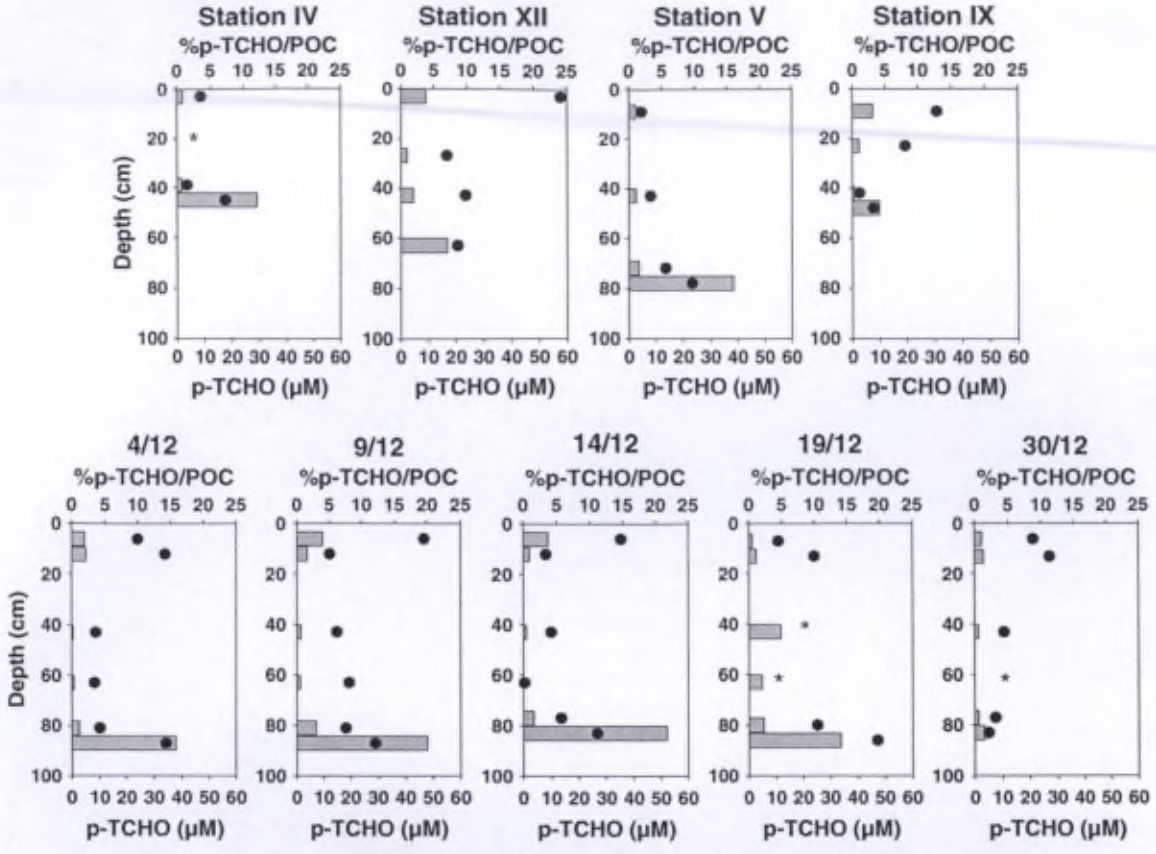

Fig. 2 Concentrations of particulate total saccharides (p-TCHO) along the ice core (*bars*) and contribution in % of p-TCHO to particulate organic carbon (POC) (*closed circles*) for ARISE (stations IV, XII, V and IX in the *upper panel*) and ISPOL (4/12, 9/12, 14/12, 19/12 and 30/12 in the *lower panel*) expeditions. The *asterisk* stands for missing values

Smith et al. 1997; Granskog et al. 2005). Moreover, high abundance of gel-like particles (TEP) has also been observed in Arctic sea ice (Krembs and Engel 2001; Krembs et al. 2002; Meiners et al. 2003; Riedel et al. 2006, 2007) and Antarctic sea ice (Meiners et al. 2004; this study). TEP is formed from polysaccharides exuded copiously by algae and bacteria (Passow 2000). The high concentrations of organic matter combined with the high salinities encountered in sea ice could further favour the abiotic formation of TEP by aggregation (Chin et al. 1998; Simon et al. 2002). Because of their viscous nature, TEP certainly play a central role in the dynamics of the sea ice ecosystem, as they cause the profound modification of the habitat of the microbial community. Indeed the brine channels turn into a gel-filled system with special characteristics with regards to retention of nutrients, organisms' attachment and mobility, and space availability (Decho 1990, Passow 2002). TEP could also protect organisms within ice floes from ice-crystal damage, and buffer the brine environment against pH and salinity changes (Thomas and Dieckmann 2002). Above all, the presence of TEP could highly modify our conception of the food web relationships. In fact, these gel-like molecules, from EPS-sized (<0.4 µm) to TEP-sized (>0.4 µm) particles, represent intermediate stages at the interface between DOC and POC, as recently highlighted by many studies investigating the organic matter size continuum (Verdugo et al. 2004). The classical/practical distinction made between dissolved and particulate fraction (GF/F, 0.7 µm) of the organic matter might not therefore be sufficiently adequate for studies of ecosystems such as the sea ice, where life conditions are comparable to those in biofilms (Mock and Thomas 2005). To make it even more complicated, TEP, classically measured on the 0.4-µm fraction, would thus operationally belong at the same time to the dissolved and particulate fractions. Strikingly, the trends in the distribution of organic constituents along a single ice core at a given station of ARISE and of ISPOL look very similar, regardless of their form (dissolved or particulate). This is also the case for TEP distribution, emphasizing the continuity of the matter and the importance to consider the organic matter pool as a whole, despite the arbitrary operational partition applied.

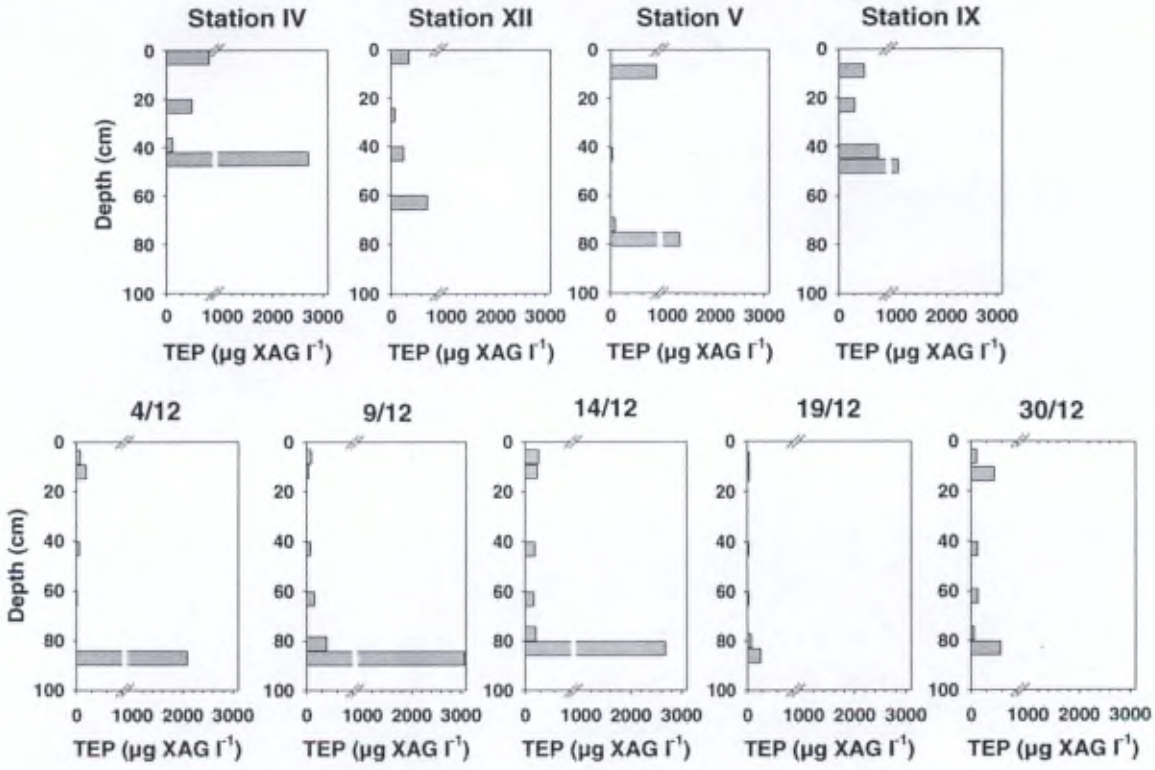

Fig. 3 Transparent exopolymeric particles (TEP) in µg XAG l^{-1} along the ice core for ARISE (stations IV, XII, V and IX in the *upper panel*) and ISPOL (4/12, 9/12, 14/12, 19/12 and 30/12 in the *lower panel*) expeditions

Concentrations and composition of organic matter

Particulate organic carbon concentrations reached up to 470 µM C, comparably to the values previously reported for Antarctic sea ice (Table 3). POC may be associated with microorganism biomass, but a non-negligible part may nevertheless be detritus, non-associated with living organisms. A large fraction of detritus has already been observed at the end of winter in Antarctic sea ice (Garrison and Close 1993) and in our ARISE samples where detritus can represent up to 99% of the POC in some layers of the winter-type station (Becquevort et al. 2008, in revision). DOC in ice cores was the major fraction of total organic carbon (TOC), accounting for 77 ± 16% of TOC. This value is slightly lower than that observed in oceans where the dissolved form typically represents 97% of TOC (Benner 2002). Our finding can be viewed as a consequence of the peculiarity of this highly organically concentrated and saline system, favouring coagulation of DOC to POC. The concentrations of DOC measured in this study (from 80 to 717 µM C) are in the same range as the few measurements previously reported for Antarctic sea ice (Table 4). In order to consider TEP values expressed in µg XAG l^{-1} in a broader context, they were converted into µg C l^{-1} (TEP-C), thanks to an empirical conversion factor of 0.75 µg C μg^{-1} XAG (Engel and Passow 2001). Caution should however be taken while using this conversion factor because the carbon content of TEP strongly depends on the origin of TEP precursors and has so far been observed to vary between 39 and 88% (w/w) (Engel 2004). The value of 75% used here was determined for a diatom culture (Engel and Passow 2001) and has been selected since the sea ice environment is usually diatom-dominated. On this basis, estimated TEP-C made up on average 7.1 ± 8.5% of the TOC and would roughly represent 25.8 ± 18.9% of the POC, keeping in mind the difference in the filtration porosity (0.4 vs. 0.7 µm) in this case. Crude estimates of TEP-C have been found to reach contributions ranging between 14 and 32% of integrated POC in Antarctic sea ice (Meiners et al. 2004) and were considered as an important fraction of the particulate pool, and of the carbon cycle. Particulate saccharides, dissolved saccharides and amino acids values measured in this study were also in agreement with the few data earlier reported in sea ice (Tables 3, 4).

Concentrations of organic matter in Antarctic sea ice are usually up to 10- to 100-fold greater than those measured in Antarctic seawater (Thomas and Papadimitriou 2003). During ARISE and ISPOL, the concentrations of DOC and POC in the underlying water column were indeed up to ten times lower than those observed in sea ice. DOC and POC values measured during this study were in agreement with the values found in the literature for Antarctic seawater

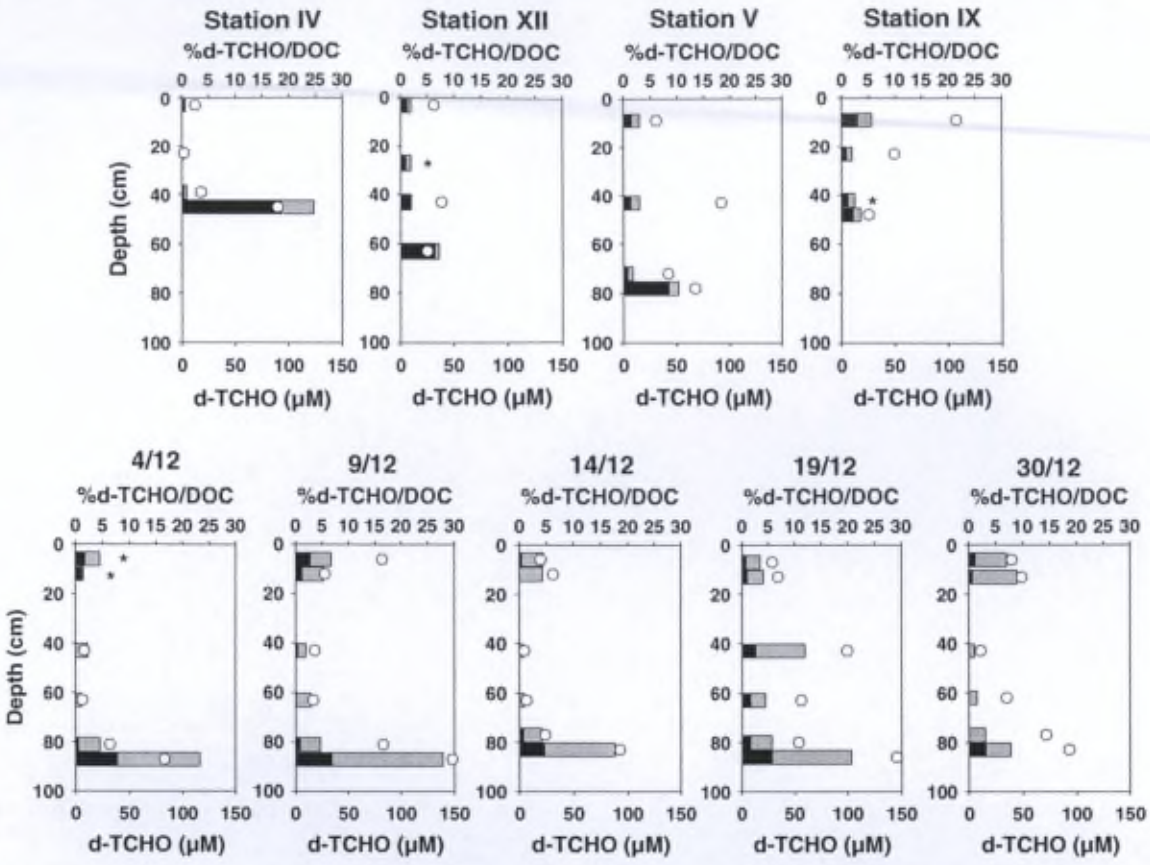

Fig. 4 Concentrations of dissolved total saccharides (d-TCHO) along the ice core, separated into monosaccharides (d-MCHO, *black bar*) and polysaccharides (d-PCHO, *grey bar*) and contribution in % of d-TCHO to dissolved organic carbon (DOC) (*closed circles*) for ARISE (stations IV, XII, V and IX in the *upper panel*) and ISPOL (4/12, 9/12, 14/12, 19/12 and 30/12 in the *lower panel*) expeditions. The *asterisk* stands for missing values

(Tables 3, 4). Although measurements for TEP, saccharides and amino acids were not available for seawater samples collected during ARISE and ISPOL (except for TEP of ARISE, see Table 3), the comparison of our sea ice values with literature data for Antarctic waters (Tables 3, 4) seems to show that the same trend of enrichment could also hold for these compounds.

Until now, only a few studies have investigated the composition of the dissolved and particulate organic matter in sea ice (Tables 3, 4), and, to our knowledge, dissolved and particulate fractions have never been reported together for the same samples. In sea ice, the organic matter is still largely uncharacterized but the presence of EPS, TEP, saccharides and amino acids has already been evidenced (Herborg et al. 2001; Amon et al. 2001; Meiners et al. 2004; Riedel et al. 2006, 2007). We found a contribution of p-TCHO to the POC pool which ranged from 0.2 to 24.1% in sea ice cores, which is similar to aquatic systems where the contribution of p-TCHO was reported to account for 5 to 20% of the POC (Wakeham et al. 1997; Volkman and Tanoue 2002). Total dissolved saccharides contributed to 0.4–29.6% of the DOC. We observed a much smaller contribution of dissolved free amino acids, ranging from 0.1 to 4.4%, in our samples. Herborg et al. (2001) reported, in sea ice core samples, an averaged saccharide contribution of 29% to the DOC. Thomas et al. (2001a) found, for various sea ice environments, a mean saccharide contribution of 36% to the DOC, although their values ranged from 1 up to 99%. These sea ice literature data cover obviously a wide range of contributions, which can be explained, however, by the multitude of ice environments sampled (surface ponds, ice cores, gap layers, platelet ice and multiyear ice). The ranges found in the present study are consistent with those given for oceanic systems, where saccharides and amino acids generally account for, respectively 10–25 and 1–3% of the DOC in surface waters (Benner 2002). As mentioned before, saccharides are important precursors of polymers and thus of exopolymeric particle formation (Passow 2000). In this study, the TEP distribution was linearly positively correlated with total (dissolved + particulate) saccharides distribution ($r^2 = 0.83$, $n = 44$, $P < 0.0001$; Fig. 6), indicating that the increase of TEP was parallel to that of the saccharides pool. This trend supports the idea that TEP are mainly made of saccharides (Passow 2002; Engel 2004).

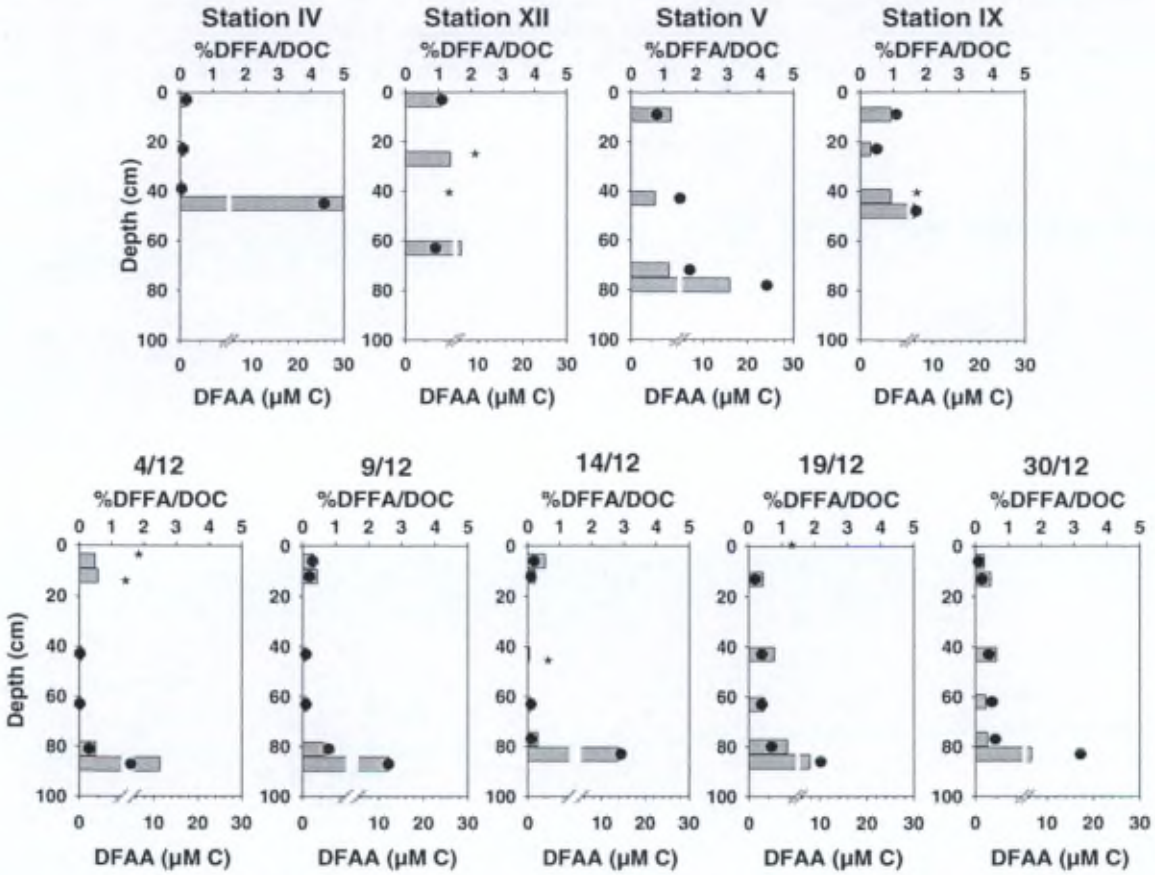

Fig. 5 Dissolved free amino acids concentrations (DFAA) along the ice core (*bars*) and contribution in % of DFAA to dissolved organic carbon (DOC) (*closed circles*) for ARISE (stations IV, XII, V and IX in the *upper panel*) and ISPOL (4/12, 9/12, 14/12, 19/12 and 30/12 in the *lower panel*) expeditions. The *asterisk* stands for missing values

Processes involved in organic matter distribution in sea ice

The enrichment of organic matter in sea ice is the result of different processes, certainly co-acting. First, organic matter (either microorganisms or detritus) can be physically concentrated by the mechanisms of sea ice formation (Garrison et al. 1983, 1989; Grossmann and Dieckmann 1994; Giannelli et al. 2001), involving harvesting or scavenging of cells by frazil ice crystals rising through the water column (Weeks and Ackley 1982) or by the increase of available crystal substrate in the skeletal layer of the columnar ice or in the platelet ice layer (Dieckmann et al. 1986). Second, the in situ production, by microorganism growth and subsequent organic matter release, contribute to this high pool of organic material. High biomasses of microorganisms can indeed occur in the sea ice. In particular, in the autumn and in the late winter, favourable conditions such as light and nutrient availability can be encountered in the sea ice as compared to the water column, leading to a large accumulation of biomass. Moreover, the narrow brine channels exclude large heterotrophs from the sea ice matrix and therefore limit the consumption of this high biomass. Finally, limitation of bacterial activities and/or low substrate quality (Thingstad et al. 1997) can also potentially hamper the use of the available organic matter pool.

Production pathways

For both cruise, POC concentrations increased with chl *a* concentrations ($r^2 = 0.74$, $n = 45$, $P < 0.0001$), a common proxy for algal biomass. This relation suggests that the increase of POC was mainly associated with in situ algal production and thus that the particulate organic fraction could be significantly influenced by the algal biomass. However, when no chl *a* was recorded, a certain amount of POC (around 40 µM C) was nevertheless estimated to be present in the ice (intercept of the regression line). Possibly, a part of the POC in the ice was therefore not directly related to the in situ algal activity, which would represent a "background" concentration. Such a kind of matter has already been observed in sea ice samples (Garrison and Close 1993). This residual fraction could be associated with the physical incorporation of organic matter during ice formation, linked to organisms other than algae or result from previous microbial activities. Interestingly, this POC concentration lies around the levels found in the internal layers

Table 3 Concentrations of particulate organic carbon (POC, μM C), particulate saccharides (p-TCHO, μM C), and transparent exopolymeric particles (TEP, μg XAG l^{-1}) measured in samples from Antarctic sea ice and seawater, compiled from literature

Location, season and sample type	POC (μM C)	p-TCHO (μM C)	TEP (μg XAG l^{-1})	Reference
1. Antarctic Sea ice				
Weddell Sea,				
Winter young pack (20–50 cm)	98 ± 69	–	–	Garrison and Close (1993)
First year and older pack (<100 cm)	184 ± 127	–	–	
Ross Sea, autumn				
Infiltration ice	39	–	–	Garrison et al. (2003)
Frazil ice	134	–	–	
Congelation ice	46	–	–	
Weddell Sea, summer/early autumn	175 ± 240	–	–	Kennedy et al. (2002)
Bellingshausen Sea, late autumn	216 (16–832)	–	*	Meiners et al. (2004)
Ross sea (Terra Nova Bay), spring, pack ice	–	38 ± 8 (18–77)	–	Guglielmo et al. (2000)
Australian sector (64–65°S, 112–119°E), end winter–early spring	29–399	<DL-38	20–2,703	This study, ARISE
Western Weddell Sea, Antarctic Peninsula (68°S, 55°W), spring–summer	14–470	<DL-52	3–3,071	This study, ISPOL
2. Antarctic Seawater				
Weddell Sea, winter	5	–	–	Garrison and Close (1993)
Weddell Sea, summer/early autumn	17 ± 8	–	–	Kennedy et al. (2002)
Bellinghausen Sea, late autumn	9 (3–30)	–	–	Meiners et al. (2004)
Terra Nova Bay (Ross Sea), summer, under pack ice	20–100	1–7 (19% POC)	–	Pusceddu et al. (1999)
Ross Sea, summer	19		–	Fabiano et al. (1993)
Terra Nova Bay (Ross Sea), summer	12.64 ± 8.91 (1.01–39.23)	2.27 ± 2.17 (0.28–14.80) (18% POC)	–	Fabiano et al. (1996)
Bransfield Strait, summer	–	–	56.77 ± 54.50	Corzo et al. (2005)
Ross Sea, Nov–Dec	–	–	308 (0–2,800)	Hong et al. (1997)
Australian sector (64–65°S, 112–119°E), end winter–early spring	5.0 ± 2.5 (1.7–9.2)	–	133–853	This study, ARISE
Western Weddell Sea, Antarctic Peninsula (68°S, 55°W), spring–summer	3.56 ± 2.87 (1.02–10.83)	–	–	This study, ISPOL

Mean values ± standard deviation and ranges are given when available

<DL below detection limit

* TEP were quantified in abundance and not in concentration in μg XAG l^{-1} in the study of Meiners et al. (2004)

of the ARISE winter/spring ice cores (mean, 42.4 μM C and see Table 1), suggesting that this POC had been incorporated or produced earlier in the sea ice.

Maximal concentrations of particulate saccharides were observed in the bottom layer where algal biomass dominated, while minimal values were found in the internal layers of the ice cover. Particulate saccharides are likely originating from algae since saccharides are major components of algal cells, for storage and structural functions and since algae are major sources of organic matter (Bertillson and Jones 2003). The co-occurrence of chl *a* maxima with p-TCHO maxima suggests the association of p-TCHO with algal activity. Furthermore, the contribution of the particulate saccharides to the POC pool (% p-TCHO/POC) paralleled the p-TCHO distribution relatively well (Fig. 2), displaying higher values in the bottom and surface layers and lower (minimal) values in the internal part of the ice core. The higher % p-TCHO/POC coincided with the layer where higher chl *a*, POC and p-TCHO concentrations were recorded. Thus, where there is higher algal production, p-TCHO would contribute to a larger extent to the POC. The p-TCHO/POC ratio would then give an indication on the relative "freshness" of the particulate organic matter.

Along the entire ice core, the p-TCHO/chl *a* ratio was rather variable but this ratio was always smaller in the bottom ice (mean value, 16 ± 8) as compared to internal and surface layers. This p-TCHO/chl *a* ratio is very close to the value of 17 found in the literature and associated with living

Table 4 Concentrations of dissolved organic carbon (DOC, μM C), dissolved saccharides (d-TCHO, μM C), and dissolved free amino acids (DFAA, μM C) measured in samples from Antarctic sea ice and seawater, compiled from literature

Location, season and sample type	DOC (μM C)	d-TCHO (μM C)	DFAA (μM C)	Reference
1. Antarctic Sea ice				
Perennial pack ice	0–2,000	–	–	Thomas et al. (1998, 2001a)
Weddell Sea, April/May	109 ± 83.5 (16–556)	–	–	Thomas et al. (2001a)
Weddell Sea, January/March	207 ± 239.6 (16–1,842)	–	–	Thomas et al. (2001a)
Weddell Sea, platelet	100–200	–	–	Thomas et al. (2001b)
Weddell Sea, summer	254 ± 133	53 ± 22 (29% DOC)	–	Herborg et al. (2001)
Davis Station, Nov	–	–	8–30	Yang (1995)
Australian sector (64–65°S, 112–119°E), end winter–early spring	80–717	1.3–124	0.1–29.8	This study, ARISE
Western Weddell Sea, Antarctic Peninsula (68°S, 55°W), spring–summer	106–701	3.3–139	0.1–13.8	This study, ISPOL
2. Antarctic Seawater				
Antarctic Ocean average	<60	–	–	Ogawa and Tanoue (2003)
6°W meridian between 47° and 60°S, surface, early spring	38–55	–	–	Kähler et al. (1997)
Weddell Sea, summer	78 ± 50	8 ± 4 (18% DOC)	–	Herborg et al. (2001)
off Queen Maud's Land (69°38′S, 03°54′E), Jan	–	8–9	–	Myklestad et al. (1997)
Drake Passage, Bransfield Strait, Weddell Sea, Dec	–	–	0.44 ± 0.16 *	Hubberten et al. (1995)
Gerlache Strait, spring	–	–	0.3–0.6	Tupas et al. (1994)
Davis Station, May–Feb	–	–	0.30	Yang et al. (1990)
Australian sector (64–65°S, 112–119°E), end winter–early spring	102.5 ± 66.7 (43.3–271.7)	–	–	This study, ARISE

Mean values ± standard deviation and ranges are given when available

* Expressed in μM N

phytoplankton (Parsons et al. 1961 in Hung et al. 2003). The mean ratio observed in the bottom layer is thus in agreement with a living algal community. Variations of p-TCHO/chl *a* ratio are linked to the quantity of p-TCHO produced by algae, which depends on (a) the type of the species present (diatoms vs. flagellates; Hecky et al. 1973; Hung et al. 2003), (b) the growth stage (Biddanda and Benner 1997) but also (c) the limitation status, by nutrient or light, (Alderkamp et al. 2007). In our samples, complex interactions of these different parameters may explain the observed ratios. For example, a nutrient limitation tends to increase the specific (per cell) production of carbohydrates. The higher p-TCHO/chl *a* found in internal and surface layers could be explained by an additional production of saccharides by algae, potentially linked to a nutrient limitation. Indeed the internal and surface layers are more likely limited by the availability of macro-nutrients as replenishment from seawater does not occur in this closed system. The even higher p-TCHO/chl *a* ratio observed in the surface layer might, in the case of ISPOL samples, be linked to the presence of *Phaeocystis* cells (Table 2), which can form colonies having a matrix of polysaccharides (Alderkamp et al. 2007).

Sea ice is considered as à favourable system for the abiotic formation of TEP since aggregation depends on the concentration of divalent cations (Simon et al. 2002) and precursors (Chin et al. 1998) and since DOC and salinity increase during freezing. Nevertheless, the abiotic transformation of DOC to TEP via freeze-concentration has not been supported by observations in sea ice (Krembs et al. 2002; this study). TEP can also be actively produced by microorganisms such as algae (Krembs et al. 2002; Meiners et al. 2003) or bacteria (Meiners et al. 2003). Hence, TEP distribution along the ice core followed the chl *a* pattern, with the largest accumulation at the ice–water interface. We observed an increase of TEP when chl *a* increased ($r^2 = 0.65$, $n = 46$, $P < 0.0001$), whereas no relationship between TEP and bacterial numbers was found. This suggests that TEP were primarily produced by algae as observed previously by Meiners et al. (2003, 2004) in sea ice. Pennate diatoms have been shown to be the dominant producers of TEP in sea ice (Krembs and Engel 2001). Pennate diatoms were indeed present in every layer sampled and dominated the autotrophic biomass in all our bottom ice samples, with the exception of the bottom layer at station IX (Table 2).

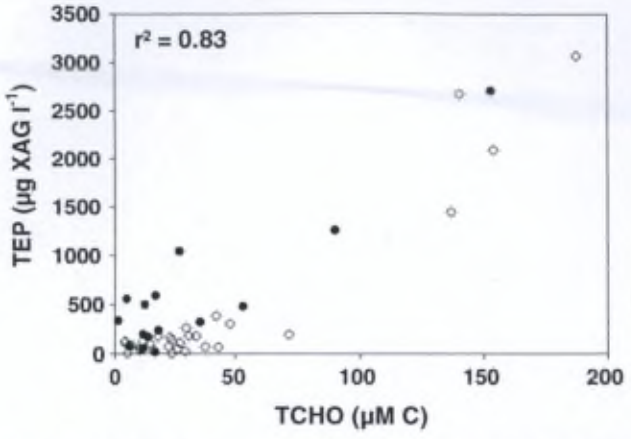

Fig. 6 Relationship between transparent exopolymeric particles (TEP) and total saccharides concentrations (dissolved + particulate, TCHO) for sea ice samples from ARISE (*black circles*) and ISPOL (*open diamonds*) expeditions

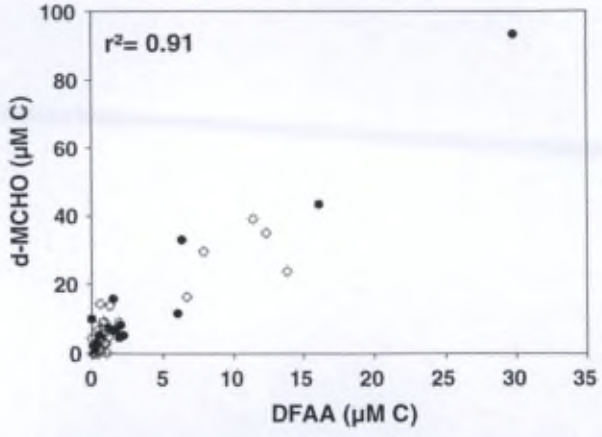

Fig. 7 Relationship between dissolved monosaccharides (d-MCHO) and dissolved free amino acids (DFAA) for sea ice samples from ARISE (*black circles*) and ISPOL (*open diamonds*) expeditions

In contrast with POC and TEP observations, there was no significant relation between DOC and chl *a* ($r^2 = 0.18$, $n = 42$, $P = 0.0057$). This supports the view that the algal production and its directly associated trophic networks were not the only factors controlling the DOC distribution; other indirect "spatio-temporal" delayed processes being equally involved. Indeed, even if some studies have found a high correlation between DOC and chl *a* in Arctic bottom sea ice (Smith et al. 1997), the explanation of this relationship is not necessarily straightforward in sea ice (Thomas et al. 1995, 1998, 2001a). The DOC pool is in fact a mixture of compounds of different origins and in various degradation states. A certain amount, like polysaccharides, for example can be freshly produced by the algae, but the remaining fraction can be made of more refractory molecules. In the bottom layers, high DOC concentrations were associated with high chl *a* levels (Table 1) but there were other layers where high DOC values were recorded together with low chl *a* levels. DOC did not seem to result from massive breakage of POC by bacterial activities as no inverse relationship existed between POC and DOC concentrations. Conversely, no evidence of transfer from DOC to POC was obvious from our data. Nevertheless, we cannot exclude that this mechanism has occurred as the high DOC concentrations measured in sea ice suggest that coagulation of DOC to TEP and POC is likely to occur in these environmental conditions (Simon et al. 2002).

Dissolved total saccharides and amino acids reached their maximal concentrations where chl *a* concentrations were maximal. The d-TCHO and DFAA pools seemed thus related to the in situ algal production and then, may be considered as relatively fresh compounds of the DOC. d-TCHO and DFFA are indeed internal components of a cell, playing the role of osmolytes regulating vital functions in sea ice (Lizotte 2003) or can also be excreted by algae. The high positive regression between monosaccharides and amino acids ($r^2 = 0.91$, $n = 46$, $P < 0.0001$, Fig. 7) strongly suggests a similar production pathway of these compounds, likely cell lysis. In addition, the contribution of d-TCHO to DOC and of DFFA to DOC was higher in layers where d-TCHO, DOC and chl *a* concentrations were higher (Fig. 4, 5). These patterns, also noticed for % p-TCHO/POC, could be easily related to the distribution of the algal production in sea ice with internal layers, potentially more limited and displaying less fresh production. Similarly the % d-TCHO/DOC and % DFAA/DOC provide information about the nature of the organic matter pool.

Removal pathways

The most labile components of DOM are monosaccharides and amino acids, the latter constituting a significant source of C and N for bacteria (Fuhrman and Ferguson 1986). The monosaccharide pool is therefore more dynamic than the polysaccharide one (Pakulski and Benner 1994). Low background concentrations (at nanomolar level) of monosaccharides and DFAA are typically observed in oceanic waters because these compounds are directly usable (turnover time, minutes to hours) by bacteria and generally taken up too rapidly to build up a detectable pool in the system (Carlson 2002). Nevertheless, accumulation of these compounds has sometimes been observed in seawater (Obernosterer and Herndl 1995; Williams 1995), e.g. in response to nutrient limitation, which affects both algae and bacteria. In this study, an accumulation of dissolved monomeric amino acids and sugars was recorded at the four stations of the ARISE cruise. In particular, concentration of monosaccharides up to 100 µM C (76% of d-TCHO) was observed in the bottom layer of station IV (winter type). During ISPOL, the monomer concentrations stayed always below 40 µM C

and the large accumulation of d-TCHO was due to the increase of polysaccharides, representing 78 ± 17%. High monosaccharide pools could result from massive bacterial ectoenzymatic hydrolysis of polysaccharides into monosaccharides. The occurrence of such a process would be indicated by a negative correlation between d-MCHO and d-PCHO. No correlation was found for our samples. An important cell lysis phenomenon due to the extreme environmental conditions of the winter period could also explain this accumulation of monomers (Thomas and Papadimitriou 2003). The lysis hypothesis is actually supported by the high phosphate concentrations that we observed during ARISE and by the high proportion of detrital organic matter (Becquevort et al. 2008, in revision). Still regardless of the production pathway of these monomers, such a transient accumulation would only be possible if their consumption by bacteria was limited during the same period. All factors contributing to the reduction of bacterial growth rate, biomass and carbon demand would lead to the accumulation of biodegradable DOC (Thingstad et al. 1997).

The unbalanced elemental composition of organic carbon in parallel to an inorganic nutrient limitation might hamper bacterial growth. For these monomeric organic substrates, we calculated a C/N ratio, estimated from d-MCHO and DFFA concentrations, using their carbon and nitrogen content ($[C_{d\text{-}MCHO} + C_{DFAA}]/N_{DFAA}$). This ratio can only be regarded as a rough estimate of available substrates for bacteria, since proteins and polysaccharides were not included in these calculations. The C/N ratio varied between 5 and 12 in the bottom layer, whereas the C/N ratio ranged from 8 to 50 in the surface layers and from 2 to 37 in the internal sections. If we consider the C/N ratio of bacteria being around 5 (Bratbak 1985; Nagata 1986; Goldman et al. 1987; Lee and Fuhrman 1987) and an estimated bacterial growth efficiency (i.e. the ratio between the C incorporated into bacterial biomass compared to the C taken up by bacteria, the rest being respired into CO_2) of 20% (del Giorgio and Cole 1998), the range of C/N ratio estimated in the bottom ice can be considered as a source of organic matter of very good quality for the consumption by bacteria. In the internal and surface layers, the C/N ratio can be higher meaning that the substrates were less well balanced regarding the bacterial metabolic requirements (i.e. C is in excess compared to N). Consequently, inorganic N amendment could sometimes be needed to satisfy bacterial growth in these layers. The P availability for bacteria has been estimated from PO_4 concentration data (Tison et al. 2005; Becquevort et al. 2008, in revision) as phosphorus is indeed preferentially used under its inorganic form by bacteria. The C/P ratio ranged between <1 and 32, with higher values in intermediate layers. In regard to the highly variable C/P ratio of bacteria (8–464) depending on growth conditions (Kirchman 2000), the bacteria seems not limited by the availability in P.

The lack of use of organic matter carbon could also be explained by the limitation of bacteria growth due to the harsh physico-chemical conditions such as low temperature, high salinity or presence of toxic compounds (Lizotte 2003). This hypothesis of bacterial growth limitation leading to monomers accumulation is reinforced by comparing the winter-type station (station IV, ARISE) to the spring-type station (station IX, ARISE). A net decrease of dissolved saccharides and amino acids in the bottom layer between these two stations was observed. Although keeping in mind the possibility of spatial heterogeneity or of organic matter leakage into seawater, this decrease can also be attributed to enhanced microbial growth as evidenced by the different bacterial biomass observed at these two stations (Table 1). Bacteria were indeed in higher abundance in the bottom layer of station IX compared to station IV. If we consider a temporal scale, this suggests that bacteria would have been capable of using the substrates accumulated during winter when the environmental conditions became more favourable for growth. Unfortunately, our data did not enable us to conclude about which particular limiting factor was acting at that time. The "bacterial limitation" hypothesis is nevertheless consistent with melting experiments (Kähler et al. 1997; Giesenhagen et al. 1999), in which dissolved organic matter released by sea ice has been observed to be rapidly consumed by pelagic bacteria, suggesting that this material was very labile. During the ISPOL cruise, conducted in December, the ice characteristics were representative of the spring–early summer period with the environmental conditions potentially more favourable for microbial growth. Accumulation of monomeric substrates was not observed and dissolved saccharides were mainly composed of polysaccharides, which are the main excretion product of algae (Myklestad 1995). This situation probably reflects both active excretion by algae and efficient utilization by bacteria. With the gradual warming of the ice cover, all organic compounds concentrations decreased in the bottom ice, flushed down with brines into the seawater. In this context, our observations about, (1) the presence and importance of TEP in sea ice and (2) the lability of the organic matter, stressed the need to undertake more studies concerning the fate of the ice-derived organic matter in order to conclude on its impact on carbon export (through, e.g. marine snow or faecal pellets), potential retention or export and bioavailability of the sea ice accumulated iron (Geider 1999).

Conclusion

This study provided a new set of data on dissolved and particulate organic matter compounds in pack ice collected at two Antarctic locations. Accumulation of dissolved and particulate

organic matter was observed in first year sea ice environment and the importance of the size continuum, especially the role of EPS and TEP, was pointed out through simultaneous measurements of all organic fractions. This gel matrix was thought to be central to the concept of the trophic relationships within the ice. Evaluation of the contribution of saccharides, amino acids and TEP to the organic matter pool has enabled us to highlight a fraction of the organic matter pool, called "fresh", which was related to the algal production in the layers where algal biomass was maximal, i.e. mainly surface and bottom layers. This "fresh" production was directly reflected by the increase of saccharides, amino acids and TEP concentrations and by the simultaneous increase of their relative contribution to the total organic matter pool. The remnant part of the organic pool was probably made of older compounds, which eventually had already undergone transformations. As postulated, the saccharides represented a non-negligible part of the organic matter and showed a trend along the ice core matching that of TEP. Accumulation of monomers in winter is suggested to result from the limited bacterial activities rather than from the chemical nature of the substrates. Temporally as the season evolved, a decrease in the organic pool was observed, likely due to the melting of the ice and/or the enhancement of the heterotrophic activities. If, like in oceanic systems, a large part of the organic matter still remains unidentified, our study has highlighted the importance of TEP, saccharides and amino acids as key constituents to investigate in the sea ice environment.

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Chapter 3

Distribution and chemical characterization of organic matter in the Antarctic pack ice zone, Bellingshausen Sea

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Distribution and chemical characterization of organic matter in the Antarctic pack ice zone, Bellingshausen Sea.

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Abstract

Concentrations and distribution of organic carbon (dissolved and particulate organic carbon, dissolved and particulate saccharides and transparent exopolymeric particles) were measured in pack ice and underlying seawaters in the Bellingshausen Sea in October 2007. The two pack ice sampling stations (Brussels and Liège), having different ice and snow thicknesses and different textures, presented a contrasted distribution of the organic matter, even if the range of values of each parameter was not very different between each station. The vertical distribution of organic matter was rather homogeneous throughout the 60 cm of Brussels ice while higher concentrations were found in the upper half of the 120-cm-thick Liège ice cover. The composition of organic matter (with a high proportion of saccharides) suggested an algal production of this organic matter. Nevertheless, the thermodynamical evolution of sea ice cover, characterized by a flood-freeze cycle, seemed to control the organic matter concentration and distribution in sea ice.

Keywords: sea ice, organic matter, saccharides, TEP, bacteria

1. Introduction

Antarctic sea ice is a very dynamic ecosystem and one of the largest biome on Earth. A highly diverse microbial community composed of algae, bacteria and protozoa live inside the brines channels of pack ice (Lizotte 2003). High concentration of organic matter (OM) has already been observed in sea ice with dissolved organic matter (DOM) concentrations up to 450-fold greater than that of surface waters (Thomas et al. 2001b). Saccharides have been observed to be major components of the dissolved and particulate organic fractions in sea ice (Amon et al. 2001, Herborg et al. 2001, Dumont et al. 2009). Similarly, high concentrations of exopolymeric substances (EPS) have been measured in sea ice (Krembs et al. 2002, Meiners et al. 2004, Riedel et al. 2007). These EPS, mainly composed of polysaccharides, are excreted by microorganisms in response to changes in e.g. temperature, salinity or inorganic nutrients. Their production by bacteria and algae is thus likely in the extreme sea ice environment. A particular type of EPS, namely the transparent exopolymeric particles (TEP), has also been observed in sea ice (Meiners et al. 2004, Riedel et al. 2007, Dumont et al. 2009). TEP are made of acid polysaccharides, have a gelatinous consistency and are changeable in shape (Passow 2002). TEP are involved in attachment of cells to surface and formation of microhabitats. This kind of particles is of prime importance in the comprehension of the interactions between OM and microorganisms as they greatly modify the chemical properties of the interior ice habitat; change the diffusive properties of ions and gases and influence the trophic relationships inside sea ice (Decho 1990, Krembs and Deming 2008, Becquevort et al. 2009).

It is now well recognized that the production and transformation of DOM in sea ice is central to any biogeochemical cycling within the ice (Thomas and Papadimitriou 2003) and that the large amounts of DOM are needed to mediate the observed ecology of algae, bacteria and protozoa within the ice (Gleitz et al. 1996, Günther et al. 1999). The accumulation of DOM in sea ice clearly suggests a disconnection of the production and consumption pathways of OM (Thomas and

Papadimitriou 2003). Reduced substrate affinity of bacteria at low temperatures has been proposed to be responsible for the poor exploitation of the high pool of available organic substrates (Pomeroy and Wiebe 2001). Accordingly, the presence of OM in the form of monomeric substrates in sea ice (Herborg et al. 2001, Dumont et al. 2009) also points towards a potential limitation of the bacterial activities by an external factor, which is likely temperature but also salinity or toxins such as acrylic acid (Brierley and Thomas 2002). The organic substrates present in sea ice seem to be freshly produced by ice algae (Amon et al. 2001, Dumont et al. 2009) and available to bacteria once released in seawater (Kähler et al. 1997, Pusceddu et al. 2009). Still Calace et al. (2001) propose that humification of OM derived from sea ice algae could occur within the sea ice. For these authors the refractory nature of humic compounds could be the reason of the under-utilization of the sea ice OM.

Further investigations of the distribution and composition of the sea ice organic matter are clearly needed to understand the dynamics of the OM pool within sea ice. The behaviour and enrichment pattern of OM during ice formation OM is also poorly documented although it seems that the concentrating effects of dissolved organic carbon (DOC) in brines might be different as compared to salts (Giannelli et al. 2001).

In this study, we present observations about the concentrations of dissolved and particulate organic matter, dissolved and particulate saccharides and transparent exopolymeric particle in pack ice, brines and seawater samples collected at two stations (Brussels and Liège) located in a same pack ice floe in the Bellinghausen Sea during austral spring. Our main objective is to study the distribution of OM in relation with the physical characteristics (ice and snow thicknesses, ice texture salinity, brine volume,) and biological parameters of sea ice (algae-Chl *a* and bacteria) and the thermodynamical evolution of the ice cover.

2. Materials and Methods

2.1. Study site and sampling

Sea ice, brines and under-ice seawater sampling was carried out in October 2007 in the Bellingshausen Sea during the Sea Ice Mass Balance in the Antarctic (SIMBA) research cruise onboard the RV *N.B. Palmer*. During all the 1-month sampling period, the vessel was anchored to an ice floe, the Belgica station. Sampling was conducted at two sites respectively named Brussels and Liège stations. These stations had contrasted physical characteristic for ice and snow thicknesses (Tison et al. 2008, Table 1). At each station, the sampling was repeated 5 times at a constant 5-days interval (Table 1).

The sampling was conducted in a 100 m x 60 m sampling site, located 1 km away from the ship, using precautions to avoid trace metal and organic matter contamination (Lannuzel et al. 2006, Dumont et al. 2009). Clean room garments (Tyvek overall, overshoes and polyethylene gloves) were worn by the operators on the site and an electropolished stainless-steel corer (14 cm diameter) was used to collect the ice cores. Brines were collected by the sackhole technique, i.e. by gravity drainage of brines into a hole drilled into the sea ice cover (Thomas and Dieckmann 2003). Brines (from 2 depths) as well as under-ice seawater (0 m, -1 m and -30 m) were collected using a portable peristaltic pump (Cole-Parmer, Masterflex E/P) and acid cleaned tubing. Brine and seawater samples were transferred into acid-washed bottles abundantly rinsed with the collected samples.

Table 1: Main physical characteristics of the 2 sampled sites during SIMBA (from Tison et al. 2008) and sampling dates at each station

	Brussels	Liège
Ice thickness (cm)	50 - 70	90 - 120
Snow thickness (cm)	8 - 25	28 – 38
Texture	Mainly columnar with some thin granular layers Granular top 15 cm	Mainly granular with variable size Rafting
Freeboard (cm)	+ 0.7 - + 4	>0 then flooding
Sampling date (Julian Days)	274, 279, 284, 289, 294	276, 281, 286, 291, 296

For chlorophyll *a* and microscopic investigations, the full-length of ice cores were cut into 10 cm thick sections and transferred into acid washed polyethylene containers and further treated as described below. The ice cores dedicated to the organic matter determinations were cut into 6 sections of 10 cm each and the sea ice sections were transferred into glass beakers pre-combusted at 450°C for 4 h.

2.2. Ice texture, salinity and temperature

The ice structure was determined by thin section analysis and photographs taken under polarized light. Based on ice crystal size and orientation, two stratigraphic units were distinguished: granular ice and columnar ice. High-resolution analyses of the $\delta^{18}O_{ice}$ allowed further discrimination between snow ice and frazil ice within the granular facies (Jeffries et al. 1989, Eicken 1998). Ice temperature was measured on site using a calibrated probe (TESTO 720) inserted every 5 cm along the freshly sampled core. Bulk ice salinity was determined by conductivity using WP-84-TPS meter. The brine volume fraction in the sea ice (V_b/V = brine volume/bulk sea ice volume ratio) was calculated on the basis of temperature and salinity values following the equations of Cox and Weeks (1983) and Leppäranta and Manninen (1988), revisited in Eicken (2003).

2.3. Organic Matter

All the material and glassware used for organic carbon sampling and measurements were either made of glass cleaned by ashing (4 h at 450°C), or washed with chromic-sulphuric acid (Merck), or of Teflon cleaned by 10% HCl soaking and rinsed with ultra high purity water (UHP) (18.2MΩ Millipore milli-Q system) obtained from a water purification system equipped with a UV-lamp and organic cartridge (Milli-Q Element, Millipore). All handling was performed in a class 100 laminar flow hood.

For organic matter samples, ice sections were thawed in precombusted glass beaker at 4°C in the dark and then

directly treated as for seawater samples as explained hereafter. For transparent exopolymeric particles measurements, ice sections were melted with addition of filtered seawater amended with formaldehyde (2% final concentration), to avoid the consumption of TEP by bacteria during melting.

2.3.1. Dissolved and particulate organic carbon

Particulate organic carbon (POC) was collected on pre-combusted (450°C, 4h) Whatman GF/F filters, stored at -20°C until analysis. After drying at 60°C, POC was analyzed with a Fisons NA-1500 elemental analyzer after carbonate removal from the filters by HCl fumes overnight. Filtered samples for dissolved organic carbon (DOC) were stored in pre-combusted (450°C, 4 h) 20-ml glass ampoules with 25 µl H_3PO_4 (concentration 50 %), which were sealed to avoid contact with air. Samples were kept in the dark at 4°C until analysis. The DOC was measured by high temperature catalytic oxidation (HTCO; procedure of Sugimura and Suzuki 1988) with a Dohrmann Apollo 9000 analyzer. Carbon concentration was determined using a five-point calibration curve performed with standards prepared by diluting a stock solution of potassium phthalate in UHP water. Each value corresponds to the average of at least five injections. Samples were measured in duplicate and the relative standard deviation never exceeded 2%. The accuracy of our DOC measurements was tested by analyzing reference materials provided by the Hansell laboratory (University of Miami). We obtained an average concentration of 45.1 ± 0.7 µM C (n=10) for deep-ocean reference material (Sargasso Sea Deep water, 2600 m) and 1.4 ± 0.7 µM C (n=10) for low-carbon reference water. Our values are within the nominal values provided by the reference laboratory (44.0 ± 1.5 µM C and 2.0 ± 1.5 µM C, respectively).

2.3.2 Dissolved and particulate saccharides

Subsamples were filtered through pre-combusted GF/F filters (Whatman, 450°C, 4 h). Filters and filtrates were stored frozen (-20°C) until analysis. Total particulate saccharides (p-TCHO) and total dissolved saccharides (d-TCHO) were determined following the colorimetric TPTZ (2,4,6-tripyridyl-*s*-triazine) method of Myklestad et al. (1997). The whole procedure was carried out in darkness because the reagents are light-sensitive (van Oijen et al. 2003). d-TCHO includes mono- (d-MCHO) and polysaccharides (d-PCHO) which are respectively the concentration before and after hydrolysis (d-TCHO = d-MCHO + d-PCHO). Dissolved and particulate samples were hydrolysed with HCl 0.1 N at 100°C, during 20h (Burney and Sieburth 1977). Calibration curves were obtained with D(+)-glucose and the values of saccharides were expressed as glucose equivalent. They were converted into carbon concentration using a conversion of 6 mol of C per mole of glucose, since each glucose molecule contains 6 carbon atoms. The coefficient of variation (CV, standard deviation/mean) of all triplicate measurements was below 5% and the detection limit was 1.0 µM C. Three blanks per batch of samples were always treated and analyzed in the same way as the samples and subtracted from the concentration of the samples.

2.3.3. Transparent Exopolymeric Particles (TEP)

Melted sea ice samples and unfiltered seawater samples poisoned with formaldehyde (2% final conc.) were filtered onto 0.4µm pore size polycarbonate filters (Nuclepore) using a vacuum pressure <150mm Hg. Transparent exopolymeric particles (TEP) were stained with Alcian Blue solution (0.02% Alcian Blue, pH 2.5) according to Passow and Alldredge (1995). Filters were stored at - 20°C until further processing. Filters were then extracted with 6ml of 80% H_2SO_4 for 2 hours, in the dark with constant stirring. Absorbance of triplicate samples was measured against a distilled water blank at 787 nm. The calibration was made using Xanthan Gum solution. Results are expressed as Xanthan Gum weight equivalent per litre (XAGeq L^{-1}).

2.4. Microorganisms

For the determination of chlorophyll *a* and enumeration of microorganisms, ice core sections were melted in the dark at 4°C in seawater prefiltered through 0.2 µm polycarbonate filters (1:4 v:v).

2.4.1. Chlorophyll *a* and Algae

Samples were filtered on 10 µm Nuclepore filter and then on 0.8 µm Nuclepore filter to collect algae > 10 µm on the first filter and 0.8 µm < algae < 10 µm on the second filter. Chlorophyll *a* (Chl *a*) was extracted in 90% v:v acetone in the dark at 4°C for 24 h, and quantified fluorometrically in each size-fraction according to Yentsch and Menzel (1963). Samples for microscopic observations of algae were preserved with gluteraldehyde-Lugol's solution (1% final concentration). Algae taxonomic composition were studied by inverted light microscopy (100 x magnification and 320 x magnification) according to the method of Utermöhl (1958).

2.4.2. Bacteria

Samples for bacterial analyses were preserved with 40 % buffered formaldehyde (final concentration 2 %). Bacteria were enumerated by epifluorescence microscopy after DAPI staining (Porter and Feig 1980). A minimum of 1000 cells was counted in at least 10 different fields at 1000x magnification. A relative standard deviation of 15% (n = 20) was estimated on the bacterial abundance determination. Bacterial biovolumes were determined by image analysis (Lucia 4.6 software) and calculated by treating rods and cocci as cylinders and spheres, respectively (Watson et al. 1977). They were converted to carbon biomass by using the relation established from data measured by Simon and Azam (1989): $C = 92\ V^{0.598}$ where C is the carbon per cell (fg C $cell^{-1}$) and V is the biovolume (μm^3).

3. Results

3.1. Particulate fraction

In bulk sea ice, concentrations of particulate organic carbon (POC) ranged between 10.2 and 117.9 µM C (median 37.4 µM C) at Brussels Station and between 10.1 and 113.5 µM C (median 25.1 µM C) at Liège Station (Tables 2 and 3). At Brussels Station, the distribution of POC in sea ice was relatively homogeneous on a vertical scale, meaning that no large accumulation as compared to interior layers was observed in surface and bottom layers (Figure 1). Concentrations increased from the first to the fourth visit at Brussels, where concentrations have doubled in upper layers compared to the third visit. POC is still high on the last visit to Brussels. By contrast, the distribution of POC in Liège sea ice presented higher concentration in the surface layer and lower levels in the internal or bottom layers (Figure 2). At Liège, POC decreased in the bottom layer during the period of investigation. POC measured in shallow (15 cm) and deep (40 cm) brines ranged between 10.1 and 55.3 µM C (Brussels, median 30.4 µM C) and between 7.6 and 144.4 µM C (Liège, median 30.8 µM C) (Tables 2 and 3). In seawaters, average POC varied between 0.7 and 1.5 µM C (Brussels) and between 0.8 and 1.6 µM C (Liège) (Table 4).

In bulk sea ice, particulate saccharides (p-TCHO) made up in average 10.1% (median) of POC (range 5.9 – 16.1%) at Brussels and 5.8% (median) of POC (range 3.0 – 10.3%) at Liège. The %p-TCHO/POC was thus ca. twice at Brussels as compared to Liège. Concentrations varied between 0.60 and 12.81 µM C (median 3.93 µM C) and between 0.42 and 10.07 µM C (median 1.75 µM C) at Brussels and Liège respectively (Tables 2 and 3). The vertical distribution of p-TCHO was similar to that one of POC. In brines, p-TCHO ranged between 0.8 and 5.4 µM C (Brussels) and between 1.4 and 29.2 µM C (Liège) (Tables 2 and 3). In seawater, p-TCHO accounted for 3.6% (0.4 – 10.6%) and 6.8% (0.3 – 11.8%) of POC at Brussels and Liège respectively with concentrations ranging from <dl to 0.14 µM C (median 0.02 µM C) and from <dl to 0.19 µM C (median 0.08 µM C) (Table 4).

Table 2: Main sea ice physical properties (depth, temperature, salinity and brine volume) along with sea ice concentrations of chlorophyll *a* (Chl *a*), particulate organic carbon (POC), dissolved organic carbon (DOC), particulate saccharides (p-TCHO), dissolved saccharides (d-TCHO), transparent exopolymeric particles (TEP) and bacterial biomass (BB) for Brussels station sampled during SIMBA in bulk sea ice (bulk SI) and brines (BR). ND: not determined

		depth Chl *a*	depth OM	temp	sal	V_b	Chl *a*	POC	DOC	TEP	p-TCHO	d-TCHO	BB
		cm	cm	°C		%	µg L^{-1}	µM C	µM C	µg XAG L^{-1}	µM C	µM C	µg C L^{-1}
Brussels 1	**Bulk**	0-10	0-10	-2.8	9.6	17.1	8.4	37.4	62	28.1	3.2	14.0	36.1
JD: 274	**SI**	10 20	10 20	-1.9	8.7	23.1	12.8	61.9	97	10.8	5.6	11.9	23.0
		20-30	20-30	-1.8	5.8	15.9	6.1	13.5	37	5.5	2.0	4.4	24.8
		30-40	30-40	-1.7	5.3	15.8	3.4	17.2	27	3.9	1.0	7.0	6.9
		40-51.5	40-52.5	-1.7	4.9	14.9	4.2	26.4	40	4.2	2.3	8.2	19.6
		51.5-61.5		-1.6	4.7	14.9	8.4						
	BR	15			36.7		3.3	30.0	ND	15.0	2.1	3.2	ND
		40			36		3.8	38.2	ND	17.6	3.2	5.0	ND
Brussels 2	**Bulk**	0-10	0-10	-3.6	11.5	15.8	11.9	34.0	80	ND	4.3	18.1	17.7
JD: 279	**SI**	10 20	10 20	-3.0	5.2	8.4	1.9	12.4	39	ND	1.2	5.9	11.7
		20-30	20-30	-3.1	5.0	7.8	1.7	10.4	48	ND	0.9	6.4	13.4
		30-40	30-40	-2.3	3.9	8.2	1.4	10.2	50	ND	0.6	10.0	22.8
		40-53.5	40-55	-2.0	4.0	9.7	7.0	17.3	78	ND	1.9	21.2	25.6
		53.5-63.5	55-65	-1.6	4.6	14.3	12.2	14.9	57	ND	1.8	18.9	17.0
			65-75	-1.6	4.6	14.3		29.8	60	ND	3.9	12.9	26.1
	BR	15			65.7		1.4	28.6	ND	10.8	2.6	4.6	ND
		40			65		0.9	19.7	ND	28.2	2.3	4.9	ND
Brussels 3	**Bulk**	0-10	0-10	-3.2	11.1	17.0	6.6	40.4	96	173.4	4.2	22.0	23.8
JD: 284	**SI**	10 20	10 20	-2.9	9.7	16.3	1.9	38.4	99	147.7	4.1	13.8	32.3
		20-30	20-30	-2.5	5.1	9.9	2.3	30.1	55	60.5	2.9	14.3	9.4
		30-40	30-40	-2.3	5.6	11.9	2.7	25.0	46	49.8	2.3	9.4	19.6
		40-52.5	40-48.5	-2.3	4.6	9.9	4.6	16.6	41	24.4	2.7	3.8	9.6
		52.5-62.5	48.5-58.5	-2.1	4.0	9.4	16.0	38.4	57	40.0	3.9	7.1	16.0
	BR	15			64.1		1.6	25.1	ND	16.8	2.2	4.7	ND
		40			60		1.9	37.7	ND	14.7	2.5	6.3	ND
Brussels 4	**Bulk**	0-10	0-10	-5.8	8.1	6.9	13.0	89.3	232	97.3	10.6	84.5	77.9
JD: 289	**SI**	10 20	10 20	-4.8	4.5	4.7	2.6	96.1	172	74.5	12.8	76.3	51.4
		20-30	20-30	-4.3	4.7	5.4	2.3	61.1	137	60.5	7.4	44.1	14.6
		30-40	30-40	-3.3	4.8	7.1	4.9	71.7	132	22.8	7.2	41.1	29.3
		40-48	40-49.5	-2.7	4.1	7.4	4.0	32.3	69	23.3	3.7	14.0	36.1
		48-58		-2.3	3.6	7.8	8.3						
		58-68		-2.1	4.6	11.1	7.0						
	BR	15			86.4		2.9	55.3	ND	31.2	6.4	14.8	ND
		40			36		0.8	10.1	ND	11.6	0.8	6.3	ND
Brussels 5	**Bulk**	0-10	0-10	-4.0	12.4	15.3	7.6	117.9	140	100.8	11.6	49.7	24.8
JD: 294	**SI**	10 20	10 20	-3.4	7.4	10.6	1.6	85.8	111	47.6	9.1	38.5	17.7
		20-30	20-30	-2.9	5.9	9.8	1.4	50.6	54	23.5	4.7	18.9	17.2
		30-40	30-40	-2.6	4.6	8.6	2.3	47.8	52	29.6	4.8	22.3	53.4
		40-50	40-49.5	-2.8	3.9	6.8	2.9	89.0	75	40.5	5.3	35.0	24.0
		50-60	49-59	-1.8	3.5	9.8	10.9	49.8	50	31.5	4.2	20.5	28.9
		60-70		-1.6	3.9	12.1	8.1						
	BR	15			66.1		1.1	30.7	ND	41.2	5.4	16.7	ND
		40			66		1.4	38.7	ND	43.9	5.2	9.9	ND

Table 3: Main sea ice physical properties (depth, temperature, salinity and brine volume) along with sea ice concentrations of chlorophyll *a* (Chl *a*), particulate organic carbon (POC), dissolved organic carbon (DOC), particulate saccharides (p-TCHO), dissolved saccharides (d-TCHO), transparent exopolymeric particles (TEP) and bacterial biomass (BB) for Liège station sampled during SIMBA in bulk sea ice (bulk SI) and brines (BR).

		depth Chl *a* cm	depth OM cm	temp °C	sal	V_b %	Chl *a* µg L^{-1}	POC µM C	DOC µM C	TEP µg XAG L^{-1}	p-TCHO µM C	d-TCHO µM C	BB µg C L^{-1}
Liège 1	**Bulk**	0-10	0-10	-2.3	10.3	22.2	25.7	94.1	198	149	7.5	27.6	23.4
JD: 276	**SI**	10-20					12.9						
		20-30					12.6						
		30-40	30-40	-1.9	3.9	10.2	19.6	24.3	34	138	1.3	19.7	19.2
		40-50	40-50	-1.9	3.0	7.8	8.2	25.6	56	181	2.1	18.9	15.6
		50-52					11.7						
		52-62					12.6						
		62-72	62-72	-1.9	2.7	7.1	15.3	24.6	27	142	1.5	4.3	35.5
		72-82					5.3						
		82-92	82-92	-1.8	2.9	7.8	2.1	13.1	45	130	0.6	2.2	5.3
		92-102					1.5						
		102-112	102-112	-1.7	2.9	8.4	1.4	46.2	45	117	3.6	4.6	20.9
	BR	15			38.2		45.9	144.4	ND	119	29.2	14.5	ND
		60			38		29.8	141.2	ND	112	26.7	17.3	ND
Liège 2	**Bulk**	0-10	0-10	-2.2	12.1	28.2	10.7	109.2	195	129	6.3	55.8	46.1
JD: 281	**SI**	10-20					8.5			189			22.2
		20-30					9.9			164			6.0
		30-40	30-40	-1.8	4.7	12.9	9.7	23.3	65	44	1.4	18.2	14.5
		40-50	40-50	-2.1	3.8	8.9	7.4	35.7	90	31	2.1	27.1	7.2
		50-54.5					6.3			41			
		54.5-60					2.1						
		60-70	62-72	-2.0	3.1	7.5	6.5	20.8	43	47	1.1	6.7	26.4
		70-80					1.8			40			
		80-90	82-92	-1.4	3.1	11.0	2.2	30.1	60	79	2.2	22.2	46.5
		90-100		-1.5	3.1	10.6	1.8			30			27.3
		100-110	102-112	-1.5	3.1	10.6	1.4	36.8	71	28	2.5	10.3	47.8
	BR	15			46.0		1.8	55.4	ND	53	5.8	9.5	ND
		60			34		1.6	30.6	ND	16	3.6	15.8	ND
Liège 3	**Bulk**	0-10	0-10	-3.0	8.8	14.4	9.2	79.9	155	86	7.3	66.4	38.1
JD: 286	**SI**	10-20					7.6						
		20-30					9.0						
		30-40	30-40	-2.3	3.8	8.3	5.3	48.8	125	28	2.8	43.6	21.4
		40-50	40-50	-2.2	3.7	8.4	4.8	42.1	112	4	2.5	43.4	21.2
		50-66	57-67	-2.0	4.2	10.2	4.4	18.8	41		1.1	5.1	
		66-76		-1.9	3.1	8.1	2.1			7			24.9
		76-86	77-87	-1.8	2.7	7.5	1.1	10.5	23		0.8	2.1	
		86-96		-1.9	2.7	6.9	2.0			11			13.6
			97-107	-2.1	3.1	7.3		13.8	23	8	0.4	1.6	14.3
	BR	15			45.0		1.6	32.8	ND	34	9.0	16.4	ND
		60			44		3.3	31.0	ND	48	8.7	22.7	ND
Liège 4	**Bulk**	0-10	0-10	-3.7	8.9	11.9	4.8	73.1	122	125	7.5	34.5	76.6
JD: 291	**SI**	10-20					3.8			-			
		20-30					2.2						
		30-40	30-40	-2.7	3.9	7.1	4.1	20.5	47	59	1.8	17.5	23.9
		40-50	40-50	-2.2	3.4	7.5	5.9	21.2	37	17	1.2	16.0	17.8
		50-63.5		-1.8	3.3	8.9	6.5						
		63.5-73.5	65-75	-1.8	3.9	11.0	2.6	19.4	25	23	1.4	4.2	37.9
		73.5-83.5	75-85	-1.8	3.2	8.7	2.2	10.1	11	28	0.4	3.6	25.7
		83.5-93.5		-1.7	3.4	9.7	3.3						
		93.5-103.5	95-105	-1.7	3.1	9.2	2.5	16.1	9	33	0.5	1.1	20.8
	BR	15			65.4		0.4	8.8	ND	21	1.4	17.8	ND
		60			59		0.4	7.6	ND	16	1.9	11.5	ND
Liège 5	**Bulk**	0-10	0-10	-1.9	10.0	26.5	9.7	113.5	119	182	10.1	51.2	15.7
JD: 296	**SI**	10-20					11.9	-					
		20-30					9.6						
		30-40	30-40	-1.8	3.7	10.5	7.3	94.0	102	62	5.5	68.5	45.2
		40-50	40-50	-1.9	3.1	8.1	5.1	64.5	80	53	3.7	42.1	28.2
		50-61		-2.0	2.4	5.9	4.8						
		61-71	62-72	-1.9	2.6	6.6	5.8	35.7	35	26	1.7	8.7	4.7
		71-81	72-82	-1.9	3.3	8.5	4.2	20.1	41	65	1.2	8.1	24.7
		81-91		-1.9	2.9	7.6	2.1						
		91-101	92-102	-1.8	4.0	11.0	8.2		23	92		5.1	35.1
	BR	15			40.7		0.3	14.2	ND	49	2.8	15.5	ND
		60			44		0.7	11.3	ND	22	1.8	14.9	ND

ND: not determined

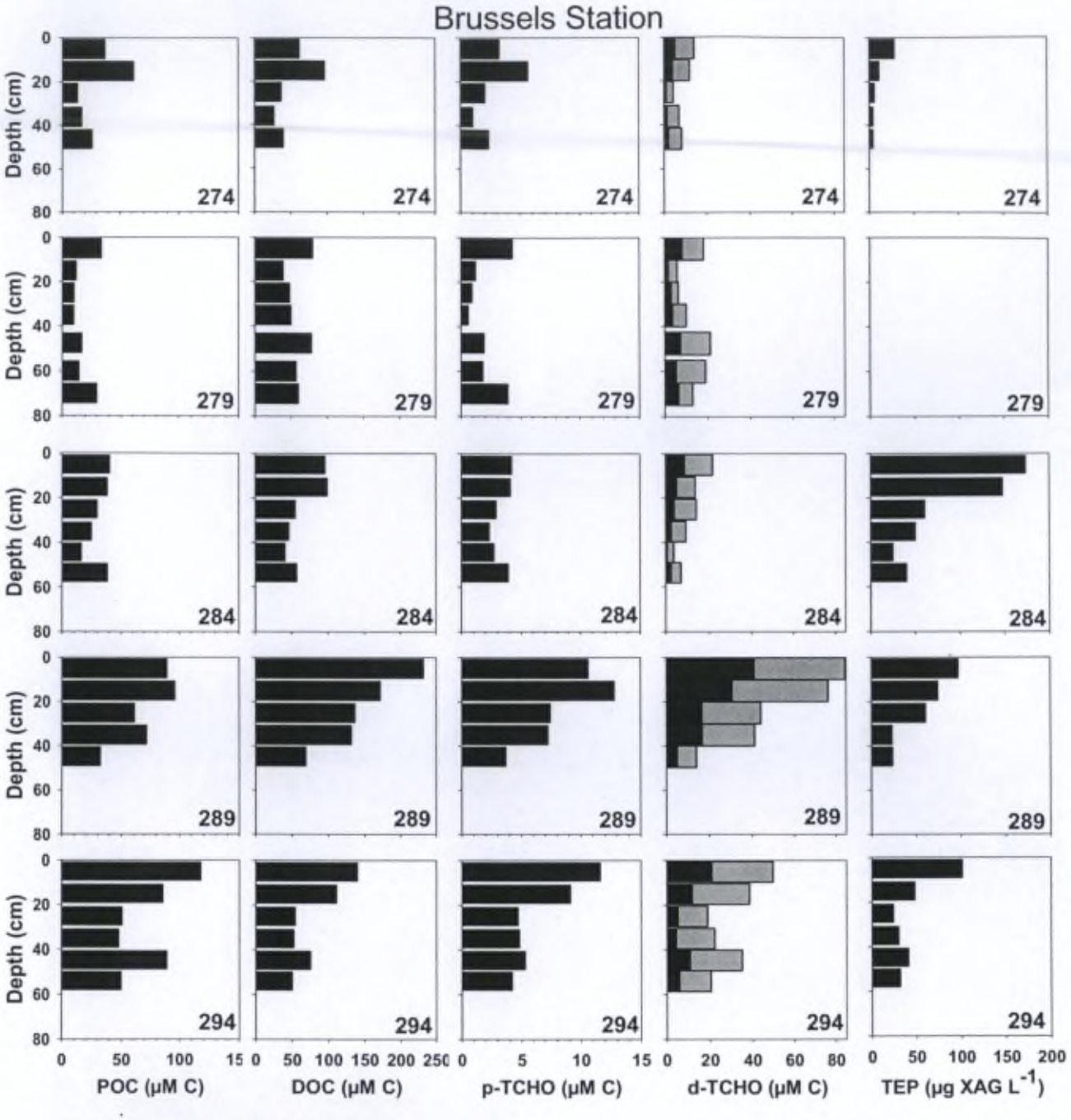

Figure 1: Distribution of a) particulate organic carbon (POC), b) dissolved organic carbon (DOC), c) particulate saccharides (p-TCHO), d) dissolved saccharides (d-TCHO) and e) transparent exopolymeric particles (TEP) in sea ice at Brussels for the 5 sampling times.

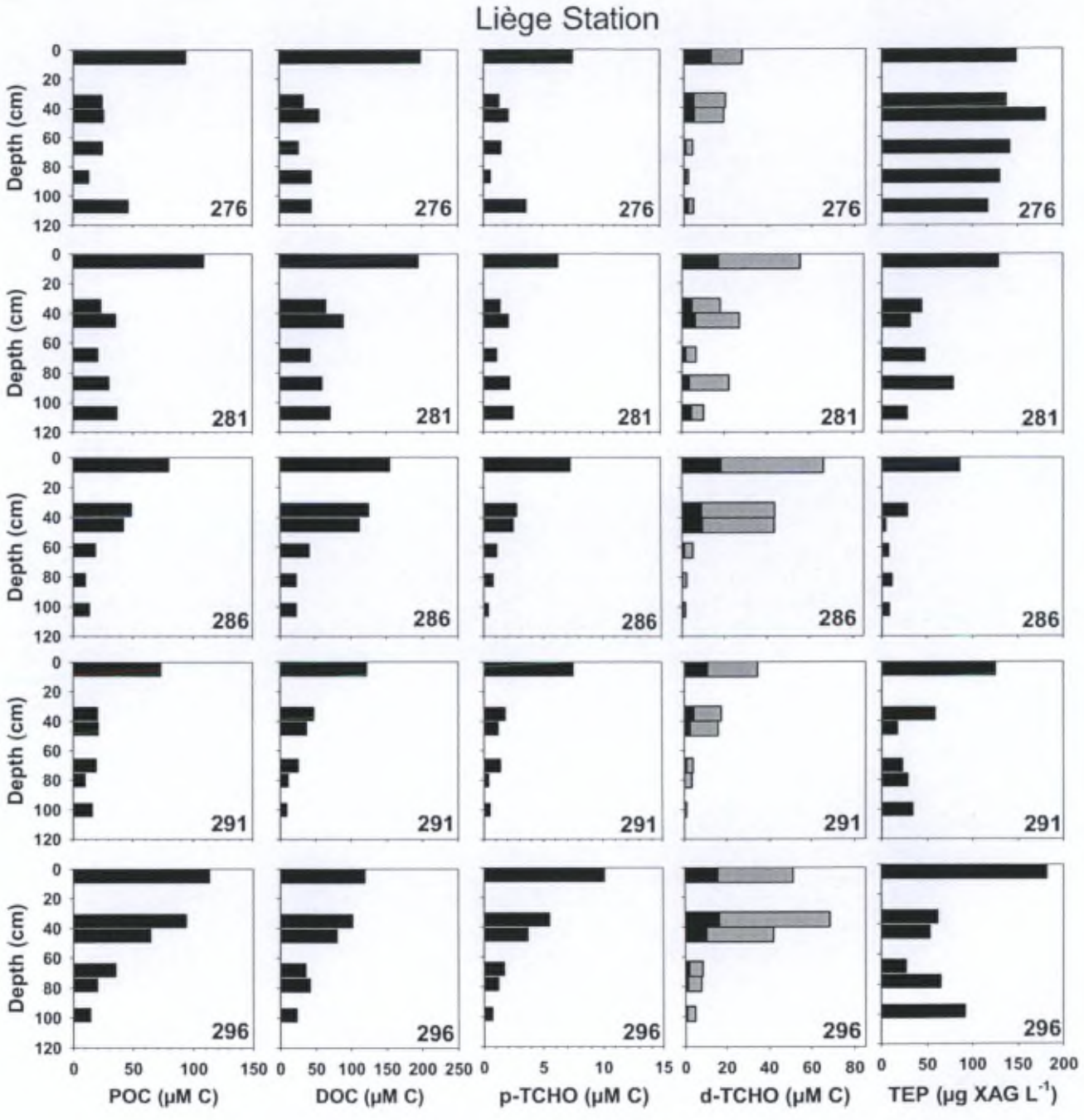

Figure 2: Distribution of a) particulate organic carbon (POC), b) dissolved organic carbon (DOC), c) particulate saccharides (p-TCHO), d) dissolved saccharides (d-TCHO) and e) transparent exopolymeric particles (TEP) in sea ice at Liège for the 5 sampling times.

Table 4: Concentrations of chlorophyll *a* (Chl *a*), particulate organic carbon (POC), dissolved organic carbon (DOC), particulate saccharides (p-TCHO), dissolved saccharides (d-TCHO), transparent exopolymeric particles (TEP) and bacterial biomass (BB) for seawaters from 0 to 30 m (average +/- standard deviation) of Brussels and Liège stations sampled during SIMBA.

	Julian Days	Chl *a* $\mu g\ L^{-1}$	POC $\mu M\ C$	DOC $\mu M\ C$	p-TCHO $\mu M\ C$	d-TCHO $\mu M\ C$	BB $\mu g\ C\ L^{-1}$
Brussels 1	**274**	0.01 ± 0.00	0.70 ± 0.13	54 ± 6	0.02 ± 0.01	4.2 ± 0.3	2.2 ± 0.4
Brussels 2	**279**	0.03 ± 0.00	0.71 ± 0.11	54 ± 1	0.01 ± 0.00	5.2 ± 1.0	3.4 ± 1.5
Brussels 3	**284**	0.12 ± 0.00	1.09 ± 0.07	52 ± 0	0.01 ± 0.00	4.5 ± 1.5	1.5 ± 0.5
Brussels 4	**289**	0.17 ± 0.01	1.54 ± 0.25	55 ± 6	0.11 ± 0.03	4.9 ± 0.3	1.4 ± 0.5
Brussels 5	**294**	0.04 ± 0.00	0.63 ± 0.12	52 ± 11	0.05 ± 0.02	5.0 ± 0.6	2.2 ± 1.3
Liège 1	**276**	0.04 ± 0.02	1.05 ± 0.15	52 ± 5	0.04 ± 0.01	3.3 ± 0.6	2.4 ± 0.4
Liège 2	**281**	0.05 ± 0.03	1.05 ± 0.53	53 ± 6	0.04 ± 0.04	4.1 ± 0.5	2.4 ± 0.8
Liège 3	**286**	0.14 ± 0.01	1.61 ± 0.19	54 ± 4	0.16 ± 0.06	4.3 ± 0.3	1.5 ± 0.5
Liège 4	**291**	0.10 ± 0.00	0.96 ± 0.18	56 ± 8	0.09 ± 0.00	4.1 ± 0.9	2.7 ± 0.5
Liège 5	**296**	0.06 ± 0.00	0.75 ± 0.14	50 ± 4	0.05 ± 0.02	3.8 ± 0.1	1.5 ± 0.4

3.2. Dissolved fraction

In sea ice, concentrations of dissolved organic carbon (DOC) ranged between 27 and 232 µM C (median 60 µM C) at Brussels Station and between 9 and 198 µM C (median 46 µM C) at Liège Station (Tables 2 and 3). Here also, the vertical distribution of DOC in sea ice of Brussels and Liège was similar to the one of POC (Figures 1 and 2). As for POC, no accumulation in a particular layer is observed at Brussels Station (Figure 1). Similarly DOC increased from the first to the fourth visit where concentrations were maximal, twice the levels of the third visit. At Liège, the distribution of DOC in Liège sea ice presented higher concentration in the surface layer and they decreased towards bottom of the ice core. Between the second and fourth visit, a decrease of the concentrations in surface and bottom layers was observed. In the internal layers, the profiles varied more randomly. In seawaters, average DOC ranged from 52 to 55 µM C (Brussels) and from 50 to 56 µM C (Liège) (Table 4).

In sea ice, dissolved saccharides (d-TCHO) made up in average 22.8% (median) of DOC (range 9.1 – 46.9%) at Brussels and 28.2% (median) of DOC (range 5.0 – 67.2%) at Liège. Concentrations varied between 3.8 and 84.5 µM C (median 14.3 µM C) and between 1.1 and 68.5 µM C (median 16.8 µM C) at Brussels and Liège respectively (Tables 2 and 3). At Brussels, concentrations highly increased at the fourth visit, about 4 times greater than the values measured on the third visit (Figure 1). On the last time, concentrations were still higher than at the beginning but smaller than on visit 4. At Liège higher concentrations were observed in surface and lower values in the bottom half ice (Figure 2). In brines, d-TCHO ranged between 3.2 and 16.8 µM C (Brussels) and between 9.5 and 22.7 µM C (Liège) (Tables 2 and 3). In seawater, d-TCHO accounted for 8.4% (6.6 – 12.8%) and 7.3% (5.4 – 9.0%) of DOC at Brussels and Liège respectively with concentrations ranging from 3.5 to 6.3 µM C (median 4.7 µM C) and from 2.7 to 5.0 µM C (median 3.9 µM C) (Table 4). The dissolved saccharides were composed of monosaccharides for 37.8% (median, range 21.8 – 52.9%) and 28.9% (median, range 15.0 – 63.7%) in sea ice of Brussels and Liège. In the corresponding seawaters, monosaccharides accounted for 55.4% (median, range 31.1 – 83.1%) and 40.6% (median, range 26.3 – 74.0%).

3.3. Transparent Exopolymeric Particles

TEP concentrations in sea ice ranged from 3.9 to 173.4 µg XAG eq L^{-1} (median 35.7 µg XAG eq L^{-1}) and from 4.3 to 189.4 µg XAG eq L^{-1} (median 52.8 µg XAG eq L^{-1}) at Brussels and Liège respectively (Tables 2 and 3). In Brussels sea ice, the smallest concentrations were observed on first visit and the highest concentrations were observed at the third visit of Brussels (Figure 1). At Liège, the highest concentrations were recorded at the first visit in all the ice layers and then TEP were higher in surface layers (Figure 2). In brines TEP ranged between 10.8 and 43.9 µg XAG eq L^{-1} (Brussels) and between 16 and 119 µg XAG eq L^{-1} (Liège) (Tables 2 and 3). In seawaters, TEP do not reach more than ca. 5 µg XAG eq L^{-1}.

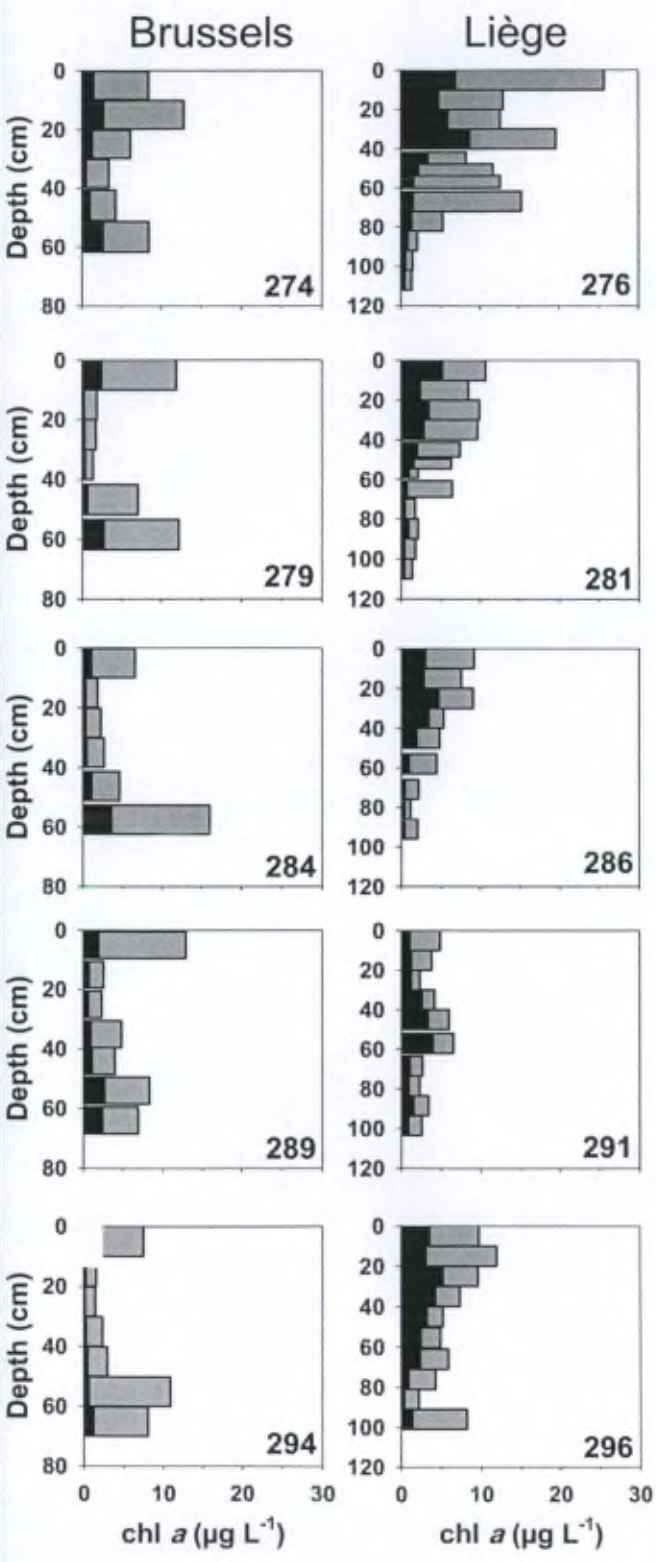

Figure 3: Distribution of chlorophyll *a* (Chl *a*, µg L^{-1}) in sea ice at Brussels (left) and Liège (right) for the 5 sampling times. Chl *a* associated to algae with a size between 0.8 - 10 µm is in black and Chl *a* associated to algae with a size > 10 µm is in grey.

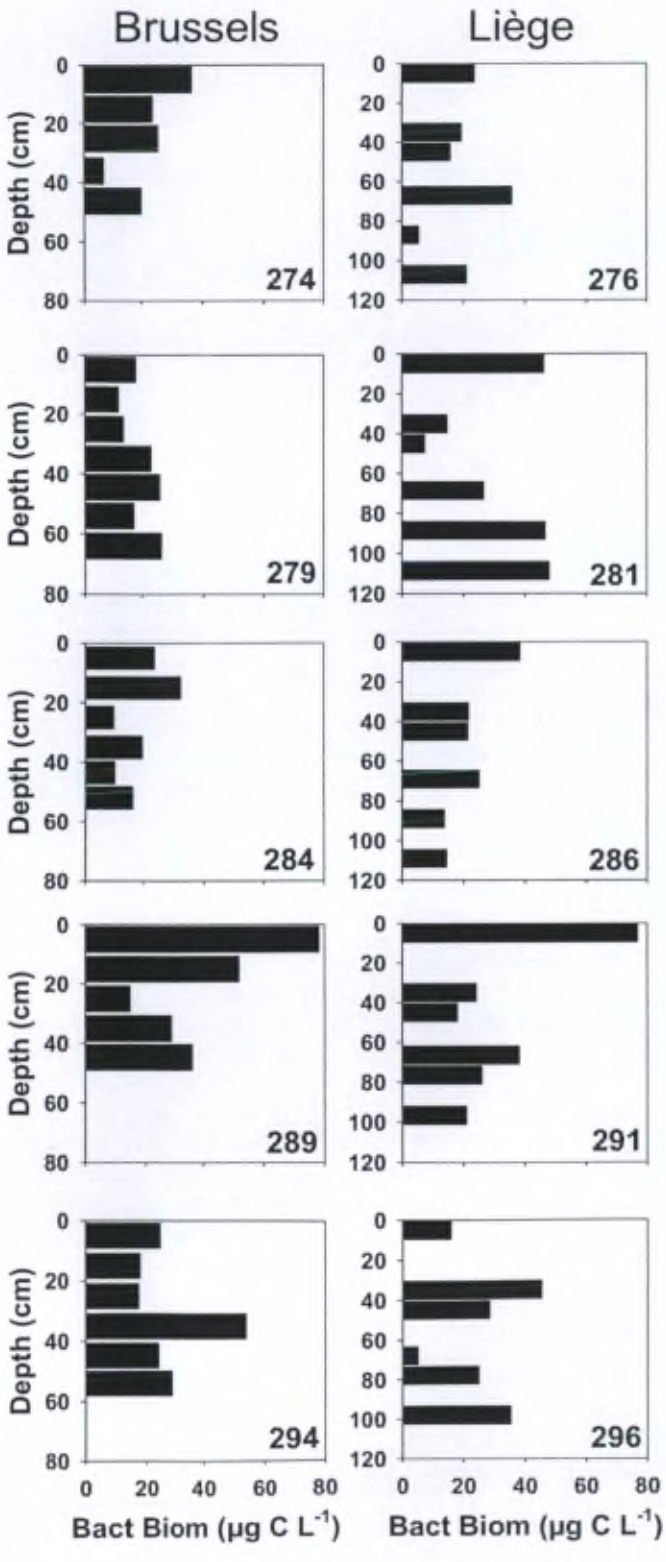

Figure 4: Distribution of bacterial biomass in sea ice at Brussels (left) and Liège (right) for the 5 sampling times.

3.4. Algae

The chlorophyll *a* (Chl *a*) ranged respectively from 1.4 to 16.0 µg L^{-1} (median 4.7 µg L^{-1}) and from 1.1 to 25.7 µg L^{-1} (median 5.3 µg L^{-1}) at Brussels and Liège stations (Tables 2 and 3). The vertical distribution of Chl *a* was rather homogeneous along the ice core of Brussels with still lower values in internal layers (Figure 3). At Liège the highest concentrations were observed in the surface layers of the first visit and then decreased towards the bottom of the ice cover (Figure 3). In brines Chl *a* ranged between 0.8 and 3.8 µg L^{-1} (Brussels) and between 0.3 and 45.9 µg L^{-1} (Liège) (Tables 2 and 3). In seawaters from Brussels and Liège, concentrations varied respectively between 0.01 and 0.19 µg L^{-1} (median 0.04 µg L^{-1}) and between 0.02 and 0.16 µg L^{-1} (median 0.07 µg L^{-1}) (Table 4). Higher seawater concentrations were measured at the third and fourth sampling time of both stations. The Chl *a* associated with algae > 10 µm dominated in sea ice (median 77% for Brussels and 61% for Liège) while algae < 10 µm dominated in seawaters (median 70% and 79% for Brussels and Liège). At Brussels, the surface algal community was composed of small diatom and nanoflagellates (dinoflagellates and naked flagelattes) and the bottom community was dominated be large diatoms, mainly *Corethron sp.* At Liège, the surface community was composed of dinoflagellates and *Phaeocystis sp.* cells while the bottom community was diatoms, mainly empty cells (frustules).

3.5. Bacteria

The bacteria were relatively equally distributed in all ice layers at Brussels and Liège, except at the fourth visit where bacterial biomass was higher in the surface layers (Figure 4). Bacterial biomass ranged between 6.9 and 77.9 µg C L^{-1} (median 23.0 µg C L^{-1}) at Brussels and between 4.7 and 76.6 µg C L^{-1} (median 23.4 µg C L^{-1}) at Liège (Tables 2 and 3). In seawater bacterial biomass represented between 0.7 and 4.7 µg C L^{-1} (median 1.9 µg C L^{-1}) and between 0.9 and 3.3 µg C L^{-1} (median 2.0 µg C L^{-1}) at Brussels and Liège respectively (Table 4).

4. Discussion

4.1. Description of sea ice physical properties and impacts on distribution of algae

The 2 sampled stations had contrasted physical characteristics in regards with ice texture and ice and snow thicknesses. Brussels ice cores were 60 cm thick, mainly composed of columnar ice while Liège ice cores were twice as thick (120 cm), and composed of granular ice with different crystal sizes. The snow layer was also thicker at Liège than at Brussels. These contrasted characteristics mean that the genesis of the ice cover was different and possibly resulted in different initial incorporation/enrichment of the microbial community and organic matter (Weissenberger and Grossmann 1998). Granular ice, associated with dynamic turbulent conditions, forms usually at high growth rates where very high concentration of cells are concentrated, by nucleation of frazil ice crystal or by scavenging of cells as a frazil crystal float up through the water column (Eicken 2003). In contrast, slow columnar ice formation rejects algal cells in the initial growth period (Palmisano and Garrison 1993, Weissenberger and Grossmann 1998). These differences in the ice formation could influence/control the distribution of sea ice microorganisms. Nevertheless, the initial concentration contrasts between textural units were probably damped as time goes by from autumn to the spring when the stations were sampled. In spring, the vertical distribution of algae might be controlled by the following key factors light, temperature, salinity and nutrients (Arrigo and Sullivan 1994). The major difference between Brussels and Liège was not temperature, salinity, or inorganic nutrients concentrations but it was the availability of light within the ice core. Indeed the available light depends on the type of ice, the ice interior structure (quantities of inclusions) but also on the snow accumulation on top of the ice (Eicken 2003). Accordingly, the vertical distribution of algae biomass (Chl *a*) was contrasted between both stations. Chl *a* was more homogeneously distributed in ice layers at Brussels while a maximum concentration was observed in the top ice layer and very low values in the bottom half ice at Liège. The thicker ice and snow accumulation at Liège clearly explain the low algal presence in the bottom part of the ice cover. Besides the presence of the algal community in the surface ice layer of Liège further contributed to the light attenuation by the self-shading effect of algae (Palmisano et al. 1987). The algae observed in the bottom ice layers at Liège were little diverse and a lot of empty frustules were observed. Oppositely the algae observed in the bottom ice layer at Brussels were diverse and dominated by a large diatom species (*Corethron sp.*).

Moreover, at the beginning of the sampling period, corresponding to the austral spring, the ice cover of Brussels and Liège was warm (isothermal) and porous; temperatures were above - 4°C and brine volumes were above 5% (Tison et al. 2008). During the investigation period, the ice cover was submitted at a flood-freeze cycle caused by the cooling of the air temperature which then cooled down the surface ice temperature, between the third and fourth visits of Brussels (Tison et al. 2008). Brine salinities (initially similar to seawater value) drastically increased (Table 2), leading to increased brine instability. Brines moved downward the ice column and initiated a convection process (Tison et al. 2008) which might have contributed to the vertically-spread location of Chl *a* at Brussels. At Liège this cold episode was less marked because of the thicker snow layer, which buffer the heat transfer.

4.2. Concentration and composition of organic matter in sea ice and seawater

The dissolved organic carbon (DOC) and particulate organic carbon (POC) measured in sea ice at both stations were present in excess of those levels predicted from dilution curves (results not shown). Their concentrations were up to respectively 4 and 128 times higher than the mean DOC and POC concentrations in seawaters, in agreement with literature data reporting a general accumulation of organic matter (OM) in sea ice

(Thomas and Papadimitriou 2003, Dumont et al. 2009). Still the maximal concentrations measured in the present study were not as high as some previously reported data from pack ice (Thomas et al. 2001, Dumont et al. 2009). Even low values similar or lower than seawater concentrations have been measured in some ice layers from Brussels and Liège. The same remark also holds for saccharides and transparent exopolymeric particles (TEP) values.

The sea ice DOC:POC ratio, ranging from 1:1 to 5:1, was in the same range than the ratios already observed in the East Antarctic sector (1:1 to 4:1, Becquevort et al. 2009). These sea ice DOC:POC ratios were also far lower than average DOC:POC ratio estimated for seawaters, respectively, 64:1 and 52:1 for Brussels and Liège and low in comparison to the oceanic value of 15:1 (Millero 1996, Kepkay 2000a, b). This shows a difference in the partition of the organic carbon between the dissolved and particulate phase and the importance of the coagulation (DOC to POC) processes in sea ice. As reported by Vertugo et al. (2004), high polysaccharides concentration may spontaneously assemble into hydrogels, and forms TEP. TEP concentrations were indeed positively correlated with the total saccharides (dissolved + particulate) particularly in brines (Figure 5; Brussels r^2=0.64, Liège: r^2= 0.79) as found by Dumont et al. (2009) in sea ice, showing the saccharidic content of TEP (Passow 2002).

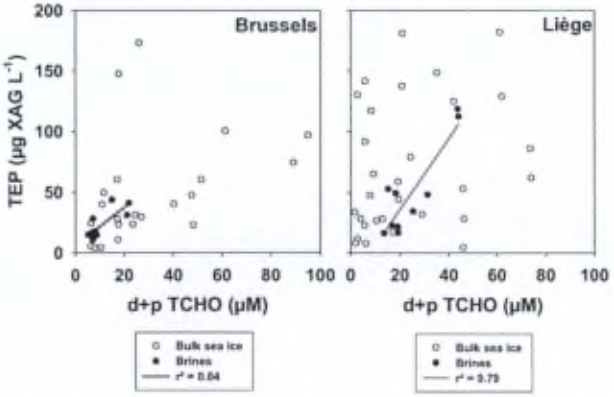

Figure 5: Relationship between transparent exopolymeric particles (TEP) and total saccharides concentrations (dissolved + particulate, d+p TCHO) for sea ice and brines samples at Brussels (left) and Liège (right).

The composition of the organic matter in sea ice is not particularly different as compared to the oceanic observations. In sea ice, particulate saccharides corresponded to 3 to 16 % of the POC in agreement with oceanic average (Volkman and Tanoue 2002) and previous ice studies (Dumont et al. 2009). The average contribution of dissolved saccharides to the DOC in sea ice, respectively 22.8 % (Brussels) and 28.2 % (Liège), was in the upper range of the oceanic literature average (10 - 25 %, Benner 2002) and in agreement with the work of Herborg et al. (2001) who found a mean contribution of 29 % for sea ice samples. Still percentages of dissolved saccharides up to 67% of DOC were observed in the present study. So a high variability of this percentage has already been reported in sea ice studies (Thomas et al. 2001), where the % ranged between 1% and 99% of DOC. The saccharides usually constitute a major portion of POM as well as DOM produced by phytoplankton (Benner 2002). The percentages observed here in sea ice OM are in agreement with estimations of the composition of phytoplankton exudates which reported that saccharides represent about 18-45% of POC and about 23-80% of DOC (Biddanda and Benner 1997). The contribution of the saccharides to the OM pool in ice layers increased where higher algal biomass was present (Dumont et al. 2009). Nevertheless, the high contribution of saccharides to the dissolved organic matter pool observed here as compared to the study of Dumont et al. (2009) is suggested to be linked to a weaker degree of degradation of the organic matter. Indeed when organic matter is remineralized by heterotrophic microorganisms, the saccharides content decreases as it is suggests by the decrease of the saccharides content of OM with depth in ocean (Benner 2002). The contributions of p-TCHO to the POC and of d-TCHO to the DOC tended to decrease towards the bottom of the Liège ice column. Under the same rationale, it could be linked to the low algal production in the bottom layer and thus presence of older OM in the bottom ice, with smaller percentage of saccharides.

The dissolved saccharides were composed of monosaccharides and polysaccharides. In sea ice, the monomeric compounds composed on average 37.8% (Brussels) and 28.9% (Liège) of the dissolved total saccharides. Polysaccharides were thus generally the dominant form as it was observed in spring/summer pack ice (Dumont et al. 2009). Still monosaccharides reached concentrations up to 40 µM C (Brussels 4) which is an unusual situation. Indeed usually dissolved free compounds such as neutral monosaccharides are considered as highly labile (Carlson 2002) and are found at nanomolar concentrations in aquatic systems. In sea ice, such an accumulation of monosaccharides has already been reported by Herborg et al. (2001) and Dumont et al. (2009). This suggests that the conditions found in the sea ice environment would be favourable to the accumulation of small organic molecules. The building up of the monomer concentration could be enhanced by the production of protective compounds by microorganisms and result from the limitation of heterotrophic activities (Thomas and Papadimitriou 2003). No relationships between bacteria and OM were observed. The high bacterial biomass present in sea ice seems not able to consume all the available labile organic substrates. A likely limiting factor would be temperature as proposed by Pomeroy and Wiebe (2001) but also salinity or toxins as discussed in Dumont et al. (2009).

4.3. Distribution of organic matter in sea ice and controlling parameters

For a given station, the vertical distribution and time evolution were relatively similar for all the fractions of OM (DOC, POC, d-TCHO and p-TCHO) (Figures 1 and 2). Also relatively good positive linear correlations were observed between p-TCHO and POC [Figure 6; Brussels: r^2 = 0.86 (sea ice) and 0.63 (brine); Liège: r^2 = 0.90 (sea ice) and 0.95 (brines)], d-TCHO and DOC (Figure 6; in sea ice: Brussels r^2 = 0.86; Liège r^2 = 0.65) and also between DOC and POC (Figure 6; Brussels: r^2

=0.60; Liège: r^2 = 0.76). Still the vertical distribution of OM was different between Brussels and Liège stations. At Brussels, the OM was rather homogeneously distributed along the ice core and at Liège, the OM presented a maximum in surface layers and low values in the bottom layers, in agreement with the general Chl *a* distribution. At Brussels, no accumulation in the bottom layer was observed, as previously observed for a similar length and texture ice core (Dumont et al. 2009). At Liège, the formation process of the ice cover, including rafting events as suggested by the various granular ice types observed (Tison et al. 2008), may also be responsible for peaks of concentrations observed in some internal layers.

The algal activity is the main production process of organic matter in aquatic systems (Nagata 2000). Despite a similar vertical distribution (see above), no significant correlation between any organic matter pools and Chl *a* was observed as it could sometimes be observed in sea ice (Dumont et al. 2009). An important OM consumption by bacteria could explain that algae and OM not correlated together but as discussed here above this case seems not to apply here. It could then result from a variation of the OM production by algal cells: indeed different abiotic factors such as light, nutrients, temperature and salinity modified the physiological conditions of algal cells and subsequently the production of OM (Nagata 2000).

A close parallelism between the evolution of temperature (and associated brine volume and salinity parameters) and the behaviour of the organic compounds (peak of concentration at Brussels 4) suggests a control of the OM production by the thermodynamic of the system. As already presented the ice cover relatively warm and permeable to gas exchanges at the beginning of the sampling period and was then cooled down between the third and fourth visits of Brussels (Tison et al. 2008). The Liège station did not present such a clear trend in the evolution of the OM; indeed the snow accumulation on top of the ice buffered the heat transfer as also seen in the physical parameters (Tison et al. 2008). The cold episode has indeed the effect to decrease the brine volume (Table 2) and to concentrate the brines as showed by the higher salinity measured inside the shallow brines (15 cm) at Brussels 4 as compared to previous times (Table 2, Tison et al. 2008). Similarly the concentrations of OM, and in this case also Chl *a*, were higher in the shallow brines of Brussels 4 (Table 2). We also observed the downward movement of brines with the salinity and concentrations in deep brines (40 cm) increasing at Brussels 5. Still as the OM measurements were made on bulk ice samples (including brine and crystal ice matrix), the higher bulk ice concentrations observed at Brussels 4 could not just be explained by the physical concentration process of brines. Indeed this process only changes the ratio of brine compared to the total ice volume and do not justify the large increase of bulk ice concentrations. It therefore implies that a supply of matter from somewhere should have occurred. At Brussels, the freeboard was always slightly positive at period of investigation but maybe flooding of the surface layers could have occurred between 2 consecutive visits (Tison J-L person. comm.). Nevertheless considering that the concentrations of OM in seawater were smaller than in sea ice, such an important enrichment of OM in sea ice seems unrealizable, unless sea ice could act as a filter concentrating organic matter deriving from seawater, by gelation or coagulation processes (Verdugo et al. 2004). Extreme physico-chemical conditions encountered in the sea ice could favour these processes.

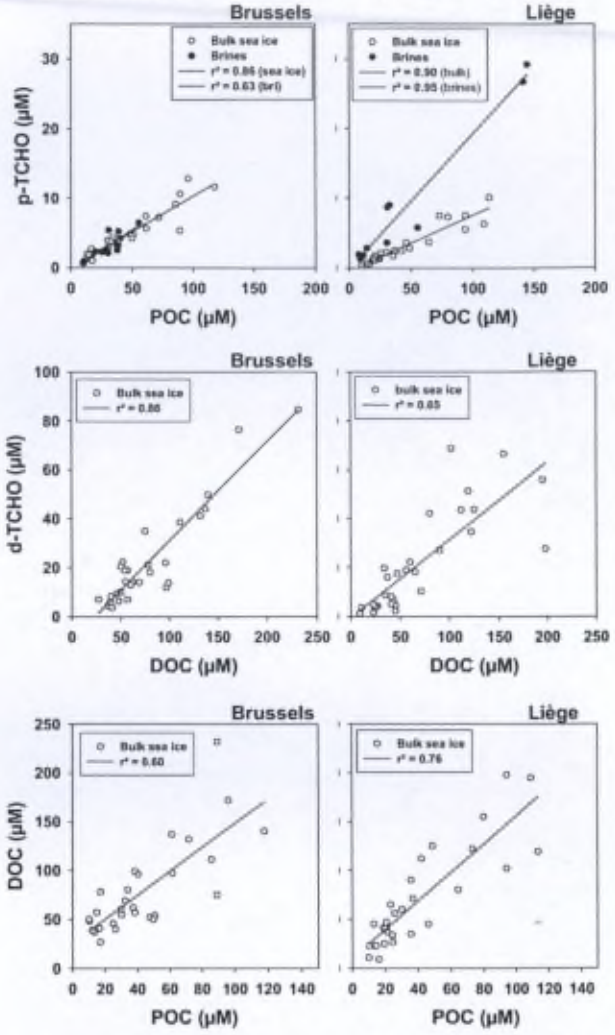

Figure 6: Relationships between particulate saccharides (p-TCHO) and particulate organic carbon (POC), total dissolved saccharides (d-TCHO) and dissolved organic carbon (DOC) and DOC and POC in sea ice and brines samples at Brussels (left) and Liège (right).

Alternatively, such a cooling down of the ice temperature and increasing brine salinity likely stressed the sea ice microorganisms. The stress maybe triggered protective reactions from microorganisms which potentially secrete more protective compounds in response to environmental stress (Thomas and Papadimitriou 2003, Krembs and Deming 2008). The production of TEP by algae is indeed enhanced in response to external stress in order to protect the cells from damages (Krembs and Deming 2008). TEP are present in sea ice (Krembs and Engel 2001, Meiners et al. 2004, Mancuso-Nichols et al. 2005, Riedel et al. 2007) and has been observed as an important extracellular component for cold adaptation in ice by protecting microorganisms and altering sea ice environment (Krembs et al. 2000, 2002, Collins et al. 2008). The ratios of TEP/Chl *a* were smaller in the

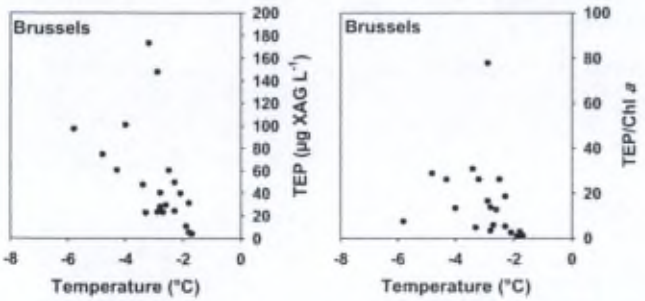

Figure 7: Relationships between transparent exopolymeric particles (TEP) and sea ice temperature and between ratio TEP/Chl *a* and temperature in sea ice from Brussels

present study (mean 14.9, range 0.9 - 77.8 at Brussels; mean 15.7, range 0.9 - 86.3 at Liège) than previously observed in end winter/early spring (mean 535, range 19.8 - 2529, Dumont et al. 2009) and spring/summer (mean 168, range 0.7 - 959, Dumont et al. 2009), still these ratios highly increased at Brussels 3 at the beginning of the cooling event. We observed that at Brussels, TEP concentrations increased when temperature decreased from - 2°C to - 6°C (Figure 7, r^2=0.67 when excluding 2 data corresponding to surface ice layer of Brussels 3). No such trend was visible at Liège (not shown) which can be explained by the less cold temperature (ca. - 2°C) of the ice column there. Similarly the ratio TEP/Chl *a* also tend to increase with decreasing temperature (Figure 7, r^2= 0.26 excluding the same 2 data as above). A similar increase of TEP/Chl *a* with decreasing temperature was observed in the pack ice region off East Antarctica (110°E-130° E) (van de Merwe under review). The abrupt cooling and subsequent brine salinity increase likely strongly enhanced the production of TEP by algae (high TEP/Chl *a* at Brussels 3). Strikingly higher concentration of OM but also of dimethylsulfoniopropionate (DMSP) and its breakdown product dimethylsulfide (DMS) (Brabant et al. person. comm.) were measured in all sea ice of Brussels 4. Oppositely the concentration of Chl *a* seems less affected by the thermodynamic of the sea ice system. As reported by Stefels et al. (2007), the production of DMSP by algae is controlled by abiotic parameters too. In *Phaeocystis* cultures, an increase of salinity resulted in an exponential increase of intracellular DMSP (Stefels et al. 2007). And as for TEP, the ratio of DMSP per C algal biomass increased at low temperature (van Rijssel and Gieskes 2002).

5. Conclusions

The vertical distribution of the dissolved and particulate organic matter was observed to be different between two ice covers having contrasted ice and snow thicknesses and texture. The presence of algae seemed to have influenced the composition of the organic matter. Nevertheless the organic matter concentration seems regulated by the thermodynamical evolution of sea ice cover characterized by a flood-freeze cycle.

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Chapter 4

Impacts of Antarctic pack ice melting on planktonic microbial communities in the Western Weddell Sea

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Impacts of Antarctic pack ice melting on planktonic microbial communities in the Western Weddell Sea

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Abstract

The influence of the melting of sea ice on planktonic microorganisms (algae, bacteria, protozoa) from the ISPOL drifting station in the Weddell Sea was studied, thanks to microcosm experiments realized under controlled and trace-metal clean conditions. A "direct melting" experiment was performed onboard to study the effects from the release of ice microorganisms, organic and inorganic nutrients on the planktonic community and to follow the evolution of the ice-derived microorganisms. The sea ice inoculum was an important source of microorganisms, organic matter and iron (Fe) for the seawater ecosystem as indicated by the ability of sea ice microorganisms to thrive in seawater and the enhanced microbial growth in presence of ice-derived components. A "sequential melting" experiment was then realized in the laboratory to study the timing of release of ice components. Brine drainage and the maximum release of Fe occurred first, but Fe release lasted longer until maximum release of particulate and dissolved organic carbon (POC and DOC) were observed. This timing of release of the different elements present in the ice cover is likely favourable to the development of blooms in the marginal ice zone in Antarctica.

Keywords: sea ice; microorganisms; melting; microcosms; iron

1. Introduction

In polar ecosystems, the annual formation and subsequent melting of the ice sheet closely couples sea ice and planktonic ecosystems. Both physical and biological processes are largely influenced by these interactions and finally result in huge implications not only for the Southern Ocean but also for the global climate (Brierley and Thomas 2002). At the time of ice formation, microorganisms present in seawater are incorporated into the ice sheet and can further develop into this new environment. Despite extreme conditions (e.g. temperature, salinity, pH...), the sea ice environment brings some advantages such as physical support for growth, favourable conditions of light, hibernation ground as well as protection against grazers (Lizotte 2003), which lead to large biomasses of microorganisms. When sea ice melts, these microorganisms are released into the water column. Because the sea ice sheets drift (e.g. Heil and Allison 1999) the place where microorganisms are released can be different from the initial location of entrapment of microorganisms (Smetacek et al. 1992 in Leventer 2003).

The annual sea ice retreat has a major impact on the formation of ice-edge plankton blooms (Smith and Nelson 1986, Lancelot et al. 1993), especially in the High Nutrient Low Chlorophyll (HNLC) Southern Ocean, where phytoplanktonic biomass remains low throughout the year. The circumpolar marginal ice zone has been reported as a region of important phytoplanktonic development (Arrigo et al. 2008), owing to the formation, at the time of sea ice melting, of a shallow vertically stabilized upper layer resulting from the production of meltwater and the subsequent release of sea ice microbial assemblages. Yet, the fate of the ice microorganisms released in the water column is uncertain (Kuosa et al. 1992, Froneman et al. 1996).

Some studies reported a similarity between ice and water microbial community (Mathot et al. 1991, Knox 1994, Gleitz et al. 1996), suggesting the possibility for ice algae to thrive in the water column and initiate phytoplanktonic blooms. Still other fates like sedimentation of large or aggregated cells (Riebesell et al. 1991) or consumption by pelagic grazers like krill (Marschall 1988) or copepods (Fransz 1988) could hamper the initiation and duration of these blooms.

Besides microorganisms, the annual ice melting also releases organic matter (Giesenhagen et al. 1999) and micro-nutrients, such as iron (Fe) (Lannuzel et al. 2008), in the water column. Indeed within the sea ice ecosystem, a highly concentrated system in terms of microorganisms, organic matter and Fe is found as compared to the water column (Thomas and Papadimitriou 2003, Lannuzel et al. 2007, 2008). In particular, the organic matter pool of the sea ice is characterized by the presence of transparent exopolymeric particles (TEP) which form a gel phase into the ice (Thomas and Papadimitriou 2003, Krembs and Deming 2008, Dumont et al. 2009). This kind of organic matter may be involved in the modification of the trophic relationships (Becquevort et al. 2009) and in Fe solubility and bioavailability (Hassler and Schoemann 2009) but also in aggregation processes (Passow 2002). In sea ice, dissolved iron could be complexed to organic ligands (Schoemann et al. 2008) as for more than 90% of dissolved iron (dFe) in ocean surface water (Wu and Luther 1995, Boyé et al. 2001). Such a complexation could influence the bioavailability of Fe (Maranger and Pullin 2003) and keeps Fe into solution, avoiding loss by precipitation and adsorption onto particles. The pulse of nutritive elements at the time of ice melting certainly influences the pelagic microbial community but the extent of such a process remains unknown.

Diverse laboratory experiments aiming to understand the mechanisms of control of ice-edge plankton blooms have been reported in the literature (Mathot et al. 1991, Riebesell et al. 1991, Kuosa et al. 1992, Giesenhagen et al. 1999). These scarce studies focussed on the Atlantic sector of the Southern Ocean and on the Weddell Sea. In each of these studies, the experimental protocols were adapted according to the specific goals: Mathot et al. (1991) simulated the seeding of sea ice algae in filtered seawater and provided information on the potential photosynthetic activity and growth of the sea ice algae released in a planktonic environment, while Riebesell et al. (1991) assessed the aggregation potential of ice algae by using rolling tanks. Kuosa et al. (1992) performed aquarium experiments involving addition of unfiltered brown ice/slush into seawater to study the impact of sea ice microorganisms on the pelagic system. Similarly, Giesenhagen et al. (1999) realized microcosm experiments in order to discriminate the effects of the supply of organic matter from the effects of microorganisms, and further investigated the loss by sedimentation and/or grazing by copepods.

However, none of these studies have been conducted under trace-metal clean conditions, and their results therefore have to be considered cautiously as Fe availability is known to co-limit primary production in the Southern Ocean. In addition, the sequence at which the nutritive elements (OM and Fe) and the microorganisms accumulated in sea ice are released into the underlying seawater, while being crucial, has not been documented so far. The balance and the time elapsed between each of these processes still need further investigation to elucidate the ice-ocean interactions as it is essential to understand the Antarctic ecosystem as a whole.

This study presents results from two types of microcosm experiments realized under trace-metal clean conditions, using sea ice and seawater collected during the Ice Station POLarstern (ISPOL) drifting station (Hellmer et al. 2008). First, at two different times during the spring-summer transition, we performed onboard microcosm experiments designed to study the impact on the pelagic ecosystem of microorganisms, organic matter and Fe released from sea ice. Then, a complementary experiment was conducted in the home laboratory to study the sequence of release of each of these components during the ice melting. The temporal evolution of physical and biological properties of sea ice and underlying seawater in the Western Weddell Sea (i.e. ISPOL drifting station) is also presented in the present paper.

2. Materials and Methods

2.1. Field sampling and biological properties of the study area

Samples were collected in the pack ice zone during the ISPOL (Ice Station POLarstern) cruise aboard the RV *Polarstern* in the Western Weddell Sea, Antarctic Peninsula (*68°S, 55°W*) during spring-summer, in November-December 2004. During the ISPOL cruise, the RV *Polarstern* was anchored to a large ice floe (few km in size) in order to follow the temporal variations of the physical and biological atmospheric-ice-ocean processes at a drifting station during the transition from austral spring to summer (Hellmer et al. 2008). The sampling was conducted in a 20 m x 20 m sampling site, located 1 km away from the ship, using precautions to avoid trace metal and organic matter contamination (Lannuzel et al. 2006, Dumont et al. 2009). The operators on-site were donned with clean room garments (Tyvek overall, overshoes and polyethylene gloves) and an electropolished stainless-steel corer (14 cm diameter) was used to collect the ice cores (Lannuzel et al. 2006).

Ice cores were cut into 6 sections of 6 cm thick each, transferred into acid washed polyethylene containers and further treated as described below for the analysis of inorganic nutrients, chlorophyll *a* (chl *a*), and microscopic investigations. For dissolved organic carbon (DOC) and particulate organic carbon (POC) determination, sea ice sections were transferred into glass beakers pre-combusted at 450°C for 4 h. Under-ice seawater (0 m, -1 m and -30 m) was collected using a portable peristaltic pump (Cole-Parmer, Masterflex E/P) and acid cleaned tubing. Seawater samples were transferred into acid-washed bottles abundantly rinsed with the collected samples. Samples were collected on seven occasions, between the 29.11.04 and 30.12.04, at regular intervals (usually every five days) for the analysis of the distribution of the microbial communities.

2.2. Experimental design of melting experiments

2.2.1. "Direct" melting

The set-up of this microcosm experiment was realized according to the procedure of Mathot et al. (1991) and Giesenhagen et al. (1999) in order to simulate the melting of the ice. All precautions as described in Lannuzel et al. (2006, 2008) were taken in order to prevent trace metal contamination during sampling in the field and further handling onboard was conducted in a clean container within a class 100 laminar flow hood. The experiment has been conducted twice, using sea ice (bottom ice) and seawater (- 30 m depth), sampled on 09.12.04 (series a) and on 25.12.04 (series b). Seawater was pre-filtered by means of 0.2 µm membrane cartridges (Sartobran 300 sterile capsule). A section of 5 cm of bottom sea ice was thawed in filtered seawater (1:4, v:v) at room temperature in the dark in an acid cleaned container. One litre of this homogenized sea ice solution (sea ice + seawater) was then filtered onto 0.2 µm polycarbonate filter.

Four treatments were applied concomitantly in 20 L polycarbonate bottles during this experiment (see Table 1). The first microcosm, M1, only contained unfiltered seawater and served as a control to simulate the evolution of the planktonic community. The second microcosm, M2, consisted of a mix of 1L of sea ice solution and 20L of seawater (1:100, v:v) and was meant to mimic "natural" conditions, i.e. the melting of 10 cm of bottom ice in a water mixed layer of 10 m. The third microcosm, M3, aimed at studying the fate of sympagic organisms without the influence from the planktonic organisms. In this case, 1L of sea ice solution was added to 19.5L of filtered seawater. Finally, the fourth microcosm, M4, contained 20L of seawater amended with 1L of filtered sea ice solution, in order to simulate the seeding of dissolved components such as dissolved organic matter (DOM), and dissolved iron from the ice cover into the pelagic system. The microcosms were incubated at -1°C and under a continuous light intensity of 45 µE m^{-2} s^{-1}. The microcosms have been sub-sampled for about 10 days in order to follow the evolution of DOC, POC, and microorganisms (algae, bacteria and protozoa). Major inorganic nutrients and total dissolvable Fe (TDFe, unfiltered, pH 1.8) were measured at the start of the two experiments.

Table 1: Set-up of the direct microcosms experiment, series a (09.12.04) and b (25.12.04)

Microcosm	Type of water	Type of addition of melted ice	Comments
M1	unfiltered	none	Pelagic biota evolution without sea ice
M2	unfiltered	unfiltered	"Natural" simulation
M3	filtered (0.2 µm)	unfiltered	Ice biota evolution in seawater
M4	unfiltered	filtered (0.2µm)	Impact of dissolved constituents from the ice (dFe and DOM) on pelagic biota

2.2.2. Sequence of melting

In order to study the temporal sequence of release of ice components (microorganisms, organic matter and iron), an experiment under controlled and trace-metal clean conditions was realized in the laboratory. The conceived device (Figure 1) enabled to realistically simulate the physical melting of sea ice, i.e. in triggering melting due to the increase of the air temperature without increasing the seawater temperature. Seawater (13.5 L, collected during the CLIVAR SR3 cruise in the Antarctic Pacific sector (*135-150°E*) in December 2001, [dFe] = 1.05 nM) was transferred in a high density polyethylene (HDPE) container, at 2°C, in the dark, under constant mixing and refrigerated using a tube filled with ethanol to maintain seawater temperature around -1.9°C. A 20 cm thick bottom ice section (diameter = 14 cm) sampled on 04.12.04 during ISPOL was transferred into this HDPE container for melting. All parts in contact with the sample were acid cleaned prior to the experiment. Meltwater was sub-sampled with a peristaltic pump (Masterflex) and a tube placed at the bottom of the container. Salinity, temperature, total dissolvable iron (TDFe), DOC and POC have been followed until complete melting of the ice piece (ca. 5 days). For each sub-sampling at time (t), a second meltwater sample (noted t') was collected and left in the incubator until the next sub-sampling time (t+1). This allows discriminating between the contribution of sea ice melting only ($C_{t+1} - C_{t'}$) and the evolution of the seawater compounds between the 2 sub-sampling times ($C_{t'} - C_t$). For each parameter, fluxes from the sea ice were estimated by the equation $F_{t+1} = (C_{t+1} - C_{t'})/ t$, with C, the concentration of the compound and t, the time elapsed between t+1 and t.

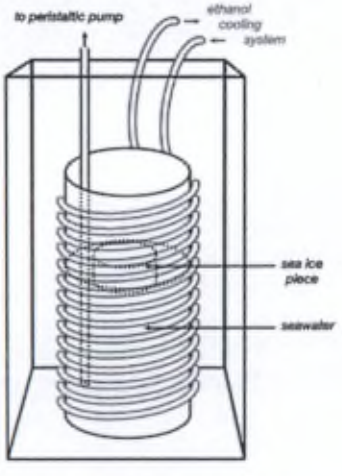

Figure 1: Schematic of the melting device used during the sequential melting experiment

2.3. *Measured Parameters*

2.3.1. Physical parameters

The ice texture, temperature and salinity of the ice cores were determined as explained in details in Tison et al. (2008). In the case of the sequential melting experiment, the temperature was measured with a general-purpose laboratory thermometer and the salinity was determined using a portable salinometer PC300.

2.3.2. Nutrients

Ice sections were melted in the dark at 4°C and then filtered together with "sack hole" brine and seawater samples through 0.4 µm polycarbonate filters. The filtrates and the microcosm sub-samples were determined onboard for inorganic nutrients (NO_3^-/NO_2^-, NH_4^+, PO_4^{3-} and $Si(OH)_4$) following the methods described in Papadimitriou et al. (2007). To avoid matrix effects, standards used for calibration were prepared in artificial seawater solutions with salinities similar to those of the samples analyzed. Brine volume-normalized concentrations were calculated by dividing the bulk ice concentration by the brine volume.

Samples collected for Fe analysis were stored in polyethylene bottles and acidified to pH 1.8 with ultrapure HNO_3 (Ultrex, JT Baker). Total dissolvable Fe (TDFe, unfiltered) and dissolved Fe (dFe, filtered on 0.2 µm Nuclepore polycarbonate filters) samples from the "direct" melting experiments were measured according to Lannuzel et al. (2006) by flow injection analysis. TDFe samples from the sequential experiment were analyzed according to de Jong et al. (2008) by isotopic dilution combined with multiple collector inductively coupled plasma mass spectrometry (ID-MC-ICP-MS) using nitrilotriacetic acid chelating resin for pre-concentration and matrix separation.

2.3.3. Dissolved and particulate organic carbon

Particulate organic carbon (POC) was collected on pre-combusted (450°C, 4 h) Whatman GF/F filters, stored at -20°C until analysis. After drying at 60°C, POC was analyzed with a Fisons NA-1500 elemental analyzer after carbonate removal from the filters by HCl fumes overnight. Filtered samples for dissolved organic carbon (DOC) were stored in pre-combusted (450°C, 4 h) 20-ml glass ampoules adding 25 µl H_3PO_4 (concentration 50 %), which were sealed to avoid contact with air. Samples were kept in the dark at 4°C until analysis. The DOC was measured by high temperature catalytic oxidation (HTCO; procedure of Sugimura and Suzuki 1988) with a Dohrmann Apollo 9000.

2.3.4. Chlorophyll *a*

For the determination of chl *a*, ice core sections were melted in the dark at 4°C in 0.2 µm-prefiltered seawater (1:4, v:v). Melted samples and microcosms sub-samples were sequentially filtered onto 10 µm and 0.8 µm filters (Nuclepore). Both filters were extracted in 90% (v:v) acetone in the dark at 4°C for 24 h, and quantified fluorometrically according to Yentsch and Menzel (1963).

2.3.5. Abundance and biomass of bacteria, algae and protozoa

Ice core sections sampled for the determination of abundance and biomass of microorganisms were melted in the same manner as for the chl *a* analysis described above. Algae, bacteria and protozoa were enumerated by epifluorescence and inverted light microscopy as explained in details in Becquevort et al. (2009). Algae and protozoa biomasses were estimated from cell biovolumes using geometric correspondences (Hillebrand et al. 1999) and specific carbon biomass relationships (Menden-Deuer and Lessard 2000). Bacteria biomasses were determined from cell volume using geometric (Watson et al. 1977) and carbon to volume relationships (Simon and Azam 1989).

3. Results

3.1. *Pack ice characteristics and microbial community distribution*

Detailed physical properties of the ISPOL drifting station for the investigation period are presented in Lannuzel et al. (2008) and Tison et al. (2008). Briefly, the ice sheet was approximately 0.9 m thick and presented a structure typical of first-year sea ice with a 0.1 m layer of granular (frazil) ice underlain by columnar ice (Tison et al. 2008). The freeboard was positive during the period of investigation. Temperature varied between - 2.8°C and - 0.5°C and salinity ranged from 1 to 10, with values generally around 5. Brine volume was always higher than the permeability threshold of 5% (Golden et al. 1998) and increased due to the warming of the atmosphere and ranged between 8 and 25%. At the beginning of the sampling period, drainage of salts by gravity occurred followed by the brine salinity becoming equal to or lower than seawater salinity, consequently stopping the gravity-driven brine drainage. Molecular diffusion is then the only process at work to allow exchanges between sea ice and seawater (Tison et al. 2008).

Nutrients concentrations in sea ice and seawater are summarized in Tables 2 and 3 respectively. Brine volume-normalized concentration for silicate ranged between < D.L. (below detection limit) and 61.4 µM (median 10.27 µM), for phosphate between < D.L. and 22.3 µM (median 1.63 µM), for nitrate/nitrite between < D.L. and 55.8 µM (median 4.83 µM) and for ammonium between < D.L. and 50.8 µM (median 4.72 µM). Bulk ice total dissolvable iron (TDFe) concentrations ranged between 2.3 and 97.8 nM in sea ice and between 0.5 and 4.1 nM in the underlying seawater (Lannuzel et al. 2008). In the case of Fe concentrations, its gradual decrease in the ice is paralleled by the increase of the Fe concentrations in the underlying seawater.

The dissolved organic carbon (DOC) and particulate organic carbon (POC) concentrations in sea ice ranged respectively between 1273 and 8413 µg C l^{-1} (median 4131 µg C l^{-1}), and between 93 and 5640 µg C l^{-1} (median 496 µg C l^{-1}) (Table 2, Dumont et al. 2009). In seawater,

POC ranged between 12 and 130 µg C l^{-1} (median 31 µg C l^{-1}).

Autotrophic microorganisms dominated the biomass in sea ice (Figure 2), especially in the surface and bottom layers. In seawater, the biomass was almost always dominated by heterotrophic organisms, except on the 30.12.04 when it represented not more than 45%. Chlorophyll *a* ranged from 0.08 to 28.41 µg l^{-1} in sea ice with the maximum values observed in the bottom layer (Table 2, Lannuzel et al. 2008). In the underlying seawater, chl *a* values were lower and ranging from 0.03 to 0.21 µg l^{-1}. The chl *a* concentrations in seawater increased between the beginning (29.11.04) and the end of the survey. In surface sea ice layers, *Phaeocystis* single cells were the major algal taxonomic group in terms of abundance and biomass (Figure 2). Some colonies were also observed although their biomass remained low. In contrast, pennate diatoms dominated the abundance and the biomass of the autotrophic microorganisms in the bottom ice layer (Figure 2). In seawater, the numbers of *Phaeocystis* single cells were the highest but in biomass the species pattern was patchier (Figure 2), with autotrophic dinoflagellates significantly contributing to the biomass. The pennate diatom species observed were contrasting between the different layers of sea ice but homogeneous within a particular layer along the period of observation. In the surface sea ice layers, *Fragilariopsis* sp. was the main pennate diatom present in abundance and biomass. In contrast, in the bottom sea ice layers, others pennate species were mainly present: in term of abundance, *Cylindrotheca* sp. and *Nitzschia* (<10µm) were the most abundant whereas in term of biomass *Amphiprora* sp. became predominant.

Table 2: Physical (temperature; salinity and brine volume fraction), and chemical (brine volume-normalized concentrations of silicates, nitrates/nitrites, phosphates and ammonium) characteristics along with chlorophyll a, dissolved organic carbon, particulate organic carbon concentrations in sea ice core sections during ISPOL Organic matter data from Dumont et al. (2009).

Sea ice	depth	T	Sal	Brine vol	chl *a*	POC	DOC	$[PO_4]$	$[Si(OH)_4]$	$[NO_3/NO_2]$	$[NH_4]$
		°C		%	µg l^{-1}	µg l^{-1}	µg l^{-1}	µM	µM	µM	µM
29.11.04	3-9	-3.1	8.0	15	0.36	684	ND	6.3	41.0	8.0	50.8
	9-15	-2.4	6.9	16	0.41	541	ND	1.7	11.9	3.1	31.8
	40-46	-2.3	6.5	13	0.18	172	ND	1.7	18.1	1.7	20.8
	60-66	-2.1	5.0	11	0.08	112	ND	11.7	14.0	2.9	24.5
	78-84	-2	4.8	14	3.98	621	ND	1.9	14.1	2.9	33.8
	84-90	-1.9	7.0	26	23.57	3766	ND	11.4	43.0	30.5	15.6
04.12.04	3-9	-1.7	5.9	16	0.44	565	ND	-0.3	1.8	2.3	2.3
	9-15	-2.1	5.8	13.5	0.44	428	ND	0.7	4.9	4.0	ND
	40-46	-1.9	5.3	13	0.45	167	8004	0.0	0.0	0.0	2.7
	60-66	-1.6	3.4	8	0.16	232	4662	2.9	8.7	6.3	1.6
	78-84	-1.8	5.3	14.5	3.44	741	4109	-1.4	19.6	5.6	4.6
	84-90	-1.8	8.9	25	26.47	3175	8413	11.2	32.4	29.6	3.2
09.12.04	3-9	-1.2	ND	15	0.97	595	2452	0.8	14.7	9.6	9.0
	9-15	-1.4	ND	16	2.22	903	5669	0.0	11.2	7.9	ND
	40-46	-1.9	ND	14.5	0.40	279	3104	-0.2	10.6	3.1	0.0
	60-66	-1.8	ND	14.5	0.25	191	5212	0.0	8.8	6.0	3.4
	78-84	-1.8	ND	17.5	2.56	1138	1656	0.9	4.7	3.1	4.3
	84-90	-1.8	ND	14.5	21.48	4754	5649	22.3	61.4	50.2	21.9
14.12.04	0-9	-1.5	3.4	25	0.69	725	6845	1.6	3.7	5.6	8.2
	9-15	-1.4	7.1	17	1.23	766	4152	3.0	10.9	10.9	4.9
	40-46	-1.7	3.9	11	0.27	327	4483	3.8	10.6	6.4	4.8
	60-66	-1.7	3.4	10	0.30	451	3856	3.2	5.5	2.8	17.6
	74-80	-1.8	4.8	12.5	2.19	773	5305	5.0	14.0	15.1	5.5
	80-86	-1.9	7.8	21	24.23	5640	5717	15.3	47.2	55.8	7.6
19.12.04	4-10	-0.4	0.9	33	0.78	282	3629	1.3	1.4	2.5	2.0
	10-16	-1.3	2.1	30	1.06	306	3561	2.1	2.4	4.5	4.0
	40-46	-1.4	5.0	17	0.20	181	3615	3.2	9.3	9.7	6.8
	60-66	-1.5	3.8	11	0.65	223	2408	4.0	3.9	6.0	4.7
	77-83	-1.6	4.7	10	4.21	618	3179	4.4	6.5	2.4	11.2
	83-89	-1.6	7.5	16	28.41	2061	4242	13.6	49.0	42.9	9.1
25.12.04	3-9	-0.2	0.9	11	0.70	93	ND	0.6	5.1	3.1	8.1
	9-15	-0.7	2.1	14	0.66	242	ND	0.6	8.6	5.1	3.9
	40-46	-1.2	5.0	21	0.69	146	ND	0.1	8.1	0.0	2.0
	60-66	-1.2	3.8	16	0.29	148	ND	0.1	7.2	0.0	3.1
	74-84	-1.2	4.7	15	3.30	496	ND	0.6	9.9	0.0	3.6
	84-90	-1.4	7.5	17	16.33	2515	ND	8.9	44.5	17.6	5.2
30.12.04	3-9	-1.1	0.9	20	1.69	357	5255	0.5	0.8	2.2	3.4
	9-16	-1.2	2.1	16	0.97	342	5488	2.0	17.1	2.7	0.0
	40-46	-1.4	5.0	13	0.59	364	2762	1.2	41.1	7.0	0.0
	59-65	-1.4	3.8	9	0.95	ND	1273	0.3	9.5	0.2	1.4
	74-80	-1.6	4.7	11	3.62	534	1273	0.9	13.5	5.6	5.4
	80-86	-1.8	7.5	17	24.77	2008	2494	0.5	8.2	2.0	0.0

Table 3: Chemical and biological characteristics of seawater during ISPOL. Median values of seawater 0 m, 1 m and 30 deep and standard deviation are given (n=3 except for * n=1).

Seawater	Chl a $\mu g\ l^{-1}$	$[PO_4]$ μM	$[Si(OH)_4]$ μM	$[NO_3/NO_2]$ μM	$[NH_4]$ μM
29.11.04	0.03 ± 0.00	1.7 ± 0.3	30.6 ± 7.6	20.0 ± 5.3	0.7 ± 0.2
04.12.04	0.05 ± 0.00	1.9 ± 0.2	45.1 ± 2.8	30.3 ± 2.0	0.8 ± 1.0
09.12.04	0.06 ± 0.00	2.1 ± 0.2	39.9 ± 6.0	23.9 ± 3.7	0.8*
14.12.04	0.09 ± 0.02	2.2 ± 0.2	37.8 ± 4.4	26.5 ± 3.2	0.5 ± 0.2
19.12.04	0.11 ± 0.02	2.0 ± 0.5	37.5 ± 2.4	25.4 ± 2.4	0.3 ± 0.1
25.12.04	0.16 ± 0.05	1.7 ± 0.5	28.7 ± 5.2	26.1 ± 5.1	0.5 ± 2.3
30.12.04	0.14 ± 0.01	2.1 ± 0.3	43.9 ± 5.8	30.3 ± 1.9	0.3*

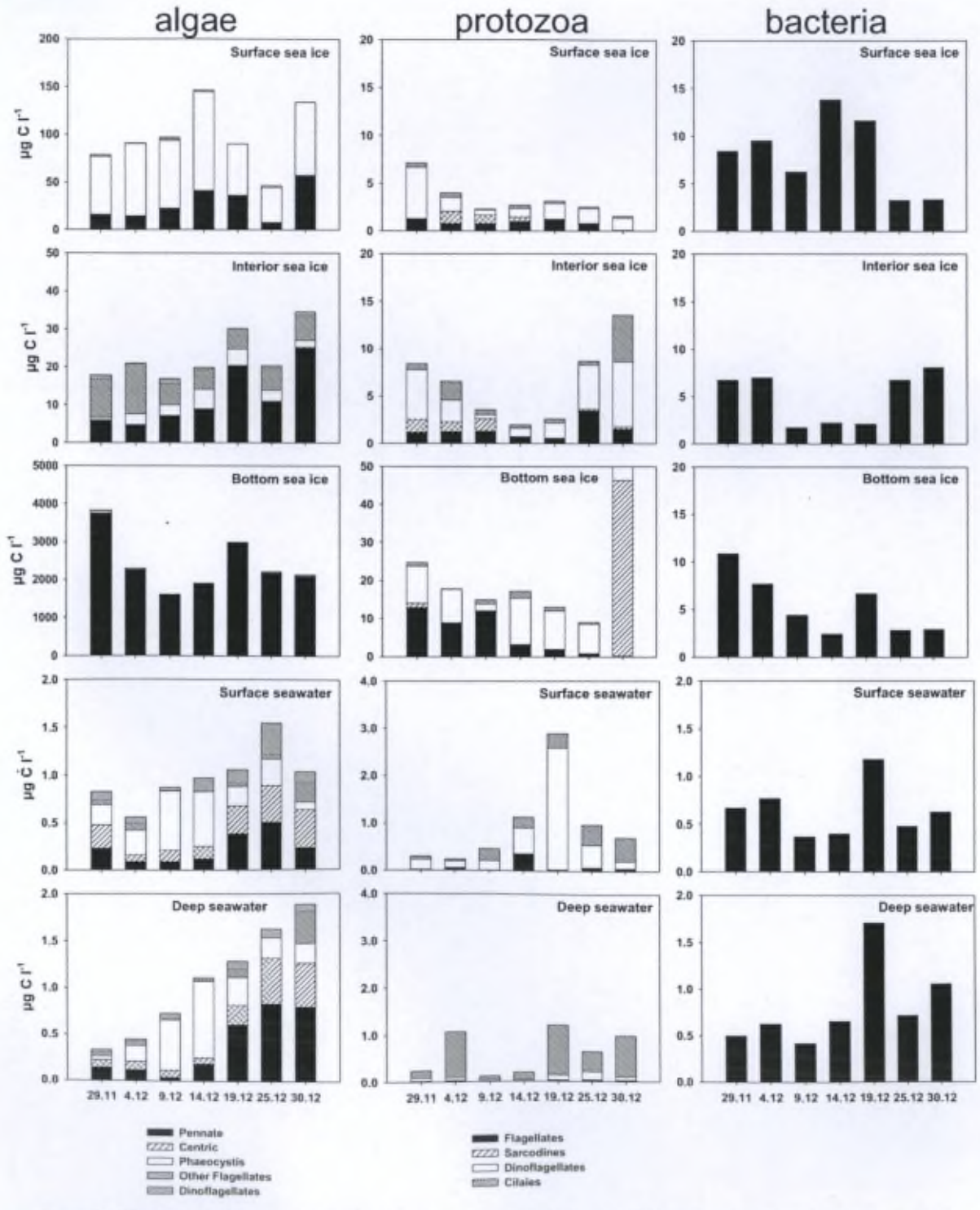

Figure 2: Distribution of biomass of a) algae, b) protozoa and c) bacteria (in µg C l^{-1}) in surface, internal and bottom layers of sea ice and in surface (mean for 0 m and - 1 m) and deep (- 30 m) seawaters during the ISPOL cruise, from November 29 to December 30, 2004 .

In seawater, a more heterogeneous distribution of pennate diatoms (e.g. *Fragilariopsis* sp., *Chaetoceros* sp.) and some centric diatoms (e.g. *Biddulphia* sp., *Corethron* sp.) were observed. In deep seawater (- 30 m), autotrophic biomasses increased ca. 5-fold between the 29.11.04 and the 30.12.04 (Figure 2).

Heterotrophic microorganisms, both bacteria and protozoa, were present in the ice with the latter accounting in average for 53% of the heterotrophic biomass. Bacteria distribution along the ice core was rather homogeneous in all the ice layers with a less contrasted trend than for the algae, which always presented a maximum in the bottom (Figure 2). Bacteria biomass ranged between 1.14 and 19.3 µg C l^{-1} (median 5.30, average 6.14 µg C l^{-1}) in sea ice and between 0.33 and 1.71 µg C l^{-1} (median 0.63, average 0.70 µg C l^{-1}) in seawater. In surface and bottom sea ice, biomass was lower towards the end of the study period than in the beginning. In seawater, bacterial biomass was maximal on the 19.12.04 but no trend was observed for the whole period. Protozoa distribution showed maximum concentrations in the bottom ice layers (Figure 2). Values ranged between 0.36 and 557 µg C l^{-1} (median 4.84, average 22.6 µg C l^{-1}) with an exceptionally high maximum value reached on the last sampled station (30.12.04). With the exception of this extreme datum, the protozoan biomass did not exceed 24.8 µg C l^{-1} in sea ice. In seawater, protozoa biomass varied between 0.11 and 3.22 µg C l^{-1} (median 0.71, average 0.85 µg C l^{-1}). In sea ice, protozoa biomass was mainly composed of flagellates, dinoflagellates and sarcodines with also ciliates present in the interior ice layers (Figure 2). In seawater, the same species were found except for the sarcodines. Dinoflagellates clearly dominated the surface waters (0 m and - 1 m) while ciliates were mainly present in deep waters (- 30 m). In sea ice, the protozoan biomass did not show a particular trend with time except an increase on the 30.12.04 in the interior and bottom ice layers.

Table 4: Initial concentrations of microorganisms, organic matter and total dissolvable iron in M1 (seawater) and M2 (seawater + sea ice) for the series a (09.12.2004) and b (25.12.2004).

		09.12.2004 Series a		25.12.2004 Series b	
		M1 SW	M2 SW+SI	M1 SW	M2 SW+SI
Algae	µg C l^{-1}	0.16	4.45	0.64	6.28
Bacteria	µg C l^{-1}	0.26	0.14	0.51	0.68
Protozoa	µg C l^{-1}	0.05	0.08	0.82	1.01
DOC	µg C l^{-1}	ND	ND	2,000	13,000
POC	µg C l^{-1}	167	318	95	127
TDFe	nM	1.6	1.7	2.3	2.4

ND: non determined

Although flagellates were the main species until this time, dinoflagellates and ciliates highly increased. In seawater, the protozoa biomass increased from the 29.11.04 to the 30.12.04, reaching a particularly high value on the 19.12.04, when the dinoflagellates biomass was dominating.

3.2. Sea ice "direct" melting experiments

3.2.1. Contribution of sea ice melting for the underlying water column

In the M1 microcosm (SW), microorganisms biomasses (algae, bacteria and protozoa) were higher at the start of the experiment on the 25.12.04 (series b; M1-b) as compared to the previous experiment performed on the 09.12.04 (series a; M1-a; Table 4). This trend is in agreement with the evolution of the microorganisms within the natural deep (- 30 m) seawater ecosystem between these two dates (see Figure 2). For both M1-a and -b, heterotrophs largely dominated the total biomass of the seawater assemblage. The heterotrophic to autotrophic ratio was similar for M1-a and M1-b and yield around 2. The contribution of protozoa to the heterotrophic biomass was higher in M1-b than in M1-a, mainly caused by the increase of ciliates as observed in Figure 2c. In the microcosm M2 (SW+SI), the 1% inoculum of sea ice in seawater constituted a major supply of algal biomass for both series (9- to 27-fold increase). Yet the supply of heterotrophs (bacteria and protozoa) was clearly less important (2- to 6-fold increase) with even a decrease of bacterial biomass observed in M2-a. Consequently, this inoculum of ice changed the initial heterotroph to autotroph ratios from ca. 2 for M1 to respectively 0.05 and 0.28 for M2-a and M2-b. The inoculum of sea ice supplied algae, mainly diatoms; the dino- and nano- flagellates biomass staying constant after the addition of sea ice. In M1, the diatom biomass was mainly composed of pennates (mainly *Fragilariopsis* sp.) with some centric (mainly *Chaetoceros* sp.) being present on the 09.12.04 whereas more centric were observed on the 25.12.04. In M2, pennate diatoms *Amphiprora* sp. (on the 09.12.04) and *Cylindrotheca* sp. (on the 25.12.04) became the dominant diatom species. These taxa were indeed the dominant ones in the corresponding natural sea ice samples (Figure 2). The sea ice inoculum also represented a high supply of organic matter (corresponding to an increase from 34% to 550% of the seawater concentration), in the particulate and dissolved fraction (Table 4). The supply of total dissolvable iron (TDFe) by the sea ice inoculum contributed only to an increase of 4 to 6% of the seawater concentration. The iron concentrations in the seawater were already relatively high particularly on the 25.12.04 likely due to ice melting events that already occurred.

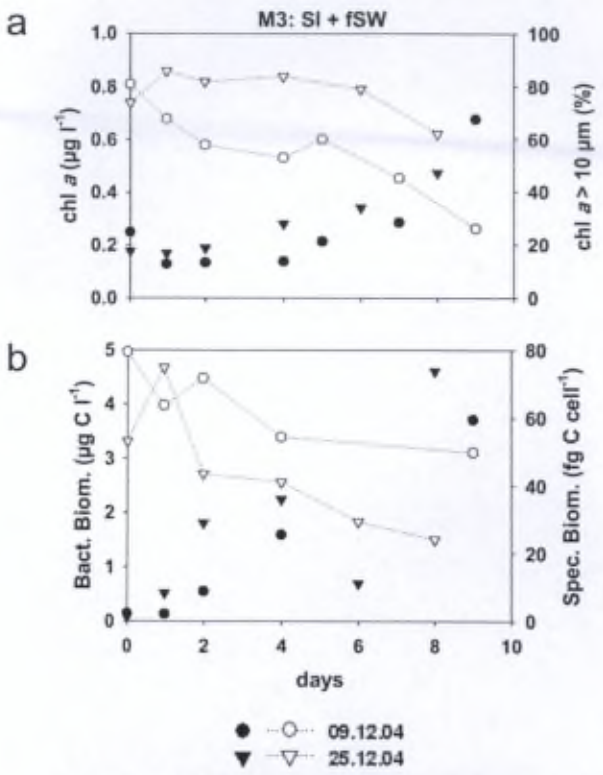

Figure 3: Evolution of a) chlorophyll *a* and percentage of chl *a*>10µm and b) bacterial biomass and specific bacterial biomass in the microcosm M3, series a (09.12.04) and b (25.12.04)

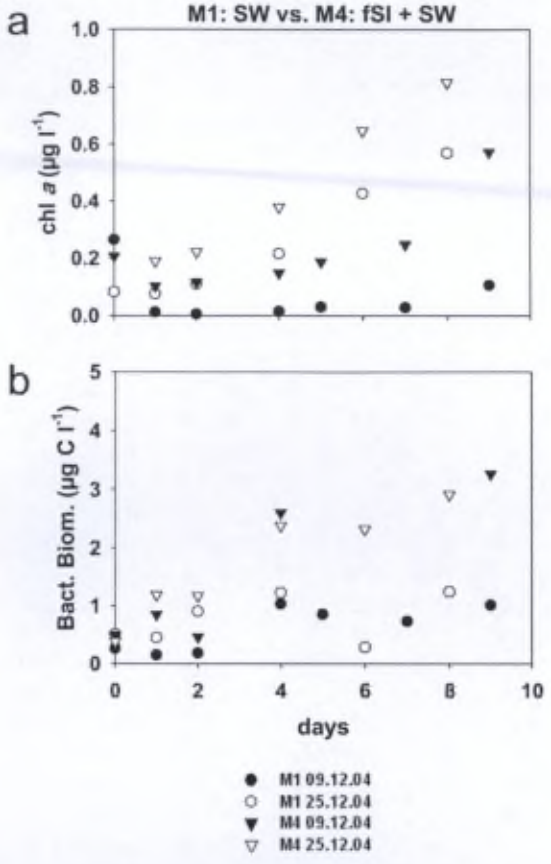

Figure 4: Evolution of a) chlorophyll *a* b) bacterial biomass in the microcosms M1 and M4 for series a (09.12.04) and b (25.12.04)

3.2.2. Evolution of sympagic microorganisms in the seawater

The third microcosm (M3) was designed to study the fate of the sympagic microorganisms when sea ice melts without the potential interactions with the pelagic microorganisms, by following the evolution of the ice-microorganisms inoculated in filtered seawater. After a latent period of ca. 2 days, chl *a* (thus associated to ice-derived microorganisms) increased from 0.25 to 0.68 µg l^{-1} and from 0.18 to 0.47 µg l^{-1} after respectively 9 and 8 days in M3-a and M3-b (Figure 3a). This suggests that, at least some of the sympagic autotrophic organisms were able to thrive and grow under the conditions present in natural filtered seawater. Yet chl *a* associated with algae >10 µm decreased with time, particularly in M3-a (Figure 3a). The taxonomic composition of the algal assemblages indeed shifted from *Amphiprora* sp.-dominated (at time zero) to *Fragilariopsis* sp.-dominated after 9 days, which fits exactly the shift from algae>10 µm to algae <10 µm as shown for chl *a*. Similarly, sea ice bacteria seemed able to grow in seawater as shown by the increases of biomasses from 0.14 to 3.72 µg C l^{-1} and from 0.07 to 4.71 µg C l^{-1} after respectively 9 and 8 days in M3-a and M3-b (Figure 3b). A decrease of the size of bacteria was observed as for algae; specific carbon content decreased from 79 to 50 fg C $cell^{-1}$ and from 53 to 24 fg C $cell^{-1}$ respectively in M3-a and M3-b (Figure 3b). If the protozoa biomass decreased from 2.92 to 0.82 µg C l^{-1} in the M3-a, it did increase from 1.27 to 3.83 µg C l^{-1} in M3-b when the highest bacterial size attenuation was observed.

3.2.3. Evolution of planktonic microorganisms with supply of dissolved compounds from the sea ice

As shown previously by comparison of M1 and M2, the melting of the sea ice constitutes a major supply in organic matter and iron into the underlying seawater. The impact of these compounds on the growth of pelagic microorganisms has further been evaluated by comparing the evolution of microorganisms in seawater only (M1) and in seawater amended with filtered (melted) sea ice (M4). For both series (09.12.04 and 25.12.04), chl *a* associated with planktonic algae reached higher concentrations in M4 than in M1 (Figure 4a). This clearly suggests a stimulation of planktonic algal growth by dissolved nutrients supplied by the filtered sea ice inoculum. The chl*a* values increased from 0.20 to 0.57 µg l^{-1} and from 0.08 to 0.82 µg l^{-1} in respectively 9 and 8 days in M4-a and M4-b (Figure 4a). Note that the increase of chl*a* observed in M1-b even without any sea ice inoculum (Figure 4a) could result from the high initial iron concentration in seawater and favourable conditions inherent to bottle experiments (such as light conditions and exclusion of mesozooplankton grazing). Planktonic bacterial growth was similarly stimulated by the sea ice inoculum (Figure 4b). After respectively 9 and 8 days, bacterial biomass indeed increased 7 to 8 times in M4-a and M4-b whereas that biomass only increased 2 to 4 times in M1-a and M1-b. In parallel to increases of chl *a* and bacteria, the protozoa biomass, in

particular heterotrophic dinoflagellates, increased from 0.06 to 1.21 µg C l^{-1} and from 0.79 to 3.62 µg C l^{-1} in respectively 9 and 8 days in M4-a and M4-b.

3.2.4. Interactions between ice-derived and planktonic microorganisms after ice melting

The previous microcosms (M1, M3 and M4) intentionally exclude potential direct interactions between sympagic and planktonic microorganisms in order to study their effects separately. Nevertheless competition between microorganisms for e.g. inorganic and organic nutrients can occur *in situ* and result in a decrease of the levels of biomass reached. In order to study these interactions at the time of sea ice melting, the evolution of sea ice and planktonic microorganisms' biomasses were followed in parallel in M2. This microcosm aims at mimicking the probable natural scenario of sea ice melting (10 cm of brown ice in a 10 m deep mixed layer). Comparing the values obtained in M2 to the sum of the values obtained in M3 and M4, is a rough way to estimate the strength of the interaction between microorganisms. The chl *a* values in M2 were very similar to that estimated by the sum of M3 and M4 (Figure 5a). A more significant difference was noted between bacterial biomass levels in M2 as compared to that estimated from M3+M4, especially at the final time of the experiments (Figure 5b). Heterotrophic nanoflagellates biomass, grazers of bacteria, increased 11 times in M2-a after incubation day 9.

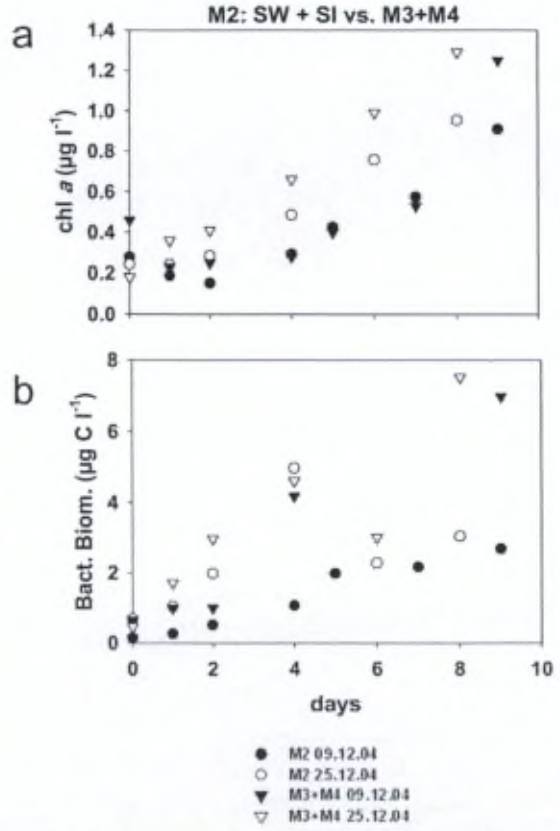

Figure 5: Evolution of a) chlorophyll *a* b) bacterial biomass in the microcosms M2 and M3+M4 for series a (09.12.04) and b (25.12.04)

3.3. Sea ice sequential melting experiments

During the entire experiment, the temperature within the container was stabilized around the *in situ* seawater temperature value of -1.9°C. Salinity fluxes show a supply in salinity after 5 h (Figure 6), due to brine drainage. This was followed by a supply of fresher water corresponding to the melting of the ice structure by itself, less saline than seawater. The iron fluxes from sea ice into seawater showed a rapid increase and stabilization after ca. 10 h, period during which the iron supply was maximum (2 nmoles h^{-1}). A null FE flux value is reached after 30 h of melting (Figure 6). The maximal organic matter flux, dissolved as well as particulate, was reached after 15 h (Figure 6). After 20 h, the organic matter fluxes had already strongly decreased, especially for the dissolved organic matter. In this case the flux even became negative, suggesting a quicker degradation of DOC in the melting container than in the control bottle.

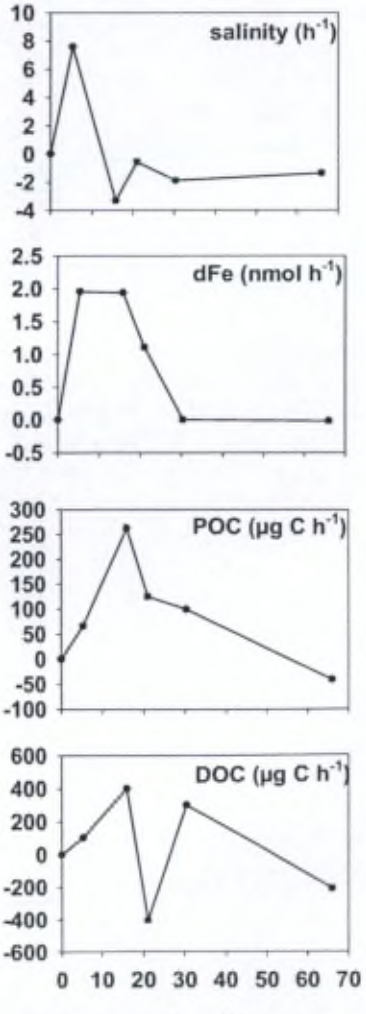

Figure 6: Fluxes of salinity, dissolved iron, dissolved and particulate organic carbon during the sequential melting experiment

4. Discussion

4.1. Evolution of pack ice and underlying seawaters in the Weddell Sea

The period of investigation of the ISPOL drifting station corresponded to the spring-summer transition. The permeability of the ice cover was already high (above 5% Vb/V) meaning that the ice cover was permeable to exchanges. As described in more detail in Tison et al. (2008), the beginning of the period corresponded to a phase of brine drainage (with exchanges from ice into seawater) followed by a phase of equilibrium and of transport by convection mechanisms. Thereby, the nutrient transfer from seawater to sea ice could have been reduced at some moments due to a limitation of the brine movement (Tison et al. 2008). Consequently the nutrient availability may have controlled the algal growth. Indeed concentrations of $Si(OH)_4$ and NO_3 in sea ice and in brines were below the theoretical dilution line (TDL, corresponding to a situation where mainly physical control on nutrient distribution is occurring, i.e. a "conservative" behaviour with salinity, Figure 7). This suggests that an important consumption of these nutrients by sea ice algae occurred and that a limitation was possible, except in the bottom ice layer. By contrast, bulk sea ice PO_4 and NH_4 concentrations were generally above the TDL (Figure 7) indicating that an accumulation would have occurred in the sea ice. Surprisingly, concentrations of PO_4 and NH_4 in brines show the opposite trend and were below the TDL. This discrepancy could result from brine sampling methodology or could mean that nutrients would be associated to the organic matter attached to the walls of the brine pockets. Dissolved Fe concentrations in sea ice were presumably still sufficient to sustain microorganism growth within the sea ice habitat (Lannuzel et al. 2008). The nutrient status of sea ice implies that, once released into seawater, the sea ice microorganisms will experience a fully different nutrient stress: seawater is, compared to sea ice, a low PO_4, NH_4 and iron environment (Lannuzel et al. 2008) but high in $Si(OH)_4$ and NO_3.

In the surface and interior ice layers, the algal biomass was rather varying during the sampling period but slightly higher on the 30.12.04 than on the 29.11.04, while in the bottom layer, a slight decrease was observed. A similar decreasing trend is observed for bacteria and organic matter concentrations in sea ice (see also Dumont et al. 2009). The algae did not really develop in sea ice between the 29.11.04 and the 30.12.04 despite a potential favourable physical environment with regards to temperature, light and brine volume. Indeed, as recently observed by Becquevort et al. (2009) and in agreement with previous work by Krembs et al. (2001), algal development in sea ice seems to be controlled by space availability. An algal increase is only observed above a brine volume of 8%, roughly corresponding to a temperature above -4°C. Considering the high brine volumes measured during ISPOL, the internal and surface space availability would then not hinder algal development. The stagnation of the algal stock could rather be due to nutrient limitation, as explained above, as well as to loss of biomass towards seawater and/or grazing. A very high biomass of heterotrophic dinoflagellates was measured in bottom layer on the 30.12.04.

A contrasted vertical distribution of algal species was observed in sea ice during ISPOL. Still within a particular ice layer, the algal species were very similar during the whole survey, showing that spatial variability was low in our sampling area. The microalgae species composition within Antarctic sea ice communities is a result of physical processes of ice formation, transformation and destruction, as well as biological processes of colonization, survival, growth, succession, grazing and death (Lizotte 2001). Single cell *Phaeocystis* was the dominant taxon in the surface ice layer of the ISPOL station. The bottom ice layer hosted mainly exclusively a pennate diatom community. High biomasses of *Amphiprora* sp., *Cylindrotheca* sp., *Fragilariopsis* sp., *Nitszchia* sp. were present in the bottom ice layer. The interior ice layers hosted a mixed community of pennate diatoms and flagellates. It is known that some organisms (e.g. *Fragilariopsis cylindrus*) can grow relatively well under the extreme conditions found in sea ice, while other species (possibly *P. antarctica*) dominate sea ice assemblages only when conditions are similar to the water column (e.g. ice surfaces washed by waves or flooded by seawater; Lizotte 2001).

In the underlying seawater, a global increase of the microorganism biomasses was recorded. Observations only do not allow discrimination between the growth of pelagic microorganisms and the contribution of microorganisms released from sea ice. However, considering the thermodynamical properties of the ice cover at that time together with the general decrease of sea ice concentration, it seems valuable to relate the increase of pelagic biomass with the melting of sea ice. In particular, the species pattern of pelagic algae observed at 30 m deep clearly showed an increase of the pennate diatom component, which is the main algal species observed in bottom sea ice. During the entire sampling period a diverse microalgal community was observed in the surface seawaters. Some algal species present in the ice were also present in the seawater but their relative dominance was different. The large *Amphiprora* sp. seems to be not adapted, or directly consumed by krill in the seawater environment contrastingly to others taxa such as *Cylindrotheca* sp. or *Fragilariopsis* sp. *Chaetoceros* sp., observed in seawater. *Phaeocystis* sp. and other flagellates cells can also develop in seawater. Such a similarity between ice and under-ice algal communities has already been observed in numerous studies and is the basis of the seeding hypothesis (Krebs 1983, Garrison and Buck 1985, 1989, Smith and Nelson 1985, 1986, Garrison et al. 1987). Conversely, a higher dissimilarity could be observed, as reported by Mathot et al. (1991) where diatoms dominated (65-95%) the ice community while accounted for less than 15% of the biomass in the water column. These results do not necessarily exclude the role of sea ice as an inoculum but stressed the role of other factors, like selective and fast grazing or sedimentation, which might hamper the seeding effect of sea ice. The capacity of adaptation of each species to environmental changes

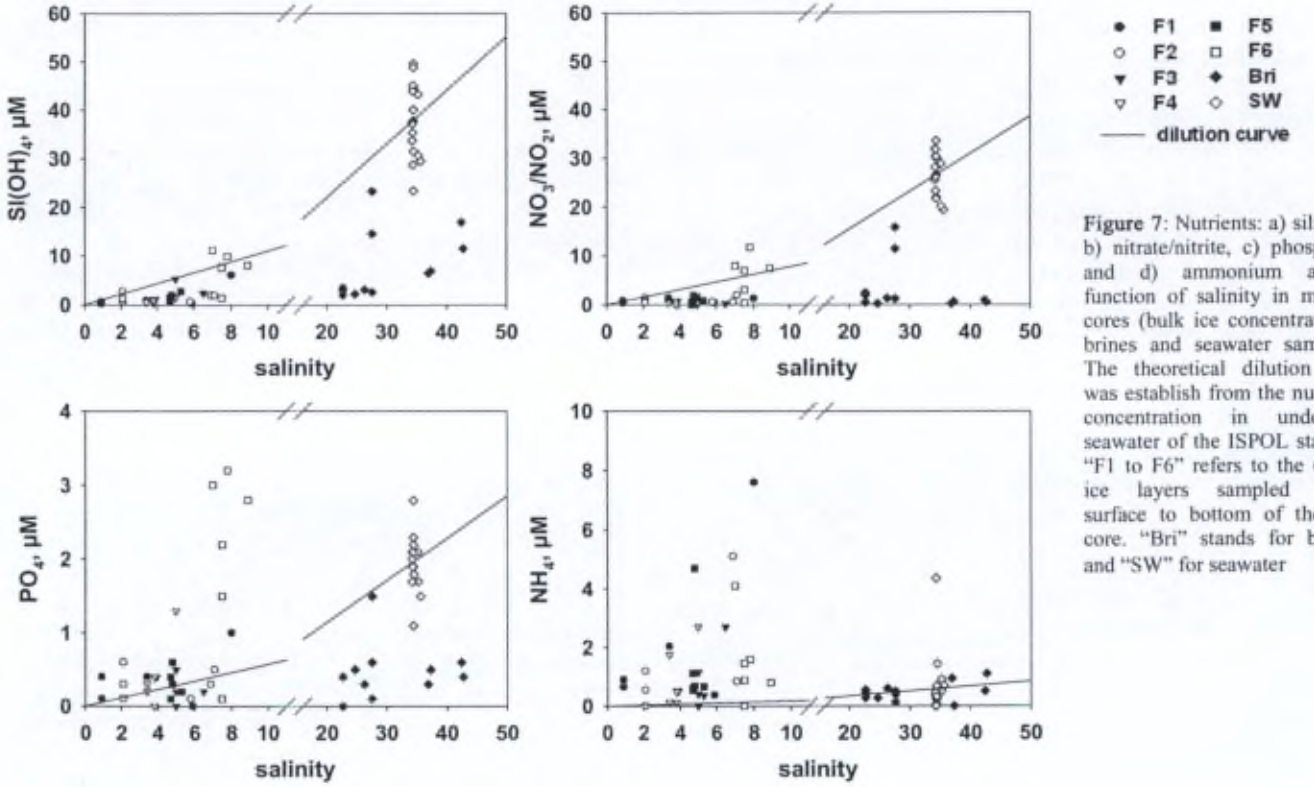

Figure 7: Nutrients: a) silicate, b) nitrate/nitrite, c) phosphate and d) ammonium as a function of salinity in melted cores (bulk ice concentration), brines and seawater samples. The theoretical dilution line was establish from the nutrient concentration in under-ice seawater of the ISPOL station. "F1 to F6" refers to the 6 sea ice layers sampled from surface to bottom of the ice core. "Bri" stands for brines and "SW" for seawater

is also a determining point. In a recent work of Mangoni et al. (2009), the acclimation capacity of different ice diatoms has been tested using mesocosms experiments. Authors conclude that some species like *Fragilariopsis cylindrus* have a versatile physiology which is able to adapt to extreme environmental conditions while others like *Amphiprora kufferathii* could not grow in pelagic conditions and probably sediment out.

4.2. Fate of ice-derived components during and after sea ice melting

4.2.1. Significance of the inoculum of sea ice microorganisms for the development of phytoplanktonic blooms

By comparing the initial algal biomasses in the microcosm containing seawater with the one containing seawater amended with sea ice, the inoculum from the sea ice clearly supplies microorganisms into the water column, mainly diatoms. As also observed in natural samples, a partial taxonomic similarity between the sympagic and pelagic communities has been observed in the microcosms. After some days of experiments, we observed a chl *a* increase due to the ice-derived organisms suggesting that at least a fraction of the algal community is able to adapt and grow in the seawater environment, as also observed by Kuosa et al. (1992). Still, increases of algal biomass are less important in our Fe-clean experiments than in theirs. Our experiments show that autotrophic biomass increase seems to be mainly attributable to species <10 µm as their proportion increases with time. The decrease of >10 µm algae could be explained by the lower Fe concentrations in seawater. While dFe concentrations were relatively high in seawater (1.7 and 2.3 nM respectively, Lannuzel et al. 2008), such levels could still be limiting for large size diatoms (Timmermans et al. 2001, 2004), used to sea ice Fe-rich conditions (up to two orders of magnitude higher than in seawater). We further observed that ice-derived algae did not seem to compete with phytoplanktonic ones. Thus sea ice algae, mainly the small size ones, seem well able to grow in the diluted seawater. Yet we need to keep in mind that in such microcosm experiments, processes of mesozooplankton grazing and sedimentation are artificially excluded although they are considered as major removal processes for sympagic algae after ice melting.

Apart from these loss factors, the ability of the sympagic species to develop and survive in the dilute seawater environment determines their fates (Garrison et al. 1987). Some large diatom species typical of the sea ice (e.g. *Entomoneis kjellmanii* or *Pleurosigma sp.*) are not competitive in the seawater and in spite of their high abundance in sea ice, they contribute weakly to the

phytoplanktonic community (Leventer 2003). The survival capacity of sympagic species in seawater is related to the advantages or disadvantages that the new environmental (physico-chemical) conditions offer to microorganisms. These conditions are driven by the concentrations of major nutrients, iron, temperature, salinity, toxic compounds, physical substrate or light exposition. For example, sea ice community is generally dominated by pennate diatoms, an algal group that also dominates pore waters of sandy sediments. Adaptation to confinement in narrow spaces is clearly a requirement for sea ice habitats (Smetacek and Nicol 2005). Many large pennate diatoms require a substrate to grow on, a condition found in sea ice but not in seawater. Also, some algal species are physiologically adapted to specific light levels and could be disadvantaged by the change of luminosity between ice and water (Palmisano and Sullivan 1985, Grossi et al. 1987) even if some species seems to be adapted to such extreme changes (Mangoni et al. 2009).

Furthermore, the production by many sea ice algae of extracellular polysaccharides (EPS) to attach themselves to brine walls (Palmisano and Sullivan 1985a, Krembs et al. 2001) likely stimulates the formation of aggregates once released in the seawater (Passow 2002) which will lead to a rapid sedimentation to the seafloor as observed in rolling tanks by Riebesell et al. (1991) or through silicon isotope measurements, by Fripiat et al. (2007). They showed that ice diatoms made up an insignificant fraction in surface seawater but were then largely exported below the mixed layer. The intensity of grazing by copepods, amphipods or krill also greatly influences the recycling of organic material including iron in surface waters. Heavily grazed blooms are likely to retain more biogenic elements in the surface layer whereas ungrazed diatoms tend to sink out (Smetacek and Nicol 2005). Faecal pellets from these organisms still contribute to the downward vertical flux.

The microcosm experiment where bacteria from sea ice were transferred in seawater also showed an increase of their biomass suggesting they are able to develop. Yet a simultaneous decrease in the bacterial cell volume is observed. This size decrease can be attributed to a decrease of the concentration of labile substrates such as the DOC (Roszak and Colwell 1987, Berman et al. 1994) but also to a stronger grazing pressure by the ice derived protozoa which preferentially consumed large bacteria (Gonzales et al. 1990). As a matter of fact, protozoa biomass increased at the time we observed the highest decrease in the bacterial biovolume. A stronger competition for substrates between sea ice- and seawater-based bacteria is also suggested by the results from the microcosms. Effect of the seeding on bacteria is poorly documented. Phylogenetic studies showed that sea ice bacterial population are fairly similar to the pelagic community (Bowman et al. 1997), metabolically active (Brinkmeyer et al. 2003) and that psychrophilic species were preferentially present in sea ice (Delille 1992, Helmke and Weyland 1995). Kaartokallio et al. (2005) reported, in Baltic sea ice, that ice-derived bacterial communities were able to adapt to salinity changes while under-ice bacterial assemblages seemed to suffer from osmotic stress. Physiological and community structure changes in response to the fluctuation of the environment (e.g. salinity) are thus likely to occur during the melting of sea ice (Kaartokallio et al. 2005).

4.2.2. Significance of the supply of organic matter and iron from sea ice for the development of blooms

Similarly to other Antarctic sea ice field studies (e.g. Lannuzel et al. 2007, Becquevort et al. 2009, van de Merwe et al. under review, Lannuzel et al. submitted), large accumulation in sea ice of both organic matter (Dumont et al. 2009) and iron (Lannuzel et al. 2008) were reported during the ISPOL survey. Our experiments clearly showed that sea ice can supply an important quantity of organic matter and iron in the Antarctic surface waters. It has been already stressed that in under ice seawater, dissolved organic matter can originate from excretion by phytoplankton, sea ice algal cell lysis, excretion or sloppy feeding by zooplankton, but also from sea ice meltwaters (Kähler et al. 1997). Similarly, the role of iron released from melting sea ice has already been addressed by several authors (e.g. Sedwick and DiTullio 1997) and the contribution of iron from sea ice has been quantified by Lannuzel et al. (2007, 2008, submitted). In the Fe-limited Antarctic waters this transitory pulse of nutrients from sea ice melting could trigger phytoplanktonic bloom, as we observed 1) annually in the marginal ice zone from satellite picture as well as 2) from the increase of algal biomass after filtered sea ice amendment in our microcosms. The stimulation of pelagic microorganisms growth after the addition of the filtered sea ice inoculum can actually be triggered by: a) the supply of micro-nutrients such as iron or b) the stimulation of heterotrophic processes owing to the input of labile sea ice DOM, supplying then autotrophs with essentials molecules such as cytokines (Brandini and Baumann 1997). Kähler et al. (1997) indeed showed that a large part of the DOM in the surface water was biologically labile and could be efficiently transformed into bacterial biomass. It was also suggested that an allopathic effect of algae could occur and prevent organic matter utilization by bacteria (Pusceddu et al. 2009). We observed an increase of the bacterial biomass in our experiments after dissolved sea ice inoculum, suggesting that either DOM and/or dFe stimulate bacterial growth. In HNLC waters, carbon seemed to be the first growth-limiting factor of the bacteria although Fe quickly became limiting when carbon limitation was alleviated (Church et al. 2000, Kirchman et al. 2000, Becquevort et al. 2007).

Moreover, the complexation of organic matter and iron in sea ice and their relation could stimulate algal blooms when sea ice melts. Although more than 99% of dFe is complexed to organic ligands in surface waters (Wu and Luther 1995, Boyé et al. 2001), the exact role of that association is still poorly known. Organic ligands, particularly saccharides, can maintain iron into solution by avoiding loss by precipitation and absorption onto particles and could influence iron bioavailability (Hassler and Schoemann, 2009). Until recently inorganic iron was considered as only form available for phytoplankton, but recent studies showed that dissolved and colloidal organic complexed iron could also be available for some species (Chen et al. 2003, Hutchins et

al. 1999, Maldonado and Price 1999, Hassler and Schoemann 2009). Various acquisition systems have been developed by some algal and bacterial species to access iron, such as production of ligands (e.g. siderophores), membrane reductases or mixotrophy/phagotrophy (Maranger and Pullin 2003).

In addition, it is important to note that interactions exist between these controlling factors, e.g. light and iron co-limit algal growth in the marginal ice zone (Boyd 2002). Raven (1990) hypothesized that when mixed layer increased and consequently the mean light level available for microorganisms decreased, the cellular iron requirements would increase because of the photoadaptation to low light of phytoplanktonic microorganisms. The thinning of the mixed layer due to freshwater input from ice melting could therefore reduce the cellular iron demand of pelagic algae in addition to supplied from decaying sea ice.

4.3. Impact of the sequence of release of the ice constituents

Inherent to their set-up, the first group of microcosms ("direct" melting) released all the sea ice components (microorganisms, OM and Fe) simultaneously into the seawater. Nevertheless in reality the melting process of sea ice is not instantaneous and the release of the sea ice constituents depends on the thermodynamical stage of the ice cover and is thus driven by the brines movement (such as drainage, convection or diffusion processes). The mattering question is in which proportion convection and diffusion affect the release of the organic and inorganic elements embedded into the gel-matrix present inside sea ice channels and pockets. Indeed the sequential release of the different components of the ice into the seawater may have great implications regarding the planktonic ecosystem. In the case of iron, if it would be released with the salty and dense brines and rapidly exported to the deep ocean and therefore not available to the phytoplankton. If iron is released from melting sea ice before the sympagic microorganisms, then this nutrient supply is not beneficial to the sea ice community once in the water column. But if the release of iron is slow, then it could be maintained in the surface water owing to the enhanced stratification and therefore be used by water-based microorganisms. Riebesell et al. (1991) observed the release of the ice algae before the complete melting of the ice sheet, hence before the stabilisation of the mixed layer. In the laboratory, we observed rather clearly that the brine drainage by convection mechanisms is the first process at work. An important fraction of the iron is released at that time but the iron flux remains constant for ca. 10 hours. Interestingly the DOC and POC maximum fluxes are not synchronized with this brine drainage, suggesting that the microorganisms stay attached to the brine walls as well as DOC, as suggested by Becquevort et al. (2009). The iron flux is still maximal when the DOC and POC fluxes are maximal, implying the possibility for sympagic microorganisms to access these substrate (Figure 6). Our experimental setting does assess whether the stabilisation of the mixed layer occurs before the release of iron, organic matter and microorganisms. Nevertheless some low-salinity water was released at the same time as the maximal fluxes of DOC and POC (around 15 hours), possibly inducing a stabilisation of the mixed layer (Figure 6). This timing of release of the key elements present in the ice cover seems thus favourable to the development of blooms in the marginal ice zone in Antarctica.

5. Conclusion

The present paper presents the first results on microcosms experiments performed under trace-metal clean conditions. The results presented here suggests that the sea ice inoculum was an important source of microorganisms, organic matter and iron for the seawater ecosystem as indicated a) by the ability of sea ice microorganisms (mainly whose size is < 10 µm) to thrive in seawater and b) the enhanced microbial growth in presence of ice-derived components. Moreover observations suggest that the timing of release of the different elements present in sea ice (especially iron) is likely favourable to the development of blooms in the marginal ice zone in Antarctica. During the ISPOL survey, the observed similarity between sea ice and pelagic algal community would support the seeding role of sea ice at the time of melting.

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The Pelagic Realm

Microbial network, organic matter and controlling mechanisms

Foreword

This second section focuses on the microbial network, organic matter and controlling mechanisms in ocean. Especially **Chapter 5** presents results concerning the bacterial remineralization of the organic matter in surface and mesopelagic depth layers of the Sub-Antarctic and Polar Front Zone of the Southern Ocean during the SAZ-Sense cruise.

***Chapter 5**: Bacterial remineralization in epipelagic and mesopelagic waters in Sub-Antarctic and Polar frontal zones south of Tasmania*
Dumont I., Masson F., Schoemann V., Jacquet S.H.M., Becquevort S.
Deep-Sea Research II, submitted

Note that a companion paper will be published together with this paper in the DSR II SAZ-Sense special issue and could be found in the annexe 1 of the present work. The sampling strategy during this cruise was elaborated in such a way that data collected could be used to compare bacterial production and Ba-proxy C mineralization.

***Annexe 1**: Twilight zone organic carbon remineralization in the Polar Front Zone and Sub-Antarctic Zone south of Tasmania*
Jacquet S.H.M., Dehairs F., Dumont I., Becquevort S., Cavagna A.-J., Cardinal D.
Deep-Sea Research II, submitted

A contribution to the paper of Schoemann et al. (in prep.) will also appear through the measurements of bacterial production during their experiments:

Control of organic ligands on Fe bioavailability, carbon production and export
Schoemann, V., Hassler C., Dumont I., Masson F., Bowie A., Lannuzel D., de Jong J.T.M., Becquevort S.
In prep.

Chapter 5

Bacterial remineralization in epipelagic and mesopelagic waters in Sub-Antarctic and Polar frontal zones south of Tasmania

I. Dumont, F. Masson, V. Schoemann, S.H.M. Jacquet, S. Becquevort

Deep-Sea Research II, submitted

Submitted to Deep Sea Research II

Bacterial remineralization in epipelagic and mesopelagic waters in Sub-Antarctic and Polar frontal zones south of Tasmania

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Abstract

Heterotrophic bacteria influence the carbon export and consequently the efficiency of the biological carbon pump through the remineralization of organic matter (either particulate or dissolved organic matter). Bacterial remineralization was investigated during the summer SAZ-SENSE cruise (January-February 2007), in the sub-Antarctic and Polar Front zones (SAZ and PFZ) of the Australian sector (Southern Ocean), by combining bacterial biomass (BB), ectoproteolytic activity (EPA) and bacterial production (BP) measurements in the epipelagic (0-100 m) and mesopelagic (100-700 m) zones. Bacterial phylogenetic composition (FISH) and viability (SYTOX) were also determined. All these parameters were studied in relation with dissolved organic matter, saccharides and chlorophyll *a* (chl*a*). Bacterial carbon demand (BCD) was also estimated by use of different conversion factors (CFs) and bacterial growth efficiency (BGE) and compared to primary production and export production estimates based on ^{13}C- ^{15}N-uptakes and the ^{234}Th deficit method, respectively. In the surface layer, bacterial biomass and its associated processes increased from SAZ waters west of Tasmania to SAZ waters east of Tasmania. Results at the Polar Front show interestingly maximum values in subsurface waters. Bacterial parameters followed chl*a* and organic matter distributions. Bacterial abundance, biomass and activities drastically decreased below depths of 100-200 m. Nevertheless, depth-integrated rates through the thickness of the different water masses showed that the mesopelagic contribution of bacteria represents a non-negligible fraction: up to 53%, 43% and 33% of the total water column BB, EPA and BP respectively. Mesopelagic remineralization seems more important in the PFZ compared to the SAZ. Despite some uncertainties in carbon flux estimations and discrepancies between methods, the present study highlights the importance to study bacterial dynamics in the twilight zone and their preponderant role in shaping the carbon fluxes through the water column.

Keywords: Bacteria; bacterial production; carbon; BGE; mesopelagic

1. Introduction

In oceans, a large part of dissolved and particulate organic carbon produced through primary production in the surface waters is exported to the deep waters. During its downward transfer, the sinking organic carbon is submitted to degradation, eventually resulting in regeneration of nutrients and CO_2 release, with only 1% of the C export estimated to reach the seafloor (Lampitt and Antia, 1997; Hansell, 2002; Lee et al., 2004). That organic material sinks out of the upper layer as particulate organic carbon (POC) in the form of faecal pellets, marine snow or sinking phytoplankton (Turner, 2002) but also as dissolved organic carbon (DOC) (Ducklow et al., 2001). The efficiency of the carbon transfer and ultimately the quantity of carbon sequestered in the deep ocean (Boyd and Newton, 1999) will depend first on the magnitude of the primary production and on the ecosystem structure (Buesseler, 2007). The vertical transport of the organic material is also influenced by particle characteristics such as size, morphology, sinking rate or mineral ballasting and by grazing (Wassmann, 1998). Finally, the strength of the remineralization processes through the water column determines the fate of the sinking carbon.

The degradation of the organic matter (OM) happens not only in the surface layer but through the whole water column, and especially within the mesopelagic zone (also called "twilight" zone, ~100-1000 m; Hansell, 2002). This zone receives the organic matter in excess from surface primary production but also from lateral inputs and migrant zooplankton active transport (Aristegui et al., 2005; Steinberg et al., 2008). Sinking particles entering the mesopelagic waters are submitted

to degradation, mainly due to local heterotrophic activity mediated by zooplankton and bacteria (Ducklow et al., 2001; Tanaka et al., 2004). The twilight zone is considered as the depth layer where most of the changes in sinking organic matter occur (Aristegui et al., 2005). For the Southern Ocean, Schlitzer (2002) reports from inverse modelling results that 70% of the C exported from the mixed layer is remineralized in the upper 450 m and 17% between 450 and 1000 m. This is reflected not only by the steep decrease of POC content with depth (see e.g. Martin et al., 1987; Buesseler et al., 2007), but also by a depth-depending change in biochemical composition of the organic material (Lee et al., 2004). Today, only a few studies have investigated the heterotrophic compartment of the mesopelagic zone in the Southern Ocean (Moriarty and O'Donohue, 1995; Lochte et al., 1997; Moriarty et al., 1997; Ducklow et al., 2001; Aristegui et al., 2002; Pedros-Alio et al., 2002; Simon et al., 2004). Even if heterotrophic activities are generally lower at depth than in surface, the integrated activities over the much thicker layer covered by mesopelagic waters might represent a non negligible fraction of the whole water column (del Giorgio and Duarte, 2002). It is therefore essential to take mesopelagic bacterial processes into account when investigating the C sequestration capacity of a system and especially, when studying cold oceanic systems with high rates of export production, i.e. delivering a high amount of organic matter below the surface layer (Simon et al., 2004). In spite of the uncertainties inherent to the different methods used to calculate the remineralisation in mesopelagic waters, results suggest that bacteria can consume major fractions of sinking fluxes of POC in that zone (Cho and Azam, 1988; Simon et al., 1992; Simon et al., 2004). Furthermore, in some areas POC fluxes to depth do not appear to be large enough to support the mesopelagic bacterial carbon demand so that additional mechanisms supplying OC (e.g. lateral or vertical transport of DOC, transport of OC by zooplankton) to the mesopelagic bacteria are required (Ducklow, 1993; Nagata et al., 2001; Reinthaler et al., 2006; Steinberg et al., 2008).

Heterotrophic bacteria, as the primary consumers of DOM, control its remineralization (Azam et al., 1983; Kirchman, 1990). They are able to remineralize the sinking particulate and dissolved organic matter by synthesizing enzymes to decompose polymeric substrates to make them directly assimilable by the cell. A fraction of the C uptake will be respired into CO_2 (bacterial respiration, BR) while the other fraction will serve to produce biomass (bacterial production, BP). The carbon fluxes through bacteria are particularly difficult to estimate correctly due to methodological difficulties to measure *in situ* bacterial activities, especially bacterial respiration. Yet the knowledge of the bacterial growth efficiency (BGE), expressed by BGE = BP/(BP+BR), allows to determine the ecological or biogeochemical roles of bacteria in the sea (Carlson et al., 2007). BGE reported in literature vary greatly across marine ecosystems, from <1 to >60% (del Giorgio and Cole, 1998; 2000) and different factors controlling BGE and bacterial metabolism (such as temperature or substrate concentration) have being proposed. However, there is no consensus on which values and controlling factors are most appropriate (Lopez-Urrutia and Moran, 2007). During the downward transfer of organic matter, the variation of these diverse parameters influences bacterial processes and consequently shape and determine the pattern of carbon export.

The Sub-Antarctic Zone (SAZ) and the Polar Front Zone (PFZ) of the Southern Ocean are mostly responsible for a large fraction of the total Ocean uptake of anthropogenic CO_2 (Inoue and Ishi, 2005). The SAZ waters east of Tasmania (150-160°E, **E-SAZ**) typically harbour higher iron levels than the SAZ waters west of Tasmania (135-145°E, **W-SAZ**) and their comparison may help understand different carbon export patterns (Trull et al., 2001b; Sedwick et al., 2008; Lannuzel et al., this issue). Also larger POC export fluxes have been observed in the PFZ (diatom dominance) than in the SAZ (no diatom dominance) (Buesseler, 1998; Boyd and Newton, 1999) while the deep fluxes exhibit the inverse pattern with lower fluxes in the PFZ than in the SAZ (Trull et al., 2001a). This suggests intense mesopelagic remineralization in the PFZ and this is corroborated by particulate mesopelagic excess-barium, a proxy for mesopelagic C remineralization. This showed that the C remineralized is 1.5 to 2 times higher in the PFZ as compared to the SAZ (Cardinal et al., 2005). There is a need to apply direct measurements of bacterial activities from surface to deep waters in order to ascertain the mechanisms of organic matter degradation and the efficiency of carbon transport in the water column including at mesopelagic depths.

That is the reason why we investigated bacterial remineralization within the water column (0-700m) during the SAZ-SENSE expedition (January-February 2007) in the Australian sector of the Southern Ocean (SAZ and PFZ regions). We performed combined measurements of bacterial abundance, phylogenetic composition, viability, ectoproteolytic activities and production. These parameters are studied in relation with chlorophyll *a*, organic carbon and saccharides concentrations. Bacterial carbon demand (BCD) has been calculated from the range of published conversion factors and BGE. BCD is compared to primary production (Westwood et al., this issue) and export fluxes (Jacquet et al., this issue, b) and their impacts are discussed in terms of carbon fluxes.

2. Materials and Methods

2.1. Study site and sampling strategy

The sample collection was conducted during the SAZ-SENSE (AAV0307 of R.V. *Aurora Australis*) from 17 January to 20 February 2007 (mid-summer) in the Sub-Antarctic Zone (SAZ) and Polar Front Zone (PFZ) of the Australian sector of the Southern Ocean (Fig. 1). Stations of short (12 h stay, 6 "transect" stations) and long (5 days stay, 3 "process" stations) duration were sampled. Seawater samples were collected using a CTD cast system, mounted on a rosette sampler equipped with 24 12L-Niskin bottles. Sub-sampling was performed carefully to avoid organic contamination. Seawater was collected into acid cleaned polycarbonate bottles and immediately treated. For bacterial production and counting, samples were collected at 6 transect stations

(SS2, SS9, SS10, SS12, SS21 and SS24) and 3 process stations (P1, P2 and P3). Seawater was collected at 15 depths, from 0 to 700 m. At each of the process station, bacterial production and abundance measurements were repeated on different days (e.g.: P1d1, d3 and d5). Organic matter parameters (DOC, total dissolved saccharides, POC) and chl*a* were measured at 3 transect (SS2, SS9 and SS12) and 3 process (P1, P2 and P3) stations. In the case of organic parameters, seawater was collected only at 7 depths (0-700 m). At long-term stations, phylogenetic composition and viability of the bacterial assemblages but also bacterial ectoproteolytic activity were studied. For these measurements, weather conditions encountered at P1 precluded operation of the CTD at depth.

The sites sampled during this study can be grouped into three main zones: a) the SAZ west (135-145°E) of Tasmania (Stns SS2 and P1; **W-SAZ**); b) the PFZ (Stn P2) and c) the SAZ east (150-160°E; **E-SAZ**) of Tasmania (Stns SS9, SS10, SS12, P3, SS21 and SS24). The main physical and biological characteristics of these regions are briefly described in Table 1. Detailed description of the physical properties of the study site is given in Griffiths et al. (this issue) and Mongin et al. (this issue).

Briefly, sea surface temperatures (SST) ranged between 5.3°C and 16.9°C, with higher surface temperatures occurring in the eastern SAZ, i.e. at stations P3, SS21 and SS24. Temperature profiles were very similar for the majority of the stations with a smooth decrease with depth except for P2 which was characterized by a marked thermocline. At P2, surface temperatures were lower (around 5.5°C) than at any other stations and the thermocline was at about 2°C, typical of Polar Front Waters. SS21 and SS24 (i.e. sites located north in the STZ and the EAC/subtropical water) have higher temperatures through the water column. Chlorophyll *a* (chl*a*) concentrations ranged from 0.09 to 0.77 µg l^{-1} in surface waters (Table 1). Maximum surface chl*a* concentrations were observed in E-SAZ, at P3 (Table 1; Wright et al., this issue). At P2, the chl*a* maximum (0.23 µg l^{-1}) was recorded below the surface, around 75 m, being a typical feature of the PFZ area reported also by others (e.g. Parslow et al., 2001). Iron concentrations were higher in the E-SAZ than in the W-SAZ (see Lannuzel et al., this issue).

2.2. Organic carbon and dissolved saccharides

All the material used for organic carbon sampling and analysis was either made of glass cleaned by combusting (4 h at 450°C), or washed with chromic-sulphuric acid (Merck), or of Teflon cleaned by 10% HCl soaking and rinsed with ultra high purity water (UHP, 18.2 M cm) obtained from a water purification system equipped with a UV-lamp and organic cartridge (Milli-Q Element, Millipore).

Seawater samples collected in acid-cleaned carboys were filtered onto pre-combusted (450°C, 4 h) Whatman GF/F filters under a fume hood inside a clean room. Filters for particulate organic carbon (POC) were kept frozen (-20°C). Filtered subsamples for dissolved organic carbon (DOC) were taken in duplicate. These were acidified with H_3PO_4 (0.05% final conc.) and stored

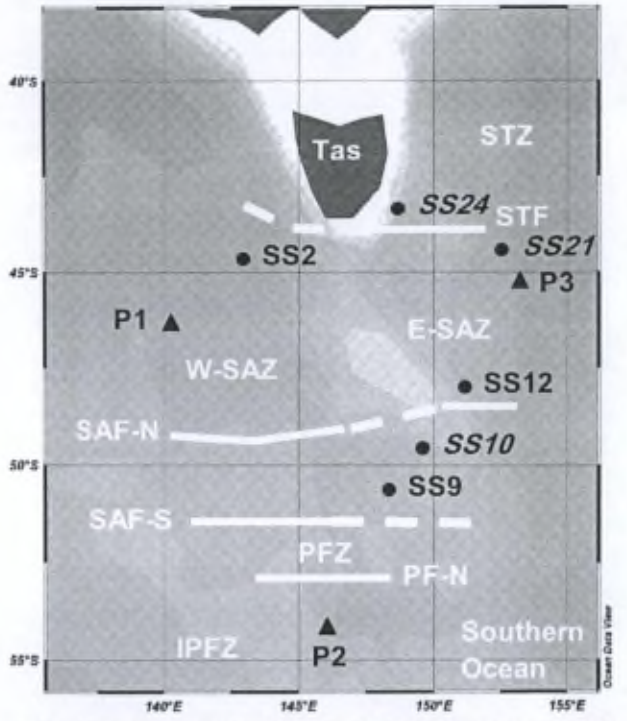

Fig. 1. Sampling stations during SAZ-SENSE. Tas: Tasmania, STZ: SubTropical Zone, STF: SubTropical Front, W- and E-SAZ: west and east SubAntarctic Zone, SAF-N: SubAntarctic Front-north, SAF-S: SubAntarctic Front-south, PFZ: Polar Front Zone, PF-N: Polar Front-north, IFPFZ: Inter Frontal Polar Zone. Station names which appear in italics have been sampled only for bacterial abundance and production but not for organic matter, see Material and Methods.

in precombusted glass vials sealed with Teflon lined screw caps. They were kept in the dark at 4°C until analysis in the home laboratory. POC was analyzed with a NA-2000 Fisons Instrument elemental analyzer (detection limit: 0.8 µmol C). DOC was measured by high temperature catalytic oxidation (HTCO; procedure of Sugimura and Suzuki, 1988) with a Shimadzu TOC-5000 analyzer. DOC concentration was calculated using a five-point calibration curve based on standards prepared by diluting a stock solution of potassium phthalate in UHP water (Milli-Q Element system, Millipore). Each DOC value corresponds to the average of at least five injections per duplicate sample. Samples were measured in duplicate and the relative standard deviation never exceeded 2%. The accuracy of our DOC measurements was tested by analyzing reference materials provided by the Hansell laboratory (University of Miami). We obtained an average concentration of 45.1 ± 0.7 µM C (n = 10) for deep-ocean reference material (Sargasso Sea Deep water, 2600 m) and 1.4 ± 0.7 µM C (n = 10) for low-carbon reference water. Our values are within the nominal values provided by the reference laboratory (44.0 ± 1.5 µM C and 2.0 ± 1.5 µM C, respectively).

Total dissolved saccharides (d-TCHO) were determined following the colorimetric TPTZ (2,4,6-tripyridyl-s-triazine) method of Myklestad et al. (1997) as modified by Hung et al. (2001). The whole procedure was carried out in the dark since the reagents are light-sensitive (van Oijen et al., 2003). d-TCHO includes mono- (d-MCHO) and polysaccharides (d-PCHO) which are respectively the concentration before and after

Table 1: Main physical and biological characteristics of the stations sampled during the SAZ-SENSE cruise. SST: sea surface temperature; deep temperature refers to the value at 500 m. W- and E-SAZ: west and east Sub-Antarctic Zone, PFZ: Polar Front Zone, SAF: Sub-Antarctic Front, EAC: East Australian Current, STZ: SubTropical Zone.

Stn.	CTD #	Long. °E	Lat. °S	Water masses	SST °C	Surf chl*a* $\mu g\ l^{-1}$	Deep Temp °C
SS2	5	143.053	44.889	W-SAZ	12.2	0.09	8.6
P1 d3	19	140.307	46.496	W-SAZ	12.8	-	8.5
P1 d5	30	140.641	46.580	W-SAZ	12.1	-	8.6
P2 d1	40	145.867	54.014	PFZ	5.4	-	2.4
P2 d2	44	146.128	54.020	PFZ	5.5	-	2.5
P2 d3	51	146.303	54.138	PFZ	5.4	0.12	2.3
P2 d6	59	147.103	54.447	PFZ	5.3	-	2.5
SS9	64	148.650	50.875	E-SAF	8.4	0.17	6.4
SS10	66	149.423	49.989	E-SAF	9.5	-	8.5
SS12	71	151.194	48.058	E-SAZ	11.7	0.41	8.4
P3 d1	78	153.176	45.548	E-SAZ	13.2	0.13	7.5
P3 d3	86	153.349	45.465	E-SAZ	13.5	0.77	8.1
P3 d5	104	153.678	45.590	E-SAZ	12.8	0.49	7.8
SS21	93	152.493	44.934	E-SAZ	14.4	-	8.8
SS24	108	148.581	43.687	EAC/STZ	16.9	-	9.4

hydrolysis (TCHO = MCHO + PCHO). Dissolved samples were hydrolysed with HCl 0.1 N at 100°C, for 20 h (Burney and Sieburth, 1977). Calibration curves were obtained with D(+)-glucose and the values of saccharides were expressed as glucose equivalent. They were converted into carbon concentration using a conversion factor of 6 mol of C per mole of glucose. The coefficient of variation (CV, standard deviation/mean) of the triplicate measurements was 5%. The detection limit was 1.0 µM C. To account for possible contamination during handling, 3 blanks per analytical session were treated and analyzed in the same way as the samples and subtracted from the concentration of the samples.

2.3. Bacterial parameters

We used in the present paper the general term "bacteria" which refers to both *Bacteria* and *Archaea*. Just after collection, seawater samples for the enumeration of bacteria were fixed with formaldehyde (2% final conc.). They were then filtered onto 0.2 µm polycarbonate black *Nuclepore* filters. Bacterial cells were stained with DAPI (10 mg l^{-1}) for 15 min and the filters were mounted in immersion oil on microscope slides according to Porter and Feig (1980). Bacteria were counted by epifluorescence under blue light excitation with a Leica DMRXA microscope fitted with Cy3 and DAPI filters. A minimum of 1000 cells were counted in at least 10 different fields at 1000 x magnification. A relative standard deviation of 15% (n = 20) was estimated on bacterial abundance determination. Bacterial abundance was converted into biomass by using the published carbon conversion factor (CCF) of 12 fg C $cell^{-1}$ (Fukuda et al., 1998).

Bacterioplankton community structure was examined by FISH (fluorescence *in situ* hybridization) using probe EUB338 for *Eubacteria*, ALF1b for the α-subclass of the *Proteobacteria*, BET42a for the β-subclass of the *Proteobacteria*, GAM42a for the γ-subclass of the *Proteobacteria*, CF319a for the *Cytophaga-Flavobacter* group, ARCH915 for *Archaea* and a negative control probe for nonspecific probe binding (Table 2). Bacterioplankton samples were prepared for FISH using a modification of the method described by Glöckner et al. (1996). Seawater samples were fixed with formaldehyde (2% final concentration). After fixation during 12-24 h, bacteria were stained with DAPI and filtered onto a 0.2-µm-pore-size polycarbonate membrane (Poretics), rinsed with 0.2-µm-pore-size-filtered seawater and stored at -20°C. A piece of the filter was placed on a Parafilm-covered glass slide, overlaid with 30 µl of hybridization solution containing 2 ng/µl of Cy3-labeled oligonucleotide probe, and incubated in a sealed container for 24 h at 42°C. The hybridization solution contains 0.9 M NaCl, 20 mM Tris-HCl (pH 7.4), 0.01% sodium dodecyl sulfate, 50 mM EDTA and varying formamide concentrations determined to achieve specificity for the targeted group of bacteria (Table 2). After hybridization, the sample was washed during 15 min at 38°C in a solution containing 20 mM Tris-HCl (pH 7.4), 10 mM EDTA, 0.01% sodium dodecyl sulfate and appropriate concentrations of NaCl i.e. 102 mM for EUB338, BET42a and GAM42a, 440 mM for ALF1b, 308 mM for ARCH915 and 80 mM for CF319a. Stained cells were determined by image analysis (Lucia 4.6 software) from epifluorescence observations of 10 fields using a Leica DMRXA microscope fitted with Cy3 and DAPI filters and equipped with a Nikon DXM 1200 camera.

The proportion of bacterial non-viable cells was estimated from Sytox-positive cells (Roth et al., 1997). A stock solution of Sytox Green stain (Molecular probes) was prepared in dimethylsulfoxide to a final concentration of 5 mM. The cells were stained with 1 µM SYTOX Green (final conc.) for at least 10 min at room temperature in the dark, as proposed on the product information sheet. The Sytox-positive cells were determined by epifluorescence microscopy (see above).

Table 2: Oligonucleotide probes used in this study. %FA = percentage of formamide in the hybridization buffer. 1: Amann et al. (1990), 2: Manz et al. (1992), 3: Manz et al. (1996), 4: Stahl and Amann (1991)

Probe	Specificity	Probe sequence (5'–3')	Target site 16S or 23S rRNA position (nucleotide)	% FA	Refs.
EUB338	*Eubacteria*	GCTGCCTCCCGTAGGAGT	16S (338–355)	30	1
ALF1b	alpha-*proteobacteria*	CGTTCGYTCTGAGCCAG	16S (19-35)	40	2
BET42a	beta-*proteobacteria*	GCCTTCCCACTTCGTTT	23S (1027–1043)	30	2
GAM42a	gamma-*proteobacteria*	GCCTTCCCACATCGTTT	23S (1027–1043)	30	2
CF319a	*Cytophaga-Flavobacterium*	TGGTCCGTGTCTCAGTAC	16S (319–336)	30	3
ARCH915	*Archaea*	GTGCTCCCCCGCCAATTCCT	16S (915–934)	25	4

Ectoproteolytic activity (EPA) was determined fluorimetrically (Kontron SFM 25 fluorimeter) as the maximum hydrolysis rate of model substrate for leucine-aminopeptidase (L-leucine-7-amino-4-methyl-coumarin) added at saturating concentration (40 mM). Wavelengths for excitation and emission were 360 and 445 nm. Fluorescence in the samples was measured as a function of time at *in situ* temperature in the dark. Increase of fluorescent units with time was converted into activity from a standard curve prepared with the end product of the reaction, 7-amino-4-methylcoumarin (Sigma). The relative standard deviation was 12% (n = 20).

Bacterial production (BP or more precisely referring to heterotrophic prokaryotic production) was determined by incorporation of ^{3}H-labelled thymidine into macromolecules precipitated by cold trichloroacetic acid (TCA) according to Fuhrman and Azam (1982). Three 20 ml (for depths <200 m) and three 40 ml (for depths >200 m) aliquots of water sample were dispensed into plastic vials. All samples received methyl-[^{3}H]-thymidine (specific activity: 74.5 Ci mmol^{-1}; Amersham), to a final concentration of 10 nM, by addition of 100 or 200 µl ^{3}H-thymidine. One of these subsamples served as a blank (t0) and the two others were incubated for 4 h (<200 m) and 8 h (>200 m) at *in situ* temperature. The thymidine incorporation was stopped by precipitating the samples with trichloroacetic acid (TCA, 5% final concentration). The samples were filtered through 0.22 µm pore size cellulose acetate filters (Sartorius) and rinsed 5 times with 2 ml 5% ice cold TCA. The filters were immediately stored at -20°C. Back to the home laboratory, they were placed in scintillation cocktail (EcoLume, MP Biomedicals), and the radioactivity incorporated in the cold TCA precipitable material was measured by a Packard Tricarb 1900TR Liquid Scintillation Analyzer. Corrections for quenching were made by transformed spectral indices of external standards and stored quench curves. Radiotracer incorporation (nmol l^{-1} h^{-1}) was converted into bacterial production (cell l^{-1} d^{-1}) using the published thymidine conversion factor (TCF) of 8.6 x 10^{17} cell per mol ^{3}H incorporated estimated by Ducklow et al. (1999). Biomass production rate was obtained by use of the CCF of 12 fg C cell^{-1} (Fukuda et al., 1998).

The measurements of BP at discrete depths throughout the water column can be used to estimate depth integrated bacterial respiration (BR) and carbon demand (BCD) from the following relations: a) BR= [(BP/BGE) – BP] and b) BCD = BP + BR = BP/BGE. To calculate this, we rely on the use of a thymidine conversion factor (TCF) and a bacterial growth efficiency factor (BGE). But the values of each of these conversion factors (CFs) found in literature are rather variable and consequently greatly influence the estimation of the carbon fluxes. We tested, and discuss below, the impact of three different ranges of TCFs and BGEs. We applied a constant carbon conversion factor (CCF) of 12 fg C cell^{-1} and used a low (TCF = 0.5 x 10^{18} cells mol^{-1} and BGE = 0.38), a middle (TCF = 0.86 x 10^{18} cells mol^{-1} and BGE = 0.15), and a high estimate (TCF = 2.0 x 10^{18} cells mol^{-1} and BGE = 0.09) to calculate bacterial respiration and bacterial carbon demand. The value of 0.15 for BGE is the median of 239 oceanic studies (del Giorgio and Cole, 2000) and the lower and higher ones are the extreme values found by Carlson et al. 1999. The choice of the TCF is made after Ducklow et al., 1999 and Ducklow, 2000. In addition, we also use the model of del Giorgio and Cole (1998) to estimate BGE from BP (in µg C l^{-1} h^{-1}) at each depth, following the relation: BGE = (0.037 + (0.65 x BP)) / (1.8 + BP).

3. Results

3.1. Distribution of organic carbon

Dissolved organic carbon (DOC) concentrations ranged between 47 and 86 µMC (Fig. 2). DOC concentrations were higher in the upper 100 m of the water column compared to underlying waters. Higher surface DOC concentrations (84 µM C) were observed at P3 (E-SAZ) than at P1 (56 µM C) and P2 (55 µM C). At P2 (PFZ), a subsurface maximum (± 50 m deep, 75 µM C) was present (Fig. 2), roughly coinciding with the deep chlorophyll maximum (DCM) (± 75 m, 0.23 µg l^{-1}). Concentrations of dissolved saccharides showed a rather similar trend with depth as DOC. They ranged between 1.3 and 13.4 µM C along the water column. They accounted for between 2.4% and 25.6% of the DOC but this percentage did not show any particular trend with depth or region. Dissolved saccharides were mainly on the polymeric form (69 ± 19%) except in the surface layer of P2, where they accounted for less than 49% of total saccharides. As for DOC, the highest concentrations were measured at P3 as compared to P2 and P1 and the same subsurface maximum was observed at P2. Particulate organic carbon (POC) concentrations were in average 10 ± 6 times smaller than DOC and

ranged between 3.3 and 14.9 µMC in the surface layers. The maximum POC value was observed at P3.

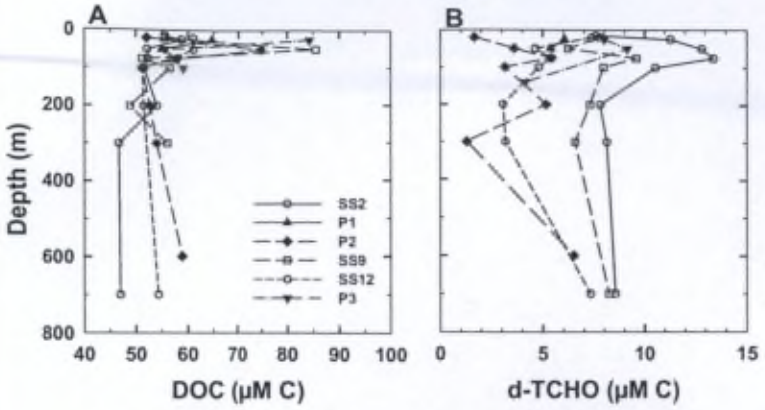

Fig.2. Vertical profiles (0-700 m) of dissolved organic carbon (DOC, µM C, left side) and, of dissolved total saccharides (d-TCHO, µM C, right side) for stations SS2, P1, P2, SS9, SS12 and P3 of the SAZ-SENSE cruise

3.2. *Bacterial standing stocks and activities*

Vertical distributions of bacteria abundance are shown in Fig. 3. For all the stations, bacterial numbers ranged from 0.03 to 4.95 x 10^9 cell l^{-1} for the 0-700 m water column, which correspond to bacterial biomasses varying between 0.48 and 59.4 µg C l^{-1}. In general, bacterial abundances and biomasses were higher at the surface, decreased continuously up to about 200 m and then stayed relatively constant below 200 m. The depth profile at the Polar Front (P2) made exception and showed interestingly maximum values below the surface, around 100 m (Fig. 3B). From 200 to 700 m, bacterial numbers remained essentially constant around 0.09 ± 0.04 x 10^9 cell l^{-1}. Deep values were slightly higher (0.3 x 10^9 cell l^{-1}) in the east SAZ compared to other regions. In the epipelagic zone, bacterial abundances and biomasses were higher at P3 (W-SAZ) compared to P1 (E-SAZ) (Fig. 3). Values at P2 fit the same range as those for the W-SAZ. The repeat sampling at each site was separated by several days but the results from the repeat samplings were rather close to each other except for P1, which showed some variability in the surface layer between days 1 and 5 (Fig. 3A). Integrated (0-700 m) bacterial biomass in the west SAZ and PFZ ranged between 64 (P1d5) and 184 (SS2) mmol C m^{-2}. Integrated bacterial biomass was higher in the E-SAZ and ranged between 138 (SS10) and 409 (SS21) mmol C m^{-2} (Table 3). Mesopelagic bacterial biomass (100-700 m) represented an important fraction of total water column bacterial biomass (0-700 m) which ranged between 41 and 68% (Table 3). The integrated biomass in the surface layer (0-100 m) and in the mesopelagic layer (100-700 m) was higher in the east, at P3, compared to P1.

At process stations, *in situ* hybridization with oligonucleotide probes showed that between 70 and 83% in surface layer (Fig. 4A) and between 51 and 65% in mesopelagic layer (Fig. 4B) of all picoplanktonic cells stained with DAPI hybridized with the probe EUB338 specific for *Eubacteria*. The proportions of *Archaea* ranged between 0 and 25% of total DAPI counts with the highest values reported in mesopelagic waters, particularly in the PFZ (Figs 4A and B). Among the *Bacteria*, the *Cytophaga/Flavobacteria* subgroup was the most represented in all the samples, with percentages ranging between 7 and 33% in the surface layer (Fig. 4C) and between 36 and 52% in the mesopelagic layer (Fig. 4D) of EUB338 cells. The α-subclass of *Proteobacteria* was also significant for P2 and P3 in the surface layers (range: 13-23% of EUB338 cells; Fig. 4C). The proportion of γ- and β-subclass of *Proteobacteria* remained fairly similar in all samples and did not exceed 8% and 2% of EUB338 cells, respectively.

Almost all bacteria were viable in the samples from the 3 process stations at all depth (Table 4). In average, they accounted for >97% of total cells at P1, >93% at P2 and >87% at P3. No difference with depth was seen.

Ectoproteolytic activities (EPA) ranged between 0.36 and 41.6 nmol l^{-1} h^{-1} for the three process stations, with the maximum value at P1. Highest bacterial ectoproteolytic activities were measured in the surface waters and they decreased with depth (Fig. 5). At P2, a subsurface maximum for EPA was observed around 75 m, coinciding with chl*a* and organic matter distributions. Specific (per cell) ectoproteolytic activities ranged between 1.7 and 54.0 x 10^{-18} mol $cell^{-1}$ h^{-1} (Table 4). Surface maximal values were observed at P1 and minimum ones at P3. Specific EPA was variable over depth (0-700 m) but average values tended to be higher at the surface, except at P3 (Table 4). Integrated values (0-700 m) were 31.3 and 61.3 mmol m^{-2} d^{-1} at P2 and P3 respectively. Integration was not possible for P1 as only surface data were available. From these integrated values, mesopelagic activities (100-700 m) represented respectively 33% and 54% for P2 and P3.

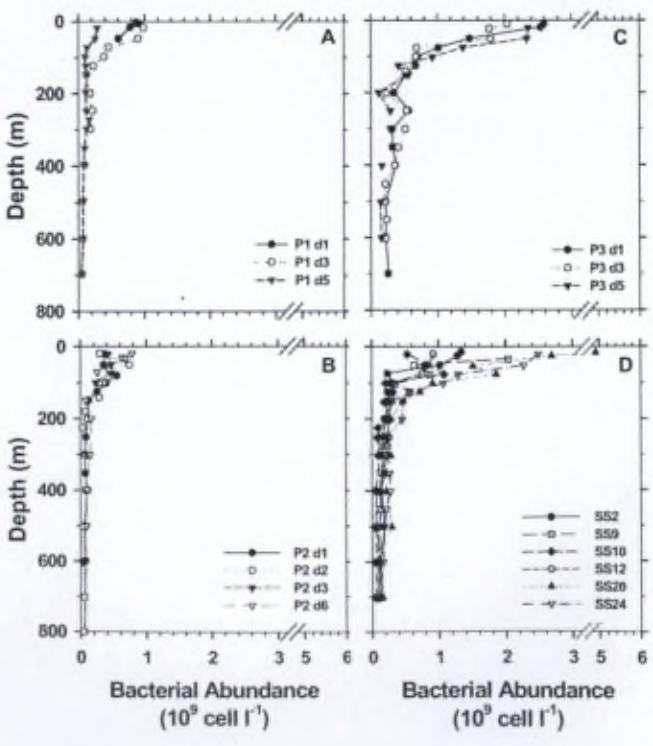

Fig. 3. Vertical distributions of bacterial abundance (109 cell l^{-1}) at process stations P1 (A), P2 (B), P3 (C) and transect stations SS2, SS9, SS10, SS12, SS20 and SS24 (D). At each process stations, measurements have been realized on different days (d).

Table 3
Depth-integrated bacterial biomass and production over the surface layer (0-100 m), the mesopelagic layer (100-700 m) and the total water column (0-700 m), for all the stations sampled during SAZ-SENSE. Contribution of the mesopelagic is given in %. Process Station P1, P2 and P3 have been sampled on different days (d). Values (middle estimates, see text) are given for a CCF = 12 fg C cell^{-1} and a TCF = 0.86 x 10^{18} cell mol^{-1}. All values are expressed in mmol C m^{-2} d^{-1}.

	Integrated BB (mmol C m^{-2})				Integrated BP (mmol C m^{-2} d^{-1})			
	0-100 m	*100-700 m*	*total*	*%$_{meso}$*	*0-100 m*	*100-700 m*	*total*	*%$_{meso}$*
SS2	85	99	184	**54**	3.3	1.9	5.3	**36**
P1 d3	74	64	138	**47**	6.7	1.7	8.4	**20**
P1 d5	20	43	64	**68**	4.8	2.6	7.3	**35**
P2 d1	42	59	101	**58**	4.6	2.0	6.6	**30**
P2 d2	44	57	101	**56**	3.7	3.0	6.6	**46**
P2 d3	43	59	101	**58**	3.1	8.8	11.9	**74**
P2 d6	52	76	128	**60**	4.7	2.0	6.7	**30**
SS9	127	112	239	**47**	2.2	1.8	4.0	**46**
SS10	72	66	138	**48**	2.5	1.9	4.4	**43**
SS12	79	105	184	**57**	4.0	1.4	5.4	**26**
P3 d1	163	209	372	**56**	11.2	1.8	13.0	**14**
P3 d3	141	209	350	**60**	7.2	2.3	9.5	**25**
P3 d5	193	142	335	**42**	13.8	2.9	16.8	**17**
SS21	242	166	409	**41**	11.5	1.3	12.8	**10**
SS24	197	174	371	**47**	13.7	2.3	15.8	**14**

Depth profiles of bacterial production for transect and process stations are presented in Fig. 6. Bacterial production during the investigation period ranged from 0.47 to 322 x 10^6 cell l^{-1} d^{-1}. Bacterial production was higher in surface waters and decreased sharply below depths of 100 m, except at P2 where there was a peak around 100 m (on day 1) and around 200 m (on day 3 of this process station) (Fig. 6B). In the surface layer, higher values were measured in E-SAZ than in W-SAZ while values at P2 were similar to those at P1. Below 200 m, values were relatively stable over depth, ca. 2 x 10^6 cell l^{-1} d^{-1}. Temporal variability was observed at P1 and P2 but not at P3 (Figs 6A, B and C). At P1, surface production is 3 to 4 times smaller on day 1 than on day 3 and 5. At P2, a subsurface maximum of bacterial production was observed at different depths on the different sampled days. Growth rates ranged between 0.004 and 0.684 d^{-1} (Table 4). They were rather variable with depth but in average they were higher in the surface layer than below this depth. The average growth rates were similar in the surface layer of P1 and P2 and were greater than at P3. Below 200m, growth rates were more constant, varying between 0.004 and 0.090 d^{-1}. In the mesopelagic layer (100-700 m), the average growth rates were the highest at P2 (Table 4). Integrated bacterial production (0-700 m) ranged between 4.0 (SS9) and 16.8 (P3d5) mmol C m^{-2} d^{-1} (Table 3). The highest values were observed in the E-SAZ, at P3, SS21 and SS24. The integrated bacterial production in the surface layer (0-100 m) was higher in E-SAZ compared to the W-SAZ while the integrated mesopelagic production (100-700 m) was rather constant for all the stations (excluding P2d3). Consequently the contribution of integrated mesopelagic activities (100-700 m) was highly variable between stations, accounting for between 10 and 74% of the total bacterial production (0-700 m) (Table 3). The proportion of the bacterial production in the mesopelagic zone (%BP$_{meso}$) was lowest in the E-SAZ and the highest at P2 on day 3. At P2, the high variability in the partition of the bacterial production between surface and mesopelagic (see Table 3) is constrained by the location of the subsurface maximum observed (Fig. 6).

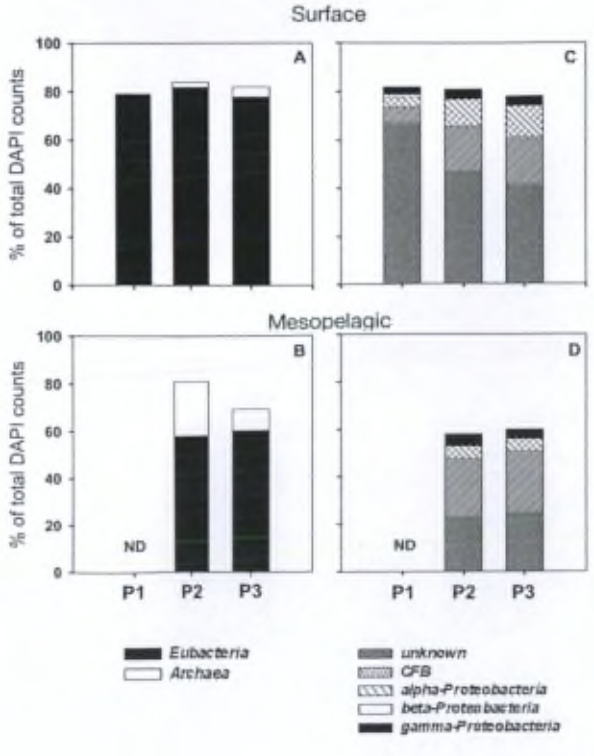

Fig. 4. Major bacterial phylogenetic groups as determined by FISH (see Methods) at the 3 process stations (P1, P2, P3). Probe data are given as percent of cells detectable after DAPI staining. Distribution of total DAPI counts between Eubacteria and Archaea in surface (A) and mesopelagic (B) layers; distribution of Eubacteria in the main groups in surface (C) and mesopelagic (D) layers.

Table 4
Percentage of viable cells as determined by the Sytox green dye, average and range (min-max) of specific ectoproteolytic activity (EPA) and bacterial growth rate for the surface (0-100 m) and mesopelagic layer (100-700 m) for the 3 process stations. ND means no data.

	P1		*P2*		*P3*	
	0-100 m	*100-700 m*	*0-100 m*	*100-700 m*	*0-100 m*	*100-700 m*
viable cells *%*	97.8	ND	92.6	93.4	83.7	93.2
Spec. EPA 10^{18} *mol cell^{-1} h^{-1}*	48.7 (43.3-54.0)	ND	18.6 (10.5-23.0)	9.3 (3.3-18.0)	4.5 (1.7-10.8)	14.0 (7.8-19.5)
Growth rate d^{-1}	0.108 (0.022-0.255)	0.041 (0.015-0.132)	0.090 (0.031-0.176)	0.069 (0.008-0.684)	0.053 (0.020-0.121)	0.016 (0.004-0.138)

3.3. Bacterial carbon demand and BGE

Assuming a thymidine conversion factor (TCF) of 0.86 x 10^{18} cells mol^{-1} and a bacterial growth efficiency (BGE) of 0.15, the middle estimate of integrated Bacterial Carbon Demand (BCD) (0-700 m) varied between 14.5 (SS9) and 92.1 (P3d5) mmol C m^{-2} d^{-1} (Table 5). When applying a TCF = 0.5 x 10^{18} cells mol^{-1} and a BGE = 0.38, the BCD values were reduced by 77%. The use of a TCF = 2.0 x 10^{18} cells mol^{-1} and a BGE = 0.09 gave very high BCD values, 3.9 times higher than the middle estimates (Table 5). The bacterial growth efficiency (expressed in %) estimated by the del Giorgio's model gave low BGE values, ranging between 2.1% and 7.2%, for all the samples when using middle conversion factors. Average BGE for W-SAZ, PFZ and E-SAZ were respectively of 2.8%, 2.7% and 3.4% in surface and of 2.1%, 2.2% and 2.1% in mesopelagic layer.

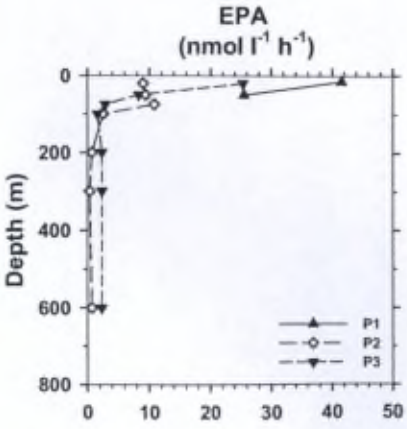

Fig. 5. Vertical distribution of bacterial ectoproteolytic activities (EPA, nmol l^{-1} h^{-1}) at the process stations (P1, P2, P3).

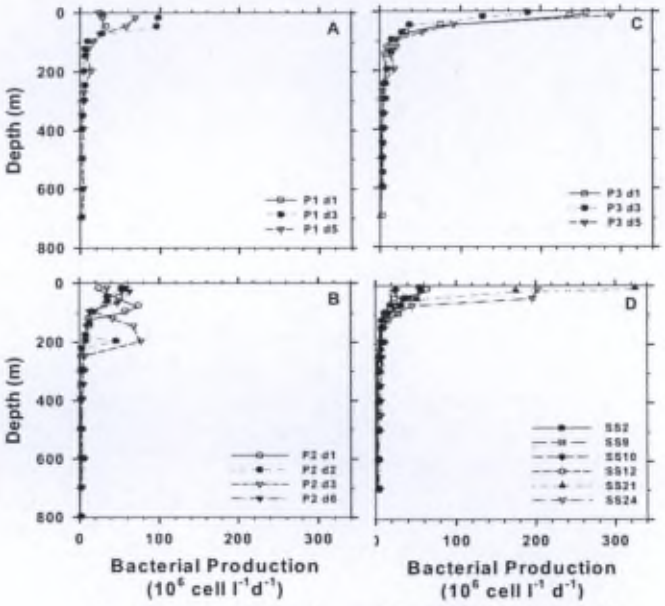

Fig. 6. Vertical distributions of bacterial production (10^6 cell l^{-1} d^{-1}) at process stations P1 (A), P2 (B), P3 (C) and transect stations SS2, SS9, SS10, SS12, SS20 and SS24 (D). At each process stations, measurements have been realized on different days (d).

4. Discussion

4.1. Choice/variability of the conversion factors and BGE

Although the estimation of the bacterial carbon demand from measured bacterial production (BP) and BGE is routinely practised in marine studies, it involves some crucial assumptions to be made. On the one hand, the incorporation rate of labelled compounds such as ^{3}H-thymidine, are only a proxy for BP as the transformation of these incorporation rates into cell and carbon production values require the use of conversions factors (CFs; Ducklow, 2000; Carlson et al., 2007). On the other hand, BGE is difficult to measure and very few experimental data are reported, in particular for the mesopelagic zone. The fixed BGE values we used in our calculations were selected as representative and fit the range of published values for Antarctic and marine studies. BGE has been estimated to vary between 9 and 38% at coastal sites (Bjornsen

and Kuparinen, 1991; Carlson et al., 1999) and around 26-30% at the Polar Front (Kahler et al., 1997). However recent studies have also reported very low BGEs (Moran et al., 2002; Reinthaler et al., 2006; Obernosterer et al., 2008), especially in the mesopelagic zone. A low BGE might result from the increase of maintenance costs in oligotrophic conditions (Carlson et al., 2007). Given the lower concentration and lower labile fraction of OM in the twilight zone, it seems likely that BGE would be lower there than in the surface layer (Williams, 2000). But Tamburini and colleagues (2003) stressed the need to assess prokaryotic activities at *in situ* pressure as they observed that pressure affects bacterial activity and that stress caused by decompression induces an increase in energy cost, and resulting in systematic underestimates of BGE.

Another approach to estimate the bacterial growth efficiency is to use models relating BGE to another parameter which is easier to measure, as described for instance by del Giorgio and Cole (1998). Applying this model (*see section 3.3*) to our bacterial production data suggests that BGE is lower in the mesopelagic layer. Nevertheless our BP values lie in the very low range of BP values used in the empirical transfer function proposed by del Giorgio and Cole (1998). Since this transfer function was constructed from surface data only, it is possible that it is not suitable for deeper water column which has different characteristics than the photic layer. Although most BGE measurements relate to free-living bacteria and assume a homogenous DOM distribution, the mesopelagic zone is characterized by the presence of aggregates. These important microenvironments introduce some heterogeneity, being literally hotspots, for the bacteria. Indeed very high interstitial DOC concentration can be found within these aggregates (Alldredge, 2000) due to very high bacterial hydrolytic activities which convert aggregated organic matter into non-sinking DOM. Furthermore, this DOM can diffuse in the surrounding waters as plumes of high OM (Azam and Malfatti, 2007). In this organically-enriched microenvironment, bacterial growth might occur with high growth efficiency (Azam and Long, 2002) and a study on aggregates has shown BGE can be as high as up to 0.50 (Grossart and Ploug, 2001). A "bulk" BGE value obtained from a given volume of water may not reflect the reality. In summary, the application of extreme conversion factors performed here highlights the large variation of the results as depending on the choice of the CFs and BGE values, and stresses the need to derive conclusions with caution (*see section 4.4*).

4.2 Bacterial standing stock and activity

The abundances of bacteria we observed for Antarctic and sub-Antarctic waters are similar to those of temperate regions, which usually range between 10^7 to 10^9 cells l^{-1} (e.g. Karl et al., 1991). We also observe that the numbers of bacteria are the highest in surface waters and decrease with depth as is usually the case (Ducklow, 1993; Nagata et al., 2000; Tamburini et al., 2002). Likewise, bacterial activities (EPA and BP) are higher at surface than deeper. Comparisons of values of bacterial activities measured in the present study with literature data for Antarctic and sub-Antarctic Oceans show that they fall within the same range than previously reported in the literature, for total and specific ectoproteolytic activity (Christian and Karl, 1992; 1993; 1995; Talbot and Bianchi, 1997, Talbot et al., 1997; Becquevort and Smith, 2001; Misic et al., 2002), bacterial production (Lochte et al., 1997; Moriarty et al., 1997; Church et al., 2000; Simon et al., 2004) and growth rates (Karl et al., 1991, Pedros-Alio et al., 1996; Carlson et al., 1998; Ducklow et al., 1999; 2001; Obernosterer et al., 2008). The FISH analysis indicates that the bacterial community includes large numbers of the *Cytophaga-Flavobacterium-Bacteroides* (CFB) group and of the *alpha-proteobacteria* throughout the studied region. These groups are indeed the most frequently observed in the surface ocean (Kirchmann, 2002). But Abell and Bowman (2005) reported, for the same region and for similar bacterial communities as observed in our study, the occurrence of clades (i.e. subdivisions of a group) that appear to be different between SAZ and PFZ.

In the surface layer, from a spatial point of view, both bacterial abundance and activities show a clear gradient between west and east SAZ, with values at the PFZ being similar to those of W-SAZ. Temperature regulates most of bacterial processes such as enzymatic activity, growth and respiration (Pomeroy and Deibel, 1986; Kirchman and Rich, 1997; Lopez-Urrutia and Moran, 2007), but the influence of temperature on bacterial carbon metabolism is complex. First, cold temperatures or the combination of low temperature and low substrate concentration, have been pointed out as controlling bacterial growth in high-latitude oceans (Pomeroy and Deibel, 1986; Cole et al., 1988; Pomeroy et al., 1991). However, considering the high diversity of bacteria (Karl and Proctor, 2007) and their faculties to adapt to their environment (e.g. enzymes, Russel, 2000), there is no reason to suspect that bacterial numbers and processes in cold ecosystems would be lower than in warmer ecosystems (see discussion in Rivkin et al., 1996). Bacterial abundances, activity and viability were not significantly reduced in the colder water column temperatures at the P2 (PFZ) site compared to these abundances and rates at the warmer SAZ sites. Furthermore, although east and west SAZ have similar sea surface temperatures (Table 1), bacterial abundance and activities were higher in E-SAZ. This suggests that temperature is not solely controlling bacterial distribution and that other factors play key roles in shaping bacterial distribution and activities pattern.

The abundance and the quality of the organic and inorganic (such as iron) substrates can limit bacterial processes (Pakulski et al., 1996; Kirchman et al., 2000; Church et al., 2000). As iron concentrations were higher in the E-SAZ than in the W-SAZ (Lannuzel et al., this issue) we can not exclude a direct effect on the bacterial metabolism. Nevertheless, several controlled experiments (one of them being realized on seawater from the same region as in our study) have concluded that carbon seemed to be the first growth-limiting factor for bacteria even if iron quickly became limiting when carbon limitation was alleviated (Church et al.,

2000; Kirchman et al., 2000; Becquevort et al., 2007). Phytoplanktonic production in the surface layer is the main source of labile organic matter in the ocean (Nagata 2000). We indeed observed that the distribution of organic matter was consistent with the chl*a* distribution: higher phytoplanktonic biomass (as indicated by chl*a* concentrations) and higher quantity of organic substrates recorded in the E-SAZ and lower ones in W-SAZ (Table 1). Even the subsurface chl*a* maximum at P2 was reflected in the concentrations of DOC and d-TCHO. The pool of OM seems thus relatively directly related to phytoplankton in these regions resulting in a correlation between DOC and chl*a* ($r^2 = 0.49$, $n = 23$; $p = 0.0002$). The DOC values in surface waters of the PFZ ranged from 51 to 75 µM C and are in agreement with literature data (60-90 µM C, Carlson and Ducklow, 1995; Guo et al,. 1995; Thomas et al., 1995; Peltzer and Hayward, 1996; Sharp, 1997; Ogawa et al,. 1999), while they reached up to ca. 90 µM C in E-SAZ. To the best of our knowledge, no DOC data have been published for this area. A fraction of the DOC in surface water constitutes "labile" organic compounds (turnover time: minutes to days), such as dissolved saccharides (d-TCHO), which are substrates available for bacteria, and rapidly removed from the system. Another fraction of the surface DOC represents "semi-labile" organic compounds (turnover time: weeks to months), which is estimated as the DOC stock in excess of the refractory pool present in the deep ocean (Carlson, 2002). Significant spatial variability in excess DOC (excess DOC=DOC concentration–background Antarctic DOC, i.e. 42 µM C as assessed by Wiebinga and de Baar, 1998) was observed in the surface waters of the different studied areas: the excess DOC was 12 ± 3 µM C, 17 ± 11 µM C and 36 ± 21 µM C in P1 (W-SAZ), P2 (PFZ) and P3 (E-SAZ) respectively. This semi-labile pool, which escapes rapid microbial degradation in the upper 100 m, could thus constitutes a significant direct C export to mesopelagic depths by overturning (thermohaline) circulation at high latitudes. In the area of this study, vertical movement of water is mainly due to the breakdown of the seasonal thermocline and the subduction of PFZ and SAF surface waters to form the Subantarctic Mode Water in SAZ (Rintoul and Trull, 2001; Parslow et al., 2001).

The distribution of the organic substrates, with higher contents in E-SAZ as compared to W-SAZ appears thus well reflected in the magnitude of bacterial abundance and activities in each region. The organic substrates seem therefore to directly influence bacterial activity, as it is often the case in open-ocean areas where bacteria are directly depending on the autochthonous resources supply (Moran et al., 2002).

In addition to the main factors studied here, we cannot exclude that predation and viral infection, which control the bacterial abundance could also have played a role (Fuhrman, 2000; Strom, 2000). The lower growth rates observed in the surface waters at P3 as compared to P1 could be related to grazing or viral infection as the abundance of the grazers and of viruses are higher there (Evans et al., this issue). Interestingly, it also appears that for an "organic-poor" system such as P1, the proportion of non-identified cells like CF319 was higher than in the case of P2 and P3.

Of course, through the water column, the same factors are influencing the distribution of the bacterial processes. However, our knowledge of controlling factors on bacteria in the mesopelagic zone is limited. We observed a decrease of the bacterial numbers and activities with depth and also a decrease of the concentration of organic substrates. While this trend not always held for the specific (per cell) ectoproteolytic activities, these do not show a general decree se with depth (Table 4). The synthesis of enzymes is a complex mechanism implying a lot of different regulating factors (Chrost 1991). The decrease of direct labile monomeric substrates in the mesopelagic zone and the need to extract carbon and energy sources necessary for the bacterial metabolism from particulate or semi-labile DOM, could lead to an over-expression of their ectoenzymatic systems (Tamburini et al., 2003). Increase of iron concentrations with depth can also play a role in the expression of the enzymes (Becquevort et al. 2007). Moreover the pattern of enzyme expression seems to change with species (Martinez et al., 1996; Kirchman et al., 2004) and the variability in bacterioplankton communities at depth is influenced by organic matter supply and patchiness (Hewson et al., 2006). Although in surface, *CFB* and *Proteobacteria* are mainly present and typical of the presence of fresh DOM (Tamburini et al., 2006), the proportion of *Archaea*, for which the metabolism is still poorly known, increases in the mesopelagic zone, as also observed by other studies (Karner et al., 2001; Church et al., 2003; Reinthaler et al., 2005). The relatively constant specific ectoenzymatic activity even at depth suggests that bacteria seem adapted and able to decompose the available substrates. By contrast, growth rates were higher in surface waters and decline with depth: bacteria populations seem to be more actively metabolizing OM in surface than at depth, which is indicative of a higher availability and a fresher OM. Even more, these trends mean that there are more small substrates directly assimilable by the bacteria in surface, without any need for enzymatic breakdown. This situation is in agreement with excretion processes by algae occurring in surface waters, providing labile substrates such as saccharides to bacteria.

4.3. Significance of mesopelagic processes

We have seen that bacterial abundance and total activities are lower at depth but that specific enzymatic activities can still be significant, within the range of values observed in the surface waters. The cumulative activities over the much thicker layer covered by mesopelagic waters do represent a non-negligible fraction of the whole water column activity. Depth-integrated biomass, proteolytic activity and production integrated between surface and 700 m showed that mesopelagic contribution (100-700 m) reached on average respectively, 53 ± 7%, 43 ± 14% and 33 ± 19% of the total activities estimated from 0 to 700 m. Previous studies have also highlighted the importance of the twilight zone for the bacterial degradation of the

Table 5
Column-integrated Gross Primary Production for the euphotic layer (GPP, from Westwood et al., this issue), Export Production at 100 m (EP_{100m}, from Jacquet et al., this issue b) and bacterial carbon demand (BCD) over the surface layer (0-100 m), the mesopelagic layer (100-700 m) and the total water column (0-700 m) for all the stations sampled during SAZ-SENSE. Process Station P1, P2 and P3 have been sampled on different days (d). All values are expressed in mmol C m^{-2} d^{-1}. * GPP have been determined from CTD close in time but not exactly from the same CTD as EP and BCD

	GPP*	EP_{100m}	BCD_{0-100m}	$BCD_{100-700m}$	BCD_{0-700m}
SS2	-	4.3	**22.4** 5.1-86.8	**12.5** 2.9-49.2	**34.9** 8.1-136.0
P1 d3	162	4.8	**44.7** 10.3-173.1	**11.2** 2.6-43.5	**55.9** 12.8-216.7
P1 d5	59	4.1	**31.5** 7.2-122.1	**17.3** 4.0-67.0	**48.8** 11.2-189.1
P2 d1	90	-	**30.3** 7.0-117.5	**13.4** 3.1-52.0	**43.7** 10.0-169.5
P2 d2	96	2.9	**24.2** 5.6-94.0	**19.8** 4.8-80.6	**44.0** 10.3-174.6
P2 d3	-	6.6	**20.8** 4.8-80.5	**58.4** 13.4-226.5	**79.2** 18.2-307.0
P2 d6	72	-	**31.2** 7.1-120.3	**13.4** 3.2-53.4	**44.7** 10.3-173.7
SS9	-	6.4	**14.5** 3.3-56.1	**12.4** 2.9-48.7	**26.9** 6.2-104.8
SS10	-	7.1	**16.9** 3.9-65.4	**12.5** 2.9-48.4	**29.3** 6.7-113.7
SS12	-	6.3	**26.7** 6.1-103.3	**9.4** 2.6-43.6	**36.1** 8.7-146.9
P3 d1	141	3.2	**74.5** 17.1-288.9	**12.0** 3.1-51.8	**86.5** 20.2-340.7
P3 d3	117	6.5	**47.6** 10.9-184.5	**15.6** 3.9-61.1	**63.2** 14.8-245.6
P3 d5	80	6.5	**92.1** 23.4-354.5	**19.5** 6.4-73.4	**111.5** 29.7-428.0
SS21	-	5.9	**76.7** 17.6-297.4	**8.8** 2.0-34.0	**85.5** 19.6-331.5
SS24	-	-	**90.9** 20.9-352.3	**14.8** 3.4-57.3	**105.7** 24.3-409.6

sinking OM in the Southern Ocean (Lochte et al., 1997; Moriarty et al., 1997; Ducklow et al., 2001; Aristegui et al., 2002; Pedros-Alio et al., 2002; Simon et al., 2004). The partition of BP between the epipelagic and mesopelagic layers is very variable between regions. This variability is due to the surface depth-integrated bacterial production, BP_{0-100m}, which has different values between stations. Consequently even if depth-integrated BP was interestingly fairly stable over the mesopelagic layer for all the locations (Table 3), the variation of BP at surface affects the final contribution of $BP_{100-700m}$. P3 has the highest BP_{0-100m} and concomitantly, the lowest mesopelagic %BP. While the algal community was diatom-dominated at P2, this was not the case at P3 and P1 (Wright et al., this issue). Thus the diatom-dominated P2 presents the highest mesopelagic contribution of BP. This corroborates some previous results (Buesseler, 1998; François et al., 2002; Cardinal et al., 2005) which stated that diatom-dominated systems export more labile organic matter. This material has been less extensively processed by the food web in the mixed layer and as a result, this exported material could be more effectively remineralized during its transit through intermediate depths, reducing its transfer efficiency to the deep sea (François et al., 2002; Panagiotopoulos et al., 2002). Results from excess Barium content would support this trend (Jacquet et al. this issue, a). Additionally, the higher EPA/BP ratio in the mesopelagic zone of P3 compared with the epipelagic zone of P3 suggests an intense enzymatic degradation process of a potentially high POC export along with a decrease of monomeric substrates directly available for bacterial production in the deeper waters.

4.4 Carbon fluxes

For each ecosystem, the bacterial carbon demand (BCD) can be compared with primary production (PP) in order to evaluate the coupling strength between them. Such evaluations of the strength of coupling have been made on many ocean expeditions and give very variable results. There are large net-heterotrophic regions (del Giorgio et al., 1997) where bacteria took up more OM than was present as local primary production, indicating that the spatial and temporal import of organic matter must have occurred, as some studies from the Southern Ocean indicate (Ducklow et al., 2000; Aristegui et al., 2002; Obernoster et al., 2008). As reported in Table 5, the bacterial carbon demand, estimated from BP measurements and literature BGE, gives high values as compared to local

primary production (Westwood et al., this issue). The minimal and middle estimation of BCD would be in agreement with the estimated local primary production and this holds for the surface water BCD as well as for the water column (0-700 m) integrated BCD. In the case of the minimal estimate, the BCD would use in average 13, 12 and 21% of the primary production in E-SAZ, PFZ and W-SAZ, respectively whereas in the case of the middle estimate the proportion of local PP used by bacteria rise up to 58, 52 and 85% in E-SAZ, PFZ and W-SAZ. As emphasized in *section 4.1*, the significance of conversion factors and BGE in the C budget estimations and the application of a constant value along the season and along the water column is, of course, problematic (CFs: Pulido-Villena and Reche, 2003; Alonso-Sáez et al., 2007; and BGE: Biddanda et al., 2001).

In addition, previous comparisons of BCD (from mesopelagic BP measurements) with C export suggest that BCD accounts for 14% to > 100% of C export (Cho and Azam, 1988; Nagata et al., 2000; Simon et al., 2004; Reinthaler et al., 2006; Steinberg et al., 2008). In this study, BCD is compared to C export estimates based on the ^{234}Th deficit method (Jacquet et al., this issue, b) and to mesopelagic remineralization estimated from the excess particulate barium contents (Jacquet et al., this issue, a) at the same sites. In the SAZ only the lower estimates of BCD are compatible with C export out of the surface layer. In the PFZ, however, any realistic estimation of BCD exceeds the C export. Overall, mesopelagic BCD values were higher than mesopelagic remineralization estimated from excess Ba contents, but the similarity in spatial distributions of both parameters persists (Jacquet et al., this issue, a) with highest values in the PFZ. These discrepancies could be inherent to the different methodologies involved. Indeed, C export fluxes as estimated by the ^{234}Th proxy reflect the POC export while the BCD is related to the whole OM pool (POC + DOC). Also, the formation of excess Ba-barite is related with the remineralization of OM in aggregates due to attached bacteria (see references in Jacquet et al., this issue, a) whilst bacterial communities consist of both attached and free bacteria for which the C source is POC as well as DOC. Organic carbon available to bacteria in the mesopelagic could be under- or over- estimated due to lateral and vertical transport of dissolved organic matter from nearby regions (Aristegui et al., 2002). There is no evidence, however, of large DOC accumulation in the Southern Ocean (Cadee, 1992; Kähler et al., 1997; Carlson et al., 1998; Wiebinga and de Baar, 1998; Doval et al., 2001). Nevertheless, an excess of DOC in mesopelagic waters as we observed here for the PFZ (20-29 µM C), was also reported for the PFZ in the Indian sector (Wiebinga and de Baar, 1998) and to a lesser extent in the Atlantic sector (Doval et al., 2001), coinciding with high bacterial activity (Aristegui et al., 2002). Moreover, active export of OC as POC by zooplankton occurs either via vertical migration, leading to a temporal decoupling between OC production and consumption (Ducklow et al., 2001; Steinberg et al., 2008) or via lateral migration from a more productive area leading to a spatial decoupling. Zooplankton can therefore actively increase the magnitude of the export of organic material by transporting surface-ingested material to mesopelagic waters, where this material is then metabolized (Steinberg et al., 2008).

5. Conclusion

In the present study, we first observed regional differences in surface waters with higher bacterial abundance and activities in the SAZ east of Tasmania than in SAZ west of Tasmania. The importance of the microbial processes all along the water column in controlling OM remineralization and carbon sequestration in the deeper water column was then pointed out. Indeed even if bacterial processes decreased with depth, we showed that the mesopelagic zone represents an important fraction of the total water column processes. It is thus essential to include the twilight zone remineralization in the assessment of the fate of fixed carbon and global C cycle in order to improve our vision of the biological C pump in the Southern Ocean and its influence on global climate. Still more studies along with methodological developments are needed in order to apprehend the activities in the whole water column.

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General Discussion and Conclusions

Firstly, I would like to discuss some difficulties inherent to the sampling, the analysis and interpretation of data collected in such a peculiar environment that is sea ice.

Then I will develop the main conclusions obtained along this thesis concerning the link between the organic matter and the microbial community (algae, bacteria and protozoa) paying attention to those factors that influence the biological carbon pump in the Southern Ocean. These results concern, on the one hand, the sea ice habitat where elevated concentrations of organic matter are trapped during ice formation, accumulate during winter and are released in the surface ocean at the time of ice melting. On the other hand, they address the cycling and fate of the organic matter exported below the ocean surface layer.

The observations made during this work allow to make hypotheses on biological mechanisms responsible for the accumulation of organic matter in sea ice, the related modifications of the physical and chemical environment of the sea ice microbial communities and the importance of the nature of organic matter which determines its availability for microbes not only in sea ice but also when released into the seawater. This work also quantifies the intensity of the degradation processes in the water column and during their transfer from the upper layer to the deep ocean.

From these observations, some hypotheses concerning the functioning of the biological carbon pump in the Southern Ocean are given. Finally some interesting perspectives are addressed.

1. Sampling the Southern Ocean: Methodological remarks

1.1. Spatio-temporal variability

Ecosystems in general and Southern Ocean in particular are diverse and evolve both temporally and spatially. The sampling of both ocean and sea ice is obligatory a snapshot of the system at a precise time. Data collection at different periods of the year is often the only way to investigate the annual cycle of a system.

Together the 3 independent sea ice cruises (2003, 2004 and 2007) carried out in the present work cover a period from September to December, i.e. from Austral late winter to early summer (see Materials and Methods, section 1. study sites). Unfortunately no mid-winter sampling was possible during this thesis because of the lack of cruises organised during this harsh period, due to difficulties in accessing sea ice. Nevertheless some stations sampled during the ARISE cruise had physico-chemical properties of cold ice and could be considered as typical of winter. The other samples from ARISE and ISPOL were corresponding to different states of the ice warming and melting evolution. The SIMBA cruise witnessed a special thermal cycle (flood-freeze cycle) where the ice cover becomes colder after a warm period. All samples were representative of first year pack ice, between 30 and 120 cm thick. Sampling strategy was slightly different between ARISE where different stations were sampled and ISPOL and SIMBA where a fixed station was sampled over time. Hence, the physical texture of each ice cover has enabled the classification and cautious comparison of each one (see also Lannuzel et al. 2007). The results from ARISE and SIMBA cruises have been carefully compared on a temporal / seasonal basis (chapters 1 and 2).

In sea ice methodological difficulties concerning representative and reproducible sampling of such a highly heterogeneous environment are well known by ice microbiologists. A high horizontal variability in microbial communities has been reported by Spindler and Dieckmann (1986). The ISPOL cruise allows us to study the temporal evolution of microbial communities from samples taken in a reduced area. We note that sampling was coherent with respect to microorganisms' presence, concentration and taxonomic composition during the 1 month investigation period (chapter 4). Spatial heterogeneity seems thus minimal in a narrow surrounding of one ice floe, presenting regular physical properties. Still the SIMBA sampling of 2 distinct stations in a same ice floe showed that the different physico-chemical characteristics of the ice cover (ice and snow thicknesses, texture) at each station (see Material and Methods) imply some variability in the biological parameters (chapter 3).

The oceanographic cruise took place in January-February 2007, i.e. during austral summer, at the most northern frontier of the Southern Ocean. The sampling was conducted in an open ocean area, in the Sub Antarctic and Polar Front zones, which are not directly influenced by sea ice. Here the sampling strategy was thought to compare high and low productivity waters (high or low iron availability). When studying the water column from surface to mesopelagic depths some spatio-temporal variability issues should be kept in mind. For instance, significant phase lags can exist between

production and export of organic matter that decouple surface and deep processes (Boyd and Trull 2007, Dehairs et al. 2008).

At the micro-scale in the water column, a homogenous DOM and microorganisms distribution is often assumed. Still the mesopelagic zone is characterized by the presence of aggregates. These important microenvironments introduce some heterogeneity, being literally hotspots, for the bacteria. For example, very high interstitial DOC concentration can be found within these aggregates (Alldredge 2000) due to very high bacterial hydrolytic activities which dissolve aggregated organic matter into non-sinking DOM. Furthermore, this DOM can diffuse in the surrounding waters as plumes of high OM (Azam and Malfatti 2007). In this organically-enriched microenvironment, bacteria might grow efficiently (Azam and Long 2002) and BGE as high as 0.50 have been reported (Grossart and Ploug 2001). The existence of such very active micro-environments suggests that the only determination of volume-averaged organism abundance should be cautiously used for describing the dynamics of microbial communities in the ocean (Hood et al. 2007).

1.2. Sampling

Sampling the sea ice environment is really a challenge. Two methods have been proposed: direct sampling of brines containing organisms or melting sea ice sample (Lizotte 2003).

The brine sampling was performed by the "sack holes" method during the 3 cruises. This method samples the fluid phase of brine from an unknown area/volume of ice. Sampling efficiency is affected not only by total brine volume fraction but also by the distribution of individual pores in the sea ice. This was clear from the observed disagreement between the concentration measured in brine samples and that estimated, based on total concentration in the melted ice and calculated brine volume (based on theoretical formulas) (chapter 1). This discrepancy is attributed to either the sampling efficiency of sack holes (unknown volume of ice sampling, individual pores, large cells or chain blocking open channels) or the retention of different elements on the surface of brine channels and pockets (e.g. Krembs et al. 2000, Mock and Thomas 2005).

Inevitably, the sea ice melting method implies the destruction of the physical environment of the microbial network to perform their analysis. Obviously this procedure raises critical issues about the reliability of the measurement after such a modification. One concerns the disruption of fragile algal cells by change in osmotic pressure (Garrison and Close 1986). This also holds for particles as TEP which are sensitive to the ionic strength (Passow 2002). Nevertheless, this is often the only possible method to investigate the interior ice. Still melting of sea ice may be conducted directly (as is) or with addition of filtered seawater to decrease osmotic shock as recommended by Garrison and Close (1986). During the thesis, a preliminary experiment was performed to test the OM behaviour in function of the two melting procedures. This allows us to determine the best protocol for obtaining reliable OM measurements in sea ice. The results showed in agreement with Thomas et al. (1998) that direct melting could be apply without significantly modify the OM concentration as high algal biomasses in sea ice correspond to diatom-dominated community which is resistant to osmotic shock. For microorganisms determination the addition of filtered water was applied.

The sampling of seawater could also involve a modification of the environment where microorganisms lived, e.g. pressure: in the case of deep ocean (mesopelagic and bathypelagic zone), incubation of deep-sea samples at atmospheric pressure may lead to underestimates of prokaryotic activity rates (see review of Tamburini 2006).

2. Distribution, evolution and interactions of organic matter and microbial community in sea ice

In this section, we discussed about the additional data sets on dissolved and particulate organic matter in sea ice provided by the present work. We also furnish some implications of the composition of the sea ice OM. Assumptions about the causes of the accumulation of OM in sea ice and the consequences for the microbial community are presented, along with some peculiar characteristics of the sea ice microbial community.

2.1. Composition and localisation of the microbial network in sea ice

Our study of the sea ice microbial community in term of algae, protozoa and bacteria in pack ice showed that the sea ice was very rich in microorganisms, with concentrations higher than in seawater (chapters 1 and 4). Indeed even if from their initial incorporation to the melting of ice, the sea ice microbial community has to cope with an extreme (e.g. temperature below -2°C and salinity up to 100) and an organically-concentrated environment where growth can be a challenge, microorganisms also found favourable conditions such as high iron concentration or protection against grazers which could be advantageous for them.

The sea ice living biomass was almost always dominated by algae (except for some internal layers of ARISE) as it is classically reported for sea ice (Lizotte 2003). The algal community was dominated by diatom cells, but flagellates and *Phaeocystis* sp. cells (and colonies) represented a high contribution in some ice layers (chapters 1, 3 and 4). Autotrophic dinoflagellates and ciliates were also observed reaching sometimes high biomasses (chapters 1 and 4). The diatoms included pennate and centric species with about 30 different diatom species observed for all samples. The here reported diatom species (e.g. *Amphiprora* sp., *Corethron* sp., *Eucampia* sp., *Nitzschia* sp. ...) were all already observed in sea ice (Garrison 1991, Garrison et al. 2005). Some species typical of sea ice such as small pennate diatoms *Fragilariopsis* sp. and the prymnesiophyte *Phaeocystis antarctica* (Lizotte 2001) were observed here. Large (up to > 100 µm) or long chains of diatom cells were present particularly in the bottom ice layers. A distinct algal community in surface ice and in bottom ice have been noted during ISPOL and SIMBA cruises (chapters 3 and 4). Flagellates such as *Phaeocystis sp.* dominated in surface while large pennate diatoms dominated in bottom layers. Accordingly, literature reported that *Phaeocystis* species are more usually found in sea ice habitats not constrained by the brine channel systems such as surface ponds, rotten summer ice or freeboard/infiltration layers (Brierley and Thomas 2002). That species pattern could be related to the mode of incorporation with episode of flooding which could bring microorganisms from the surface water to the surface ice layers or alternatively result from particular needs of each species such as light levels or major nutrients concentrations.

Generally abiotic factors such as light, temperature, salinity or nutrients are considered to control ice algal growth and explain spatial and temporal distribution of sea ice microorganisms (Arrigo and Sullivan 1994).

In pack ice samples from ARISE and ISPOL, the maximal concentration of algae was observed in the bottom layer of ice, at the interface with ocean water (chapters 1 and 4). That accumulation in the bottom ice is not especially typical of pack ice (Ackley and Sullivan 1994). Nevertheless the bottom ice is a favourable habitat because it could benefit from more exchanges with seawater (e.g. major nutrients replenishment...), less variation in salinity and suitable light levels (Thomas and Papadimitriou 2003). At end of winter-early spring (ARISE), the brine volume i.e. the space available for microorganisms controls the accumulation of algae (Chapter 1). During SIMBA, the algal biomass (Chl *a*) peaked in the surface layer at the Liège station (120 cm) while a rather vertically spread distribution of algae (Chl *a*) was observed at Brussels station (chapter 3). This distribution of algae at Liège station is very likely driven by the light available in function of the snow and ice thicknesses (chapter 3). The distribution of algae in sea ice was also controlled by the major nutrients availability in spring/summer (Chapter 3 and 4).

Bacteria which not depend on light were more evenly distributed along the ice core. Bacteria contribute for a small percentage of the total biomass in bottom layers but could represent a high percentage in the internal layers (chapter 1). No general relationships between algae and bacteria could have been observed even if originally they are thought to be incorporated together (Grossmann and Gleitz 1993, Grossmann and Dieckmann 1994). Heterotrophic protozoa were mainly composed of dinoflagellates and ciliates (chapters 1 and 4). Ciliates with ESD < 40 µm and dinoflagellates with ESD around 11 µm (median) dominated in ARISE. They likely grazed on bacteria and nanoflagellates (chapter 1).

Oppositely to sea ice, small (< 10 µm) algae and protozoa dominated in seawater during end of winter/spring and in seawater the heterotrophic biomass was largely higher than the autotrophic biomass (chapter 1). During the transition from spring to summer, similarities between seawater and sea ice algal species were observed likely suggesting a seeding from sea ice (chapter 4).

2.2. Concentrations and composition of organic matter in sea ice

For our 3 sea ice cruises, results showed DOC and POC accumulation in sea ice, compared to underlying seawater (Tables D.1 and D.2) in agreement with literature (e.g. Thomas and Papadimitriou 2003). The measured concentrations were in a similar range than literature values for different Antarctic locations (Tables D.1 and D.2).

Table D.1 Concentrations of particulate organic carbon (POC, µM C), particulate saccharides (p-TCHO, µM C), and transparent exopolymeric particles (TEP, µg XAG l^{-1}) measured in samples from Antarctic sea ice and seawater, compiled from literature

Location, season and sample type	*POC* *µM C*	*p-TCHO* *µM C*	*TEP* *µg XAG l^{-1}*	*Reference*
1. Antarctic Sea ice				
Weddell Sea,				Garrison and Close 1993
- Winter young pack (20-50cm)	98 ± 69	-	-	
- First year & older pack (<100cm)	184 ± 127	-	-	
Ross Sea, autumn				Garrison et al. 2003
- Infiltration ice	39	-	-	
- Frazil ice	134	-	-	
- Congelation ice	46	-	-	
Weddell Sea, summer/early autumn	175 ± 240	-	-	Kennedy et al. 2002
Bellingshausen Sea, late autumn	216 [16-832]	-	no quantif.	Meiners et al. 2004
Ross sea (Terra Nova Bay), spring, pack ice	-	38 ± 8 [18-77]	-	Guglielmo et al. 2000
East Antarctica (110°E - 130° E), sept/oct, pack ice	3.2 - 275.8	-	2.8 - 2690	van de Merve et al. under rev
Australian sector (64-65°S, 112-119°E), end winter-early spring	*29 - 399*	*<DL - 38*	*20 - 2703*	*This study, ARISE*
Western Weddell Sea, Antarctic Peninsula (68°S, 55°W), spring-summer	*14 - 470*	*<DL - 52*	*3 - 3071*	*This study, ISPOL*
Bellinghausen Sea,				*This study, SIMBA*
- *Brussels*	*10 - 118*	*0.6 - 13*	*4 - 173*	
- *Liège*	*10 - 114*	*0.4 - 10*	*4 - 189*	
2. Antarctic Seawater				
Weddell Sea, winter	5	-	-	Garrison and Close 1993
Weddell Sea, summer/early autumn	17 ± 8	-	-	Kennedy et al. 2002
Bellinghausen Sea, late autumn	9 [3-30]	-	-	Meiners et al. 2004
Terra Nova Bay (Ross Sea), summer, under pack ice	20-100	1-7 *(19% POC)*	-	Pusceddu et al. 1999
Ross Sea, summer	19		-	Fabiano et al. 1993
Terra Nova Bay (Ross Sea), summer	12.64 ± 8.91 [1.01-39.23]	2.27 ± 2.17 [0.28-14.80] *(18% POC)*	-	Fabiano et al. 1996
Bransfield Strait, summer	-	-	56.77 ± 54.50	Corzo et al. 2005
Ross Sea (bloom Phaeo), Nov-Dec	-	-	308 [0-2800]	Hong et al. 1997
Australian sector (64-65°S, 112-119°E), end winter-early spring	*5.0 ± 2.5 [1.7 - 9.2]*	-	*133 - 853*	*This study, ARISE*
Western Weddell Sea, Antarctic Peninsula (68°S, 55°W), spring-summer	*3.6 ± 2.9 [1.0 - 10.8]*	-	-	*This study, ISPOL*
Bellinghausen Sea,				*This study, SIMBA*
- *Brussels*	*0.5 - 1.8*	*<DL - 0.1*	*<DL - 5*	
- *Liège*	*0.6 - 1.8*	*<DL - 0.2*		

Mean values ± standard deviation and ranges are given when available
<DL below detection limit
* TEP were quantified in abundance and not in concentration in µg XAG l^{-1} in the study of Meiners et al. (2004)

Table D.2 Concentrations of dissolved organic carbon (DOC, µM C), dissolved saccharides (d-TCHO, µM C), and dissolved free amino acids (DFAA, µM C) measured in samples from Antarctic sea ice and seawater, compiled from literature

Location, season and sample type	*DOC µM C*	*d-TCHO µM C*	*DFAA µM C*	*Reference*
1. Antarctic Sea ice				
Perennial pack ice	0 – 2000	-	-	Thomas et al 1998, 2001a
Weddell Sea, April / May	109 ± 83.5 [16 – 556]	-	-	Thomas et al. 2001a
Weddell Sea, January / March	207 ± 239.6 [16 – 1842]	-	-	Thomas et al. 2001a
Weddell Sea, platelet	100 – 200	-	-	Thomas et al. 2001b
Weddell Sea, summer	254 ± 133	53 ± 22 (*29% DOC*)	-	Herborg et al. 2001
Davis Station, Nov	-	-	8-30	Yang 1995
East Antarctica (110°E - 130° E), sept/oct, pack ice	30.5	-	-	van de Merve et al. under rev
Australian sector (64-65°S, 112-119°E), end winter-early spring	*80 - 717*	*1.3 - 124*	*0.1 - 29.8*	*This study, ARISE*
Western Weddell Sea, Antarctic Peninsula (68°S, 55°W), spring-summer	*106 - 701*	*3.3 - 139*	*0.1 - 13.8*	*This study, ISPOL*
Bellinghausen Sea,				*This study, SIMBA*
- *Brussels*	*27 - 232*	*3.8 - 85*	-	
- *Liège*	*9 - 198*	*1.1 - 69*	-	
2. Antarctic Seawater				
Antarctic Ocean average	<60	-	-	Ogawa and Tanoue 2003
6°W meridian between 47° & 60°S, surface, early spring	38 - 55	-	-	Kähler et al. 1997
Weddell Sea, summer	78 ± 50	8 ± 4 (*18% DOC*)	-	Herborg et al. 2001
off Queen Maud's Land (69°38'S, 03°54'E), Jan	-	8-9	-	Myklestad et al. 1997
Drake Passage, Bransfield Strait, Weddell Sea, Dec	-	-	0.44 ± 0.16 *	Hubberten et al. 1995
Gerlache Strait, spring	-	-	0.3 - 0.6	Tupas et al 1994
Davis Station, May-Feb	-	-	0.30	Yang et al. 1990
Australian sector (*64-65°S, 112-119°E*), end winter-early spring	102.5 ± 66.7 [43.3-271.7]	-	-	*This study, ARISE*
Bellinghausen Sea,				*This study, SIMBA*
- *Brussels*	*44 - 60*	*3.5 - 6.3*		
- *Liège*	*46 - 62*	*2.7 - 5.0*		

Mean values ± standard deviation and ranges are given when available
* Expressed in µMN

One important result lies in the different partition of the organic carbon between the dissolved (DOC) and particulate (POC) fraction when comparing sea ice and seawater (chapters 1 and 3). We showed that the DOC:POC ratio ranged between 1:1 and 4:1 in sea ice (chapters 1 and 3) smaller than the traditional ocean ratio (15:1; Millero 1996, Kepkay 2000) but in agreement with ratios typically found in biofilms and aggregates (Giani et al. 2005). Following aggregation theory (Chin et al. 1998), this could be explained by a transfer of DOC into TEP and POC above a DOC threshold concentration. Indeed according to Verdugo et al. (2004), OM molecules form a size continuum, with TEP at the interface between DOC and POC. In sea ice, TEP reaches high concentration as compared to seawater: TEP contributed in average to 7.1 ± 8.5% of TOC or roughly 25.8 ± 18.9% of POC (chapter 2). Moreover TEP are clearly an integral part of the structural organization of a biofilm (Decho 1990).

Table D.3 Contribution of particulate saccharides in the particulate organic carbon (%p-TCHO/POC) and of dissolved saccharides in the dissolved organic carbon (%d-TCHO/DOC) in sea ice and seawater.

	%p-TCHO/POC	%d-TCHO/DOC	Ref
Sea ice			
ARISE	*1.1 - 24.1*	*0.4 - 21.5*	*This study*
ISPOL	*0.2 - 19.7*	*0.9 - 29.6*	*This study*
SIMBA Brussels	*5.9 - 16.1*	*9.1 - 46.9*	*This study*
SIMBA Liège	*3.0 - 10.3*	*5.0 - 67.2*	*This study*
Weddell Sea	-	29	Herborg et al. 2001
Weddell Sea	-	36 (1 - 99)	Thomas et al. 2001
Seawater			
Literature average	5 - 20	10 - 25	Wakeham et al. 1997, Volkman and Tanoue 2002, Benner 2002
SIMBA Brussels	*0.4 - 10.6*	*6.6 - 12.8*	*This study*
SIMBA Liège	*0.3 - 11.8*	*5.4 - 9.0*	*This study*
SAZ-Sense	-	*2.4 - 25.6*	*This study*
Terra Nova Bay (Ross Sea)	19	-	Pusceddu et al. 1999
Terra Nova Bay (Ross Sea)	18	-	Fabiano et al. 1996
Weddell Sea	-	18	Herborg et al. 2001

Note that we clearly observed a similar distribution and evolution of all organic fractions in sea ice samples from ARISE, ISPOL and SIMBA (chapters 2 and 3). We suggest that the usual subdivision made between OM pools (operationally defined by the porosity of filters) reflects only weakly functional features in sea ice. In sea ice, it seems more adequate to consider the OM pool as a whole than as distinct fractions. That being said, it is convenient to use the dissolved/particulate partition to compare our results with other studies.

One important point to investigate in sea ice is the nature of the organic matter pool as few data are available (Tables D.1 and D.2). We determine the composition of the OM by quantifying its content in particulate and dissolved saccharides (p-TCHO and d-TCHO), in dissolved free amino acids (DFAA) and transparent exopolymeric particles (TEP) by applying spectrophotometric and fluorometric methods. The contribution of particulate saccharides to the POC pool (%p-TCHO/POC) found in sea ice was relatively similar to aquatic systems (Table D.3). The dissolved saccharides contribution to the DOC pool (%d-TCHO/DOC) in sea ice was also similar to the oceanic average for ARISE and ISPOL cruises (Table D.3). By contrast, the %d-TCHO/DOC could represent up to 67% of DOC for SIMBA cruise. Such variable and very high (up to 99%) contributions of saccharides in the DOC have already been observed in sea ice (Table D.3, Thomas et al. 2001). These percentages observed in SIMBA sea ice OM are still in agreement with estimations of the composition of phytoplankton exudates which reported that saccharides represent about 18-45% of POC and about 23-80% of DOC (Biddanda and Benner 1997).

The dissolved saccharides were mainly monosaccharides (d-MCHO) in end winter/early spring and polysaccharides (d-PCHO) in spring/summer (chapters 2 and 3).

Note that the compounds that we measured constitute in average a minor part of the OM, and as in ocean a large part of the OM is still uncharacterized (Benner 2002). However our results confirm the particular importance of saccharides and TEP in the sea ice, as reported in previous studies (Herborg et al. 2001, Thomas and Papadimitriou 2003, Krembs and Deming 2008). We also found that TEP was linearly positively correlated with total (dissolved + particulate) saccharides concentrations, indicating that TEP increased in parallel with the increase of saccharides (chapters 2 and 3). This trend supports the role of saccharides as major constituent of TEP (Passow 2002, Engel 2004).

2.3. Biological and physical controls on organic matter distribution in sea ice

The observed accumulation of OM in sea ice (see 2.2) likely results from different processes. The first possible explanation is the scavenging of living and detrital organic matter by ice crystals when sea ice is formed in winter (Garrison et al. 1983, 1989, Ackley and Sullivan 1994). By the way experimental ice formation experiments showed that DOC was enriched in sea ice as compared to a salt conservative dilution (Giannelli et al. 2001). Still the enrichment process is not yet exactly understood. This problematic was not approached during the present work.

Then the accumulation of OM could be explained by the growth of autotrophic microorganisms and subsequent OM release in sea ice which contribute to the *in situ* production of OM, especially considering the high algal biomasses observed in sea ice (see 2.1, Lizotte 2003). Our observations point that the maximal OM concentrations were almost always found where

autotrophic microorganisms were observed (chapters 1, 2 and 3). For ARISE and ISPOL, positive linear relationships between POC and Chl *a* and TEP and Chl *a* suggest that the increase of POC and TEP was associated with the increase of ice algae biomass (chapter 2). Still POC could be present where very low Chl *a* was recorded (typically in the interior ice habitat). We concluded that that part of the POC pool not related to the *in situ* algal biomass represents a background concentration of about 40 µM C (intercept of the regression line POC vs Chl *a*, see chapter 2). This suggested that this POC had been incorporated or produced earlier in sea ice. The production of TEP is indeed often related to Chl *a* and diatoms (Krembs et al. 2002, Meiners et al. 2003, Riedel et al. 2006, 2007). By contrast the DOC pool was not related to Chl *a* in sea ice (chapter 2). The DOC is indeed a mixture of compounds of different origins and degradation states for which a direct link with Chl *a* is often difficult. Nevertheless observations suggest that a fraction of the OM pool composed of saccharides and amino acids could be considered as a fresh component of the whole OM pool. We indeed observed that 1) maximal concentrations of p-TCHO, d-TCHO and DFFA were found where Chl *a* was maximal and 2) the proportions of these compounds in the OM pool (i.e. %p-TCHO/POC, %d-TCHO/DOC and %DFAA/DOC) were higher where the Chl *a* was higher (chapter 2). More precisely, in sea ice surface and bottom layers which host higher algal biomass, the OM pool could be considered as highly influenced by *in situ* algae metabolism while the OM from internal layers was more slightly influenced by algae (chapter 2). By contrast, no linear relationships between any OM fractions and Chl *a* were observed during the SIMBA cruise (chapter 3). Also no such contrasted situation in the proportion of saccharides to the POC and DOC in function of Chl *a* was noticed for the SIMBA samples. During SIMBA though, we assumed from the increase of the TEP/Chl *a* ratio with decreasing temperature that TEP were produced by algae in response to the stress induced by the cooling (chapter 3).

Finally the accumulation of OM in sea ice indicates a disconnection between its production and consumption pathways (Thomas and Papadimitriou 2003). The accumulation of OM in sea ice could be explained by the limitation of the bacterial activities and/or the low substrate lability both potentially hampering the use of OM. In particular, our study further shows that monomeric compounds accumulated in sea ice at all seasons (chapters 2 and 3) in the form of dissolved monosaccharides (d-MCHO) and dissolve free amino acids (DFAA), in agreement with the study of Herborg et al. (2001). In particular, concentration of d-MCHO up to 100 µM C was measured in the most winter station of ARISE (chapter 2). Usually low concentrations (at nanomolar level) are observed in oceanic waters because d-MCHO and DFFA are very labile substrates (turnover time, minute to hour) and are usually taken up by bacteria too rapidly to build up a detectable pool in the system (Carlson 2002 All factors contributing to the reduction of bacterial growth rate, biomass and carbon demand would lead to such an accumulation of this potentially biodegradable DOC (Thingstad et al. 1997). As high pools of monomeric substrates, in the form of monosaccharides and amino acids, could build up in the different ice samples, are very likely labile, it is thus the consumption by bacteria which seems inappropriate. We indeed showed that the elemental composition and inorganic nutrient did not seem to be the main factors limiting OM consumption (chapter 2). The comparison between a winter and a spring station suggested that bacterial activities would be limited by an external factor rather than by the nature of the OM. Our observations do not enable us to conclude which environmental factor among temperature, salinity or toxic compound (such as acrylic acid) is limiting the bacterial activities. Yet these results are consistent with the works of Kähler et al. (1997) which also stated the OM from sea ice seems labile and with the assumption that the sea ice bacteria could be limited by temperature (Pomeroy and Wiebe 2001).

In addition to these biological production and removal pathways of OM in sea ice, the distribution of the organic parameters also strongly depends on the physical properties (temperature, salinity, brine volume) of the sea ice cover (Ackley and Sullivan 1994). The temperature which controls the brine salinity and volume structures the internal sea ice ecosystem (Eicken 1992). It is considered that for brine volumes above a threshold of 5%, the network of brine inclusions connects and sea ice becomes permeable to fluid transport (Golden et al. 1998). During ARISE, at the transition between winter and spring, we observed that the accumulation of algae biomass was only possible above a brine volume of 8%, corresponding to a temperature of - 4°C in agreement with Krembs et al. (2001) (chapter 1). During ISPOL and SIMBA cruises, where brine volumes were well above 5%, other temperature-driven processes controlled the distribution and evolution of microorganisms (and organic matter) in sea ice. During ISPOL the evolution of the organic parameters were controlled by brine drainage, followed by stabilization and convection mechanism (chapters 2 and 4), as also shown for the evolution of nutrients in relation with the brine dynamics (Vancoppenolle et al. submitted). Similarly the temperature and salinity evolution of the ice cover during the SIMBA cruise, characterized by a flood-freeze cycle controlled the behaviour of the microbial and organic pool (chapter 3).

2.4. Consequences of OM accumulation on biogeochemistry in sea ice

The accumulation of organic matter and particularly of TEP has a major impact on the microbial life environment of sea ice by modifying physical and chemical parameters but also by influencing the biological interactions (Brierley and Thomas 2002, Thomas and Papadimitriou 2003).

The chemical properties and viscous nature of TEP modify the brine channels into a gel-filled system. Like imagined by Krembs et al. (2000) and Mock and Thomas (2005), the sea ice internal habitat resembles that of a biofilm adhering to the brine channel walls, with an overlying flowing liquid phase i.e. the brine fraction. The formation of a biofilm was likely in our samples as first the values of DOC:POC ratios and TEP presence suggested (see 2.2). The biofilm was also suggested by the observations that microorganisms but also dissolved compounds remained attached to the brine walls and embedded in an extracellular gel-like matrix of polymeric substances which prevented them from being collected by brine sackhole sampling (chapter 1), resulting in a difference between the concentration measured in brine and concentration estimated from bulk ice concentration and brine volume (chapter 1). Besides the results from the sequential melting experiment also argued towards the biofilm presence as it is the only way to explain the timing of release of the organic compounds (see 3.1.).

The gel-filled system we observed also seems to adsorb nutrients like phosphate (chapter 1) or iron (Schoemann et al. 2008) as suggested by Decho (1990) or Davey and O'Toole (2000). We observed a parallel accumulation of TEP, DOC, Fe and PO_4^{3-} in sea ice, which can be explained by the association of OM-Fe-P complexes due to chemical bindings between Fe (oxy)hydroxides, DOM and PO_4^{3-} (Maranger and Pullin 2003). Similarly, Schoemann et al. (2008) found that the OM could have a role in the accumulation of iron in sea ice, with likely a different complexation capacity for the old (detrital) and fresh POC as suggested by two relationships between POC and PLFe (particulate iron) in sea ice (see Figure B6.20 in Tison et al. 2008). The complexation capacity of the OM has to be related to the presence of some chemical groups such as acidic polysaccharides which tend to form complex with anions (Decho 1990). Such a reservoir effect of the biofilm may buffer the direct effect of nutrient depletion and allow maintaining a nutrient-rich environment around cells (chapter 1).

TEP have also been reported to sequester toxins (Decho 2000, Krembs and Deming 2008). In sea ice, high algal toxin production such as acrylic acid which derived from the degradation of dimethylsulphonio-propionate (DMSP) can occur. High concentration of DMSP and DMS were measured in sea ice from ARISE, ISPOL and SIMBA (Tison et al. 2008). Accumulation of toxins with TEP might have inhibitory effects on the bacterial growth and therefore on the utilization of organic carbon and TEP (Brierley and Thomas 2002). Likewise, healthy *Phaeocystis* colonies are preserved from bacterial colonization by high acrylate concentrations sorbed on the colony mucus (Noordkamp et al. 2000). Hence, at the time of ice melting and sympagic biofilm release to the water column, the toxicity of acrylic acid would be diminished by seawater dilution effect, allowing then the quick consumption of organic carbon (DOC, POC and TEP) by pelagic bacteria.

Then this gel phase inside the brine channels modify the classical (meaning aquatic) microbial interactions. Indeed the movement of microorganisms may be restricted because of the TEP presence. It could act as protectant against predators (Decho 1990, Passow 2002). During ARISE, the prey-grazer (i.e. protozoa-bacteria) interactions seemed to be modified as suggested by the analysis of absence/presence of prey and grazers and numerical response curves (see details in chapter 1). This showed that the protozoa biomass only increased when a food threshold was reached (chapter 1). Indeed the presence of high TEP has been observed to change grazer mobility (Joubert et al. 2006) and regulate their interactions. This is in agreement with the study of Riedel et al. (2007) where a negative correlation was found between TEP concentration and experimentally derived ingestion rates of bacterivores in Arctic sea ice. The presence of TEP coupled with the narrow channel space might thus well be another reason for the apparent breakdown of the classic microbial loop in the sea ice and the subsequent accumulation of OM (Thomas and Papadimitriou 2003).

3. Impacts of sea ice melting

The melting of sea ice in spring release organic and inorganic nutrients as well as microorganisms in the water column. The impact of this event on the biological carbon pump was approached by combining results from observations and laboratory experiments. Firstly the sequence of melting is discussed. Then the supply of OM for the ocean below is estimated from the quantification of sea ice OM in function of the mixed layer depth. The characterization of the organic matter pool in sea ice give us clues about its potential role during melting and once released in the water column. Finally, the results of the microcosms experiments tend to support the possibility for ice microorganisms to maintain and grow once released in the seawater as developed below.

3.1. Time sequence of melting

The Antarctic sea ice is not melting at once. The gradual warming of the ice cover induces changes in the structure of the ice matrix and brine movement. The process of melting is key for the pelagic ecosystem. On the one hand, the melting of the ice cover releases a large volume of freshwater in the surface ocean which decreases its salinity and forms a shallow meltwater lenses in the upper ocean (Smith and Nelson 1985). On the other hand, the release of the organic and inorganic components present in the brine channels depends on the dynamics of the brines which is driven by gravity or convection processes (Eicken 2003). The delay between the releases of freshwater which stabilizes the water column and that of the organic components determines the possible utilization of this OM by the surface layer microbial community.

During the sequential melting experiment (chapter 4) we observed the following steps:

- brine drainage and iron flux from sea ice into seawater, occurring certainly before the stabilization of the mixed layer
- OM flux after the end of brine drainage. The stabilization of the mixed layer is thus possible at time of maximal OM flux from sea ice into seawater. At that time, iron is still released from sea ice

As said before (2.4), we suggest that the timing of release of the ice components observed in that experiment could only be explained by the presence of a gel-matrix (biofilm) which embeds microorganism but also maintained dissolved elements on the walls of the brine channels. Moreover in function of the time when iron is released (i.e. before of after the stabilization of the mixed layer), iron could stimulate or not phytoplanktonic blooms.

3.2. Significance of the OM supply for the ocean

3.2.1. Fluxes of DOM

The OM accumulated in sea ice will finally be released in the underlying surface ocean. The significance of the OM supply for the ocean depends on the volume in which OM will be diluted. Firstly, considering an integrated stock of DOC in sea ice of 27 - 416 mmol C m^{-2} (Table D.4) and a hypothetical unique supply (i.e. all DOC is released at the same time), the increase of DOC concentration would be equivalent to 0.5 - 8.3 µM C in the upper 50 m or of 2.7 - 41.6 µM C in the upper 10 m of ocean (Table D.4). So in function of the mixed layer depth, the DOM released from sea ice would represent an important supply of organic substrates for the pelagic community as compared to a winter DOC of 42 µM C (Carlson et al. 2000).

Still if we consider a gradual release of OM during the melting of the ice cover, the significance of the OM supply could be less important: considering the same integrated stock of DOC in sea ice of 27 - 416 mmol C m^{-2} (Table D.4) and a 30 day-period of melting, the daily flux to the ocean is estimated to 1 - 14 mmol C m^{-2} d^{-1} into the seawater (Table D.4). It corresponds to small (< 1 µM C) DOC inputs even in a 10 m mixed layer (Table D.4). Besides in function of the dynamics of the brines, the release the OM supply could be variable and occur by pulse. The released OM could directly be remineralized, exported with brine drainage, scavenged by aggregates or diluted in a too deep surface mixed layer (in case of strong winds).

Table D.4 Integrated stocks of DOC in sea ice and estimations of the significance of their release in a 50 m and 10 m deep underlying water column for a) a unique supply and b) a 30-days-meltng period.

		Unique supply		30 days melting		
	Sea ice DOC *mmol C/m²*	added to upper 50 m *mmol C/m³*	added to upper 10 m *mmol C/m³*	daily DOC supply *mmol C/m²/d*	added to upper 50 m *mmol C/m³*	added to upper 10 m *mmol C/m³*
ARISE Stn IV	121	2.4	12.1	4.0	0.08	0.4
ARISE Stn V	138	2.8	13.8	4.6	0.09	0.5
ARISE Stn XII	186	3.7	18.6	6.2	0.12	0.6
ARISE Stn IX	89	1.8	8.9	3.0	0.06	0.3
SIMBA BXL 1	27	0.5	2.7	0.9	0.02	0.1
SIMBA BXL 2	45	0.9	4.5	1.5	0.03	0.2
SIMBA BXL 3	39	0.8	3.9	1.3	0.03	0.1
SIMBA BXL 4	74	1.5	7.4	2.5	0.05	0.2
SIMBA BXL 5	48	1.0	4.8	1.6	0.03	0.2
SIMBA LGE 1	77	1.6	7.7	2.6	0.05	0.3
SIMBA LGE 2	99	2.0	9.9	3.3	0.07	0.3
SIMBA LGE 3	88	1.8	8.8	2.9	0.06	0.3
SIMBA LGE 4	48	1.0	4.8	1.6	0.03	0.2
SIMBA LGE 5	74	1.5	7.4	2.5	0.05	0.2
ISPOL 04.12.04	416	8.3	41.6	13.9	0.28	1.4
ISPOL 09.12.04	293	5.9	29.3	9.8	0.20	1.0
ISPOL 14.12.04	337	6.7	33.7	11.2	0.22	1.1
ISPOL 19.12.04	246	4.9	24.6	8.2	0.16	0.8
ISPOL 30.12.04	214	4.3	21.4	7.1	0.14	0.7

The microcosm experiments were designed to simulate a 1:100 sea ice/seawater melting (i.e. 10 cm of ice in a 10 m water column). Results showed that under these peculiar conditions of microcosms, the supply of DOC from the bottom sea ice was sufficient to stimulate bacterial growth (chapter 4).

3.2.2. Lability of organic matter

We conclude from our observations about the composition of the OM that the OM present in the sea ice is potentially labile because of the accumulation of monomeric compounds such as monosaccharides and amino acids observed in sea ice, for all samples and in particular in winter (chapters 2 and 3). If we assume that these monomers are easily consumed by bacteria (Carlson 2002, Nagata 2008), these organic compounds once in the water column are directly available for heterotrophic organisms. The bacteria sustained by these new organic substrates could eventually provide essential nutrients for phytoplanktonic growth by remineralizing the OM. Other studies looking at the lability of ice-derived OM conclude that DOM seems very labile (Kähler et al. 1997, Pomeroy and Wiebe 2001) while a recent study by Pusceddu et al. (2009) is more sceptical and suggests that the OM originating from algae accumulated in the bottom ice, oppositely to OM from platelet ice, could significantly reduce bacterial production, which they related to an allopathic control of sympagic algae on bacterial growth. Still it is reasonable to think that when OM is diluted in the seawater, effect of toxins may be diminished and OM would be then degraded.

3.2.3. Fe-OM complexation in surface waters

The characteristic of the sea ice OM pool constituted by saccharides and TEP (chapters 2 and 3) may have profound impact on the cycling of iron, also in high concentration in sea ice (Lannuzel et al. 2007, 2008), once they will be released in the seawater. Indeed the co-occurrence of Fe and OM in surface waters possibly leads to the formation of complexes Fe-OM as it is known that >90% of Fe is strongly bound to organic matter in the surface oceanic water (Boyé et al. 2001). These complexes may retain Fe in surface waters (Kuma et al. 1996), increase its solubility and influence the iron bioavailability for planktonic organisms. Recent experiments reported that saccharides could indeed enhance Fe bioavailability by complexing it (Hassler and Schoemann 2009). The close association of iron and OM in surface waters would allow the consumption by microorganisms which could finally lead to a phytoplanktonic bloom. During the SAZ-Sense and SIMBA cruises, experiments were realized to study the bioavailability of different Fe-OM complexes (Schoemann et al. in prep, Masson et al. in prep). Results suggest that some organic ligands decrease Fe bioavailability (i.e. EDTA, DFB), whereas others (PIX, GLU) can favour its bioavailability.

3.2.4. Aggregates formation

Alternatively the nature of the organic matter present in sea ice could enhance the formation of aggregates. These aggregates can be composed of microorganisms, detritus and could also bind iron. Being dense, the formed aggregates are exported below the surface layer. The role of TEP in aggregate formation is well known (Passow 2002); we therefore hypothesize that the significant contribution of TEP to sea ice OM (chapters 2 and 3) may plays a key role in driving exportation of the ice-derived OM and associated elements such as complexed Fe and organisms. Ice-diatoms producing high TEP concentrations (Krembs and Engel 2001) are forming aggregate (Riebesell et al. 1991).

3.3. Fate of ice microorganisms

The receding ice-edge is often associated with phytoplanktonic bloom events (Smith and Nelson 1986, Lancelot et al. 1993, Arrigo et al. 2008). Indeed the melting of sea ice promotes water stratification, increases the light available in the water column and releases microorganisms being potentially a seeding population for blooms (Mathot et al. 1991, Knox 1994, Gleitz et al. 1996). Still sea ice algae would be an inoculum to phytoplanktonic blooms only if they are not grazed (Marschall 1988), not exported (Riebesell et al. 1991) and able to survive in the seawater environment (Kuosa et al. 1992). In addition sea ice melting supplies organic matter (Giesenhagen et al. 1999) and iron (Sedwick and Di Tullio 1997, Lannuzel et al. 2008, Lancelot et al. submitted) into the water column, which can then stimulate growth of microorganisms.

The comparison between ice and pelagic algal communities at the time of ice melting along with results from the microcosm experiments (chapter 4) both suggest that at least a fraction of the ice microalgae may contribute to the bloom in the marginal ice zone. Indeed a part of the microorganisms seems able to develop in the water, mainly the smaller species (< 10 µm). The decrease of >10 µm algae could be explained by the lower iron concentrations in seawater compared to sea ice (Lannuzel et al. 2008). Iron concentrations in seawater could be limiting for large size diatoms (Timmermans et al. 2001, 2004), which are used to the higher iron-concentrated (up to two orders of magnitude) system of the sea ice. Still grazing and sedimentation were not addressed in this experimental setting and it would be difficult to draw general conclusions about the fate of the microorganisms from these experiments only.

3.4. Significance of sea ice in the biological carbon pump in the Southern Ocean

It is generally thought that primary production in sea ice is significant and could greatly contribute to the Southern Ocean productivity maybe not only quantitatively but seasonally and regionally (Lizotte 2001). The organic carbon exported from sea ice to the pelagic system (Riebesell et al. 1991) is also influencing the biological carbon pump. The episodic release of bioavailable iron from melting sea ice seems an important factor regulating phytoplankton production, particularly ice-edge blooms, in seasonally ice-covered Antarctic waters (Pasquer et al. 2005, Lancelot et al., submitted). The present study brings observations comforting these ideas that sea ice has a key role in seeding the water column at time of melting. Still to fully address the carbon cycle in ice-covered ocean, coupled sea ice-ocean ecosystem models are needed. As a step in this direction the existing ice-ocean-biogeochemical models 3D NEMO-LIM-SWAMCO (Lancelot et al. submitted) has allowed to investigate the different mechanisms of iron supply for phytoplankton blooms.

4. OM degradation in Antarctic waters

As explained in the introduction section, the efficiency of the biological carbon pump is determined by the quantity and nature of primary production but also by the strength of the organic matter remineralization along the water column. This section presents the main results concerning the composition of the dissolved organic carbon, the bacterial degradation of the OM in the epipelagic (0-100 m) and mesopelagic (100-700 m) waters (chapter 5).

During the SAZ-Sense cruise, observations made in the Sub Antarctic Zones (SAZ) and Polar Front Zones (PFZ), suggest that the OM was the main factor controlling the distribution of bacteria (chapter 5). The east-SAZ region, more productive because of iron-replete conditions, also has higher bacteria abundances in surface layer compared to the iron-deplete west-SAZ region.

4.1. OM composition along the water column

Accordingly to the Chl *a* distribution, the surface dissolved organic carbon (DOC) was higher in the east-SAZ (84 µM C) than in west-SAZ (56 µM C) and in the Polar Front (55 µM C). The pool of OM seems thus relatively directly related to phytoplankton in these regions resulting in a positive correlation between DOC and Chl *a* ($r^2 = 0.49$).

The DOC concentrations were higher in the upper 100 m of the water column than below. Concentrations of dissolved saccharides (d-TCHO) showed a rather similar trend with depth as DOC. d-TCHO ranged between 1.3 and 13.4 µM C along the water column. They accounted for between 2.4% and 25.6% of the DOC but this percentage did not show any particular trend with depth or region. Dissolved saccharides were mainly on the polymeric form (69 ± 19%) except in the surface layer of Polar Front, where they accounted for less than 49% of total saccharides.

A fraction of the DOC in surface water constitutes "labile" organic compounds (turnover time: minutes to days), such as dissolved saccharides (d-TCHO), which are substrates available for bacteria, and rapidly removed from the system. Another fraction of the surface DOC represents "semi-labile" organic compounds (turnover time: weeks to months), which is estimated as the DOC stock in excess of the refractory pool present in the deep ocean (Carlson 2002). Significant spatial variability in the excess DOC (with [excess DOC] = [DOC concentration] – [background Antarctic DOC]) was observed in the surface waters of the different studied areas. Considering a background DOC of 42 µM C as assessed by Wiebinga and de Baar (1998), the excess DOC was 12 ± 3 µM C, 17 ± 11 µM C and 36 ± 21 µM C in west-SAZ, Polar Front and east-SAZ respectively. This semi-labile pool, which escapes rapid microbial degradation in the upper 100 m, could thus constitutes a significant direct C export to mesopelagic depths by turbulent diffusion and advection.

The distribution of the organic substrates, with higher contents in east-SAZ as compared to west-SAZ appears thus well reflected in the magnitude of bacterial abundance and activities in each region. The organic substrates seem therefore to influence rather directly bacterial activity, as it is often the case in open-ocean areas where bacteria are directly depending on the autochthonous resources supply (Moran et al. 2002).

4.2. Fate of exported OM in the mesopelagic layer

At depth (> 100m), OM concentration and bacterial abundances were lower than in surface. Still the OM present in the mesopelagic zone may sustain active bacterial growth which is far from negligible. The depth-integrated values through the thickness of the different ocean layers showed that the contribution of the bacteria in the mesopelagic zone represented, respectively, up to 53 ± 7%, 43 ± 14% and 33 ± 19% of the total bacterial biomass, ectoproteolytic activity and bacterial production (chapter 5).

In the Polar Front Zone, the mesopelagic remineralization was relatively more important than in Sub- Antarctic Zone (chapter 5). In the PFZ, the algal

community is dominated by diatoms, which was not the case in the SAZ. It has already been reported in literature that diatom-dominated systems export more labile organic matter (Buesseler 1998, François et al. 2002, Cardinal et al. 2005). Diatom-derived matter is denser and sinks rapidly, so that it is little degraded when reaching the mesopelagic layer. As a result, this freshly-derived organic matter is more efficiently remineralized during its transit through intermediate depths, reducing its transfer efficiency to the deep sea (François et al. 2002, Panagiotopoulos et al. 2002).

This result stresses the importance of considering not only processes in the surface layer but along the water column to evaluate the efficiency of the biological C pump. A fast-sinking algal community does not necessarily mean a large carbon export to the deep ocean, as heterotrophic processes occur at all depths.

Note also that as the ice algal community is dominated by diatoms, a similar scenario could be considered leading to a significant export of diatom-derived matter below the surface layer.

4.3. Carbon fluxes through bacteria

In order to evaluate the efficiency of the biological carbon pump, the carbon fluxes through the bacteria have to be determined. Still a lot of uncertainties and methodological difficulties hamper to directly and easily budgeting the carbon fluxes. These issues, dealing with the choice of the conversion factors and the measurement of bacterial activities, are discussed in chapter 5.

During the SAZ-Sense cruise, the bacterial carbon demand (BCD) was determined from measurements for bacterial production (using a min-max range of conversion factors, see chapter 5) and from a min-max range of literature values for bacterial growth efficiency (BGE) (see chapter 5).

Estimated BCD was compared to primary production, C export fluxes (^{234}Thorium proxy) and remineralization (Barium proxy) (Table 5).

As reported in Table 5, estimated BCD gives high values as compared to local primary production (Westwood et al. submitted). The minimal and middle estimation of BCD would be in agreement with the estimated local primary production and this holds for the surface water BCD as well as for the water column (0-700 m) integrated BCD. In the case of the minimal estimate, the BCD would use in average 13, 12 and 21% of the primary production in E-SAZ, PFZ and W-SAZ, respectively whereas in the case of the middle estimate the proportion of local PP used by bacteria rise up to 58, 52 and 85% in E-SAZ, PFZ and W-SAZ.

In addition, previous comparisons of BCD (from mesopelagic BP measurements) with C export suggest that BCD accounts for 14% to > 100% of C export (Cho and Azam 1988, Nagata et al. 2000, Simon et al. 2004, Reinthaler et al. 2006, Steinberg et al. 2008). During SAZ-Sense, BCD was compared to C export estimates based on the ^{234}Th deficit method (Jacquet et al.-b submitted) and to mesopelagic remineralization estimated from the excess particulate barium contents (Jacquet et al.-a submitted) at the same sites. In the SAZ only the lower estimates of BCD are compatible with C export out of the surface layer. In the PFZ, however, any realistic estimation of BCD exceeds the C export. Overall, mesopelagic BCD values were higher than mesopelagic remineralization estimated from excess Ba contents, but the similarity in spatial distributions of both parameters persists (Jacquet et al.-a, submitted) with highest values in the PFZ.

The discrepancies which arose between these values can be due to many reasons cited below (and longer discussed in chapter 5 and also Jacquet et al.-a submitted, Annex 1):

- Uncertainties in the method (conversion factors)
- Heterogeneity (particles vs bulk values)
- Differences of OM pool considered: C export fluxes as estimated by the ^{234}Th proxy reflect the POC export while the bacterial carbon demand is related to the whole OM pool (POC + DOC)
- Differences in bacterial communities considered: the formation of excess Ba-barite is related with the remineralization of OM in aggregates due to attached bacteria whilst bacterial communities consist of both attached and free bacteria for which the C source is POC as well as DOC
- Differences in timescales of measurements
- Hydrodynamics

Despite some uncertainties in carbon flux estimations and discrepancies between methods, the present study highlights the importance to study bacterial dynamics in the twilight zone and their preponderant role in shaping the carbon fluxes through the water column. It is thus essential to include the twilight zone remineralization, provided methodological developments, in the assessment of the fate of fixed carbon and global C cycle in order to improve our vision of the biological C pump in the Southern Ocean and its influence on global climate.

Table 5
Column-integrated Gross Primary Production for the euphotic layer (GPP, from Westwood et al. submitted), Export Production at 100 m (EP_{100m}, from Jacquet et al. b submitted) and bacterial carbon demand (BCD) over the surface layer (0-100 m), the mesopelagic layer (100-700 m) and the total water column (0-700 m) for all the stations sampled during SAZ-Sense. Process Station P1, P2 and P3 have been sampled on different days (d). All values are expressed in mmol C m^{-2} d^{-1}. W- and E-SAZ: west and east Sub-Antarctic Zone, PFZ: Polar Front Zone, SAF: Sub-Antarctic Front, EAC: East Australian Current, STZ: SubTropical Zone

* GPP have been determined from CTD close in time but not exactly from the same CTD as EP and BCD

Stations	Long. °E	Lat. °S	Water masses	GPP*	EP_{100m}	BCD_{0-100m}	$BCD_{100-700m}$	BCD_{0-700m}
SS2	143.053	44.889	W-SAZ	-	4.3	**22.4** 5.1-86.8	**12.5** 2.9-49.2	**34.9** 8.1-136.0
P1 d3	140.307	46.496	W-SAZ	162	4.8	**44.7** 10.3-173.1	**11.2** 2.6-43.5	**55.9** 12.8-216.7
P1 d5	140.641	46.580	W-SAZ	59	4.1	**31.5** 7.2-122.1	**17.3** 4.0-67.0	**48.8** 11.2-189.1
P2 d1	145.867	54.014	PFZ	90	-	**30.3** 7.0-117.5	**13.4** 3.1-52.0	**43.7** 10.0-169.5
P2 d2	146.128	54.020	PFZ	96	2.9	**24.2** 5.6-94.0	**19.8** 4.8-80.6	**44.0** 10.3-174.6
P2 d3	146.303	54.138	PFZ	-	6.6	**20.8** 4.8-80.5	**58.4** 13.4-226.5	**79.2** 18.2-307.0
P2 d6	147.103	54.447	PFZ	72	-	**31.2** 7.1-120.3	**13.4** 3.2-53.4	**44.7** 10.3-173.7
SS9	148.650	50.875	E-SAF	-	6.4	**14.5** 3.3-56.1	**12.4** 2.9-48.7	**26.9** 6.2-104.8
SS10	149.423	49.989	E-SAF	-	7.1	**16.9** 3.9-65.4	**12.5** 2.9-48.4	**29.3** 6.7-113.7
SS12	151.194	48.058	E-SAZ	-	6.3	**26.7** 6.1-103.3	**9.4** 2.6-43.6	**36.1** 8.7-146.9
P3 d1	153.176	45.548	E-SAZ	141	3.2	**74.5** 17.1-288.9	**12.0** 3.1-51.8	**86.5** 20.2-340.7
P3 d3	153.349	45.465	E-SAZ	117	6.5	**47.6** 10.9-184.5	**15.6** 3.9-61.1	**63.2** 14.8-245.6
P3 d5	153.678	45.590	E-SAZ	80	6.5	**92.1** 23.4-354.5	**19.5** 6.4-73.4	**111.5** 29.7-428.0
SS21	152.493	44.934	E-SAZ	-	5.9	**76.7** 17.6-297.4	**8.8** 2.0-34.0	**85.5** 19.6-331.5
SS24	148.581	43.687	EAC/STZ	-	-	**90.9** 20.9-352.3	**14.8** 3.4-57.3	**105.7** 24.3-409.6

6. Conclusions and leads for future

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The Figure D.1 tries to regroup the main conclusions of this work, by pointing the important mechanisms or factors which has been found to be essential to further model the sea ice and associated pelagic ecosystem.

Firstly, we observed that the algal community was typically composed of flagellates (dinoflagellates and *Phaeocystis* sp.) in surface ice layers and of large diatoms in bottom ice layers (Chapters 1, 3 and 4). The brine volume i.e. the space available for microorganisms controls the accumulation of algae in end of winter-early spring (Chapter 1). The distribution of algae was rather controlled by the light (Chapter 3) and major nutrients (Chapter 3 and 4) availability in spring/summer.

Secondly the present thesis provided new data sets on dissolved and particulate organic carbon (DOC and POC) in Antarctic pack ice from 3 locations. We observed large concentrations of DOC and POC in sea ice, enriched as compared to underlying seawater from end of winter until early summer (Chapters 1, 2 and 3). Especially the sea ice OM was in the form of saccharides and transparent exopolymeric particles (TEP). The partition between DOC and POC was different than in ocean likely because of the transformation and coagulation between fractions. So, the transparent exopolymeric particles (TEP) were a key substance for biogeochemistry of sea ice and ocean. Indeed we point out that the high accumulation of OM in particular TEP induces the formation of a biofilm (involved in the attachment of cells to the walls of the brine channels). The gel properties of this biofilm influenced the prey-grazer interactions, the retention of nutrients and accumulation of toxins around cells (Chapter 1). We also

suggest that TEP were involved in the timing of release of organic components during the ice melting and in the complexation or aggregation (Chapter 4).

We found that the OM production was related to algae. The composition as well as the vertical distribution of OM in sea ice was linked to algae. A fraction of the OM seems freshly *in situ* produced by algae (Chapter 2). The contribution of saccharides in the OM pool increases with stronger presence of algae. We observed an unusual accumulation of monomeric, likely labile, substrates (dissolved free amino acids and monosaccharides) up to 100 µM C as compared to nanomolar concentrations typical of oceanic systems. The higher accumulation of monomers was observed in winter. We proposed that the consumption of OM by bacteria was limited by environmental conditions such as temperature, salinity or presence of toxic compounds (e.g. acrylic acid from breakdown of DMSP) rather than by the nature of the substrate. This pool of OM was indeed consumed when the environmental conditions of spring alleviated the limitation of bacteria. Besides the OM originating from sea ice was able to sustain bacterial growth in the water column (Chapter 4).

We also observed the strong influence of the physical evolution of sea ice (temperature, brine volumes and movement) on the microbial community and organic matter. The evolution of the distribution of the organic parameters was influenced during end of spring-early summer by the melting of the ice cover and associated brine movement (Chapters 2 and 4) as it is also the case during a flood-freeze cycle in spring (Chapter 3).

At the time of melting, sea ice was a major supply of iron, organic nutrients and microorganisms (algae, bacteria and protozoa) for the water column and influences the carbon retention in and/or export below the surface waters. Small microorganisms (< 10 µm) coming from sea ice were able to thrive and grow once in the water column. Dissolved organic and inorganic nutrients from sea ice have a positive effect on the growth of pelagic microorganisms. Furthermore the temporal sequence of release of each of these components during melting supports the seeding hypothesis of sea ice algae for phytoplanktonic blooms.

Finally we highlighted the importance of taking into account the bacterial remineralization of OM in the mesopelagic zone when estimating the efficiency of the biological carbon pump.

Clearly additional studies of the reasons and consequences of accumulation of OM in sea ice would be very helpful to confirm our assumptions and conclude about complementary subjects not studied here. It first involves going on with the spatial and temporal inventory of OM in Antarctic pack ice by collecting samples in a multidisciplinary approach involving glaciologists, chemists and biologists. We indeed showed that to fully understand the biogeochemistry of the sea ice it is essential to integrate the observations. The present work proved the importance of determining the chemical nature of the OM pool with e.g. the TEP or biofilm formation or the monosaccharides accumulation. It would be interesting to further study the characterization of the OM pool e.g. presence/absence of humic substances to decipher the biological processes involved in its production and consumption. Moreover measurements of bacterial activities inside sea ice would inform about their ability to consume the OM present or the limitation of their activities as we assumed. Laboratory experiments (such as in Kähler et al. (1997) or Pusceddu et al. (2009)) studying the bacterial degradation of the OM originating from sea ice would also give information about its lability.

The accumulation of OM in sea ice results from a disconnection of the production and consumption pathways (Thomas and Papadimitriou 2003) and we gave here some hypotheses concerning *in situ* production and limited consumption of OM. Still the large concentrations of OM could also partly origin from the initial enrichment during ice formation but little information is available. It would be a key subject to investigate the mechanisms of OM incorporation in sea ice. It could be achieved by laboratory experiments using tanks and ice formation device, following the method designed by Giannelli et al. (2001) or Grossmann and Gleitz (1993). The delicate step to handle would be the maintenance of an organic and trace-metal clean device to avoid contamination of the experiment.

Similarly to the microcosm experiments performed in the present work, other experimental melting experiments could be set up to study e.g. the sequential release of ice components with or without TEP.

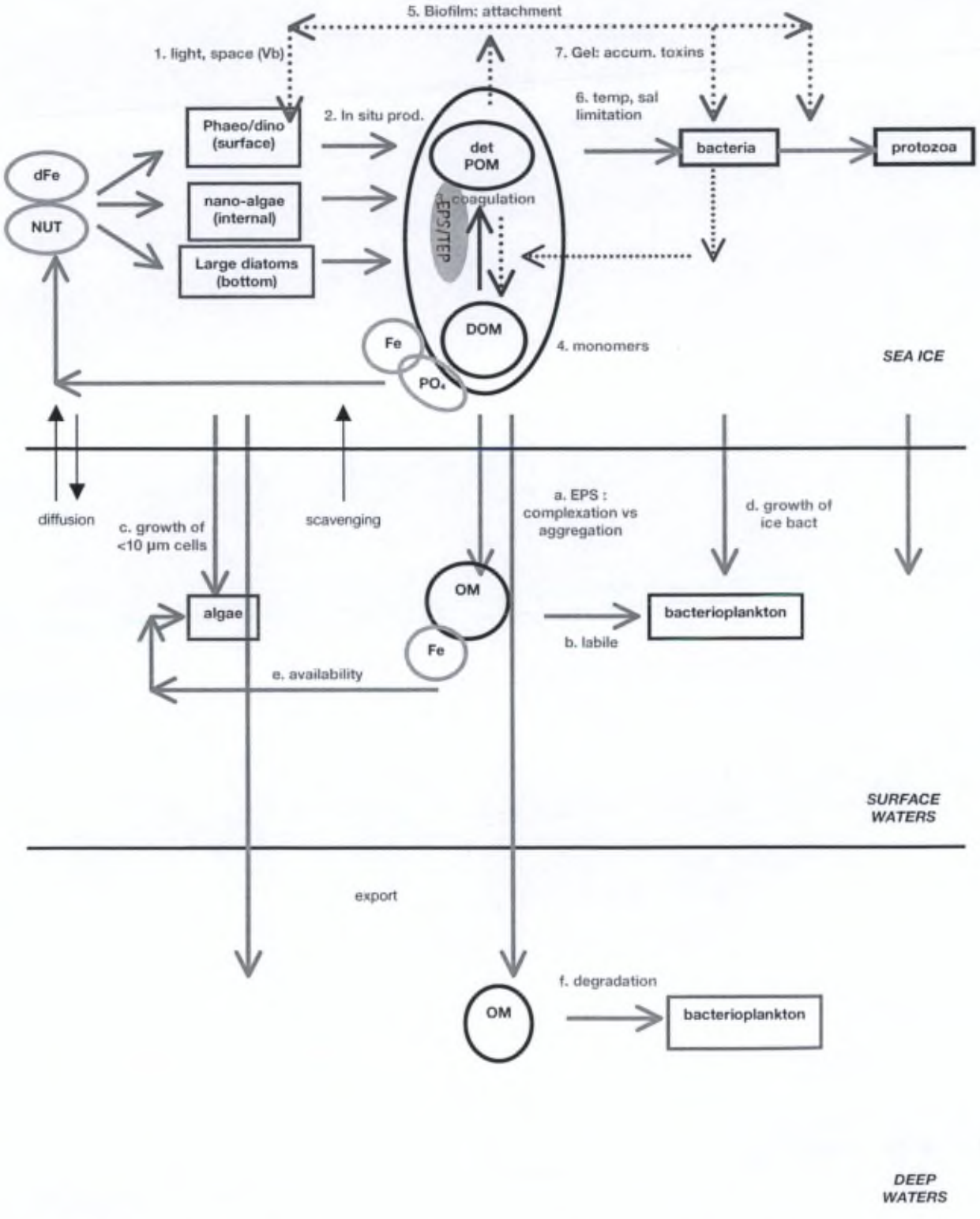

Figure D1: Main mechanisms or factors which have been found to be essential in the sea ice and pelagic ecosystems from the present thesis.

Twilight zone organic carbon remineralization in the Polar Front Zone and Subantarctic Zone south of Tasmania

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Twilight zone organic carbon remineralization in the Polar Front Zone and Subantarctic Zone south of Tasmania

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Abstract

We report on the distribution of excess, non-lithogenic particulate barium (Ba_{xs}), a proxy for twilight zone remineralization of organic matter, in the Australian sector of the Southern Ocean during the mid-austral summer 2007. This study was part of a broader investigation focusing on macro- and micronutrient availability controlling ecosystem functioning in this area. Particulate excess Ba in the twilight zone (100–600 m depth layer) proved to be significantly controlled by the vertical distribution of bacterial activity, with higher particulate Ba contents in situations where significant bacterial activity extended deeper in the water column. However, despite this covariation the magnitudes of the carbon fluxes as estimated from particulate Ba content and bacterial activity did not match, with carbon demand based on bacterial activity largely exceeding organic carbon remineralization estimated from Ba_{xs}. Possible reasons for this discrepancy are discussed, but it appeared that the magnitude of the mesopelagic carbon remineralization flux obtained from Ba_{xs} was realistic when weighted against primary, new and export production. Overall mesopelagic Ba_{xs} contents increased from SAZ (flagellate and cyanobacteria dominance) to PFZ (diatom dominance), confirming earlier results obtained in spring 2001 and summer 1998 for the same general area. These results reflect also increasing mesopelagic C remineralization toward the Polar Front. We observed furthermore that the Fe-replete Subantarctic Zone east of the Tasman Plateau (SAZ-East) had higher mesopelagic Ba_{xs} contents and also a higher remineralization efficiency (on average 77% carbon exported from the upper 100 m) than the SAZ area west of the plateau (SAZ-West) where the remineralization efficiency was on average 43%. However, unexpectedly, despite these differences in export and mesopelagic remineralization, SAZ East and SAZ West sites were equally efficient in transferring part of the net primary production to the deep ocean beyond 600m. This corroborates earlier results which indicated that Fe-replete

55 blooms (which was the case for SAZ East) are more efficient in transferring
56 surface layer-exported particulate organic carbon through the mesopelagic
57 into the bathypelagic water column (>1000 m). It is likely that next to Fe
58 availability also differences in community composition and trophic structure
59 are important control factors that set the system's efficiency for deep ocean
60 carbon sequestration.
61
62 **Keywords:** Particulate biogenic barium, mesopelagic C mineralization,
63 Southern Ocean, C transfer efficiency
64
65 **1. INTRODUCTION**
66 The efficiency of the biological pump in transferring carbon to the deep
67 sea with the potential for longer-term sequestration is strongly influenced by
68 the processing of organic matter in the mesopelagic zone, also called twilight
69 zone (~100-1000 m) [see e.g. Buesseler et al., 2007]. One of the different
70 possible approaches to study mesopelagic organic matter breakdown consists
71 in assessing the excess particulate biogenic barium Ba (Ba_{xs}) stocks in this
72 depth zone [e.g. Cardinal et al., 2005; Jacquet et al., 2007a, 2008a, 2008b;
73 Dehairs et al., 2008]. In the twilight zone particulate biogenic Ba is present
74 mainly as micro-crystalline barite ($BaSO_4$) [Dehairs et al., 1980; Jacquet et al.,
75 2007b]. The link between barite and C remineralization resides in the fact that
76 this mineral precipitates inside oversaturated micro-environments (biogenic
77 aggregates) during the process of bacterial (prokaryotic) degradation of
78 sinking particulate organic matter [Collier and Edmond, 1984; Bertram and
79 Cowen, 1997; Dehairs et al., 1980, 1992, 1997, 2000; Ganeshram et al.,
80 2003; Bishop, 1988]. Carbon remineralization fluxes are assessed via a
81 transfer function relating Ba_{xs} with oxygen utilisation rate [Shopova et al.,
82 1995; Dehairs et al., 1997]. However, direct comparisons between Ba_{xs} and
83 mesopelagic bacterial activity have been few [Dehairs et al., 1997; Jacquet et

84 al. 2008a; Dehairs et al., 2008]. Recently we reported a convergence between
85 the organic C remineralization rate (based on the Ba_{xs}-barite proxy), bacterial
86 C demand (based on bacterial production) and particulate organic carbon
87 (POC) flux attenuation (from C fluxes sampled by neutrally buoyant sediment
88 traps deployed over the upper mesopelagic layers) [Dehairs et al., 2008;
89 Buesseler et al., 2007]. This corroborated earlier statements that seasonally
90 accumulated suspended barite reflects an integral of past organic C
91 remineralization [Dehairs et al., 1980, 1997; Stroobants et al., 1991].
92 The Ba_{xs}-barite proxy approach was also applied during an artificial
93 iron fertilization experiment in the Polar Front Zone (PFZ) (EIFEX; 2004;
94 Strass et al., 2005) as well as during a study on natural iron fertilization in
95 the Subantarctic Kerguelen area (KEOPS; 2005; Blain et al., 2007). It
96 appeared that the efficiency of C-transfer through the mesopelagic layer
97 under conditions of Fe-repleteness was increased compared to Fe-depleted
98 environments [Jacquet et al., 2008a, 2008b; Savoye et al., 2008; Blain et al.,
99 2007]. Investigating whether a similar situation prevailed in the SAZ areas
100 supposedly influenced by the Tasman Plateau was part of the objectives of the
101 present study.
102 Although significant progress has been made in the assessment of
103 twilight zone C remineralization [see e.g. Boyd and Trull, 2007; Buesseler et
104 al., 2007], we are still far from being able to predict C transfer efficiency to
105 the deep water column at the global scale, because of regional differences in
106 the factors controlling export of biogenic material and its remineralization.
107 These factors need to be better constrained.
108 The present work was conducted as part of the SAZ-SENSE (Sub-
109 Antarctic Zone Sensitivity to Environmental Change) expedition (Jan.-Feb.
110 2007, R/V *Aurora Australis*). The general objectives were to assess the zonal
111 variability of the C sequestration potential in the Subantarctic Zone (SAZ)
112 southeast and southwest of Tasmania and the Polar Front Zone (PFZ), during

113 mid-summer and to identify possible causes inducing this variability. The PFZ
114 and SAZ merit particular attention because these represent a key interface
115 that mediates the global influence of the Southern Ocean through CO_2 uptake,
116 intermediate water mass formation (i.e. Sub-Antarctic Mode Water- SAMW
117 and Antarctic Intermediate Water- AAIW) redistributing nutrients to lower
118 latitudes. Moreover, there is evidence that the SAZ east of the Tasman Rise
119 exhibits relatively higher phytoplankton biomass in contrast to the area west
120 of Tasman Rise [see Chl-*a* satellite image in Griffiths et al., this issue].
121 Possible causes for this condition could include, amongst others: changing
122 patterns of the East Australia Current advecting warmer stratified waters
123 (possibly enriched with Fe) to the south or changing patterns of eolian
124 transport of dust from the Australian continent. The rationale guiding the
125 sampling pattern at sea was therefore to evaluate the possible zonal
126 variability in the SAZ region as a proxy for long-term temporal evolution,
127 potentially ongoing with climate change.
128 Our contribution to SAZ-SENSE consisted in studying the variability of
129 organic C export [see contributions by Cavagna et al. and Jacquet et al. b,
130 both this issue] as well as the fate of exported C in the mesopelagic waters.
131 The present work mainly focuses on the latter issue. The objective also was to
132 shed further light on the relationship between Ba_{xs}-barite and bacterial
133 activity in the mesopelagic water column. Therefore, we systematically
134 compared the Ba_{xs}-barite proxy with bacterial production measurements
135 [Dumont et al., this issue]. Calculated remineralization rates are compared
136 with new production [Cavagna et al., this issue] as well as export production
137 [Jacquet et al. b, this issue].
138
139 **2. EXPERIMENT AND METHODS**
140 **Study area and sampling**

141 The Sub-Antarctic Zone Sensitivity to Environmental Change (SAZ-
142 SENSE) cruise (AAV0307 of R/V *Aurora Australis*; Jan. 17 to Feb. 20, 2007;
143 mid-austral summer) was carried out in the Australian sector of the Southern
144 Ocean (43-54°S; 140-155°E) (Figure 1). Sampled zones and fronts were from
145 north to south: the SubTropical Zone (STZ), the SubTropical Front (STF), the
146 SubAntarctic Zone (SAZ), the SubAntarctic Front (SAF, northern and southern
147 branch), the Polar Front (PF, northern branch), the Polar Front Zone (PFZ)
148 and the Inter Polar Front Zone (IPFZ). Detailed descriptions of the complex
149 physical structure of the area, circulation, water masses and fronts are given
150 in Rintoul and Bullister [1999], Sokolov and Rintoul [2002], Herraiz-
151 Borreguero and Rintoul [this issue], Griffiths et al.[this issue] and Mongin et
152 al. [this issue].
153 A total of 17 CTD casts (surface to 800-1000 m) was sampled for
154 particulate barium (Table 1). Four further CTDs casts were sampled down to
155 2500 m depth. Seawater was sampled using 10 L Niskin bottles mounted on a
156 rosette. Two types of stations were occupied: (i) Process stations (P1, SAZ-
157 West: CTD casts #11, 19, 30; P2, PFZ: CTD casts #40, 44, 51, 58, 59; P3,
158 SAZ-East: CTD casts #78, 79, 86, 104), repeatedly sampled for Ba and
159 bacterial production over a period of 4 to 6 days, and (ii) Transit stations
160 (CTD casts #3, 5, 36, 61, 64, 66, 71, 93, 108) where a single cast for Ba was
161 sampled (Figure 1 and Table 1). Usually 20 depths were sampled between
162 surface and 1000 m. Four to seven L of seawater were filtered onto
163 polycarbonate membranes (diameter usually = 47 mm, but = 90 mm for
164 surface samples; all 0.4 µm porosity). Filter membranes were subsequently
165 rinsed with Milli-Q grade water (<5 mL) to remove sea-salt, dried (50°C) and
166 stored in petri dishes for later analysis in the home-based laboratory.
167
168 **Analysis**

169 We performed a total digestion of samples using an HF/HCl/HNO_3 acid
170 mixture and analyzed for Ba and other major and minor elements (Al, Sr and
171 Ca) by ICP-AES (inductively coupled plasma- atomic emission spectrometry;
172 Thermo Optek Iris Advantage) and ICP-QMS (inductively coupled plasma-
173 quadrupole mass spectrometry; VG Plasma Quad 2+). The detailed protocol is
174 described in Cardinal et al. [2001]. Average detection limits (DL) equal 0.18
175 nM for Al, 0.4 nM for Ca, 0.4 pM for Sr and 1 pM for Ba. DL were calculated as
176 3 times the standard deviation on the blank and then normalized to an
177 average factor dilution factor of 415 as for the samples (i.e. particles from ~5
178 L of seawater, dissolved in a final volume of ~12 mL).
179 Biogenic barium (hereafter called excess-Ba or Ba_{xs}) was calculated
180 normatively as the difference between total particulate Ba and lithogenic Ba
181 using Al as the lithogenic reference element [Dymond et al., 1992; Taylor and
182 Mc. Lennan, 1985]. Al concentrations range between few nM and 35.5 nM
183 (Table 2). At most sites and depths particulate biogenic Ba_{xs} represented
184 >96% of total particulate Ba. The standard uncertainty [Ellison et al., 2000]
185 on Ba_{xs} data ranges between 5 and 5.5%.
186 Organic carbon remineralization in the mesopelagic layer was
187 estimated using an algorithm relating mesopelagic Ba_{xs} contents to the rate of
188 oxygen consumption obtained from a 1-D advection diffusion model
189 established for the Southern Ocean [Shopova et al., 1995; Dehairs et al.,
190 1997]. These mesopelagic Ba_{xs} inventories build up over the growth season
191 thereby integrating past activity (see Discussion section below and Figure 6).
192 The range of oxygen utilization rates resulting from this model approach are
193 similar in magnitude as Apparent Oxygen Utilisation Rates (AOUR) based on
194 Apparent Oxygen Utilisation (AOU) and age models [see e.g., Feely et al.,
195 2004; Nakayama et al.,2008; Karstensen et al., 2008]. Relative standard
196 uncertainties [Ellison et al., 2000] on C remineralization range between 15
197 and 68% (see further Table 4). We compare organic C remineralization

198 deduced from Ba_{xs}-barite inventories with bacterial production (BP) and
199 bacterial carbon demand (BCD) as well as with new production (NP) and C
200 export production (EP). BP was calculated from [methyl-^{3}H]-thymidine (TdR)
201 incorporation using a conversion factor of 0.86×10^{18} cells mol^{-1} [Ducklow et al.,
202 1999] and a C conversion factor of 12 fg C $cell^{-1}$ [Fukuda et al., 1998]. BCD
203 was estimated assuming bacterial growth efficiency (BGE) was = 0.15 [del
204 Giorgio and Cole, 2000]. For full details concerning the assessment of BP and
205 BCD we refer to the paper by Dumont et al. [this issue]. The methods for new
206 production (NP; ^{13}C-, ^{15}N-uptake experiments) and export production (EP;
207 ^{234}Th-deficit method) are discussed in Cavagna et al. and Jacquet et al. b,
208 respectively [both this issue].
209
210 **3. RESULTS**
211 **3.1. Particulate biogenic Ba_{xs} profiles**
212 Ba_{xs} data at process, transit and deep stations are given in Table 2.
213 Process stations were sampled repeatedly (P1: 3 CTD casts, P2: 5 CTD casts
214 and P3: 4 CTD casts) over periods of 4, 6 and 6 days, respectively. Figure 2
215 shows the average Ba_{xs} profile for each process station. At P1 in the SAZ-West
216 Ba_{xs} contents are low and variable (Figure 2-a; Table 2), with concentrations
217 ranging from a few pM to 280 pM, with a few peak values reaching up to 395
218 pM (CTD #11; 450m). At station P2 in the PF Ba_{xs} contents are higher with
219 maxima of 500 to 600 pM between 150 and 800 m depth (Figure 2-b; Table
220 2). Furthermore, P2 is the only site with very high Ba_{xs} contents in the shallow
221 subsurface waters. Also, these high surface values increased between the first
222 and third CTD cast, reaching up to 1600 pM. Subsequently, for CTD #58 and
223 #59 Ba_{xs} values decreased again to 564 pM and 217 pM, respectively. We also
224 observed some very high Ba_{xs} concentrations in the deeper water column
225 (CTD #40; Ba_{xs} values up to 792 pM at 700m and 735 pM at 800 m). At
226 station P3 in the SAZ-East a consistent Ba_{xs} maximum is present between 100

and 400 m (Figure 2-c; Table 2), but maximum Ba_{xs} contents at P3 (369 to 453 pM) are lower than those reached at P2. Ba_{xs} concentrations at stations P1 and P3 are similar, except for the 100 to 400m depth interval where P3 values exceed those at P1 (Figure 2-a, -c). Note also the deep extension (to 700 m) of high Ba_{xs} values at P2 (Table 2) compared to P1 and P3.

Transit CTD #5 (STZ), process station P1 (SAZ-West) and transit CTD #93 (SAZ-East, north of P3) have Ba_{xs} profiles similar in shape and values, with values increasing between surface and 200 m and staying relatively constant deeper (Figure 2-d). Transit CTD #108 located north-west of P3 in the STZ displays the lowest mesopelagic Ba_{xs} contents (Figure 2-d; 70 to 325 pM), but surface values are relatively high (reaching 428 pM) even exceeding mesopelagic values, as was observed also at P2 (Figure 2-b).

Characteristic mesopelagic Ba_{xs} maxima are observed at transit CTD #64, #66 and #71 located on the section from P2 (PF) to P3 (SAZ-East, Figure 3-a), as well as at process station P3 (Figure 2-c). We note also that these mesopelagic Ba_{xs} maxima tend to increase and to shoal in north easterly direction (Ba_{xs} maxima reach 416, 444 and 540 pM for CTD #64, #66 and #71 respectively; Figure 3-a).

3.2. Depth-weighted average Ba_{xs} contents in the mesopelagic

Table 3 shows the mesopelagic depth-weighted average (DWAv) Ba_{xs} values, i.e. Ba_{xs} inventories divided by the height of the considered water column (100-600 m). Overall, values range from 146 to 515 pM. At P1 and P3 in the SAZ, DWAv Ba_{xs} content remains relatively constant over the duration of station occupation (DWAv Ba_{xs}: 199±21 pM and 279±19 pM, respectively). The highest and most variable DWAv Ba_{xs} values are observed at P2 in the PF. DWAv Ba_{xs} increases from about 340 pM for the two first repeat CTD casts to 440 pM (#51) and 515 pM (#58) but decreases again to 463 pM for the last CTD (#59). Ba_{xs} averages for the different repeat CTD casts at the process

256 stations show P2 (PFZ; 421±76) and P3 (SAZ-East; 279±19) to exceed P1
257 (SAZ-West; 199±21) by a factor 2.1 and 1.5, respectively.
258 After an initial decrease of DWAv Ba_{xs} between P2 and the first CTD
259 (#64) on the section between P2 and P3, a steady increase in northward
260 direction is observed: #64 (280 pM), #66 (306 pM), #71 (395 pM) (Table 3).
261 However, further north at P3 and also at transit stations beyond P3, DWAv
262 Ba_{xs} concentrations decrease again (e.g., #104, 259 pM; #93: 244 pM) with
263 the lowest value (146 pM) recorded for STZ CTD #108 located in the southern
264 extension of the East Australia Current.
265
266 **4. DISCUSSION**
267 **4.1. Ba_{xs} distributions in the STZ, SAZ and PFZ**
268
269 *Surface waters*
270 At P2 in the PFZ and to a lesser extent for CTD #108 in the STZ east of
271 the Tasman Rise we observe high Ba_{xs} contents in the upper 80 m, with
272 values reaching up to 1600 pM (CTD #44). The occurrence of quite high
273 surface ocean Ba_{xs} contents is not unusual and similar or even higher Ba_{xs}
274 contents have been reported earlier for the PFZ in the Atlantic sector [Dehairs
275 et al., 1997], Indian sector [Jacquet et al., 2005, 2008a] and Atlantic sector
276 [Jacquet et al., 2007b]. Previous studies showed that Ba_{xs} in the surface layer
277 is mainly incorporated into or adsorbed onto biogenic particles with barite
278 being a minor component [Dehairs et al., 1980; Cardinal et al, 2005; Jacquet
279 et al., 2007b]. This situation contrasts with the one at mesopelagic depths
280 where Ba_{xs} appears mainly composed of barite micro-crystals as linked to
281 remineralization processes.
282 Surface Ba_{xs} contents do not appear to follow primary production (PP),
283 as reported in Jacquet et al. [2008b]. Indeed, surface Ba_{xs} values are highest
284 at P2 in the PF (up to 1600 pM) where primary production in the euphotic

285 layer ranges between 34 and 38 mmolC m^{-2} d^{-1} (Cavagna et al., this issue and
286 Table 4) while they are low (<200 pM) at P1 and P2, where PP values reach
287 up to 88 mmolC m^{-2} d^{-1} (Cavagna et al., this issue and Table 4). However, it
288 is likely that phytoplankton community composition, besides primary
289 production is an important factor controlling surface water Ba_{xs} content.
290 Indeed, while diatoms dominated at P2 and cyanobacteria and flagellates
291 dominated at P1 and P3 [Armand et al., pers. comm.; Wright et al., this
292 issue], other plankton organisms could be involved. We observed that
293 particulate Ca and Sr contents in the upper 150m were highest and rather
294 similar at P2 and P3 (average [Ca]: 120±70 and 129±33 nM, respectively;
295 average [Sr]: 1106±591 and 926±287 pM, respectively; Table 2), while at P1
296 Ca and Sr concentrations reached only 71±25 nM and 539±385 pM,
297 respectively (Table 2). During the EIFEX expedition (PFZ, Atlantic sector) high
298 surface Ba_{xs} contents (up to 2000 pM) were observed to coincide with high
299 Sr/Ca mole ratios (50 to 164 mmol mol^{-1}) related with the presence of $SrSO_4$
300 secreting acantharia [Jacquet et al., 2007b]. However, in the present study
301 Sr/Ca mole ratios at sites with highest surface water Ba_{xs} are lower, ranging
302 between 8 and 22 mmol mol^{-1} (CTD #108 and P2 CTD #44, respectively) and
303 overall no particularly high Sr values were observed (Table 2). Present Sr/Ca
304 ratios are closer to values reported for phytoplankton (10 to 40 mmol mol^{-1})
305 [Martin and Knauer, 1973] and also suspended matter (2 to 35 mmol mol^{-1})
306 from the same SAZ sector investigated here [Cardinal et al., 2001]. Therefore,
307 in the present study there is no compelling evidence for an important
308 contribution of acantharia to the high surface Ba_{xs} values we observed at P2
309 and CTD #108.
310
311 *Mesopelagic waters*
312 We now focus on the mesopelagic zone where most of the
313 remineralization of exported C is taking place [Martin et al., 1987; Sarmiento

314 et al., 1993; Buesseler et al., 2007] and where Ba_{xs} mainly consists of barite
315 micro-crystals [Dehairs et al., 1980; Jacquet et al., 2007b]. Stations located
316 along the SAZ-East transect (P2, CTD#64, #66, #71 and P3) are
317 characterized by relatively high mesopelagic Ba_{xs} contents, the highest being
318 observed at P2 in the PF . This contrasts with stations in the SAZ-West and in
319 the STZ north of P3 where mesopelagic Ba_{xs} contents are lower and no clear
320 maximum is present. Thus our Ba_{xs} data point to the P2 - P3 transect (section
321 between 54.01- 45.55°S) as the area where mesopelagic remineralization is
322 most intense (Figure 4). In previous work the PFZ along WOCE SR3 line
323 (~145°E) was also identified as an area of more intense mesopelagic
324 remineralization compared to the STZ and SAZ [Cardinal et al., 2001, 2005;
325 Jacquet et al., 2007a] (Figure 5). Furthermore, this earlier work highlighted
326 the occurrence of a seasonal increase in mesopelagic Ba_{xs} from late-spring
327 (Nov. 2001; CLIVAR-SR3) to late-summer values (Feb.-Mar. 1998; SAZ-98)
328 (Figure 5). The N-S trend of mesopelagic Baxs values for the present early
329 summer cruise are intermediate between these late spring and late summer
330 trends, corroborating the observed seasonal trend and highlighting the
331 consistency of the Ba_{xs} signal over the past decade (Figure 5).
332 While the Ba_{xs} content in the upper 100 m and at depths >400 m, are
333 similar between SAZ-West (P1 and transit CTD #5) and SAZ-East (P3 and
334 transit CTD #93) (Figure 2-d), a salient feature of our data is the presence of
335 a clear mesopelagic Ba_{xs} maximum between 100 and 400 m at P3 in the SAZ-
336 East, while this is absent in the SAZ-West (P1 and CTD #5) but also at the
337 northernmost SAZ-East CTD #93. Such differences suggest a more intense
338 remineralization east as opposed to west of the Tasman Rise, but also that
339 sub-mesoscale variability exists in the P3 area (no mesopelagic Ba_{xs}
340 maximum observed at CTD #93), possibly related with the strong gradients in
341 phytoplankton biomass indicated by the patchiness and filamentous structure
342 of Chl-*a* distribution in the area [Griffiths et al., this issue].

343 We also observe that the mesopelagic Ba_{xs} maximum shoals from
344 stations P2 to P3 along the SAZ-East transect (Figures 3 and 4). This shoaling
345 actually follows a decrease in thickness of the upper mixed layer, from about
346 103 (CTD #64) to 71 m (CTD #71) (Figure 3-b). Moreover, a steady increase
347 of DWAv Ba_{xs} in northward direction appears associated with this shoaling of
348 the upper mixed layer (Table 3; Figure 3-b). The higher DWAv Ba_{xs} value at
349 CTD #71 (395 pM) coincides with a stronger density gradient at the bottom of
350 the mixed layer (Figure 3-b), and since sharp density gradients have been
351 reported as sites of organic aggregates and floc formation [e.g., MacIntyre et
352 al., 1995] it is possible that these conditions have led to enhanced barite
353 formation.
354 Some particularly high Ba_{xs} values were observed at CTD #40 (site P1)
355 between 700 to 800 m (up to 792 pM at 700 m) and CTD #3 (SAZ-West;
356 1991 pM at 800m) (see Table 2). Occasionally peak values of Ba_{xs} may also
357 occur deeper in the water column, below 1000 m (1453 pM at 1750 m; CTD
358 #3). High Ba_{xs} values in the deep water column are not unusual and have also
359 been reported for the Kerguelen-Crozet Basin during the ANTARES 4 cruise,
360 with values reaching up to 1700 pM at 1000 m [Jacquet et al., 2005]. This
361 suggests that remineralization is still continuing in the bathypelagic water
362 column confirming recent observations by van Beek et al. [2007]. This
363 indicates barite formation is not limited to the upper mesopelagic but does
364 also take place deeper in the water column, probably associated with bacterial
365 degradation of faster sinking fecal pellets and large aggregates. However, for
366 the deep CTD #3 cast we also note that the Ba_{xs} peak values at 797 and
367 1748m are associated with high Al contents (up to 35.5 nM; see Table 2).
368 Thus we can not exclude the possibility of an advective input of particulate
369 Ba_{xs} together with lithogenic material for the northern SAZ-West. A similar
370 conclusion was reached by Cardinal et al. [2001] who suggested that east to
371 west currents around 45°S advect material from the Tasmanian continental

372 shelf and margin to the SAZ-West. Such currents are likely associated with
373 the Tasman outflow described by Ridgeway and Dunn [2007] and Speich et
374 al. [2002, 2007], evidence for which was also revealed by the track of Argo
375 floats [Herraiz-Borreguero and Rintoul, this issue].
376
377 **4.2. Twilight zone Ba_{xs} and bacterial production**
378 Previous studies revealed that mesopelagic Ba_{xs} is closely related with
379 the vertical distribution of bacterial production. During the KEOPS (Kerguelen
380 Plateau) and VERTIGO (stations ALOHA, North Pacific and K2 Subarctic
381 Pacific) expeditions, it appeared that the shape of the water column
382 integrated bacterial production (BP) profile is important in setting the Ba_{xs}
383 signal in the mesopelagic [Jacquet et al., 2008b; Dehairs et al., 2008]. These
384 previous studies indicated mesopelagic Ba_{xs} content was lower when most of
385 the column integrated bacterial productivity was restricted to the upper mixed
386 layer, compared to situations where a significant part of integrated BP was
387 located deeper in the water column. Our results from the present study
388 corroborate these earlier findings. Figure 6 shows the ratio of mixed layer
389 integrated BP over BP integrated over the upper 600 m (BPML/BP600) vs.
390 mesopelagic DWAv Ba_{xs} (100 to 600 m depth interval). Excluding a single
391 outlier (CTD #58), a significant inverse correlation is apparent between
392 BPML/BP600 and the mesopelagic (100-600 m) DWAv Ba_{xs} maximum (R^2 =
393 0.59; $p<0.01$). Thus, for SAZ-SENSE as well, increased mesopelagic Ba_{xs}
394 contents reflect the fact that significant bacterial activity extends deeper in
395 the water column beyond the upper mixed layer. The outlier (#58) is the
396 highest DWAv Ba_{xs} value recorded during this cruise (515 pM). While such
397 value is well within the range we reported for earlier expeditions in this area
398 [Cardinal et al., 2001, 2005], the corresponding BPML/BP600 ratio (0.71) is
399 high compared to other CTD casts from the same station (P2), due to unusual

400 high BP values between 150 and 200 m for this particular cast [Dumont et al.,
401 this issue].
402 We also compared BP integrated between ML and 600 m with DWAv
403 Ba_{xs} in the 100 to 600 m mesopelagic depth interval. The regression (not
404 shown) is poor, though significant (R^2 = 0.40, $p<0.01$) when calculated
405 without the outlier (i.e. cast #58 at P2). Several reasons can be invoked to
406 explain why this particular correlation is poor, while ratios of mixed layer
407 integrated BP over 600 m integrated BP correlate well with mesopelagic Ba.
408 First of all, it should be kept in mind that processes with quite different time
409 scales are being compared. Indeed, while formation of mesopelagic Ba_{xs} is a
410 rather slow process with a time scale of a few days to weeks or so [Jacquet et
411 al., 2007a; Dehairs et al., 1997], the time scale of bacterial activity is rather
412 of the order of a few hours, at most. Secondly, conversion factors (i.e. TdR
413 CF; cell C content) used to calculate BP were kept constant at all sites and
414 depths [see Dumont et al., this issue], while it is likely that these vary with
415 pressure, temperature, food supply, ... [see e.g., Tamburini et al., 2003;
416 Moran et al., 2007]. The fact that the correlation between the BPML/BP600
417 ratio and DWAv Ba_{xs} proved significant (Figure 6), while BP integrated
418 between ML and 600m vs DWAv Ba_{xs} did not, suggests that varying ambient
419 conditions indeed had an effect, which by taking the ratio of integrated BP
420 was, at least partly, eliminated.
421 Despite increasing evidence for the existence of a causal relationship
422 between bacterial activity and mesopelagic Ba_{xs} content, the question about
423 how well both proxies reflect twilight zone organic carbon remineralization is
424 still not fully resolved. This is discussed in the next section.
425
426 **4.3. Estimates of organic remineralization**
427 The carbon remineralization rate deduced from Ba_{xs} is based on an
428 empirical relationship between Ba_{xs} and oxygen consumption established for

429 $BaSO_4$ saturated ACC waters [see discussions in Dehairs et al., 1997; 2008].
430 For zero oxygen consumption (i.e. in winter, when primary production and
431 bacterial activity are minimal) this relationship indicates a Ba_{xs} intercept of
432 180 pM, representing for $BaSO_4$ saturated waters, a background Ba_{xs} signal
433 carried over between successive seasons. For the PF station P2 located in
434 $BaSO_4$ saturated waters, we kept 180 pM as the background Ba_{xs} value. Since
435 the area north of the PF is known to be undersaturated for $BaSO_4$ over the
436 whole of the water column [Monnin et al., 1999; Monnin and Cividini, 2007],
437 we arbitrarily choose a lower intercept value of 100 pM for all stations north of
438 the PF in order to account for possible dissolution of barite (see also
439 discussion in Dehairs et al., 2008). In Table 4 we compare organic C
440 remineralization deduced from Ba_{xs}-barite inventories with bacterial carbon
441 demand (BCD; Dumont et al., this issue), primary and new production (PP,
442 NP; Cavagna et al., this issue) and C export (EP; Jacquet et al. b, this issue).
443 Note that Ba_{xs} (and BCD), PP, NP and EP were not always analyzed for the
444 same CTD cast, but casts used for comparison were taken close by in time
445 and space, limiting possible natural variability.

446 Overall C remineralization fluxes calculated from DWAv mesopelagic
447 Ba_{xs} contents and integrated over the 100-600 m water column, range from 1
448 to 7 mmol C m^{-2} d^{-1} (Table 4). Largest remineralization rates are observed at
449 P2 in the PF with values ranging between 3.2 and 7.0 mmol C m^{-2} d^{-1}. Such
450 values exceed those we reported for the WOCE SR3 transect (spring 2001)
451 and the Crozet-Kerguelen Basin (1999) during summer [Cardinal et al., 2005;
452 Jacquet et al., 2005, 2007a, 2008a]. Remineralization rates in the SAZ-West
453 (1.8 to 3.1 mmol C m^{-2} d^{-1}) are lower compared to the SAZ-East (3 to 6.1
454 mmol C m^{-2} d^{-1}) (see Table 4) but are similar to values reported by Cardinal
455 et al. [2005] for the SAZ along WOCE SR3 line (1.2 to 2.5 mmol C m^{-2} d^{-1}).

456 Taking one step further we now compare the organic carbon
457 remineralization rates based on mesopelagic Ba_{xs} with bacterial carbon

458 demand (BCD) in the 100 to 600m depth layer (Table 4). BCD was calculated
459 from BP considering a BGE value of 0.15 (see methods and Dumont et al.,
460 this issue). While the trend is of course the same as for the regression
461 between BP and Ba_{xs}, results indicate that BCD exceeds the organic carbon
462 remineralization rate based on Ba_{xs} by five-fold on average. We note however
463 that our Ba_{xs}-based remineralization rates are in the same range (but usually
464 lower, as expected) than new production and also C-export (estimated via
465 ^{15}N-uptake experiments and the ^{234}Th-deficit method, respectively), while
466 BCD values are clearly in excess of carbon export (Table 4). Furthermore, our
467 Ba_{xs}-based remineralization rates are within about a factor 2 of the POC flux
468 sampled by drifting sediment traps deployed at 150m (see Ebersbach et al.,
469 this issue), giving further credence to the order of magnitude of the calculated
470 carbon remineralization rates.

471 Several reasons can be invoked to explain the fact that BCD values are
472 systematically greater than estimates based on the different other approaches.
473 It is possible that BCD is *in fine* satisfied by dissolved organic C (DOC)
474 availability [e.g. Azam and Malfatti, 2007]. If this DOC does not originate
475 from sinking particulate organic carbon but is directly advected from the
476 surface [Carlson et al. 1994] or from nearby regions [Bauer et al., 2002;
477 Aristegui et al., 2003], or is actively exported via zooplankton migration
478 [Ducklow et al., 2001; Steinberg et al., 2008] such discrepancies could arise,
479 since the approach based on particulate Ba_{xs} does not take advection of DOC
480 into account. On the contrary, if all mesopelagic DOC finds its origin in sinking
481 POC traced by the ^{234}Th method, this would suggest bacterial carbon demand
482 to be overestimated. A better matching of BCD and Ba_{xs}-based
483 remineralization would require a BGE value larger than 0.15. While the latter
484 value is in agreement with available data in the literature, as discussed in
485 Dumont et al. [this issue], it is nevertheless a fact that most of the BGE
486 values reported in literature concern the efficiency of free-living bacteria and

487 assume a homogenous dissolved organic matter (DOM) distribution. However,
488 aggregates are reported to carry a very high interstitial DOC load [Alldredge,
489 2000] and to host significant bacterial hydrolytic activity which converts
490 particulate organic matter into non-sinking DOM which forms plumes in the
491 wake of the sinking particles [Azam and Malfatti, 2007]. Azam and Long
492 [2001] report that bacteria associated with these plumes probably have a
493 higher growth efficiency. Likewise, laboratory studies on aggregates have
494 revealed BGE values as high as 0.50 [Grossart and Ploug, 2001]. Such large
495 BGE values, if realistic for the oceanic environment, would decrease our BCD
496 values by a factor 3.3, bringing these close to organic carbon respiration
497 estimated from mesopelagic Ba_{xs}. We remind also that the mesopelagic Ba_{xs}
498 proxy is the reflection of processes integrated over a period of a few days to a
499 few weeks [Cardinal et al., 2005; Jacquet et al., 2008b] while BCD is based
500 on <8h incubations, though day-to-day variation between repeat CTD casts at
501 process stations appears limited (Table 4). Therefore, part of the discrepancy
502 may also result from these differences in time scales between both processes.
503 The largest mesopelagic remineralization (up to 7.0 mmol C m^{-2} d^{-1}) is
504 observed for the less productive P2 site (PP ranging from 34 to 37.7 mmol C
505 m^{-2} d^{-1}), while the more productive P1 and P3 sites (PP up to 87.9 mmol C m^{-2}
506 d^{-1}) are characterized by lower remineralization rates (from 1.8 to 2.6 mmol C
507 m^{-2} d^{-1}) (Table 4). It is also worth noting that carbon fluxes related with
508 production, export and remineralization, while showing internal consistency in
509 general, do not necessarily co-vary on a cast by cast basis (see process
510 stations in Table 4). For instance, at P1 the decrease of NP over time is not
511 accompanied by a similar change in EP and remineralization rate. Also, while
512 EP at P3 doubles between repeat stations (from 3.2 to 6.5 mmol C m^{-2} d^{-1}),
513 the remineralization rate remains quite constant (3.3 to 4.1 mmol C m^{-2} d^{-1}).
514 In contrast, the doubling of EP at P2 (from 2.9 to 6.6 mmol C m^{-2} d^{-1}) is also
515 seen for the remineralization rate (from 3.2 to 7.0 mmol C m^{-2} d^{-1}). Again

516 such mismatches may be related to the large differences in time scales
517 characterizing the different processes that are compared.
518 We defined the twilight zone remineralization efficiency (r-ratio in % in
519 Table 4) as the ratio of mesopelagic C remineralization (based on Ba_{xs}) over C
520 export from the 100 m horizon (the latter obtained via the ^{234}Th-deficit
521 method; see Jacquet et al. b, this issue). P1 and P3 are quite different in
522 terms of r-ratio in mesopelagic waters, which was smaller, reaching 26-68%
523 at P1 (average = 43%) compared to 48 to >100% at P2 and P3 (averages =
524 106 and 77%, respectively). The deep extension (to 1000 m) of high Ba_{xs}
525 values at P2 (Table 2) reflects remineralization of OM to extend deeper in the
526 water column compared to P3. CTD casts #64, #66, #71 along the SAZ-East
527 transect between P2 and P3 also exhibit important mesopelagic
528 remineralization efficiencies, reaching 60, 61 and 98 % respectively. Overall,
529 remineralization rates are consistent with values reported for a previous
530 cruise in the region [Cardinal et al., 2005], but rates in the PFZ and SAZ-East
531 (P2, #64, #66, #71, P3) exceed those in the SAZ-West (P1, Table 4).
532 Bacterial abundances and activities are also higher in the SAZ-East as
533 potentially linked to higher iron concentrations [Lannuzel et al., this issue],
534 availability of organic substrates [Dumont et al., this issue] and grazer plus
535 viral activities [Evans et al., this issue; Thomson et al., this issue].
536 The fate of matter exported from the upper surface layer is different
537 between SAZ-West, SAZ-East and PFZ. This can be visualized (Figure 7) by
538 plotting the ratio of POC flux at 100m (from ^{234}Th deficit; Jacquet et al.-b this
539 issue) over Net Primary Production (i.e., EP100/NP; NP as integrated over the
540 euphotic zone; data from Cavagna et al., this issue) vs. the fraction of POC
541 flux at 100m that exits the mesopelagic through the 600m horizon (i.e. T600
542 = EP600/EP100 = 1-(MR/EP100), with MR/EP100 = r-ratio; see above).
543 Hereby we follow an idea developed by Buesseler and Boyd [in review for
544 Limnology and Oceanography]. The graph highlights that the P2 site in the

545 PFZ, while having the highest fraction of NP (55%) exported from the upper
546 100 m, has all exported POC remineralized within the mesopelagic zone. This
547 feature is remarkable and indicates the poor capacity of PF-PFZ waters for
548 deep ocean (bathypelagic) carbon sequestration via the biological pump.
549 Further appreciation of the zonal extension of this condition is required, since
550 important for refining global carbon balance calculations. P3 is the site with
551 the next highest fraction of NP (50%) exported out of the surface water, but
552 in contrast to P2 about 20% of this POC is exported to depths >600 m, the
553 rest being remineralized in the mesopelagic zone. At the P1 site only 20% of
554 NP is exported from the upper 100 m, but close to 60% of that POC is
555 exported to the water column water >600 m. Despite these differences
556 between SAZ-East and SAZ-West concerning the fraction of NP that is
557 exported from the surface waters and remineralized in the mesopelagic,
558 interestingly, both SAZ sites exhibit the same efficiency in exporting
559 autotrophic fixed carbon to the deep ocean, with about 10% of NP reaching
560 beyond 600 m. Ebersbach et al. [this issue] report on the morphology of
561 sinking particles captured in free floating gel-traps deployed between 140 and
562 290 m at the process stations. It appears that particles at P2 in the PFZ were
563 smaller but denser compared to P1 and P3, and were more of the aggregate
564 type, possibly produced by fragmentation of original silica-rich fecal pellet
565 material. Significant fragmentation of sinking pellets could explain the
566 successful retention of the organic C and its subsequent re-oxidation in the
567 mesopelagic waters. At P1 and also P3 in the SAZ captured sinking particles
568 are larger and more of the intact zooplankton fecal pellet type. At P1 these
569 apparently transit the mesopelagic zone more efficiently thereby avoiding
570 complete remineralization.

571 We now investigate how the studied SAZ system, with its eastern part
572 reported as being Fe-replete [Lannuzel et al. this issue], compares with Fe-
573 replete areas elsewhere (KEOPS, a natural iron fertilization study above the

574 Kerguelen Plateau; Jacquet et al., 2008a; EIFEX an artificial iron fertilization
575 study in a mesoscale eddy detached from the Polar Front, Atlantic sector;
576 Jacquet et al., 2008b). Mesopelagic POC remineralization of exported carbon
577 from Fe replete surface waters during KEOPS and EIFEX was observed to be
578 relatively less efficient compared to HNLC reference stations [Jacquet et al.,
579 2008a,b] and this implied a more efficient transit of exported carbon through
580 the mesopelagic into the bathypelagic. To some degree our present results
581 corroborate these earlier findings, since they indicate that a larger fraction of
582 primary production ended up in the deep (>600 m) water column in the SAZ
583 (with the SAZ-East reported to be Fe replete) compared to the Fe depleted
584 HNLC PFZ area, where remineralization of organic matter exported to the
585 mesopelagic water column (100-600 m) was essentially complete. However,
586 the phytoplankton community composition of the SAZ environment studied
587 here differs from those prevailing during EIFEX and KEOPS. Indeed, in the
588 present study flagellates and cyanobacteria were dominant both in the SAZ-
589 East and SAZ-West area, while diatoms were dominant during KEOPS and
590 EIFEX.
591
592 **Conclusions**
593 This work investigated particulate excess Ba contents (Ba_{xs}), a proxy
594 for mesopelagic organic carbon remineralization, in the upper 1000 m of the
595 water column in the SAZ-East, SAZ-West, PFZ and PF areas south of
596 Tasmania which contrast strongly in terms of C production and export. The
597 vertical distributions of Ba_{xs} in the water column also were quite different
598 between these regions, with higher mesopelagic Ba_{xs} contents in the SAZ-East,
599 PFZ and PF.
600 Overall we confirm earlier results for the same sector indicating that
601 mesopelagic remineralization efficiency increases from SAZ to PFZ, following a

602 general trend of southward decreasing deep water column (> 800m) POC flux
603 [Cardinal et al., 2001; 2005].

604 We observed mesopelagic Ba_{xs} contents to be related with bacterial
605 activity, with higher mesopelagic Ba_{xs} contents in situations where significant
606 bacterial activity extended deeper in the water column. Our findings indicate
607 that the SAZ-East and PFZ are regions of relatively more important
608 remineralization of exported organic matter in the mesopelagic water column
609 (100-600 m) as compared to the SAZ-West, with PFZ even showing complete
610 mesopelagic remineralization of exported POC. The latter feature is
611 remarkable and indicates the poor capacity of PF-PFZ waters for deep ocean
612 (bathypelagic) carbon sequestration via the biological pump. However, both
613 SAZ regions, showed a similar efficiency regarding deep export of autotrophic
614 fixed carbon, with about 10% of NPP being exported deeper than 600m.
615 Further appreciation of the zonal extension of these conditions is required,
616 since important for refining global carbon balance calculations.
617

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638
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870

870 **Figure Captions**

871 Figure 1: Map of the SAZ-SENSE cruise in Australian sector of the Southern
872 Ocean area showing location of process and transit stations. STZ =
873 Subtropical Zone; STF = Subtropical Front; SAZ = SubAntarctic Zone; PFZ =
874 the Polar Front Zone; SAF-N and SAF-S = SubAntarctic Front northern and
875 southern branch; PF-N = Polar Front northern branch, IPFZ = InterPolar Front
876 Zone.

877

878 Figure 2: Average profiles of particulate excess Ba (Ba_{xs}; pM; 0-1000 m) at
879 process stations (a) P1 (SAZ-West), (b) P2 (PFZ), (c) P3 (SAZ-East) and at
880 transit stations (d) #5 (SAZ-West), #93 (SAZ-East) and #108 (STZ).

881

882 Figure 3: Profiles of (a) particulate excess Ba (Ba_{xs}; pM; 0-600 m) and (b)
883 potential density (kg/m^3; 0-200m) at transit stations #64, #66, #71.

884

885 Figure 4: ODV plot showing the depth distribution of particulate excess Ba
886 (Ba_{xs}; pM; 0-1000 m) vs. longitude (°E). Note that the southernmost station
887 (P2) is located in the middle of the panel, with the SAZ-West profiles to the
888 left and the SAZ-East profiles to the right. Graph constructed using Ocean
889 Data View (Schlitzer, 2003; Ocean Data View; http://www.awi-
890 bremerhaven.de/GEO/ODV).

891

892 Figure 5: Latitudinal distribution of depth weighted average (DWAv)
893 mesopelagic Ba_{xs} (pM) vs. salinity at 150 m; salinity is taken here as a proxy
894 for latitudinal position eliminating temporal and spatial variability in position
895 of the fronts and water masses. Shown are data for the CLIVAR-SR3 cruise
896 (squares, late-spring; Ba_{xs} values for the 100-450 m depth region; Cardinal et
897 al. 2005); the SAZ-SENSE cruise (triangles, mid-summer; 100-600 m depth

region; this study) and the SAZ-98 cruise (circles, late-summer; Ba_{xs} values for the 100-450 m region; Cardinal et al., 2001, 2005); regression lines (power functions) are shown to highlight the trend; IPFZ = Inter Polar Frontal Zone; PFZ = Polar Frontal Zone; SAZ = Subantarctic Zone; PF = Polar Front; SAF = Sunantarctic Front; STF = Subtropical Front.

Figure 6: Regression of the ratio of mixed layer (ML) integrated BP over upper 600 m integrated BP versus depth weighted average (DWAv) mesopelagic Ba_{xs} (pM; 100-600 m).

Figure 7: Y-axis: POC flux from upper 100 m as a fraction of NP; X-axis: POC flux at 600 m as a fraction of POC flux at 100 m. Isolines represent the modeled 1, 5, 10, 20 and 30% of NP export to depths >600 m.

Table Captions

Table 1: Station location and sampling depth range.

Table 2: Excess particulate biogenic Ba (Ba_{xs}; pM), Ca (nM), Sr (pM) and Al (nM) and Sr/Ca (mmol mol^{-1}) at process and transit stations.

Table 3: Depth-weighted average values of mesopelagic Ba_{xs} (pM; 100-600 m).

Table 4: Comparison of Ba_{xs} based mesopelagic organic carbon remineralization (MR) with gross primary production (GPP), new production (NP), bacterial carbon demand (BCD) and export production (EP); r-ratio (in %) = ratio of MR over EP. All fluxes in mmol C m^{-2} d^{-1}.

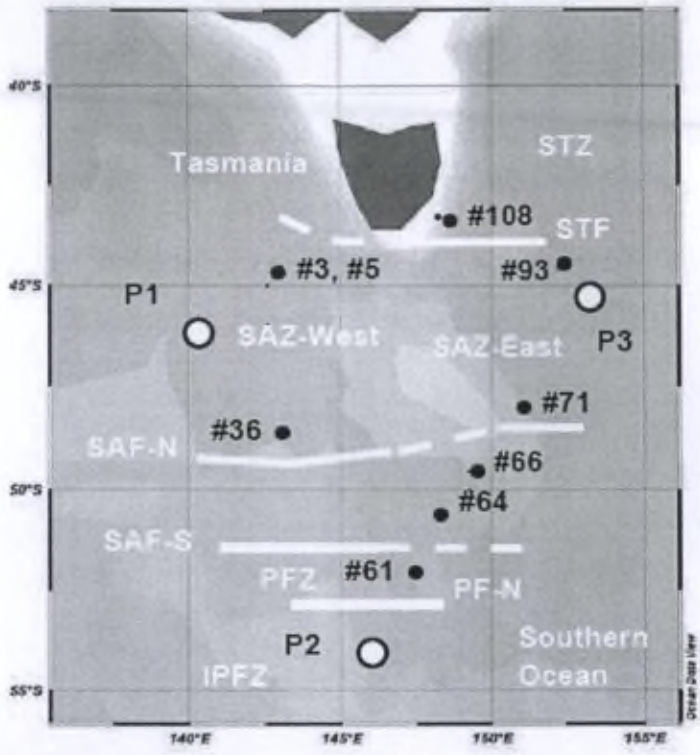

Figure 1

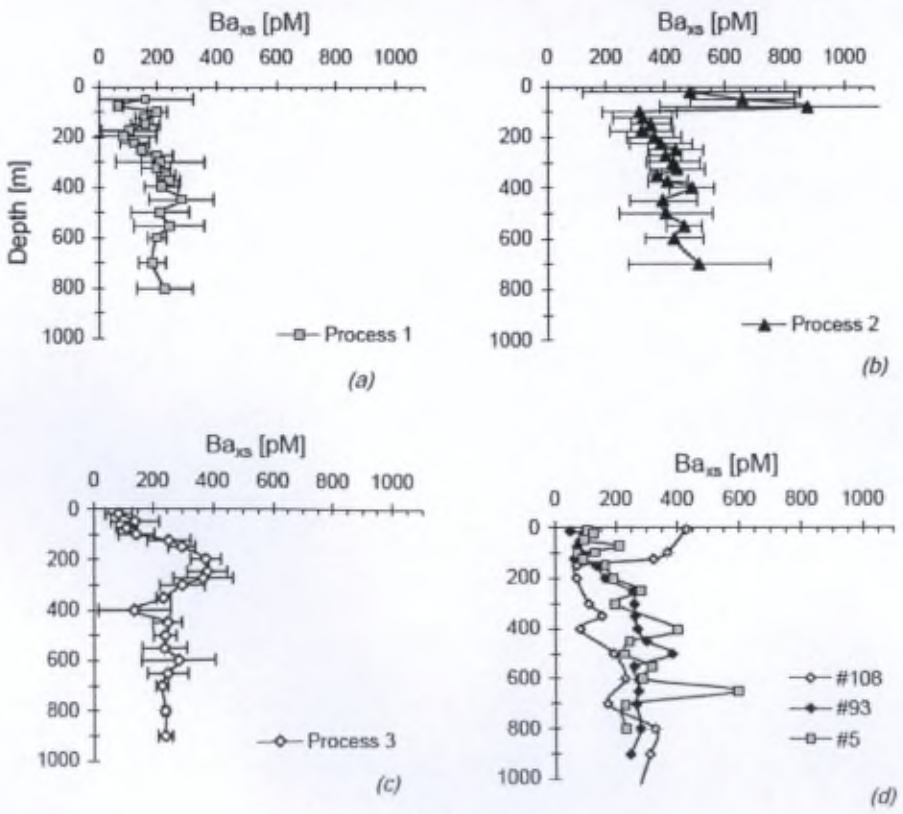

Figure 2

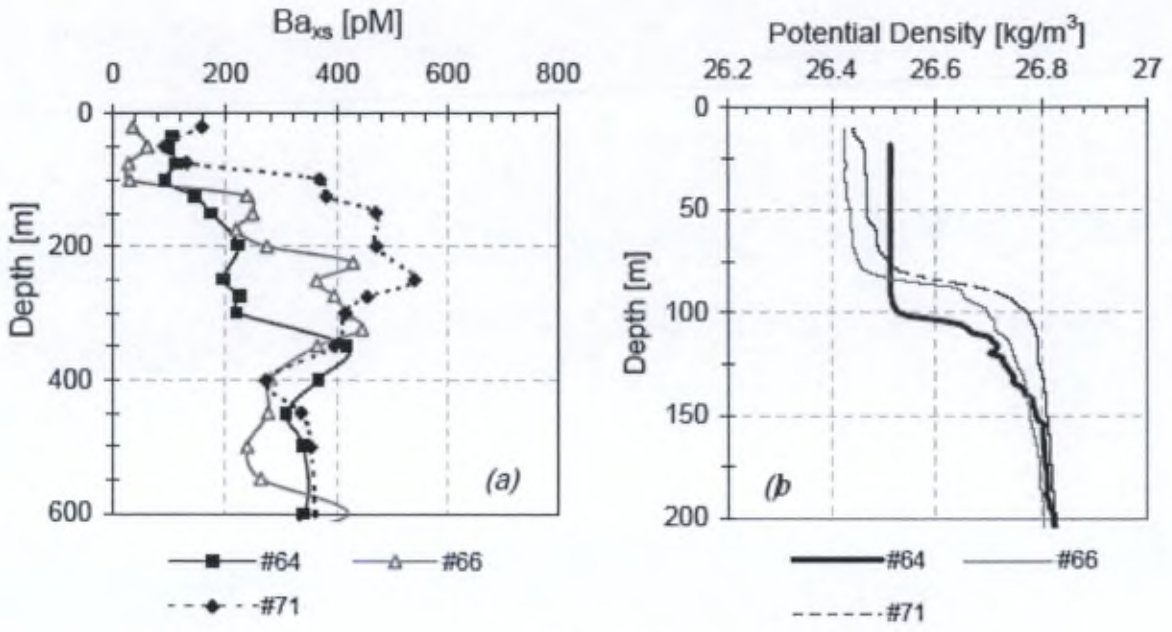

Figure 3

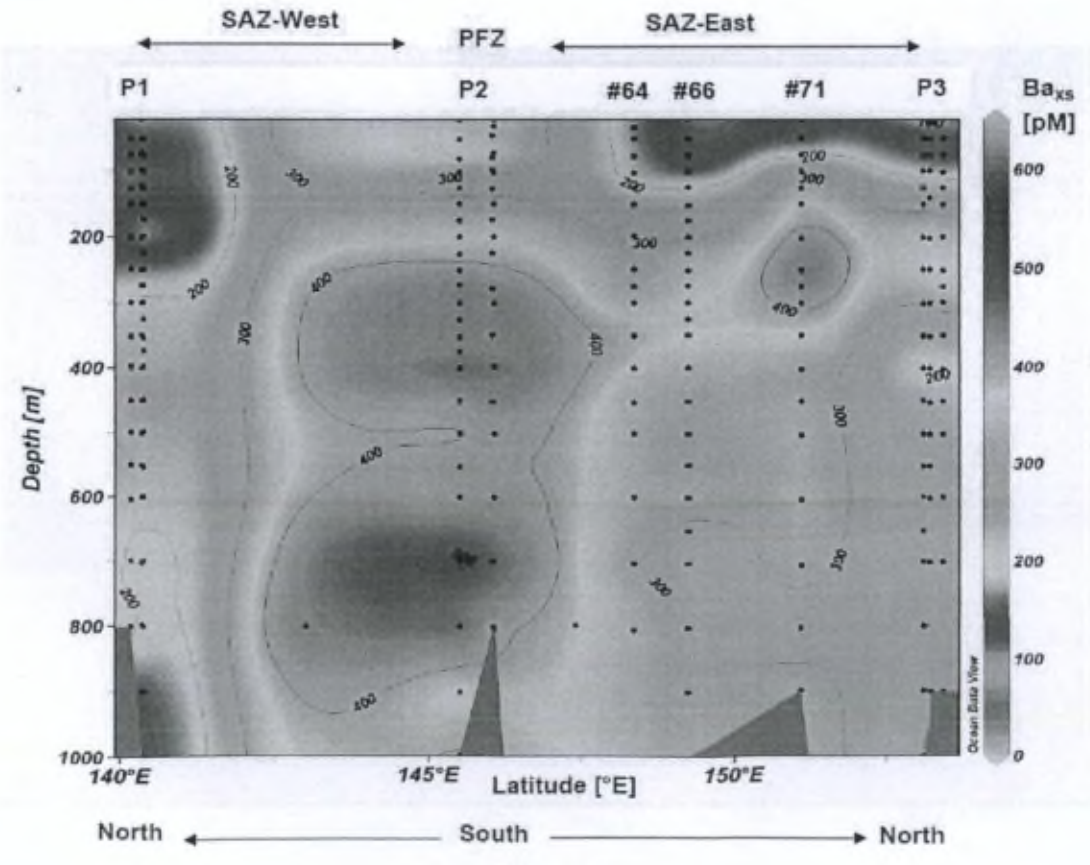

Figure 4

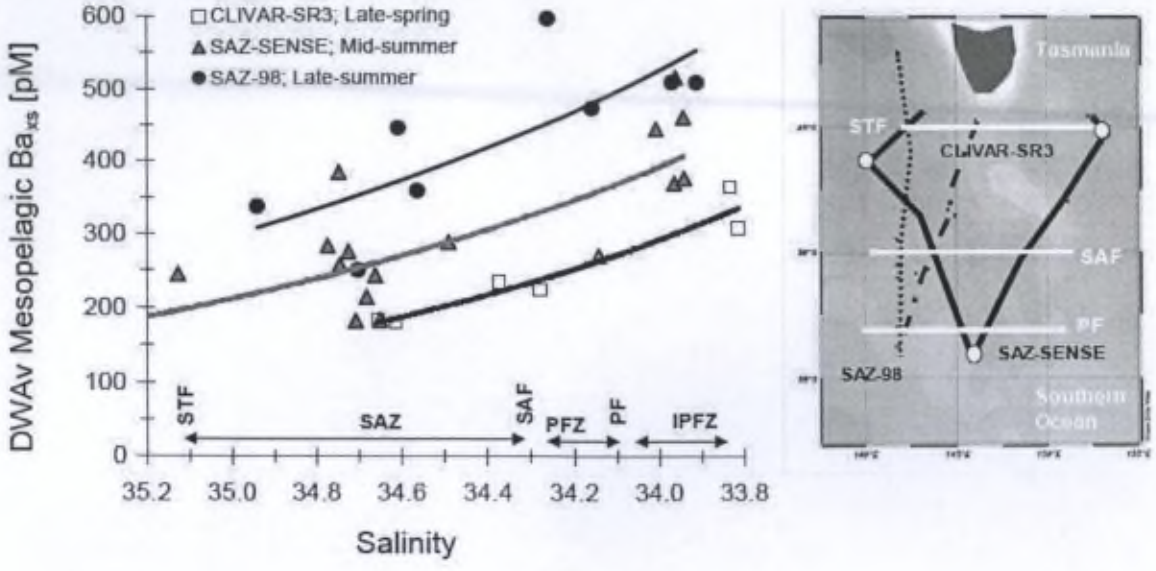

Figure 5

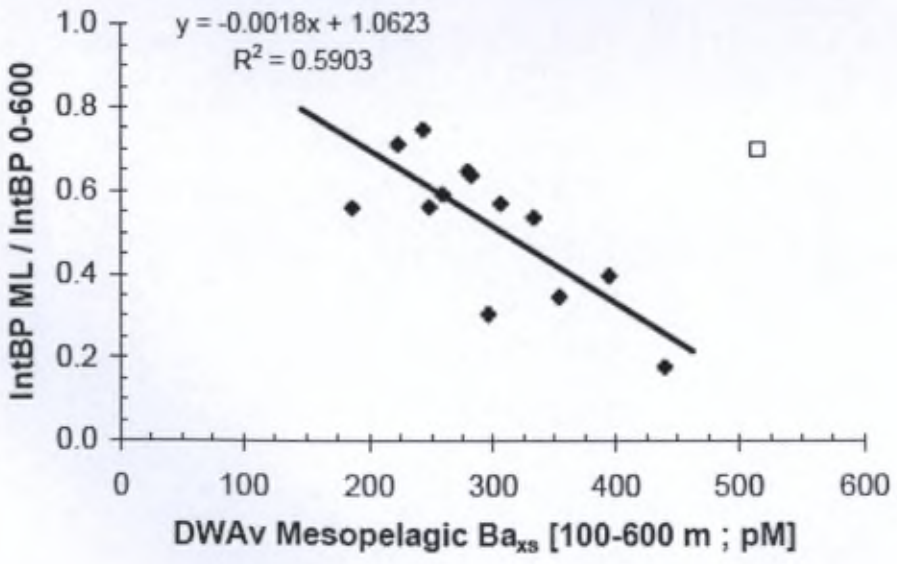

Figure 6

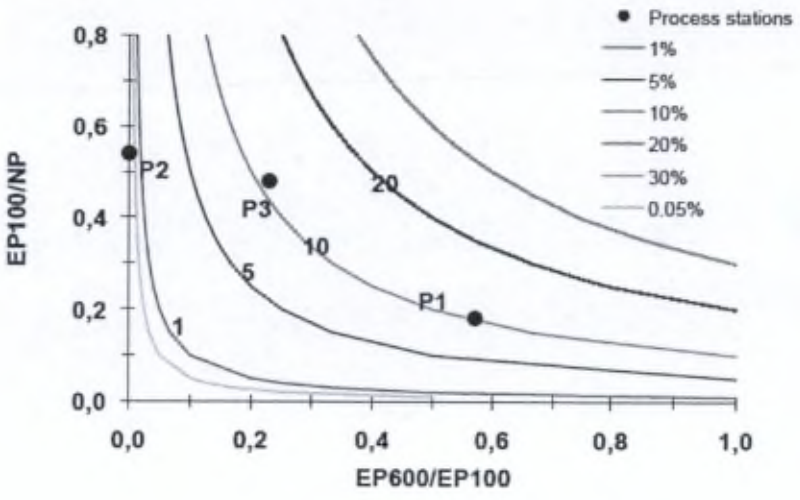

Figure 7

Table 1

Station	CTD cast #	Longitude, °E	Latitude, °S	Location	Depth range, m
Process station 1					
4	11	140.56	46.32	SAZ-West	0 - 1000
4	19	140.31	46.50	SAZ-West	0 - 1000
4	30	140.64	46.58	SAZ-West	0 - 1000
Process station 2					
6	40	145.87	54.01	PFZ	0 - 1000
6	44	146.13	54.02	PFZ	0 - 1000
6	51	146.30	54.14	PFZ	0 - 1000
6	58	146.12	54.45	PFZ	0 - 800
6	59	147.10	54.45	PFZ	0 - 800
Process station 3					
17	78	153.18	45.55	SAZ-East	0 - 1000
17	79	153.18	45.56	SAZ-East	800-2500
17	86	153.35	45.47	SAZ-East	0 - 900
17	104	153.68	45.59	SAZ-East	0 - 900
Transit stations					
2	3	142.98	45.01	SAZ-West	800 - 2250
2	5	143.05	44.89	SAZ-West	0 - 1000
5	36	143.01	48.99	SAF-West	800 - 2250
8	61	147.71	52.01	SAF-East	800 - 2500
9	64	148.65	50.88	SAF-East	0 - 1000
10	66	149.42	49.99	SAF-East	0 - 1000
12	71	151.19	48.06	SAF-East	0 - 1000
21	93	152.49	44.93	SAZ-East	0 - 1000
24	108	148.58	43.69	STZ	0 - 1000

Table 2

Process 1 CTD #11

Niskin	Depth m	Bass pM	Al nM	Ca nM	Sr pM	Sr/Ca mmol/mol
24	25	223	<DL	102	116	1.1
22	51	343	0.7	84	146	2.3
20	73	81	7.4	11	17	1.6
17	98	214	1.9	24	200	8.2
16	123	135	4.1	8	24	4.3
15	150	207	1.4	10	26	2.6
14	201	36	4.6	4	0	0.1
13	250	136	1.1	6	6	0.8
12	274	177	1.7	57	56	1.0
11	301	76	<DL	6	9	1.5
10	350	211	2.6	6	87	13.4
9	400	188	7.4	3	314	106.1
8	450	306	5.3	5	110	23.0
7	500	308	3.7	1	93	87.2
6	550	137	4.0	7	94	13.8
5	600	186	3.6	4	76	17.9
4	702	153	5.2	38	242	6.4
3	798	113	1.6	8	106	16.2
2	900	<DL	2.7	16	<DL	/
1	999	135	5.2	33	736	22.0

Process 1 CTD #19

Niskin	Depth m	Bass pM	Al nM	Ca nM	Sr pM	Sr/Ca mmol/mol
22	50	89	5.7	141	1875	13.3
19	74	54	3.2	20	238	11.9
18	100	147	0.7	25	330	13.0
17	100	221	<DL	11	436	40.0
16	125	187	0.5	16	501	32.2
14	149	160	13.6	15	268	18.1
13	201	212	1.7	19	212	11.4
11	249	144	<DL	9	110	12.7
10	300	179	3.8	13	115	9.0
8	352	264	2.2	25	186	8.3
7	398	260	<DL	27	190	6.7
6	450	184	0.1	28	123	4.6
5	499	213	<DL	18	112	8.4
4	549	359	7.5	24	158	6.3
3	603	204	<DL	41	222	5.4
2	699	234	<DL	31	75	3.7
1	801	282	0.8	39	206	5.8

Process 1 CTD #30

Niskin	Depth m	Bass pM	Al nM	Ca nM	Sr pM	Sr/Ca mmol/mol
23	50	46	13.8	140	1156	8.5
22	75	84	5.8	85	994	11.7
21	100	152	5.6	94	986	11.7
20	125	141	1.1	18	147	8.4
19	150	108	0.2	16	14	0.9
18	174	<DL	0.4	29	283	9.7
17	198	<DL	2.5	10	65	6.4
16	224	101	1.5	6	38	6.4
15	250	152	0.5	25	278	12.1
14	274	259	2.1	33	230	7.1
13	306	371	0.8	34	48	1.3
12	325	226	1.2	17	<DL	/
11	353	207	0.2	14	<DL	/
10	373	344	3.0	18	162	8.9
9	398	174	<DL	16	<DL	0.0
8	449	263	1.3	26	356	11.9
7	498	106	0.4	6	<DL	/
6	553	206	<DL	12	<DL	/
5	600	226	0.3	17	38	2.2
4	699	152	<DL	13	98	7.3
3	801	273	3.8	24	255	9.8
2	901	226	2.9	37	53	1.4

Process 2 CTD #40

Niskin	Depth m	Bass pM	Al nM	Ca nM	Sr pM	Sr/Ca mmol/mol
24	21	286	5.9	167	1017	6.1
23	51	534	9.2	159	826	5.2
22	80	745	3.2	232	1950	8.4
21	100	129	0.6	54	374	6.9
20	125	182	0.8	21	80	3.8
19	149	304	3.1	24	225	9.3
18	175	211	2.7	16	251	15.2
17	200	359	1.1	12	89	7.7
16	226	284	<DL	18	<DL	/
15	251	515	<DL	17	<DL	/
14	273	436	<DL	18	77	4.3
13	300	404	0.8	23	81	3.5
12	326	481	<DL	8	19	3.1
11	352	358	1.5	8	<DL	/
10	374	405	<DL	11	32	3.0
9	400	536	0.2	15	124	8.2
8	450	303	<DL	2	45	21.4
7	500	266	<DL	12	60	4.9
6	552	433	<DL	17	95	5.5
5	599	275	0.4	14	11	0.8
4	649	792	<DL	83	135	4.5
3	800	735	1.8	21	<DL	/
2	901	265	<DL	11	23	2.1
1	999	442	<DL	17	70	4.2

Process 2 CTD #44

Niskin	Depth m	Bass pM	Al nM	Ca nM	Sr pM	Sr/Ca mmol/mol
24	20	356	7.8	141	952	6.8
23	50	646	16.7	123	974	7.9
22	85	1902	10.3	114	2516	22.0
19	100	356	6.6	49	530	10.9
18	141	256	1.2	31	423	13.2
16	183	248	2.8	6	50	7.9
14	200	256	0.1	6	84	11.4
13	226	278	1.8	4	54	13.0
12	276	337	1.0	3	45	13.2
11	300	356	1.5	9	82	11.1
10	350	366	<DL	3	31	10.3
8	400	378	3.4	4	33	8.5
7	451	334	3.0	7	29	4.2
6	501	281	1.0	4	34	8.6
5	551	406	<DL	5	39	8.2
4	602	415	3.8	4	34	9.3
3	702	356	2.7	6	33	5.6
2	800	244	1.3	4	26	7.3
1	899	196	2.4	3	24	8.2

Process 2 CTD #51

Niskin	Depth m	Bass pM	Al nM	Ca nM	Sr pM	Sr/Ca mmol/mol
23	20	1035	3.2	205	1331	6.5
21	49	905	2.6	152	734	4.8
19	74	569	0.9	122	610	5.0
17	100	338	2.5	117	617	7.0
16	124	394	1.7	6	59	9.6
15	149	459	3.8	39	191	4.9
13	201	335	<DL	19	<DL	/
12	251	494	<DL	21	3	0.2
10	300	370	1.7	11	32	1.9
9	349	367	<DL	4	<DL	/
8	401	518	<DL	19	<DL	/
7	449	417	<DL	5	34	8.8
6	500	515	<DL	8	<DL	/
5	550	473	<DL	10	<DL	/
3	602	459	1.1	14	84	5.8
2	750	279	<DL	11	59	5.5
1	900	397	2.0	8	62	7.6

Process 2 CTD #58

Niskin	Depth m	Bass pM	Al nM	Ca nM	Sr pM	Sr/Ca mmol/mol
21	20	265	3.0	191	1411	7.9
13	30	562	9.3	350	2251	6.4
9	71	994	6.2	213	2150	10.1
8	100	420	<DL	278	2696	10.6
7	149	356	4.3	19	272	15.1
6	200	601	9.9	36	270	7.5
5	301	576	1.0	16	283	17.6
4	397	525	3.4	20	162	8.0
3	500	665	0.8	16	209	13.2
2	599	629	2.6	17	156	8.8
1	803	520	5.8	42	414	9.9

Process 2 CTD #59

Niskin	Depth m	Bass pM	Al nM	Ca nM	Sr pM	Sr/Ca mmol/mol
22	45	184	8.9	57	700	12.3
20	78	217	8.2	81	970	11.9
19	97	173	5.7	20	470	23.5
18	125	343	4.3	55	704	12.8
16	174	426	3.9	298	3413	11.4
14	224	436	1.1	54	398	7.4
12	276	456	0.9	10	29	3.0
10	349	521	7.2	183	2553	13.8
8	451	558	8.0	17	639	38.6
5	599	393	2.9	15	60	4.0
4	900	292	2.9	26	370	14.8

Process 3 CTD #78

Niskin	Depth m	Bass pM	Al nM	Ca nM	Sr pM	Sr/Ca mmol/mol
24	9	83	0.3	291	3423	12
22	18	53	1.6	237	1344	5.7
20	49	234	1.0	13	49	3.7
18	75	146	1.6	196	1396	7.1
17	99	211	1.0	11	94	8.7
16	124	332	0.1	18	239	13.5
15	149	316	<DL	14	108	7.8
14	200	369	0.8	28	131	5.0
13	249	305	<DL	25	254	8.9
12	301	212	0.2	21	71	3.3
11	350	310	<DL	26	91	3.2
10	400	235	0.5	26	<DL	/
9	449	270	0.2	39	167	4.2
8	500	283	2.4	21	56	2.8
7	550	322	1.2	39	251	6.4
6	598	331	1.7	39	228	5.8
5	651	254	1.7	32	148	4.6
4	699	230	2.8	31	201	6.4
3	799	252	0.4	35	62	1.8
2	899	242	0.7	33	61	1.8
1	1000	317	2.4	47	124	2.6

Process 3 CTD #79

Niskin	Depth m	Bass pM	Al nM	Ca nM	Sr pM	Sr/Ca mmol/mol
9	900	209	5.2	19	134	7.1
6	1249	174	0.8	12	66	5.8
4	1749	315	0.9	14	102	7.3
1	2501	321	3.0	15	104	6.9

Process 3 CTD #86

Niskin	Depth m	Bass pM	Al nM	Ca nM	Sr pM	Sr/Ca mmol/mol
24	9	17	5.3	61	400	6.6
23	22	137	6.4	337	2408	7.1
20	50	80	1.7	103	879	8.5
18	75	108	3.2	59	403	6.8
17	99	95	<DL	9	88	9.9
16	149	245	1.7	18	291	15.8
13	201	430	4.7	13	195	15.2
11	249	430	4.7	24	250	10.6
10	300	350	2.0	18	202	11.4
9	351	239	4.1	9	109	12.3
7	400	<DL	1.6	4	5	1.5
6	452	283	2.1	19	191	10.0
5	501	229	2.9	18	179	11.1
4	550	218	2.6	20	202	10.0
3	601	378	1.7	39	304	7.8
2	700	250	8.0	28	225	8.1
1	801	217	3.2	17	149	8.7

Process 3 CTD #104

Niskin	Depth m	Bass pM	Al nM	Ca nM	Sr pM	Sr/Ca mmol/mol
24	19	65	0.8	260	1406	5.3
22	48	107	3.5	259	1496	5.8
20	74	78	3.5	184	1339	9.0
18	101	127	2.3	38	323	8.5
16	124	240	2.0	17	215	12.5
15	150	327	0.6	16	230	14.0
14	199	329	1.8	25	189	7.6
12	250	407	2.7	32	253	8.0
11	275	453	<DL	<DL	246	/
10	296	332	1.1	34	257	7.6
8	349	258	1.6	31	181	5.9
7	401	177	2.4	19	148	7.8
6	450	193	2.2	24	139	5.8
5	501	211	0.6	23	162	7.1
4	600	147	3.0	17	104	6.1
2	700	211	4.5	34	165	4.8
1	900	284	3.5	43	184	4.3

Transit CTD #5

Niskin	Depth m	Bass pM	Al nM	Ca nM	Sr pM	Sr/Ca mmol/mol
24	13	167	21.5	307	2704	8.8
22	24	126	21.5	129	1022	7.9
20	53	94	5.9	54	384	7.1
17	73	210	8.8	59	535	9.1
16	99	73	8.8	15	166	11.3
15	99	130	1.6	26	297	11.5
14	124	93	2.3	49	926	19.8
13	149	163	2.6	22	221	10.2
12	199	191	19.7	37	267	7.2
11	249	282	8.6	17	107	6.2
10	300	197	21.6	59	377	6.4
8	401	309	5.2	24	167	7.0
7	449	242	3.6	30	724	24.8
6	499	226	4.8	22	158	7.3
5	551	314	8.1	30	170	5.7
4	599	285	6.8	19	112	5.9
3	650	593	10.1	34	183	5.4
2	703	228	4.8	55	541	9.8
1	900	232	3.4	26	197	7.6

Transit CTD #64

Niskin	Depth m	Bass pM	Al nM	Ca nM	Sr pM	Sr/Ca mmol/mol
24	33	105	0.9	163	654	4.0
22	50	99	1.8	294	1806	6.1
20	78	112	2.5	265	1612	6.4
19	102	92	1.6	212	730	3.4
16	125	145	0.1	81	606	7.4
15	150	173	3.4	32	213	6.6
14	196	232	<DL	36	144	3.8
12	249	198	<DL	23	<DL	/
11	275	224	<DL	30	<DL	/
10	300	229	1.3	21	10	0.5
8	352	418	<DL	173	1224	7.1
7	403	364	0.1	47	146	3.1
6	452	397	2.1	51	46	1.0
5	500	337	<DL	31	182	5.9
4	599	539	1.6	39	20	0.5
3	701	232	5.2	319	5729	7.0
2	804	329	8.4	31	89	2.9
1	1002	362	2.0	39	21	0.5

Transit CTD #66

Niskin	Depth m	Bass pM	Al nM	Ca nM	Sr pM	Sr/Ca mmol/mol
24	20	35	2.7	104	612	7.8
23	50	81	1.6	113	726	6.4
22	75	29	2.4	68	458	4.8
21	100	30	0.4	33	264	7.9
20	124	237	2.6	41	273	6.7
19	152	247	<DL	45	239	5.1
18	175	219	33.4	38	214	5.6
17	200	273	1.7	49	387	7.8
16	224	429	0.1	50	305	6.1
15	251	361	1.0	45	274	6.1
14	274	394	3.5	51	259	5.1
13	300	417	5.0	71	430	6.0
12	325	444	4.7	77	356	4.6
11	349	353	2.9	58	319	5.5
10	369	279	2.8	39	183	4.7
9	451	275	5.5	53	242	4.5
8	501	239	2.9	46	217	4.8
7	549	253	2.7	44	212	4.8
6	600	419	1.0	63	345	5.5
5	650	209	0.8	42	252	6.0
4	701	235	2.0	42	214	5.2
3	801	295	5.0	52	244	4.7
2	900	281	3.0	45	242	5.0
1	1001	219	2.1	37	184	4.9

Transit CTD #71

Niskin	Depth m	Bass pM	Al nM	Ca nM	Sr pM	Sr/Ca mmol/mol
24	19	158	4.9	219	3651	16.7
22	49	92	2.8	126	1150	9.0
20	74	131	4.7	111	867	7.8
19	99	367	5.2	51	437	8.5
16	125	379	3.2	29	326	11.5
15	149	459	1.9	35	326	9.2
14	201	470	3.9	35	375	10.6
12	250	540	1.5	34	302	9.0
11	275	453	0.9	35	260	7.5
10	300	413	1.3	31	244	7.8
8	349	394	1.4	29	225	7.7
7	401	370	2.4	22	229	10.4
6	450	334	1.4	31	277	8.8
5	502	352	2.5	40	278	7.0
4	601	358	1.5	40	257	6.5
3	700	358	4.9	45	244	5.3
2	801	207	1.8	34	220	6.5
1	899	378	2.3	49	272	5.5

Transit CTD #93

Niskin	Depth m	Bass pM	Al nM	Ca nM	Sr pM	Sr/Ca mmol/mol
24	15	49	<DL	227	1 636	7.2
22	24	93	1.4	256	1 246	4.9
20	52	62	<DL	298	1 263	3.8
18	75	71	<DL	102	369	3.6
17	103	84	<DL	25	83	3.3
16	124	53	<DL	13	<DL	/
15	150	134	<DL	41	202	5.0
14	199	163	<DL	16	70	4.4
13	250	252	0.1	27	238	8.9
12	301	259	1.3	60	559	9.4
11	349	280	<DL	17	28	1.6
10	401	289	1.6	43	<DL	/
9	449	298	1.6	35	136	3.9
8	503	331	0.4	79	595	7.5
7	550	257	0.0	52	298	5.8
6	602	270	0.6	36	140	3.7
5	649	271	<DL	43	303	7.0
4	703	264	0.2	46	191	4.0
3	799	277	1.5	46	116	2.5
2	901	244	1.0	41	226	5.5

Transit CTD #108

Niskin	Depth m	Bass pM	Al nM	Ca nM	Sr pM	Sr/Ca mmol/mol
24	9	428	34.1	408	7439	8.2
20	98	367	1.3	310	3416	11.0
18	125	322	1.8	298	3856	13.3
16	150	71	2.0	158	792	5.0
17	200	70	3.1	105	584	9.8
15	301	109	4.7	19	219	12.1
14	349	151	2.5	21	278	13.3
13	401	80	1.1	18	150	7.8
12	499	169	2.8	36	290	8.8
11	600	228	3.7	57	536	9.5
10	699	169	3.4	28	157	5.0
9	800	325	2.3	47	302	6.5
8	900	307	4.5	44	295	6.7
7	1001	291	2.1	41	260	6.4
6	1249	223	1.4	34	286	7.7
5	1249	172	4.3	47	642	13.7
5	1500	277	5.5	44	236	5.5
4	1750	201	2.0	30	249	8.9
3	1999	253	2.4	35	243	6.8
2	2249	217	4.5	33	202	6.2
1	2500	235	5.0	30	160	5.0

Transit CTD #3

Niskin	Depth m	Bass pM	Al nM	Ca nM	Sr pM	Sr/Ca mmol/mol
9	797	1991	35.5	57	636	11.0
6	1248	341	1.3	46	359	7.4
4	1748	1453	19.9	217	1235	5.7
2	2248	239	7.7	25	439	17.2

Transit CTD #36

Niskin	Depth m	Bass pM	Al nM	Ca nM	Sr pM	Sr/Ca mmol/mol
9	800	453	5.5	65	601	9.4
6	1247	552	9.5	66	459	6.9
4	1750	466	11.9	78	511	6.5
2	2249	475	11.2	72	568	8.2

Transit CTD #61

Niskin	Depth m	Bass pM	Al nM	Ca nM	Sr pM	Sr/Ca mmol/mol
9	796	588	1.7	109	1364	12.5
6	1252	287	0.0	71	1329	18.8
4	1750	370	0.8	44	151	3.5
1	2500	499	3.5	28	145	5.2

Table 3

Station type	CTD cast #	Longitude, °E	Latitude, °S	DWAV* Ba_{ex} [pM]
Process 1	11	140.56	46.32	188
Process 1	19	140.31	46.50	223
Process 1	30	140.64	46.58	187
				199±21
Process 2	40	145.87	54.01	354
Process 2	44	146.13	54.02	333
Process 2	51	146.30	54.14	440
Process 2	58	146.12	54.45	515
Process 2	59	147.10	54.45	463
				421±76
Process 3	78	153.18	45.55	282
Process 3	86	153.35	45.47	296
Process 3	104	153.68	45.59	259
				279±19
Transit	5	143.05	44.89	249
Transit	64	148.65	50.88	280
Transit	66	149.42	49.99	306
Transit	71	151.19	48.06	395
Transit	93	152.49	44.93	244
Transit	108	148.58	43.69	146

DWAV*= Depht weighted average value integrated between 100-600 m in the twilight zone

Table 4

All fluxes in mmolC/m²/d

Station type	CTD #	Longitude °E	Latitude °S	GPP [1] *euphotic layer*	NP [1] *euphotic layer*	BCD[2] *100-600m*	EP [3] *100 m*	MR [4] *100-600 m*	*MR Stnd Uncertainty* %	r-ratio[5] %
Process 1	7	140.65	46.39	-	-	-	7.0	-	-	
Process 1	9	140.65	46.32	87.9	44	-	-	-	-	
Process 1	11	140.56	46.32	-	-	-	-	1.8	40	
Process 1	15	140.50	46.44	-	-	-	4.8	-	-	
Process 1	17	140.40	46.47	44.2	9.5	-	-	-	-	26-68
Process 1	19	140.31	46.50	-	-	11.1	-	2.6	29	
Process 1	30	140.64	46.58	-	-	16.9	-	1.8	38	
Process 1	31	140.34	46.48	-	-	-	4.1	-	-	
Process 1	33	140.20	46.71	-	-	-	3.8	-	-	
Process 1	34	140.29	46.65	9.4	1.3	-	-	-	-	
				9.4-87.9	***1.3-9.5***	***14±4.1***	***4.9±1.4***	***2.1±0.4***		***43***
Process 2	40	145.87	54.01	-	-	16.6	-	3.6	21	
Process 2	42	146.17	54.05	34.0	6.7	-	-	-	-	
Process 2	44	146.13	54.02	-	-	19.6	-	3.2	24	
Process 2	47	146.32	54.13	34.0	10.3	-	-	-	-	
Process 2	49	146.31	54.15	-	-	-	2.9	-	-	48- >100
Process 2	51	146.30	54.14	-	-	58.9	-	5.4	17	
Process 2	58	146.12	54.45	37.7	9.0	13.2	-	7.0	15	
Process 2	59	147.10	54.45	-	-	-	6.6	5.9	16	
				35.2±2.1	***8.7±1.8***	***27.1±21.4***	***4.7±2.6***	***5.0±1.6***		***>100***
Process 3	78	153.18	45.55	-	-	12.4	-	3.8	21	
Process 3	81	153.23	45.52	39.8	8.1	-	-	-	-	
Process 3	84	153.28	45.45	-	-	-	3.2	-	-	
Process 3	85	153.34	45.45	43	11.3	-	-	-	-	
Process 3	86	153.35	45.47	-	-	15.3	-	4.1	20	51->100
Process 3	96	153.62	45.50	65.2	11.3	-	-	-	-	
Process 3	97	153.51	45.51	-	-	-	6.5	-	-	
Process 3	104	153.68	45.59	-	-	19.9	-	3.3	23	
				49.3±13.8	***10.2±1.8***	***15.9±3.8***	***4.8±2.3***	***3.7±0.4***		***77***
Transit	4	143.02	44.92	-	-	-	4.3	-	-	73
Transit	5	143.05	44.89	-	-	10.7	-	3.1	25	
Transit	64	148.65	50.88	-	-	13.7	6.4	3.7	21	60
Transit	66	149.42	49.99	-	-	12.2	7.1	4.3	19	61
Transit	71	151.19	48.06	-	-	13.6	6.3	6.1	16	98
Transit	93	152.49	44.93	-	-	8.8	5.9	3.0	25	51
Transit	108	148.58	43.69	-	-	15.8	-	1.0	68	-

1 GPP = Gross Primary Production based on ^{13}C-uptake; NP = new production based on ^{15}N-uptake; from Cavagna et al. (this issue)
2 BCD= Bacterial Carbon Demand; from Dumont et al. (this issue)
3 EP= Export Production, based on ^{234}Th-deficit data; from Jacquet et al.b (this issue)
4 MR= Mesopelagic remineralization, based on meso-Ba_{ex} data, this work
5 r ratio: ratio MR to EP

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Isabelle Dumont

Interactions between the microbial network and the organic matter in the Southern Ocean: impacts on the biological carbon pump

The Southern Ocean (ca. 20% of the world ocean surface) is a key place for the regulation of Earth climate thanks to its capacity to absorb atmospheric carbon dioxide (CO_2) by physico-chemical and biological mechanisms. The biological carbon pump is a major pathway of absorption of CO_2 through which the CO_2 incorporated into autotrophic microorganisms in surface waters is transferred to deep waters. This process is influenced by the extent of the primary production and by the intensity of the remineralization of organic matter along the water column. So, the annual cycle of sea ice, through its in situ production and remineralization processes but also, through the release of microorganisms, organic and inorganic nutrients (in particular iron) into the ocean has an impact on the carbon cycle of the Southern Ocean, notably by promoting the initiation of phytoplanktonic blooms at time of ice melting.

The present work focussed on the distribution of organic matter (OM) and its interactions with the microbial network (algae, bacteria and protozoa) in sea ice and ocean, with a special attention to the factors which regulate the biological carbon pump of the Southern Ocean. This thesis gathers data collected from a) late winter to summer in the Western Pacific sector, Western Weddell Sea and Bellingshausen Sea during three sea ice cruises ARISE, ISPOL-drifting station and SIMBA-drifting station and b) summer in the Sub-Antarctic and Polar Front Zone during the oceanographic cruise SAZ-Sense.

The sea ice covers were typical of first-year pack ice with thickness ranging between 0.3 and 1.2 m, and composed of granular and columnar ice. Sea ice temperature ranging between -8.9°C and -0.4°C, brines volume ranging between 2.9 to 28.2% and brines salinity from 10 to >100 were observed. These extreme physico-chemical factors experienced by the microorganisms trapped into the semi-solid sea ice matrix therefore constitute an extreme change as compared to the open ocean. Sea ice algae were mainly composed of diatoms but autotrophic flagellates (such as dinoflagellates or Phaeocystis sp.) were also typically found in surface ice layers. Maximal algal biomass was usually observed in the bottom ice layers except during SIMBA where the maxima was localised in the top ice layers likely because of the snow and ice thickness which limit the light available in the ice cover. During early spring, the algal growth was controlled by the space availability (i.e. brine volume) while in spring/summer (ISPOL, SIMBA) the major nutrients availability inside sea ice may have controlled algal growth. At all seasons, high concentrations of dissolved and particulate organic matter were measured in sea ice as compared to the water column. Dissolved monomers (saccharides and amino acids) were accumulated in sea ice, in particular in winter. During spring and summer, polysaccharides constitute the main fraction of the dissolved saccharides pool. High concentrations of transparent exopolymeric particles (TEP), mainly constituted with saccharides, were present and their gel properties greatly influence the internal habitat of sea ice, by retaining the nutrients and by preventing the protozoa grazing pressure, inducing therefore an algal accumulation. The composition as well as the vertical distribution of OM in sea ice was linked to sea ice algae. Besides, the distribution of microorganisms and organic compounds in the sea ice was also greatly influenced by the thermodynamics of the sea ice cover, as evidenced during a melting period for ISPOL and during a flood-freeze cycle for SIMBA. The bacteria distribution in the sea ice was not correlated with those of algae and organic matter. Indeed, the utilization of the accumulated organic matter by bacteria seemed to be limited by an external factor such as temperature, salinity or toxins rather than by the nature of the organic substrates, which are partly composed of labile monomeric saccharides. Thus the disconnection of the microbial loop leading th OM accumulation was highlighted in sea ice.

In addition the biofilm formed by TEP was also involved in the retention of cells and other compounds (DOM, POM, and inorganic nutrients such as phosphate and iron) to the brine channels walls and thus in the timing of release of ice constituents when ice melts. The sequence of release in marginal ice zone, as studied in a microcosm experiments realized in controlled and trace-metal clean conditions, was likely favourable to the development of blooms in the marginal ice zone. Moreover microorganisms derived from sea ice (mainly <10 µm) seems able to thrive and grow in the water column as also the supply of organic nutrients and Fe seems to benefit to the pelagic microbial community.

Finally, the influence of the remineralization of organic matter by heterotrophic bacterioplankton on carbon export and biological carbon pump efficiency was investigated in the epipelagic (0-100 m) and mesopelagic (100-700 m) zones during the summer in the sub-Antarctic and Polar Front zones (SAZ and PFZ) of the Australian sector (Southern Ocean). Opposite to sea ice, bacterial biomass and activities followed Chl a and organic matter distributions. Bacterial abundance, biomass and activities drastically decreased below depths of 100-200 m. Nevertheless, depth-integrated rates through the thickness of the different water masses showed that the mesopelagic contribution of bacteria represents a non-negligible fraction, in particular in a diatom-dominated system.