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Particulate organic matter release below melting sea ice (Terra Nova Bay, Ross Sea, Antarctica): possible relationships with zooplankton

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Authors' role:
AP: Conceptualization, Formal analysis; Writing - review & editing
OM, **MS**, **AG**: Conceptualization, Writing - original draft,
LG: Conceptualization, Formal analysis; Funding acquisition
AC, KMS: Investigation, Writing - original draft
DM, **FB**, **RM**, **CE**: Laboratory analyses, Writing - original draft

Highlights

- Sea ice melting released a large quantity of POM into the water column
- Zooplankton grazing influenced composition and nutritional value of sinking POM
- Sinking POM composition varied with time and influenced pteropod composition
- POM release from melting ice is a key step in the energy transfer to higher trophic levels
- Since Antarctic sea ice area is decreasing, we anticipate consequences on planktonic trophic webs

Abstract

Timing and rates of release of particulate organic matter (POM) beneath the Antarctic sea ice during the melting season are relatively unknown. To shed light on this topic, we investigated: i) quantity and biochemical composition of POM released below annual sea ice in Terra Nova Bay (TNB, Ross Sea, Antarctica) through sediment traps deployed at 10- and 30-m depth; ii) the abundance and taxonomic composition of phytoplankton; iii) the abundance and biochemical composition of two zooplankton species, namely the pteropods *Clione limacina antarctica* and *Limacina helicina antarctica*. Overall, our results show that in late spring-early summer in TNB the melting of annual sea ice determines a rapid release of particles into the underlying water column, and that those particles are transported downward at the rate of hours to days. POM fluxes were dominated by lipids, which probably resulted from zooplankton grazing activity on sea-ice algae and faecal pellets that were released into the water column. This, after the initial injection of particles likely associated with sinking sympagic microalgal biomass derived from the sea ice melting, enhanced the nutritional value of POM. POM released beneath the sea ice, in turn, had also a possible effect on the biochemical composition of the two pteropod species. Our results confirm that melting of sea ice in spring-summer each year in Antarctic coastal waters represents a fundamental step in the transfer of energy towards the higher trophic levels. Evidence of a decline in Antarctic sea ice over the last five years, plausibly as a consequence of global warming, underlines a conspicuous decline in habitat availability for sea-ice algae and, as a consequence, a drop in the availability of food for pteropods and the higher trophic levels of the coastal Antarctic Ocean.

1. Introduction

Antarctic sea ice, which varies seasonally in coverage from ca. 20 (winter) to 2 (summer) millions of km^2, represents, after the Arctic Ocean in winter, the largest portion of the Earth permanently covered by sea ice (Comiso and Nishio, 2008; Thomas and Dieckmann, 2010). The formation and melting processes of Antarctic sea ice are among the most impressive and dynamic seasonal phenomena occurring on our planet, with pivotal implications for the world's climate and ocean processes, from coastal areas to abyssal plains (Lambeck et al., 2002; Arrigo et al., 2008).

Seasonal variation in Antarctic sea ice extent triggers the tight relationship between the sympagic and pelagic domains (Arrigo et al., 1997; Brierley and Thomas, 2002; Mangoni et al., 2017). In winter, when phytoplankton production is nearly zero, sympagic algae can represent a key source of food for ice-dependent fish and zooplankton (Kohlback et al., 2018; Guidetti et al., 2015; Jia et al., 2016), while POM released by the sea ice is nil. In spring, annual sea ice hosts a huge microalgal biomass, mainly diatoms, which accumulate in thick brown-colored layers localized at the sea ice-seawater interface (Arrigo et al., 1997; Lizorre, 2001; Arrigo and Thomas, 2004; Mangoni et al., 2009a; Saggiomo et al., 2017).

During late spring-early summer, sea ice melting releases into the water column large quantities of dissolved and particulate organic matter (mostly of algal origin) which, in turn, influences the pelagic and benthic communities, and, as a whole, the Southern Ocean food webs (Loeb et al., 1997; Pusceddu et al., 1999; Pusceddu et al., 2009a; Riaux-Gobin et al., 2003; Arrigo and Thomas, 2004; Saggiomo et al., 2020).

The amount of particulate matter released from the sea ice, which typically shows a vast short-term pulsing event in late spring-early summer (Langone et al., 2000), is influenced by local variations in sympagic microalgae production, snow and ice coverage, light intensity and mixing depth of the surface ocean (Lazzara et al., 2007; Pusceddu et al., 2009a; Thomas and Dieckmann, 2010). Fluxes of particles below the sea ice, particularly in late spring-early summer, also exhibit variations on shorter temporal scales (i.e., days or weeks), associated with physical (e.g., water mass exchange, ice coverage) and biological (e.g., grazing activities, fecal pellet production) processes (Fabiano et al., 1997; Michels et al., 2008) acting at the local scale.

The Ross Sea is the most productive region of the Antarctic ocean, with a high variability in timing of production and physical constraints of different sub-systems (Bolinesi et al., 2020) and, being a major site for atmospheric carbon flux, has a pivotal role in Southern Ocean biogeochemical cycles (Arrigo et al., 2008). In the annual sea ice at Terra Nova Bay (Ross Sea), huge autotrophic and heterotrophic biomass accumulates during early spring at the ice-water interface (Guglielmo et al., 2000; Guglielmo et al., 2004; Guglielmo et al., 2007; Mangoni et al., 2009a). Such biomass is a dominant contributor to the particulate organic matter (POM) pool that is released into the water column during summer sea ice melting, fueling both pelagic and benthic habitats (Fabiano and Pusceddu, 1998; Pusceddu et al., 1999;

Guglielmo et al., 2004; Misic et al., 2017b; Saggiomo et al., 2017). POM released by sea ice melting is intercepted by zooplankton, which in summer also represent a key step in the transfer of energy from the sympagic communities to higher trophic levels (Pane et al., 2004; Granata et al., 2009; Smith et al., 2017).

To date, knowledge about timing, quantity, biochemical composition and nutritional quality of POM released during ice melting in late spring–early summer in the coastal Ross Sea is relatively poor. Also, to the best of our knowledge, no data are available on the gross (protein, carbohydrate, lipid) biochemical composition of zooplankton below the sea ice and whether and how it relates to the composition of sinking POM. In this regard, it must be noted that pteropods are a key component of Antarctic ecosystem functioning as they provide food for higher trophic levels, exert top-down control on primary production and influence Southern Ocean biogeochemical cycles (Hunt et al., 2008; Weldrick et al., 2019). There is also evidence that pteropods can be early responders to ocean acidification (Bednaršek et al., 2017) and that their decline, possibly due to climate change, could impair the biological pump (Manno et al., 2010). Thus, pteropods represent an ideal target to shed light on the potential effects of climate change on Antarctic ecosystems. However, to date, the knowledge of their ecological role beneath the sea ice is rather limited (Elliott et al., 2009),

We hypothesized that short-term variations in the biochemical composition of one of the two most abundant zooplankton taxa (along with copepods) below the Terra Nova Bay sea ice, namely the pteropods *Clione limacina antarctica* (predator) and *Limacina helicina antarctica* (herbivorous) (Pane et al., 2004; Guglielmo et al., 2007), are related to the gross biochemical composition of POM released beneath the sea ice.

To test our hypothesis, during late spring-summer in Terra Nova Bay (Ross Sea), we investigated short-term variations in: 1) the quantity and biochemical composition of organic matter fluxes and taxonomy of microalgal species released into the water column during sea ice melting; and 2) the abundance and gross biochemical composition of pteropods caught below the sea ice.

2. Materials and methods

2.1 Study Area

Terra Nova Bay (TNB; **Fig. 1**), located between Cape Washington and the Drygalski Ice Tongue, is a wide inlet, occupying an area of approximately 6000 km^2 along the coast of Victoria Land, in the western Ross Sea. TNB is characterized by the presence, in late winter-early spring, of either platelet or pack ice, which, during melting, contributes to the injection of large quantities of dissolved and particulate matter into the water column (Catalano et al., 2000).

2.2 Particle sampling

Vertical particle flux below annual sea ice was estimated using cylindrical traps with a collecting area of ca. 70 cm^2 (total volume 6 L). Two sets of three traps, each equipped with a collector cup of 1.0 L

capacity, were deployed at 10 and 30-m depth from November 12nd to December 15th 2015. During this period, the traps were left operating consecutively for 72 h, thus retrieved and redeployed every three days. The traps were deployed through an ice-hole located on the pack ice of Terra Nova Bay (74°41.02' S; 164°07.85' E; **Fig. 1**). During the recovery, though the traps remained open, the very small mouth of the collector cup (ca. 3.5 cm^2 against 70 cm^2 of the trap) limited particle resuspension inside the trap (height/diameter aspect of the traps was ca. 20).

Based on visual inspection, no swimmers were present in the traps for any of the sampling dates and depths. We did not use any preservative in the collector traps, as already done in previous experiments in the same study area (Pusceddu et al., 1999; Pusceddu et al., 2009a), to avoid the potential (though minor) interference of preservatives with the biochemical analyses used (Fabiano et al., 2001). The use of preservatives for this kind of analyses is generally used in oligotrophic deep-sea environments where traps are left in operation for weeks or months (Sanchez Vidal et al., 2015). Moreover, using the available information on the extracellular enzymatic activities (glucosidase and peptidase) in the water column of the Ross Sea polynya (Misic et al., 2002), we estimated that the time potentially needed to degrade the entire protein and carbohydrate pools in the traps (according to Pusceddu et al., 2014) would vary in the range from tens (for protein) to hundreds (for carbohydrate) of days, far longer than the 3 days of trap deployment in our study.

On each sampling date seawater aliquots of 200-250 mL from each cup (n=3) were filtered onto Whatman GF/F membranes (25 mm diameter, 0.7 μm nominal pore size, pre-combusted at 450 °C for 4 hours) using a vacuum pump (25 mm Hg) and then analyzed for POM biochemical composition, as described below. An additional 200 mL aliquot of seawater was drawn from each cup (n=3) and filtered onto GF/F Whatman filters (25 mm diameter) for the analysis of total phytoplankton biomass (expressed as Chlorophyll-*a* (Chl-*a*) concentration). Samples for the analysis of Chl-*a* were stored in liquid nitrogen until analysis with a spectrofluorometer. For the identification and enumeration of phytoplankton, 200 mL subsamples from each cup were preserved in 4% buffered (Ca carbonate) formaldehyde (final concentration).

2.3 POM biochemical composition

Protein, carbohydrate and lipid contents of POM were analyzed spectrophotometrically using albumin, D (+) glucose and tripalmitine, as standards, respectively (Hartree, 1972, Dubois et al., 1956; Bligh and Dyer, 1959; Marsh and Weinstein, 1966). Protein, carbohydrate and lipid concentrations were converted into carbon equivalents using 0.49, 0.40 and 0.75 mg C mg^{-1} as conversion factors, respectively, and reported as biopolymeric Carbon (BPC) (Fabiano and Pusceddu, 1998). We limited our analysis to BPC as, in the study area, it represents 80-90% of organic C (POC) concentrations (Misic et al., 2017a), but also because, being based on the gross biochemical composition of POM, it provides more information about the nutritional value of particles' than the sole POC as a whole (Fabiano and Pusceddu, 1998; Pusceddu et al., 2009b).

2.4 POM flux and export rates

Fluxes of each biochemical variable (g m^{-2} d^{-1}) were estimated using the following formula (Pusceddu et al. 2007):

$$F_i\ (g_i\ m^{-2}\ d^{-1}) = 1000 \bullet Cf \times [Vs \bullet Fs^{-1}] \bullet Td^{-1} \bullet Sc^{-1}$$

where *Fi* is the flux of the *i* compound, *Cf* is the concentration (mg mL^{-1}) of the *i* compound in the analytical subsample, *Vs* is the total volume of the aliquot (mL), *Fs* is the fraction of the total volume collected by the trap (1/4 or 1/5), *Td* is the time of trap deployment (days) and *Sc* is the collecting surface of the trap (m^2).

To investigate temporal variations in the export rates of POM below the pack ice, the percentage difference in fluxes at 30 m and 10 m depth was calculated for each sampling date. Positive values were assumed to be indicative of an immediate export of POM towards deeper water column layers, whereas negative values were considered as indicative of lateral particle transport or grazing activity.

2.5 Phytoplankton biomass and community composition

Chl-*a* and phaeopigment (Phaeo) contents were analyzed according to Holm-Hansen et al. (1965) using a Varian Eclipse spectrofluorometer. For comparison with BPC, total phytopigment contents were calculated as the sum of Chl-*a* and Phaeo, and converted into C equivalents, using 40 as the conversion factor (Lopez Fernandez et al. 2013). Various phaeophorbides and phaeophytins have been recognized as indicators of grazing by different herbivores (Strom, 1993; Wright and van den Enden, 2000). Here, we used the pheao:chl a ratio as proxy of grazing activity on phytoplankton cells (Shuman and Lorenzen, 1975; Collos et al., 2005).

The phytoplankton species composition and cell abundance were determined according to the Utermöhl method (Utermöhl, 1958) using an inverted light microscope (LM) Zeiss Axiophot, at 400 x magnification, and counting at least 200 cells for each sample.

2.6 Abundance and gross biochemical composition of zooplankton

A total of 25 zooplankton samples were collected, almost daily, from November 21th to December 15th 2015 with an Apstein net (100 cm total length, net aperture 40 cm, surface 0.125 m^2, 100 μm mesh size). The net was vertically towed at a speed of 0.5-1.0 ms^{-1}, from a hole in the sea ice, located ca. 10 m apart from the hole used for the deployment of traps, down to 150 m depth, which is the depth within which the maximum abundance of pteropods is typically encountered in this area (Pane et al., 2004; Guglielmo et al., 2007; Smith et al. 2017). In this regard, we also notice that we fixed the depth of zooplankton sampling well below the depth of deployment of the sediment traps as previous studies demonstrated that a large proportion (up to 75%) of POM released by melting sea ice can be found below 20-m depth (Pusceddu et al., 2009a).

Zooplankton samples were brought to the Italian station within 1 hour from collection and, once in the laboratory, the total volume (500 mL) of each tow was split into two sub-aliquots (250 mL each)

using a Motoda plankton splitter (Motoda, 1959). 30 pteropods were randomly sorted out from one of the two 250 mL sub-samples, identified at species level under a Leica Wild M10 stereomicroscope, and finally frozen at -20 ° C for subsequent biochemical analyses. The remaining volume from the first sub-sample and the whole second 250 mL aliquot were preserved in borate buffered 4%formalin-seawater solution with for subsequent qualitative and quantitative analyses of the entire zooplankton community.

From the whole sample, the mean abundance (over 150-m depth) of the pteropods *Clione limacina antarctica* and *Limacina helicina antarctica* was determined and expressed as number per m^3.

To assess the gross biochemical composition (protein, carbohydrate and lipid) of the two pteropod species, a pool of 5 specimens for each pteropod species on each sampling date were sub-sampled from the initial pool of 30 and homogenised through a manual homogenizer (kept in an ice bath to avoid sample warming) in 2 mL of reagent grade water. Biochemical analyses were conducted (in triplicate) using a variable amount (0.1-0.4 mL) of the homogenized sample, using the same analytical procedures as for POM analyses, modified for animal tissues (Perrone et al. 2003; Gastoni et al. 2010). Protein, carbohydrate and lipid concentrations were normalized to the wet weight of the sample.

2.7 Statistical analyses

Temporal variations in the quantity and biochemical composition of particle fluxes at both 10 and 30 m depth were investigated through two-way permutational analyses of variance (PERMANOVA) (Anderson, 2001; Anderson and Robinson, 2001), in either univariate (*i.e.* each variable separately) or multivariate contexts, followed by post-hoc comparison tests in cases of significant effects. Analyses were carried out separately on fluxes of particulate protein, carbohydrate, lipid and BPC using Euclidean-distance resemblance matrixes after normalization of the data. The same transformation of the data and typology of matrixes were used in the multivariate context by including protein, carbohydrate and lipid C equivalents. Also, temporal variations in the gross biochemical composition of the two pteropod species were investigated through PERMANOVA, using species and sampling date as sources of variance.

The significance of variations in the biochemical composition of POM flux on the zooplankton biochemical composition was determined by means of a distance-based redundancy analysis (Legendre and Anderson, 1999; McArdle and Anderson, 2001). The analysis was carried out on Euclidean distance-based resemblance matrixes obtained from raw data of protein, carbohydrate and lipid contents of the two pteropod species. Variance in the biochemical composition of pteropods explained by the protein, carbohydrate and lipid contents of POM fluxes (after normalization of the raw data) were then determined using a step-wise procedure and R^2 as the selection criterion.

All statistical analyses were performed using the software PRIMER 7+ (Anderson et al., 2008).

3. Results

3.1 Particulate fluxes below the sea ice

Estimates of protein, carbohydrate, lipid and BPC fluxes below the pack ice in Terra Nova Bay are reported in **Appendix A** (**Table A.1**).

Protein, carbohydrate, lipid, BPC and the biochemical composition of particles sinking below the pack ice varied significantly among sampling times and between depths (**Table 1**), though with variable patterns and some exceptions (*i.e.*, BPC and lipid fluxes between depths). Post-hoc pairwise comparison tests revealed that: 1) differences in the quantity of POM fluxes between sampling depths were significant during the first half of the sampling period (until November 27th) and became smoother or had disappeared by the end of the sampling period; 2) differences in the biochemical composition of POM fluxes between the two depths were significant during almost the entire sampling period, but tended to be smoother during the second phase of the sampling period (starting from December 3rd) (**Table 2**).

Particulate protein flux ranged from 2.6 ± 0.4 to 24.3 ± 3.6 mgC m^{-2} d^{-1} and from 4.0 ± 0.6 mgC m^{-2} d^{-1} to 18.3 ± 2.7 mg C m^{-2} d^{-1} at 10- and 30-m depth, respectively. At 10-m depth, protein flux reached the highest values between the last week of November and the first days of December, and showed a minor peak at the end of the sampling period (**Fig. 2A**). At 30-m depth, protein flux reached the highest value on November 18th, markedly dropped during the following week, then generally increased from November 24th to the end of the study period (**Fig. 2A**).

Particulate carbohydrates fluxes ranged from 1.1 ± 0.4 to 8.8 ± 3.1 mgC m^{-2} d^{-1} and from 0.7 ± 0.2 mgC m^{-2} d^{-1} to 21.0 ± 7.8 mgC m^{-2} d^{-1} at 10- and 30-m depth, respectively. At both depths, particulate carbohydrates fluxes suddenly decreased after November 15th, remained low and relatively constant till December 12th, and showed a peak at the end of the sampling period (**Fig. 2B**).

Fluxes of particulate lipids ranged from 5.0 ± 0.9 to 36.2 ± 7.2 mgC m^{-2} d^{-1} at 10-m depth and from 9.9 ± 1.9 to 52.1 ± 10.4 mgC m^{-2} d^{-1} at 30-m depth. Temporal variations in particulate lipid fluxes at both depths were similar to those observed for proteins and showed minor peaks at the beginning of the study period, on November 27th and on December 15th (**Fig. 2C**).

Vertical fluxes of BPC under the pack ice varied from 10.4 ± 1.6 to 60.8 ± 9.1 mgC m^{-2} d^{-1} and from 14.7 ± 2.2 to 75.7 ± 11.4 mgC m^{-2} d^{-1} at 10- and 30-m depth, respectively. Temporal variations in BPC fluxes resembled those observed for particulate proteins and lipids, with sub-peaks at the beginning of the study period, on November 27-30th and December 15th (**Fig. 2D**).

At both sampling depths sinking particles were consistently dominated by lipids (on average 59% of BPC), followed by proteins (32-27%) and carbohydrates (9-14%). At 10-m depth, temporal variations in the composition of fluxes were wider than those at 30-m depth, and were characterized by a noticeably progressive increase in the protein contribution to BPC from 15 to 24 November, mostly at the expense of carbohydrates. At 30-m depth, both protein and carbohydrate contributions to BPC grew slightly at the end of the sampling period, at the expenses of the lipid fraction (**Appendix B**; **Fig. B1**).

For all of the variables, particle downward export was positive during the first week of sampling

(November 15-18th), then became consistently negative till the end of the study period, with a few exceptions: 21 November and 9 December for proteins; 18 and 24 November and 3 and 15 December for carbohydrates; 21 and 27 November and 6 and 9 December for lipids; 15-21 November and 9 December for BPC (**Fig. 3**).

3.2 Total phytopigment fluxes and phytoplankton community composition below the sea ice

At 10-m depth, total phytopigment fluxes were characterized by very low values until November 24th (on average 2.1 ± 0.5 mgC m^{-2} d^{-1}), increased considerably up to 66.4 ± 5.1 mgC m^{-2} d^{-1} during the last week of November, then slightly decreased and remained relatively constant (on average 45.4 ± 7.6 mgC m^{-2} d^{-1}) till the end of the study period (**Fig. 4A**). At 30-m depth, the flux was low until November 24th (on average 2.1 ± 0.4 mgC m^{-2} d^{-1}), then remained relatively high during the remaining sampling period (on average 53.5 ± 16.7 mgC m^{-2} d^{-1}) and reached a maximum of 81.0 ± 6.5 mgC m^{-2} d^{-1} on December 3rd (Fig. 4A). At both depths, the highest value of the Phaeo/Chla ratio, a descriptor of algal senescence, occurred during the period of minimum phytopigment flux (18-24 November; **Fig. 4B**).

At both depths, the phytoplankton community was constantly dominated by Bacillariophyceae (on average 92% of the total cell count), and was characterized by variations in abundance, species richness (both peaking since November 30th) and community composition (**Appendix B; Fig. B.2**). At the beginning of the sampling activities (15-21 November), when the abundances were very low, we found a species variability within the phytoplankton assemblages larger than that observed at the end of November. In particular, at 10 m the abundance of some Bacillariophyceae species increased consistently during the study period and, among these, *Thalassiosira spp*. abundance increased by almost two orders of magnitude, from 3.7 × 10^4 cells l^{-1} (mid November) to 1.9 ×10^6 cells l^{-1} (at the end of the study period), with cells that appeared in good condition and in division. During the same period, also Prymnesiophyceae increased from 9.6 × 10^4 to 1.3 ×10^6 cells l^{-1}. At 30 m, phytoplankton abundance was generally lower than that observed at 10 m, but characterized by a larger species variability. Among Bacillariophyceae, *Nitzschia spp*. increased from 9.6 × 10^4 to 3.1 ×10^6 cells l^{-1}, with *Nitzschia taeniiformis* representing the most abundant species. *Chaetoceros* spp. increased from 3.0 × 10^4 to 1.5 ×10^6 cells l^{-1} until December 9th. Observations at the light microscope suggest that in the upper layer (10 m) there was a growth of cells rather than an accumulation as observed at 30 m. (**Appendix B; Fig. B.2**).

3.3 Pteropods' abundance and biochemical composition

Zooplankton in the upper 150 m of the water column was dominated by copepods (85-99%), followed by pteropods, which represented from 1 to 7% of the copepods' abundance. The two pteropod species were collected in all samples for the entire sampled period. The abundance of both pteropod species fluctuated widely during the study period (**Fig. 5**). The abundance of *L. helicina antarctica* varied from 0.4 to 10.2 ind. m^{-3}, whereas that of *C. limacina antarctica* varied from 0 to 0.44 ind. m^{-3} (**Fig. 5**). The

herbivorous *Limacina helicina antarctica* always dominated on the carnivorous *Clione limacina antarctica*, although the herbivorous: carnivorous percentage ratio slightly decreased from November (97: 3%) to December (88:12%).

Temporal changes in the biochemical composition of *C. limacina antarctica* and *L. helicina antarctica* are reported in **Table 3** and illustrated in **Fig. 6A**. The two species were characterized by significantly different biochemical compositions (**Appendix A; Table A.2**), with *C. limacina antarctica* dominated by lipids (72%) followed by proteins (25%) and carbohydrates (3%), and *L. helicina antarctica* characterised by a dominance of proteins (70%), followed by lipids (24%) and carbohydrates (6%). The redundancy analysis revealed that the biochemical composition of the two species was differently explained by the biochemical composition of sinking POM from the two different water depths (Appendix A; Table A.3). Particulate lipids flux generally explained most of the variations in the biochemical composition of the two pteropod species, with the unique exception for the composition of *Clione limacina antarctica* which was mostly explained by protein fluxes at 30 m depth (**Fig. 6B**).

4. Discussion

In Terra Nova Bay primary production in the pack and platelet ice typically rises at the end of November (Guglielmo et al., 2004; Lazzara et al., 2007; Saggiomo et al., 2017) and rapidly sinks to the sea bottom (Pusceddu et al. 2009a). However, only few observations of POM vertical export rates to the sea bottom are available for this area (Fabiano et al., 1997; Albertelli et al., 1998; Smith et al., 2011) and even fewer information is available about variations in the quantity, biochemical composition and nutritional value of POM released by melting sea ice (Pusceddu et al., 1999; Pusceddu et al., 2009a) and the potential linkages with zooplankton beneath the sea ice.

Overall, our results confirm that in late spring-early summer in TNB the melting of annual sea ice releases a large quantity of particles into the underlying water column, and that those particles can be transported downward very rapidly (hours-days; Pusceddu et al., 2009a). Our results reveal that during the relatively short period of sampling, the biochemical composition of sinking particles varied significantly. We infer that such variations could result partly from zooplankton grazing and also that the changing composition of sinking particles could have influenced the biochemical composition of pteropods.

We report here that lipids represented the consistently dominant fraction of POM fluxes during the entire study period, at both depths. Since lipids represent a substantial fraction of zooplankton faecal pellets (Accornero et al., 2003; Pane et al., 2004) our results would suggest that the large contribution of lipids to particle fluxes beneath the sea ice during late spring-summer in TNB could be the result of active grazing activity associated with the POM release by melting sea ice. Our hypothesis is somehow corroborated by observations made in the ice-free waters of the Terra Nova Bay polynya, where *L. helicina* faecal pellets alone can contribute 20-100% to the whole organic C flux (Accornero et al.,

2003; Manno et al., 2010). Unfortunately, we had not the possibility to visually inspect the presence of zooplankton faecal pellets in our trap samples, so that this hypothesis needs to be confirmed and must be considered with some caution. Nevertheless, our results suggest that the grazing activity by zooplankton (including pteropods) beneath the sea ice can play a key role in fostering the pelagic-benthic coupling in Antarctic waters, either during the spring-summer melting season (our data) or during winter-spring transitions (Jia et al., 2016).

At the beginning of the sampling period (November 15th), POM flux beneath the sea ice was characterized by high carbohydrate content, relative to protein, that decreased in the following days. Carbohydrates can represent, with proteins in diatoms (Finkel et al., 2016), the major constituent of living sea ice algae (McConville et al. 1985), which, during the late spring period, typically reach the highest biomass values at the sea ice-water interface (Guglielmo et al., 2000; Guglielmo et al., 2004; Saggiomo et al., 2017). The biochemical composition of sinking POM reported here at the beginning of the sampling period is therefore somehow consistent with the observed taxonomic composition of the phytoplankton community within the traps, which, on November 18th, was still dominated by sea-ice diatoms (ca. 75% of the total cell counts) and a relatively low value of the Phaeo/Chl-*a* ratio (ca. 1.5). In this regard, we must recall that sympagic algae released beneath the sea ice during ice melting are not able to seed phytoplankton blooms in sea ice-free waters because of photo-physiological constraints (Mangoni et al., 2009b); rather, they enter the heterotrophic compartment (via grazing) or are rapidly transported to deeper water layers (Pusceddu et al., 2009a). These results would suggest that, at the very beginning of our study, the initial melting of sea ice could have triggered the release of still photosynthetically active sympagic microalgae, enriched in carbohydrates, into the water column. However, we cannot exclude that the dominance or co-dominance of carbohydrates in POM released by melting sea ice could be due to a selective (and faster) degradation of proteins over carbohydrates (Misic et al., 2002; Jiao et al., 2011).

After their initial release via ice melting, POM particles retrieved within both traps showed a significant increase in the protein to carbohydrate ratio (from <1 on November 15-21th to >5 till December on 9th), which then decreased (though remaining >2) at the end of the sampling period (12-15 December; **Fig. B.3**). The protein enrichment of algal-derived material is consistent with both the microbial colonization and microbial degradation of suspended particles (Fabiano and Pusceddu, 1998; Manna et al., 2020). Since N is the most limiting element for heterotrophic nutrition and proteins are N-rich compounds, the higher the values of the protein to carbohydrate ratio the higher the nutritional value of suspended (Fabiano and Pusceddu, 1998; Pusceddu et al., 2005), sinking (Fabiano et al., 1997; Fabiano et al., 2001) and coastal and deep-sea sediment organic particles (Pusceddu et al., 1999; Pusceddu et al., 2010; Gambi et al., 2014). Typically, values of this ratio that are <1 are indicative of a poor nutritional quality of organic matter (OM), whereas values >1 are associated with OM characterized by a relatively higher nutritional value (Fabiano and Pusceddu, 1998; Pusceddu et al., 2000; Pusceddu et al., 2007; Pusceddu et al., 2010). Our results, therefore, allow us to suggest that

422 particles derived from sea-ice algae released in the water column could have determined a rapid
423 response of consumers. The highest protein enrichment of particles (on November 24th) was also
424 associated with the highest values of the Phaeo/Chl-*a* ratio and slightly preceded the highest flux of
425 phytopigments. Since the Phaeo/Chl-a ratio is an indicator of zooplankton grazing, these results suggest
426 that, after the initial melting of sea ice, particles entering the water column beneath the sea ice became
427 nutritionally more available for and, possibly, were rapidly consumed by zooplankton. Moreover, we
428 highlight here that the protein enrichment of POM during this period (November 27-30) could be also
429 the result of the putatively increased production of faecal pellets by zooplankton, including pteropods
430 (**Fig. 5**), whose abundance peaked in the same timeframe. Notably, another, though minor, peak in the
431 contribution of protein to the POM fluxes (though associated with lower protein to carbohydrate ratio
432 values) occurred at the end of the sampling period, concurrently with other peaks in abundance of the
433 pteropods. Although pteropods represented just the second most abundant taxon of the zooplankton
434 community, overall, these results corroborate our hypothesis by which the observed dominance of lipids
435 in sinking POM beneath the sea ice is suggestive of the presence of a large amount of zooplankton
436 faecal pellets, including those produced by the pteropods. This hypothesis is also corroborated by a
437 significant linear correlation between lipid fluxes below the sea ice and the abundance of both pteropod
438 species (**Fig. B.4**). On the other hand, the results of the DistLM tests suggest also that changes in the
439 biochemical composition of particulate food during the study period could have influenced that of the
440 pteropods.
441 During the entire study period, the two pteropod species under scrutiny were characterized by
442 consistently strong differences in their gross biochemical composition and by clear temporal changes
443 (**Fig. 6**). Namely, the herbivorous *L. helicina antarctica* was characterized by a dominance of proteins,
444 whereas the carnivorous *C. limacina antarctica* was characterized by the dominance of lipids. *L.*
445 *helicina antarctica* showed a sudden initial increase in its protein and carbohydrate contents, which
446 coincided with the initial observed release of either particles enriched in their carbohydrate fraction at
447 the beginning of the study period or particles enriched in their protein fraction. *C. limacina antarctica*
448 showed a progressive increase in their lipid fraction that was associated with the progressive increase
449 of the lipid fraction in sinking POM at both depths. This linkage between sinking POM and the predator
450 pteropod *C. limacina antarctica* could be related to the lipid storage ability of this species while feeding
451 on zooplankton taxa including *L. helicina antarctica* and other taxa dwelling in the platelet ice and in
452 the water below the pack ice (Guglielmo et al. 2007).
453 Our results, though based on a merely correlative approach, confirm that the spring-summer
454 annual sea ice melting process in Antarctic coastal waters represents a fundamental step in the transfer
455 of energy towards the higher trophic levels and that, besides the trophic role of the particles exported
456 to the benthos (Pusceddu et al. 2000), this phenomenon represents a key step of energy transfer also
457 through the pelagic food web.
458 From 2011 to 2015, the anomaly (vs. the average of the period 1981-2010) of the Antarctic sea

ice area in January has increased up to +27%. Since 2016 such anomalies have instead shifted towards negative values (down to ca. -30% in 2019) suggesting a progressive loss of sea ice (Copernicus Climate Change Service (C3S) Climate Bulletin, Sea Ice Cover for January 2020), which could be a signal of the consequences of current climate change. If this trend would continue, we could anticipate that the progressive loss of Antarctic sea ice extent and area will result in a conspicuous decline in habitat availability for sea-ice algae and, as a consequence, in a drop of the availability of food for pteropods and the higher trophic levels of the coastal Antarctic Ocean.

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674 2400

675 **Table 1.** Results of the PERMANOVA test for bathymetric and temporal variations in protein, carbohydrate, lipid, BPC fluxes and the biochemical composition
676 (in terms of protein, carbohydrate and lipid contents) of particles sinking below the pack ice. df = degrees of freedom; MS = mean square; Pseudo-F = statistic
677 F; p(MC) = probability level after Monte Carlo simulations; ** = p<0.01; *** = p<0.001; ns = not significant.
678

	Source	**df**	**MS**	**Pseudo-F**	***p* (MC)**	**% explained variance**
Protein	Depth	1	0.78	7.7	**	1
	Time	10	2.61	25.9	***	26
	Depth x Time	10	3.37	33.3	***	67
	Residual	44	0.10			6
Carbohydrate	Depth	1	2.37	56.4	***	5
	Time	10	4.80	114.3	***	60
	Depth x Time	10	1.28	30.6	***	31
	Residual	44	0.04			3
Lipid	Depth	1	0.05	0.4	ns	0
	Time	10	2.50	22.5	***	24
	Depth x Time	10	3.51	31.7	***	69
	Residual	44	0.11			7
BPC	Depth	1	0.06	0.5	ns	0
	Time	10	2.71	20.4	***	27
	Depth x Time	10	3.19	24.0	***	64
	Residual	44	0.13			8
POM biochemical composition	Depth	1	3.19	12.57	***	2
	Time	10	9.90	39.02	***	35
	Depth x Time	10	8.16	32.15	***	57
	Residual	44	0.25			6

679

680 **Table 2.** Results of the pairwise test comparison testing for differences in the quantity of protein, carbohydrate, lipid, BPC and biochemical composition of the
681 POM fluxes between the two sampling depths (10-m vs. 30-m) at each sampling date. t = statistic t; p(MC) = probability level after Monte Carlo simulations;
682 * = p<0.05; ** = p<0.01; *** = p<0.001; ns = not significant.

Date	Protein		Carbohydrate		Lipid		BPC		Biochemical composition	
	t	p(MC)	t	p(MC)	t	p(MC)	t	p(MC)	t	p(MC)
15-nov	78.14	**	68.25	**	0.08	ns	37.20	*	57.88	**
18-nov	98.08	***	46.48	**	10.42	**	98.63	**	10.03	***
21-nov	17.74	ns	54.77	**	44.99	**	27.24	ns	38.24	**
24-nov	87.91	**	12.12	ns	28.60	ns	55.28	**	78.89	***
27-nov	40.00	**	28.46	ns	3.83	ns	6.33	ns	24.76	*
30-nov	54.77	**	39.09	**	34.62	**	41.70	*	46.04	**
03-dic	26.96	ns	99.40	***	44.00	**	22.48	ns	51.31	**
06-dic	26.80	ns	28.74	ns	2.54	ns	4.39	ns	17.03	ns
09-dic	28.60	ns	2.25	ns	3.32	ns	2.06	ns	30.49	*
12-dic	0.25	ns	35.36	*	37.79	**	23.63	ns	23.92	*
15-dic	0.22	ns	19.24	ns	38.22	**	16.45	ns	24.21	*

683

684 **Table 3.** Concentrations of proteins, carbohydrate and lipids (mg g^{-1} wet weight ± standard error) in
685 *Clione limacina* and *Limacina helicina antartica* during the study period.
686

Species	Date	Protein mgC g^{-1}	Carbohydrate mgC g^{-1}	Lipid mgC g^{-1}
Clione limacina antarctica	21/11	34.9 ± 7.2	4.9 ± 1.5	154.0 ± 33.3
	24/11	24.4 ± 13.4	2.7 ± 1.0	110.4 ± 66.6
	27/11	27.9 ± 14.1	3.4 ± 1.7	62.9 ± 32.1
	30/11	31.3 ± 3.0	3.6 ± 0.6	74.2 ± 12.8
	4/12	26.8 ± 3.9	3.0 ± 0.6	65.7 ± 19.1
	6/12	16.6 ± 6.4	2.0 ± 0.1	22.9 19.7
	9/12	18.8 ± 0.3	2.1 ± 0.2	45.6 ± 27.7
	12/12	9.3 ± 1.9	1.5 ± 0.3	17.6 ± 14.3
	15/12	22.2 ± 8.2	1.9 ± 0.6	43.1 ± 16.8
Limacina helicina antarctica	21/11	26.9 ± 17.4	1.1 ± 0.1	23.1 ± 16.1
	24/11	27.0 ± 34.0	1.2 ± 1.3	5.2 ± 5.2
	27/11	51.0 ±31.6	3.6 ± 2.0	14.2 ± 9.4
	30/11	58.8 ±25.4	5.3 ± 4.5	12.5 ± 10.8
	4/12	31.7 ± 0.9	3.5 ± 0.8	14.0 ± 0.6
	6/12	35.8 ± 29.2	2.4 ± 1.4	15.8 ± 9.3
	9/12	46.6 ± 0.2	4.4 ± 2.5	13.0 ± 2.1
	12/12	38.2 ± 2.3	3.1 ± 0.0	12.0 ± 0.7
	15/12	35.3 ± 1.4	6.5 ± 2.8	13.3 ± 3.5

687

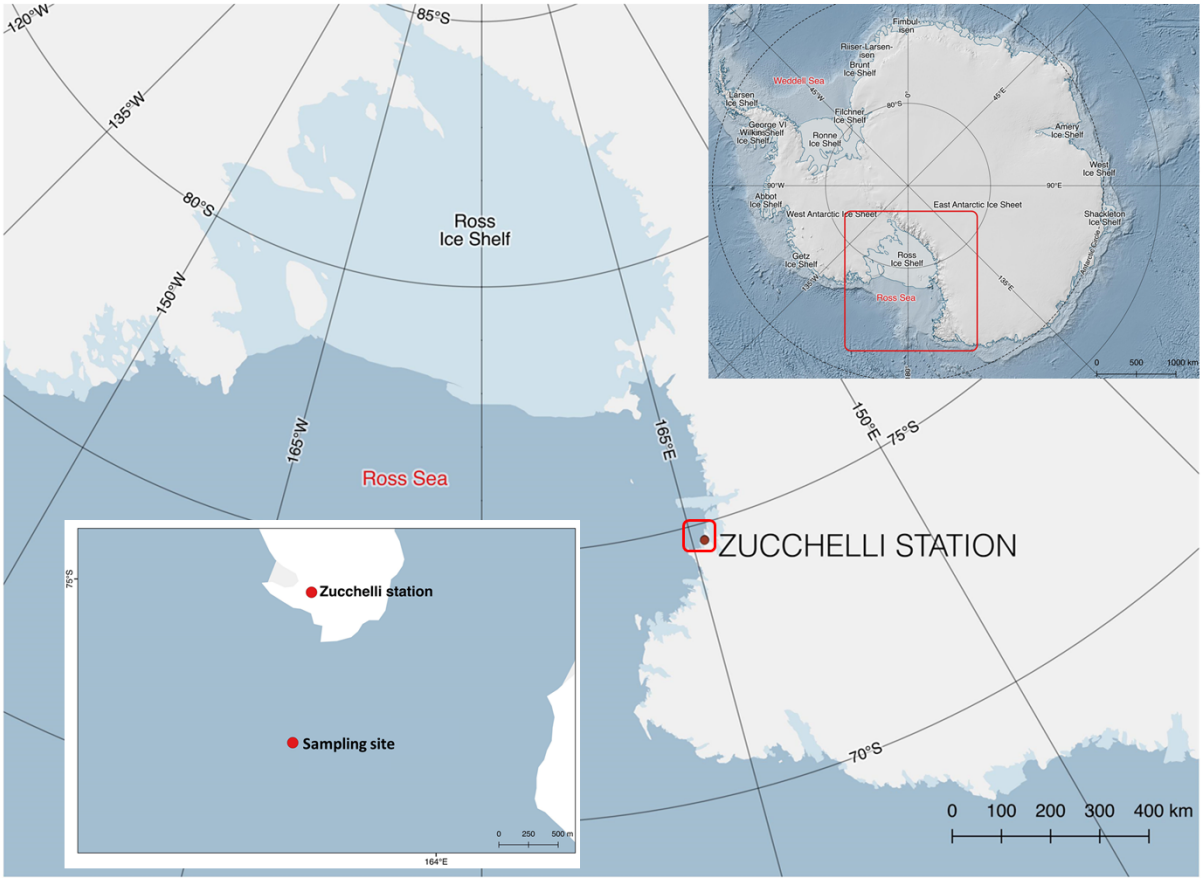
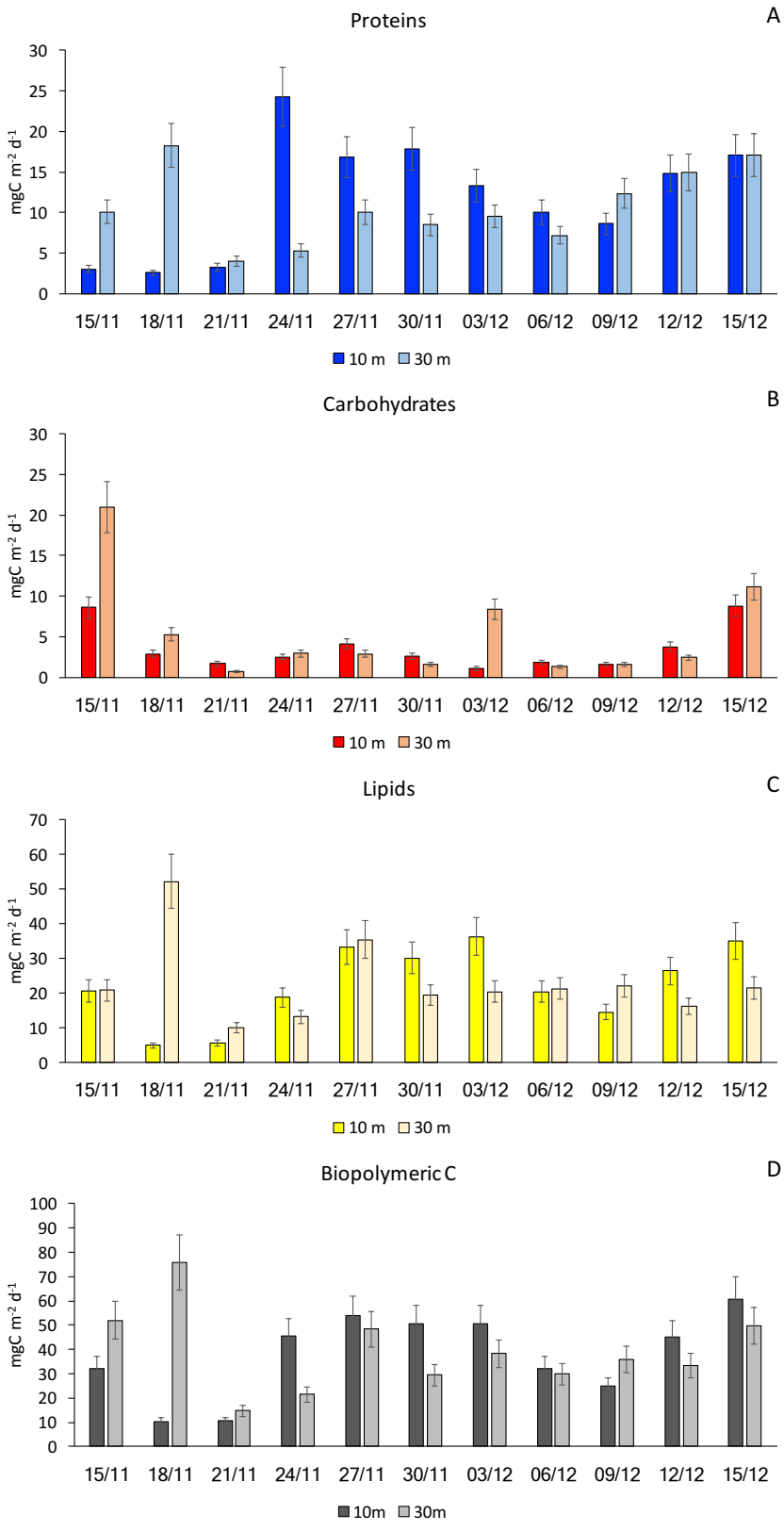
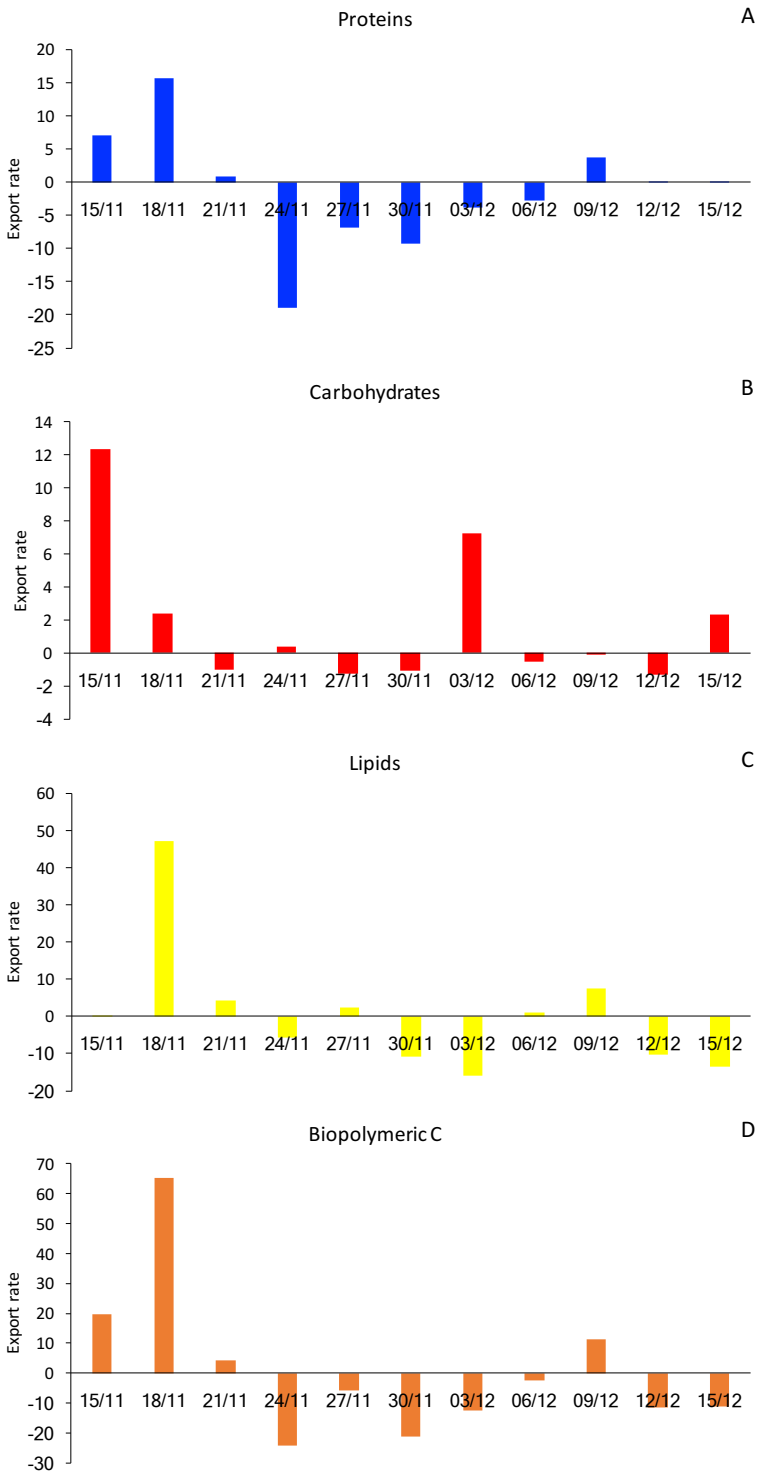


Fig. 1. Study area and location of the traps deployment.



690

691 **Figure 2.** Temporal variations in vertical fluxes of: A) proteins; B) carbohydrates; C) lipids and D)
692 BPC at 10- and 30-m depth below the sea ice at Terra Nova Bay.
693



694
695
696 **Fig. 3.** Export rates of sinking particles to deep waters (as % of the ratio between values at 30-m and
697 10-m depth): proteins (A), carbohydrates (B), lipids (C) and BPC (D).

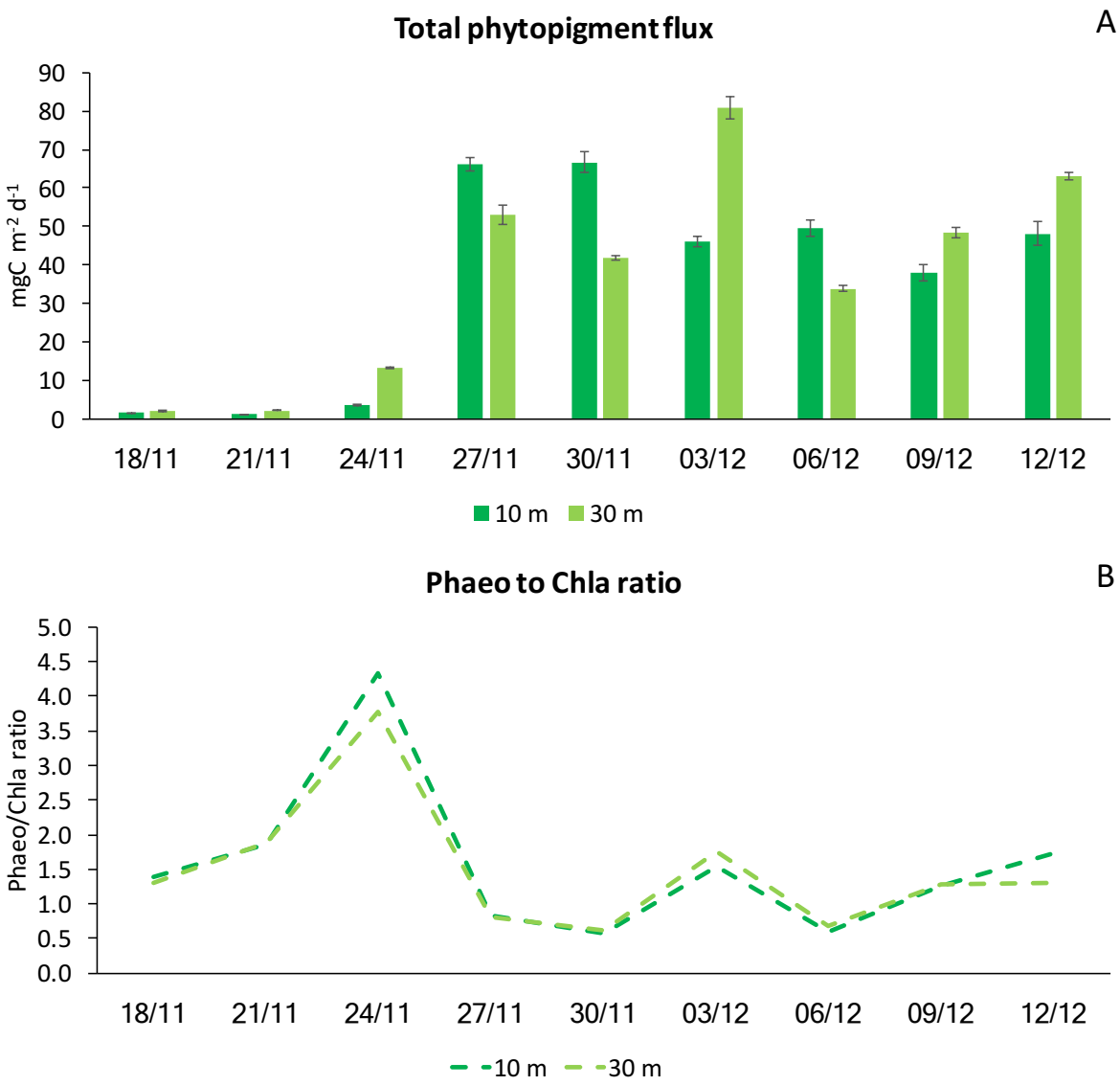


Figure 4. Variations in total phytopigment flux (A) and values of the Phaeo/Chla ratio (B) at 10- and 30-m depth below the sea ice at Terra Nova Bay.

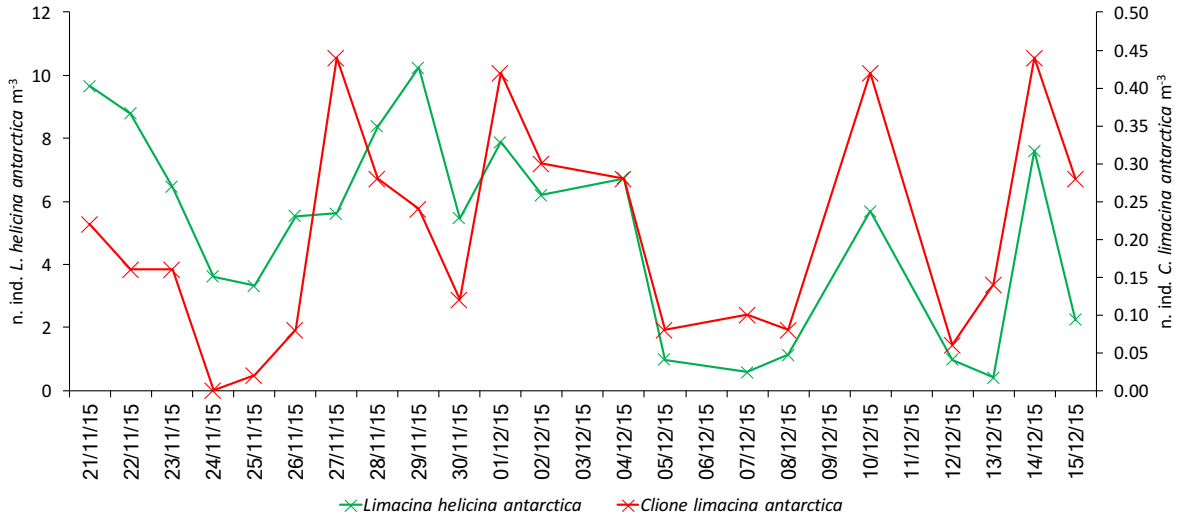


Figure 5. Abundance of *Limacina helicina antarctica* and *Clione limacina antarctica* below the sea ice in Terra Nova Bay during the study period (0-150 m water column).

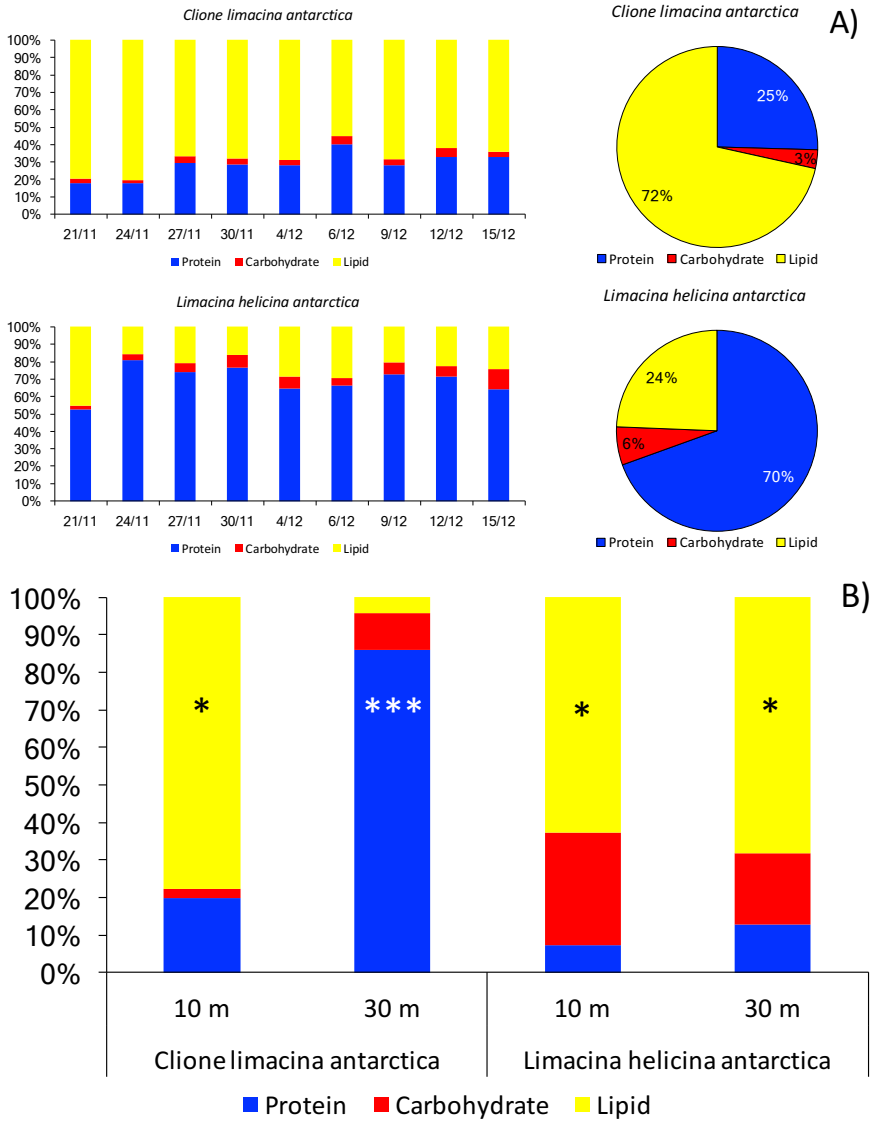


Figure 6. Temporal variations and time integrated means in the biochemical composition (% of BPC) of *Limacina helicina antarctica* and *Clione limacina antarctica* (A) and percentage of variations in the biochemical composition of the two pteropod species explained by protein, carbohydrate and lipid fluxes at 10- and 30-m depth (B); * = $p<0.05$; *** = $p < 0.001$.