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Carbon assimilation and food web relationships of Arctic zooplankton organisms revealed by fatty acid and stable isotope analyses

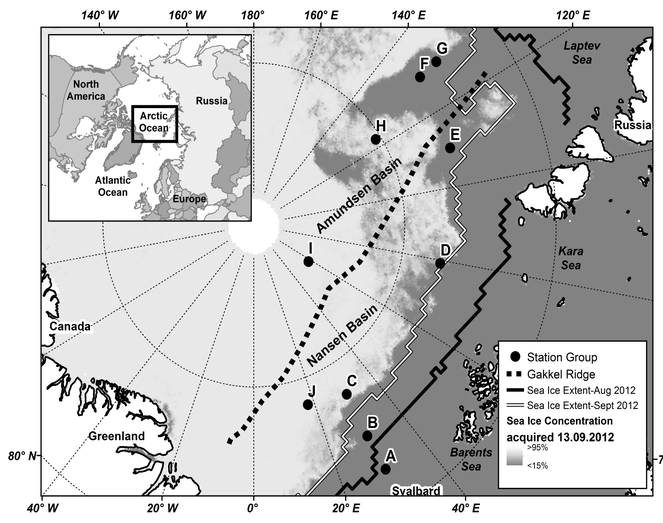

Fig. 1. Sampling location Arctic Ocean

Introduction

In Arctic marine ecosystems, zooplankton organisms are the key link for the nutritional energy flux from primary producers to higher trophic levels. However, the trophic relationships and transfer of energy within the Arctic pelagic food web is not yet fully understood. We investigated the larger *Calanus* spp., the small sized copepods *Pseudocalanus minutus* and *Oithona similis*, the amphipods *Apherusa glacialis* and *Themisto libellula* as well as the pteropod *Clione limacina* in order to obtain new insights into the carbon and energy flux through the pelagic ecosystem. The analysis of fatty acid trophic markers (FATM) provides information on the nutritional quality of the particulate organic matter (POM) produced by algae, and enables differentiation of dietary input on a broader taxon level.

Stable isotope analysis of bulk material (BSIA) and individual compounds (CSIA) permit to distinguish largely on a species level, since primary producers have distinct carbon stable isotope compositions with significant higher ^{13}C enrichment in POM produced by sea ice algae (I-POM) relative to pelagic POM (P-POM). CSIA-analysis of ^{13}C-labelled fatty acids allows to calculate the amount of carbon accumulated in the copepods and provide detailed information about their lipid biosynthesis. Combined isotope studies are an efficient tool to investigate carbon accumulation and turnover of lipids, seasonal carbon ac-

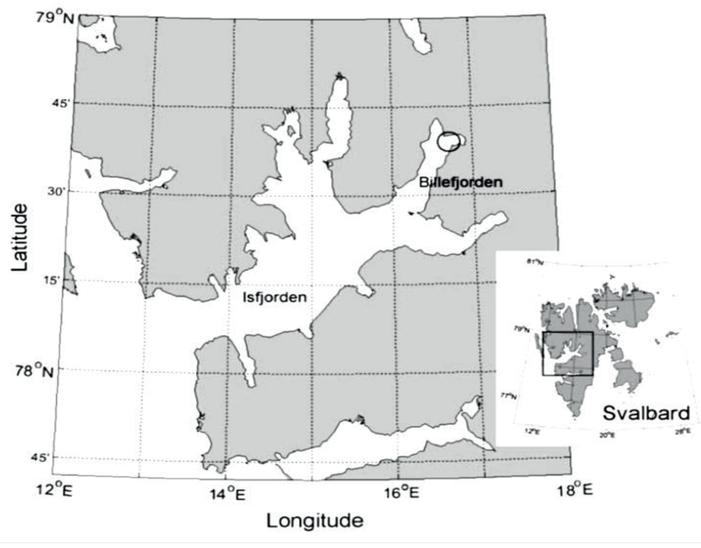

Fig. 2. Sampling location Billefjorden, Spitsbergen

Material and methods

Sampling:
Arctic I-POM, P-POM and under-ice fauna species were collected during the „Polarstern" expedition PS80 between August and October 2012 in the Eurasian Basin of the Arctic Ocean. Sampling was performed with a Surface and Under-ice Trawl (van Franeker et al. 2009)[9]. The small-sized copepods *Pseudocalanus minutus* and *Oithona similis* were collected in Billefjorden, a high Arctic fjord, in July 2014, and subjected to feeding experiments with ^{13}C labelled algae, essentially after the method described by Graeve et al. (2005)[3]

Gas chromatography (GC):
Total lipid content was extracted by homogenizing samples in dichloromethane:-methanol 2:1, modified after Folch et al. (1957)[1]. Fatty acids (FAs) were converted into fatty acid methyl esters by transesterification, and separated by GC.

Bulk Stable Isotope Analysis (BSIA):
Freeze-dried and homogenized bulk sample material was combusted in tin capsules. Isotopic ratios of nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) were determined versus atmospheric N_2 and Vienna Pee Dee Belemnite (VPDB) standard via continuous flow isotope ratio mass spectrometer (IRMS), interfaced with an elemental analyzer and connected via a Conflo IV interface (Kohlbach et al. subm.)[2].

Compound-specific Stable Isotope Analysis (CSIA):
$\delta^{13}C$ of FAs were determined using GC-c-IRMS (gas chromatography-combustion-IRMS (Graeve et al. 2005, Kohlbach et al. submitted)[2,3].

Results of fatty acid analysis

The Arctic phytoplankton bloom consists of two distinct categories of primary producers: ice algae growing within and on the underside of the sea ice, and phytoplankton growing in open waters. Long chain polyunsaturated fatty acids (omega3 PUFAs) exclusively produced by these algae, are essential to all marine organisms for successful reproduction, growth, and development. Sea ice algae communities (I-POM) are often dominated by diatoms, whereas dinoflagellates are more abundant in pelagic algae communities (P-POM) (Soreide et al.2010)[4]. Certain fatty acids (FAs) biosynthesized by primary producer, such as omega3 fatty acids, can be used to track predator-prey relationships (Fig. 3a). Marker FA proportions in abundant Arctic under-ice fauna species collected between late summer and autumn 2012 in the Eurasian Basin of the Arctic Ocean are reflecting feeding modes and importance of lipid storage (Fig. 3b).

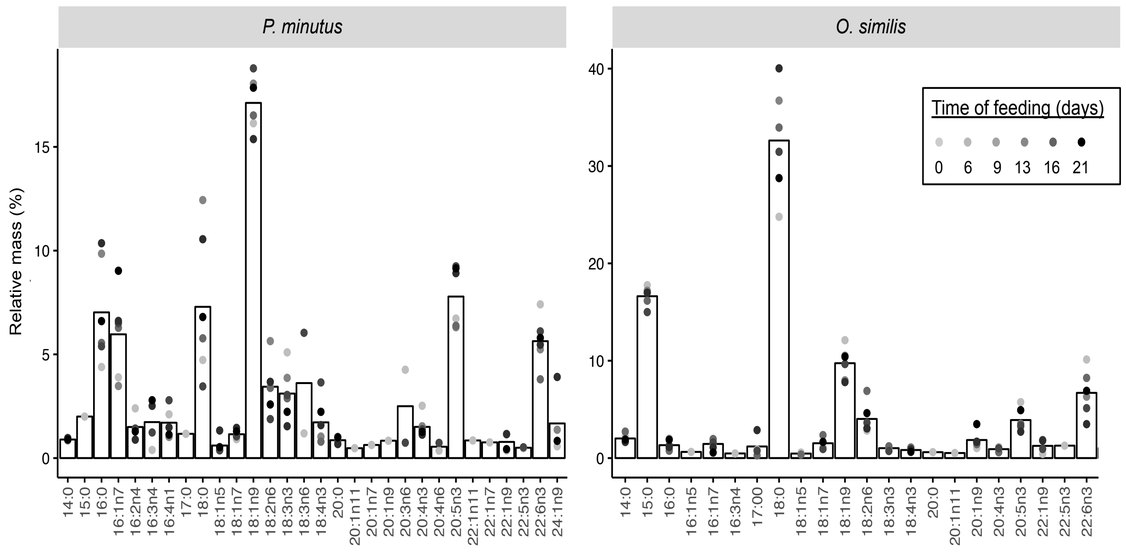

Fig. 4. FA composition of small-sized copepods

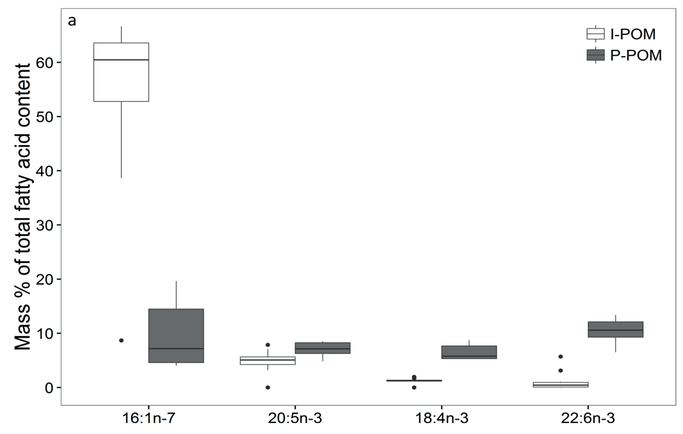

Fig. 3a. Marker FA proportions in I-POM and P-POM

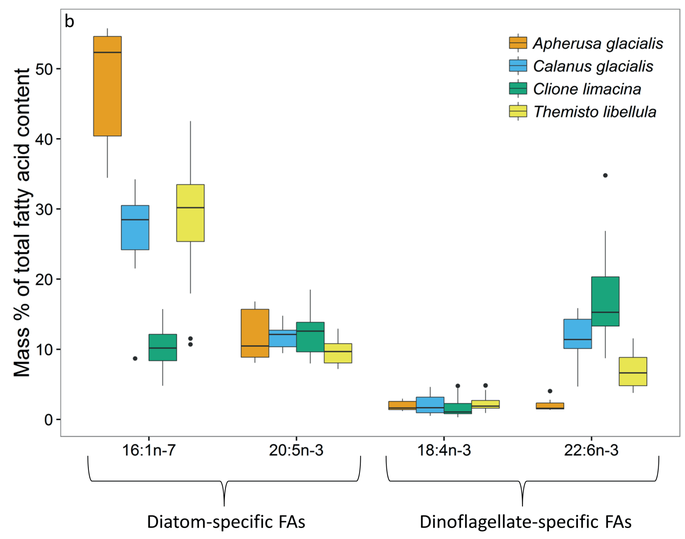

Fig. 3b. Marker FA proportions in abundant under-ice fauna species

Results of stable isotope analysis

Isotopic composition of the abundant under-ice fauna species revealed information on food web relationships and carbon sources. $\delta^{15}N$ values were used to estimate consumer's trophic levels (TL) within the food web based on the two-source food web model (Post 2002, Søreide et al. 2013)[5,6] (Fig. 5a). The $\delta^{13}C$ values of selected trophic marker FA were used to estimate the proportional contribution of ice algae-produced carbon versus carbon produced by pelagic algae to the consumer's diet by using Bayesian multi source stable isotope mixing models (SIAR; Parnell et al. 2010)[7] (Fig. 5b).

Compound specific stable isotope analysis (CSIA) was used in order to detect the incorporation of C into FA, when using a ^{13}C labelled food source. Used in complement to traditional GC methods, and taking into account the label success in the food (L, expressed as atom %), carbon assimilation into FA can be quantified and standardized (proportion of C exchanged, PE, as % FA mass) to compare the efficiency of different species in terms of carbon turnover. PE (%) = $[((R_{sample}/R_{sample}+1) \times 100)t=0 - ((R_{sample}/R_{sample}+1) \times 100)t=i]/L$. (Brenna et al. 1997)[8] (Fig. 6). Even though the copepods did not increase their lipid biomass, they assimilated FA from their diet, and used them to replace the ones used for metabolism.

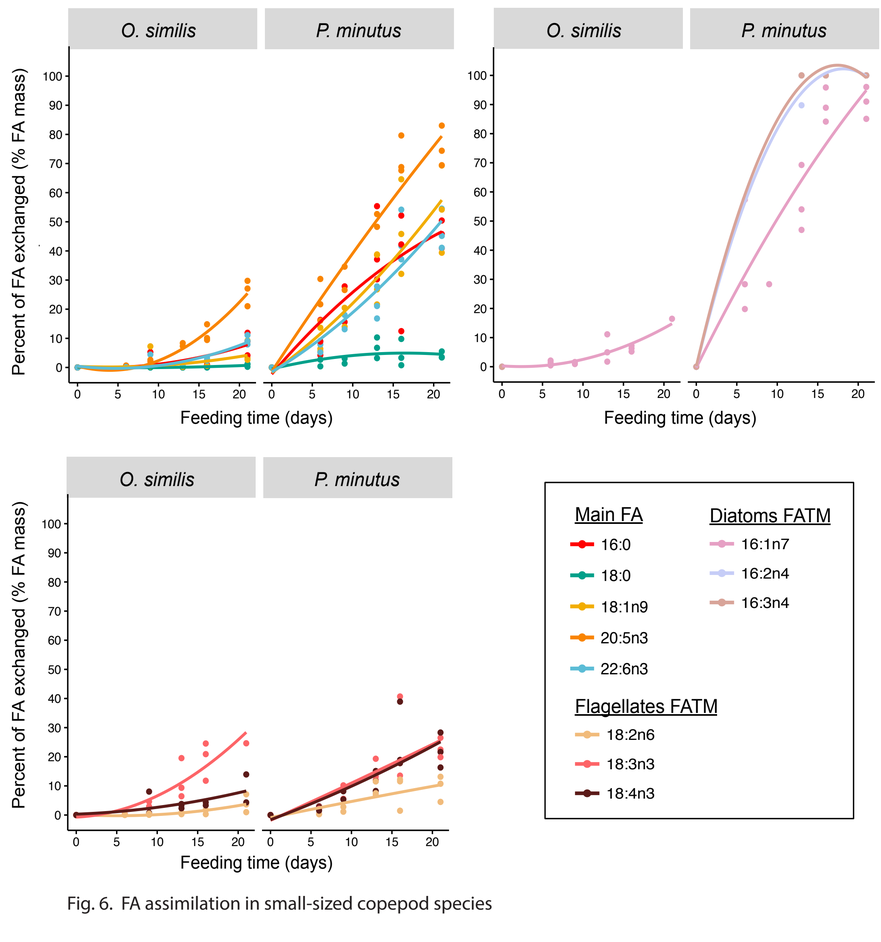

Fig. 6. FA assimilation in small-sized copepod species

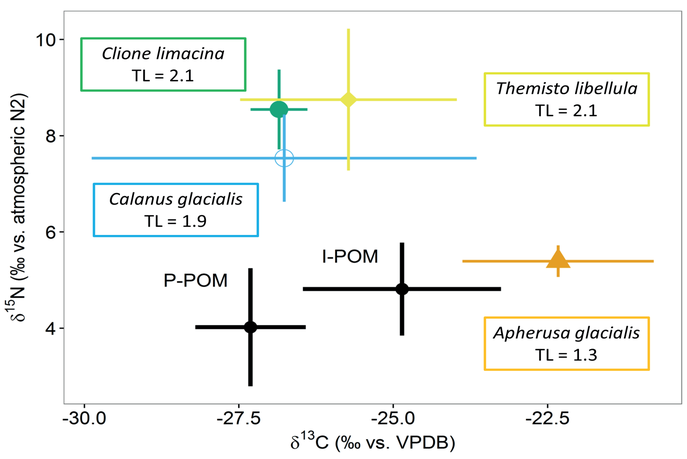

Fig. 5a. Trophic position of abundant under-ice zooplankton species

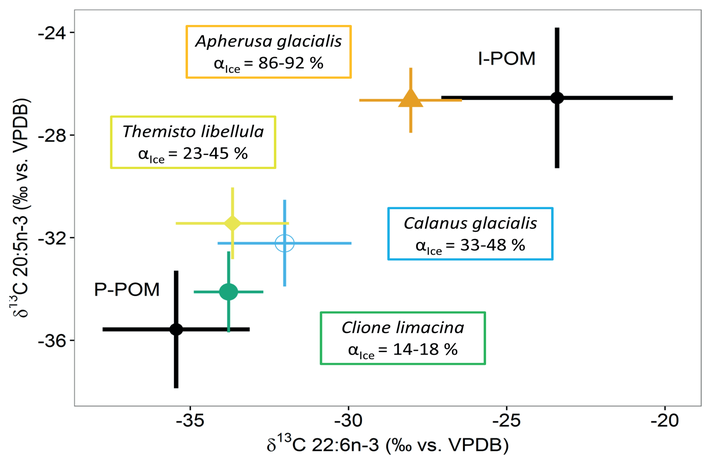

Fig. 5b. Proportional contribution of ice algae-produced carbon (α_{ice}) to the diet of abundant under-ice fauna species

Conventional FA analysis combined with stable isotope analysis is recommended to:

- study the source of the carbon assimilated by organisms
- quantify incorporation of food and turnover rates of FA
- confirm *de novo* biosynthesis of FA

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