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# Lipid and Fatty Acid Turnover of Arctic Zooplankton Organisms Revealed by Stable Isotope Analyses

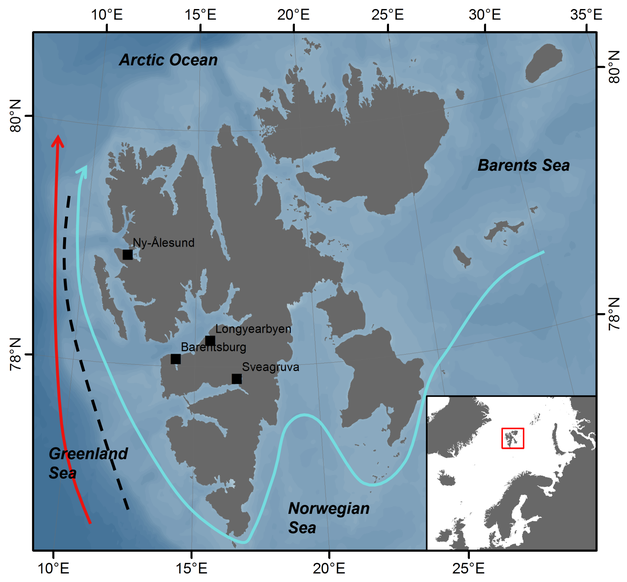


Fig. 1 Map of Svalbard showing the major currents. West Spitsbergen current (WSC, red), Arctic coastal water (ArW, blue)

## Introduction

High latitude marine ecosystems are characterized by strong seasonality in incoming light and thus primary production. In particular, the Arctic marine food web is based on primary producers represented by algae growing under the sea ice and phytoplankton in the open sea. Main taxa of Arctic zooplankton are represented by copepods, amphipods and, at times, pteropods. While some zooplankton species are herbivorous and feed strictly on phytoplankton, others are omnivorous to carnivorous and prey upon organic matter and smaller zooplankton species. These organisms have developed the ability of storing large amounts of lipid reserves to face this variable environment. Lipids are composed of fatty acids, which are transferred from unicellular algae via zooplankton to higher trophic levels.
In our experiments, a $^{13}C$ labeled diatom-flagellate mix was fed to key zooplankton species (copepods and thecosome pteropods) over some days to a couple of weeks to follow the fatty acid carbon assimilation and possible de novo synthesis of fatty acids and alcohols. Fatty acid and fatty alcohol compositions were determined by gas chromatography. Compound specific stable isotope analysis (CSIA) was used in order to detect the incorporation of carbon into FA, when using a $^{13}C$ labelled food source.

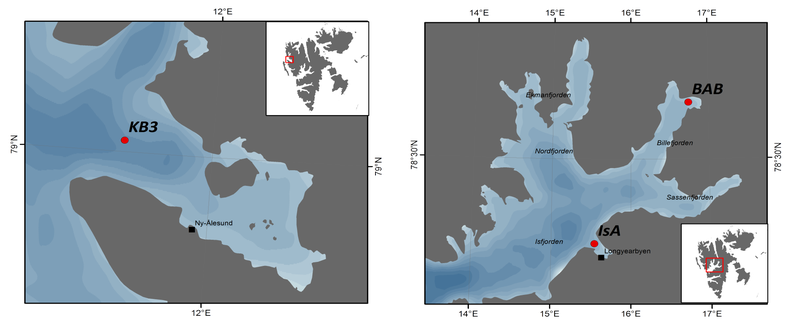


Fig. 2 Sampling stations in Adventfjorden, Billefjorden and Kongsfjorden

## Results of $^{13}C$ uptake in copepods

The overall fatty acid composition of *Calanus glacialis* stage IV and the small sized copepods *Pseudocalanus minutus* and *Oithona similis* is presented in Table 1. Lipids of large herbivorous Calanus species are mainly composed of wax esters comprising sometimes more than 90% of total lipids. They are de novo synthesized of long-chain monounsaturated fatty acids and alcohols as well as of the dietary fatty acids. In the more omnivorous copepods the shorter chain moieties with 14 and 16 carbon atoms dominate the fatty acid and alcohol composition of wax esters. The long-chain polyunsaturated omega-3 fatty acids, 20:5(n-3) and 22:6(n-3), synthesized primarily by phytoplankton, are major components of phospholipids.

The turnover of dietary marker fatty acids 16:1(n-7), 18:4(n-3), 20:5(n-3) and 22:6(n-3) is shown in Fig. 3. The younger stages of *C. glacialis* showed an assimilation of the diatom fatty acid 16:1(n-7) of about 40% after 10 days, reaching 20-30% at the end of the experiment after 21 days. In *P. minutus*, the diatom marker 16:1(n-7), were almost completely renewed from the diet within 21 days, while only 15% of the flagellate markers 18:4 (n-3) were exchanged. *O. similis*, 15% of both flagellate and diatom markers were renewed within 21 days (Fig 3c).

In this study, the production of total lipids (Fig. 5) was most efficient in in the herbivorous copepods *C. glacialis* and *P. minutus,* since they could assimilate about 1.3% and 2.6% of total lipids per day, respectively. *O. similis* had a slow turnover rate of 0.5% TL day$^{-1}$, may be explained by its omnivorous feeding mode. This species maintains its metabolic activity throughout the year, feeding on a wide variety of organisms from small flagellates to copepod nauplii and faecal pellets.

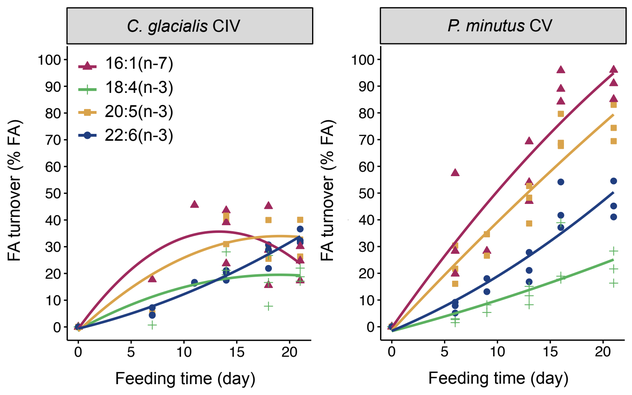


Fig. 3 $^{13}C$ assimilation of major herbivorous copepods

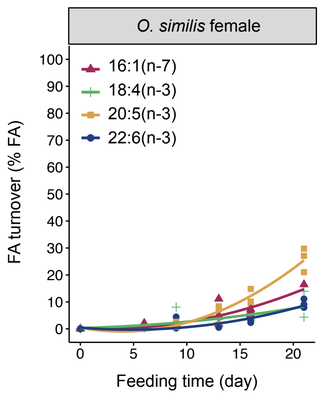


Fig. 3c $^{13}C$ assimilation of *Oithona similis*

Fig. 3d *Oithona similis*

Table 1 Fatty acid composition of Arctic copepods (mass% of total FA)

| | *C. glacialis (15)* | *P. minutus (15)* | *O. similis (15)* |
|---|---|---|---|
| **FA** | | | |
| 14:0 | 4.1 ± 2.4 | 1.2 ± 0.2 | 2.5 ± 0.6 |
| 15:0 | 0.3 ± 0.1 | 0.4 ± 0.7 | 0.5 ± 0.1 |
| 16:0 | 12.7 ± 4.4 | 7.8 ± 3.2 | 19.3 ± 1.3 |
| 16:1(n-5) | 0.3 ± 0.2 | 0.0 ± 0.0 | 0.5 ± 0.5 |
| 16:1(n-7) | 6.3 ± 4.1 | 9.0 ± 1.3 | 1.4 ± 0.5 |
| 16:2(n-4) | 1.0 ± 0.3 | 2.6 ± 1.3 | 0.0 ± 0.0 |
| 16:3(n-4) | 1.5 ± 1.2 | 2.1 ± 0.6 | 0.1 ± 0.2 |
| 16:4(n-1) | 0.7 ± 0.5 | 1.8 ± 2.3 | 0.0 ± 0.0 |
| 17:0 | 0.2 ± 0.1 | 0.3 ± 0.4 | 0.7 ± 0.3 |
| 18:0 | 10.0 ± 7.2 | 8.2 ± 1.5 | 31.0 ± 14.7 |
| 18:1(n-5) | 0.5 ± 0.1 | 0.7 ± 0.1 | 0.4 ± 0.5 |
| 18:1(n-7) | 1.0 ± 0.3 | 1.5 ± 0.3 | 1.2 ± 1.1 |
| 18:1(n-9) | 4.4 ± 3.1 | 24.8 ± 7.4 | 13.4 ± 4.2 |
| 18:2(n-6) | 2.6 ± 2.6 | 4.3 ± 1.8 | 3.0 ± 1.9 |
| 18:3(n-3) | 1.1 ± 0.4 | 5.4 ± 1.5 | 0.7 ± 0.7 |
| 18:3(n-6) | 0.7 ± 0.7 | 0.3 ± 0.4 | 0.0 ± 0.0 |
| 18:4(n-3) | 2.1 ± 1.2 | 2.3 ± 1.2 | 0.7 ± 0.3 |
| 20:0 | 0.8 ± 0.3 | 0.9 ± 0.7 | 0.5 ± 0.5 |
| 20:1(n-11) | 0.2 ± 0.2 | 0.3 ± 0.3 | 0.2 ± 0.4 |
| 20:1(n-7) | 0.7 ± 0.2 | 0.3 ± 0.4 | 0.0 ± 0.0 |
| 20:1(n-9) | 1.3 ± 0.5 | 0.6 ± 0.7 | 2.0 ± 1.3 |
| 20:3(n-6) | 1.0 ± 0.4 | 0.9 ± 1.6 | 0.0 ± 0.0 |
| 20:4(n-3) | 0.5 ± 0.2 | 2.8 ± 0.7 | 1.1 ± 0.6 |
| 20:4(n-6) | 0.4 ± 0.7 | 0.3 ± 0.1 | 0.0 ± 0.0 |
| 20:5(n-3) | 11.9 ± 3.4 | 11.4 ± 1.8 | 6.3 ± 1.9 |
| 22:1(n-11) | 2.0 ± 0.9 | 0.5 ± 0.5 | 0.1 ± 0.2 |
| 22:1(n-7) | 0.1 ± 0.2 | 0.2 ± 0.3 | 0.1 ± 0.2 |
| 22:1(n-9) | 0.4 ± 0.4 | 0.4 ± 0.4 | 0.7 ± 0.4 |
| 22:5(n-3) | 0.6 ± 0.4 | 0.7 ± 0.2 | 1.3 ± 0.8 |
| 22:6(n-3) | 12.7 ± 4.8 | 9.6 ± 2.1 | 10.1 ± 2.7 |
| **FAlc** | | | |
| 14:0 | 0.4 ± 0.3 | 36.5 ± 18.6 | 12.9 ± 5.9 |
| 16:0 | 4.2 ± 1.1 | 55.7 ± 11.1 | 47.0 ± 14.3 |
| 16:1(n-7) | 3.1 2.2 | -- | -- |
| 18:1(n-9) | 1.2 ± 0.5 | 14.1 ± 3.7 | 8.0 ± 2.9 |
| 18:1(n-7) | 2.2 ± 2.5 | 2.1 ± 1.8 | 0.0 ± 0.0 |
| 20:1 | 6.7 ± 2.5 | 3.9 ± 5.5 | 32.1 ± 22.2 |
| 22:1 | 8.5 ± 3.1 | 1.3 ± 1.0 | 0.0 ± 0.0 |

## Material and methods

**Sampling:**
The small-sized copepods *Pseudocalanus minutus* and *Oithona similis* were collected in Billefjorden in July 2014 (Fig.2). The pteropods *Limacina retroversa*, *L. helicina* and *Clione limacina* were collected on various sampling sites in Svalbard waters, e.g. Kongsfjorden, Isfjorden, and along the west coast of Spitsbergen. The samples were taken from a small boat and RV Helmer Hanssen. *C. limacina* were sampled north of Svalbard on board RV Lance. The various species were subjected to feeding experiments with $^{13}C$ labelled single cultures of diatoms (*Chaetoceros debilis* and *Contricribra weissflogii*) and flagellates (*Rhodomonas salina* and *Dunaliella salina*) essentially after the method described by Graeve et al. (2005)[1] and Boissonnot et al. (2016)[2].

**Gas chromatography (GC):**
Total lipid content was extracted by homogenizing samples in dichloromethane:methanol 2:1, modified after Folch et al. (1957)[3]. Fatty acids (FAs) were converted into fatty acid methyl esters by transesterification, and separated by GC. Fatty alcohols obtained by derivatization of wax esters were separated prior to gas chromatography.

**Compound-specific Stable Isotope Analysis (CSIA):**
$^{13}C/^{12}C$-ratios of FAs were determined using GC-c-IRMS (gas chromatography-combustion-IRMS (Graeve et al. 2005, Boissonnot et al. (2016)[1,2]. The label success in the food L [atom %] and the carbon assimilation into FA can be quantified and standardized as proportion of carbon exchanged PE [%FA mass] to compare the efficiency of different species in terms carbon turnover. PE (%) = [((Rsample/ Rsample+1) x 100)t=0 – ((Rsample/ Rsample+1) x 100)t=i]/L (Brenna et al. 1997).

Fig. 3b *Calanus glacialis*

Fig. 4a *Limacina helicina*

**Our methods allow us to estimate lipid and fatty acid turnover rates of specific Arctic key organisms to better understand the carbon und energy flux through the high latitude marine ecosystems.**

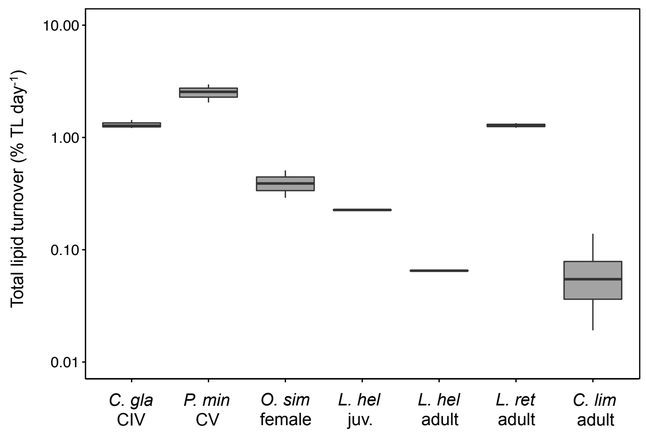


Fig. 5 Carbon turnover of Arctic zooplankton organisms

## Conclusions

*- Arctic herbivorous species exhibit a highly efficient total lipid turnover essentially for the de novo synthesis of wax esters*

*- Omnivorous species show lower total lipid turnover rates, reflecting a more independent life strategy from lipid reserves*

*- Carnivorous species exhibit during our experiment, a very slow lipid turnover, which could be related to the direct investment of energy into metabolism after a long period of starvation*

***Because Arctic zooplankton represent a crucial link between primary producers and higher trophic levels, changes in species distribution and lipid composition of zooplankton will have a decisive effect on future life in Arctic oceans.***

## Results of $^{13}C$ uptake in pteropods

Thecosome pteropods, in contrast, are less lipid-rich and less studied, although they can contribute with more than 20% to the zooplankton biomass in Arctic waters. The major fatty acids of *L. helicina* juveniles and adults as well as *L. retroversa adults* were 16:0, 20:5(n-3) and 22:6(n-3) together reaching 50-70% of total lipids. The juveniles had also considerable amounts of the 18:4(n-3) fatty acid a typical marker for summer phytoplankton. Odd-chain fatty acids such as 17:0 (4.8%), and 17:1(n-8), 19:0, and 19:1 (together 1.7%) contributed to the total lipids of gymnosome *Clione limacina* (Table 2).

The turnover of dietary fatty acids in *L. helicina* juveniles and adults was relatively low with a maximum turnover of 1-1.5% after 6 days. However, in *L. helicina* juveniles the diatom fatty acid 16:1(n-7) showed highest assimilation (4-7% FA). In contrast, the dinoflagelate marker 18:4(n-3) had a very high turnover with up to 24% on day 6 (Fig.4). The difference in FATM assimilation between *L. helicina* and *L. retroversa* , which are closely related species with a similar ecology, may be due to evolutionary traits with respect to their different natural environments (temperate versus polar hemisphere). Accumulation of fatty acid trophic marker was very low in *Clione limacina* (Fig. 6). This could be related to the direct investment of energy into metabolism after a long period of starvation.

The daily turnover rate of lipid was 0.2% day$^{-1}$ *L. helicina*, 0.1% day$^{-1}$ in *L. helicina* and 1.3% day$^{-1}$ in *L. retroversa*. In spite of slightly higher lipid turnover in the latter species, its small body mass makes it less efficient to provide lipids to higher trophic levels, even when reaching high abundances. The gymnosome pteropod *Clione limacina* showed a significant lower daily lipid turnover of 0.07% day$^{-1}$ (Fig. 5).

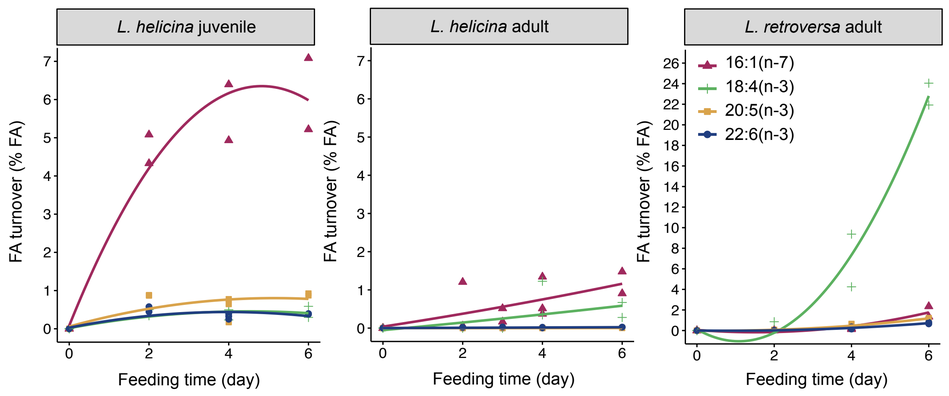


Fig. 4 $^{13}C$ assimilation of pteropods

Table 2 Fatty acid composition of Arctic pteropods (mass% of total FA)

| | *L. helicina* juv. (11) | *L.helicina* adult (10) | *L. retroversa* adult (10) | *C. limaci* |
|---|---|---|---|---|
| **FA** | | | | |
| 14:0 | 1.6 ± 0.4 | 3.1 ± 0.7 | 1.5 ± 0.6 | 0.8 ± |
| 15:0 | 1.0 ± 0.2 | 0.5 ± 0.1 | 1.0 ± 1.1 | 1.7 ± |
| 16:0 | 25.0 ± 3.9 | 12.6 ± 3.3 | 12.2 ± 1.9 | 14.3 ± |
| 16:1(n-9) | -- | -- | -- | 0.3 ± |
| 16:1(n-7) | 2.0 ± 1.2 | 3.0 ± 1.3 | 1.5 ± 0.7 | 1.6 ± |
| 16:1(n-5) | -- | -- | -- | -- |
| 16:2(n-4) | 2.2 ± 0.3 | 0.4 ± 0.1 | 0.8 ± 0.3 | 0.3 ± |
| 16:3(n-4) | 1.8 ± 1.2 | 0.2 ± 0.1 | 0.6 ± 0.1 | 1.6 ± |
| 17:0 | -- | -- | -- | 4.8 ± |
| 17:1(n-8) | -- | -- | -- | 1.1 ± |
| 18:0 | 13.3 ± 4.0 | 3.0 ± 0.8 | 3.4 ± 1.0 | 14.0 ± |
| 18:1(n-9) | 1.1 ± 0.6 | 2.0 ± 0.6 | 0.6 ± 0.1 | 4.1 ± |
| 18:1(n-7) | 1.1 ± 0.7 | 1.0 ± 0.3 | 0.8 ± 0.2 | 1.4 ± |
| 18:1(n-5) | -- | 0.1 ± 0.0 | -- | 0.6 ± |
| 18:2(n-6) | 0.9 ± 0.3 | 1.4 ± 0.4 | 0.6 ± 0.2 | 2.8 ± |
| 18:3(n-6) | -- | -- | 0.1 ± 0.1 | 0.0 ± |
| 18:3(n-3) | 1.0 ± 0.5 | 1.8 ± 0.4 | 1.2 ± 0.3 | 0.5 ± |
| 18:4(n-3) | 4.4 ± 1.4 | 2.7 ± 0.6 | 0.7 ± 0.4 | 0.8 ± |
| 19:0 | -- | -- | -- | 0.3 ± |
| 19:1 | -- | -- | -- | 0.3 ± |
| 20:0 | 3.1 ± 0.8 | 0.5 ± 0.2 | 1.2 ± 0.2 | 1.0 ± |
| 20:1(n-11) | 0.8 ± 0.3 | 0.6 ± 0.3 | 1.1 ± 0.5 | 1.0 ± |
| 20:1(n-9) | 2.1 ± 0.7 | 2.9 ± 1.1 | 2.6 ± 0.5 | 1.7 ± |
| 20:1(n-7) | 3.1 ± 1.8 | 4.1 ± 1.1 | 3.6 ± 0.5 | 2.6 ± |
| 20:2(n-6) | -- | -- | -- | 1.7 ± |
| 20:3(n-6) | 1.4 ± 1.0 | 0.9 ± 0.2 | 2.3 ± 0.5 | -- |
| 20:3(n-3) | 0.7 ± 0.5 | 2.1 ± 0.6 | 1.6 ± 0.6 | -- |
| 20:4(n-6) | 0.9 ± 0.5 | 0.8 ± 0.2 | 0.9 ± 0.3 | 3.5 ± |
| 20:4(n-3) | 1.2 ± 0.8 | 1.1 ± 0.3 | 1.4 ± 0.6 | 0.7 ± |
| 20:5(n-3) | 10.2 ± 4.5 | 23.7 ± 5.9 | 25.9 ± 4.7 | 12.2 ± |
| 22:1(n-11) | 2.1 ± 1.8 | 0.6 ± 0.5 | 0.3 ± 0.1 | 0.1 ± |
| 22:1(n-9) | 4.7 ± 4.1 | 0.5 ± 0.2 | 0.8 ± 0.4 | 0.6 ± |
| 22:1(n-7) | 3.7 ± 2.6 | -- | 0.2 ± 0.1 | -- |
| 22:5(n-3) | 1.6 ± 0.8 | 1.1 ± 0.3 | 2.3 ± 1.6 | 0.6 ± |
| 22:6(n-3) | 9.0 ± 4.0 | 28.9 ± 7.2 | 30.0 ± 7.1 | 23.0 ± |

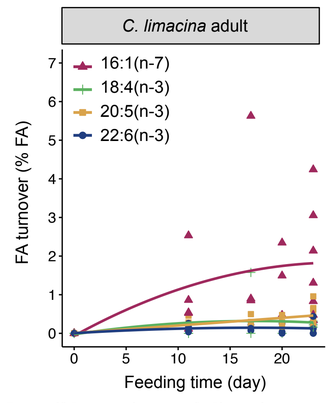


Fig. 6 $^{13}C$ assimilation of *Clione limacina*

Fig. 6a *Clione limacina*

References:

[1] Graeve M, Albers C, Kattner G (2005) Assimilation and biosynthesis of lipids in Arctic *Calanus* species based on feeding experiments with a $^{13}C$ labelled diatom. J Exp Mar Biol Ecol, 317: 109-125
[2] Boissonnot L, Niehoff B, Hagen W, Søreide JE, Graeve M (2016) Lipid turnover reflects life-cycle strategies of small-sized Arctic copepods. J Plankton Res, 38: 1420-1432
[3] Folch J, Lees M, Stanley GHS (1957) A simple method for the isolation and purification of total lipids from animal tissues. J Biol Chem, 226: 497-509
[4] Brenna JT, Corso TN, Tobias HJ, Caimi RJ (1997) High precision continuous flow isotope ratio mass spectrometry. Mass spectrometry reviews, 16: 227-258

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