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Pteropods (Gastropoda: Opisthobranchia) in the Southern Ocean: First results from fatty acid and stable isotope analyses on the SYSTCO material

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Abstract

The Antarctic Ocean is a complex ecosystem including planktonic herbivores like krill, salps, copepods as well as carnivores like amphipods, ctenophores, cnidarians and which are fed upon by birds, fish, squids, seals and baleen whales. Although there are many reports about the chemical components of the Antarctic krill species *Euphausia superba*, as well as for calanoid copepods, which are key organisms in the planktonic food web of the Antarctic Seas, there is yet little information available on the biochemical constituents of other common species, like pteropods, which are frequently found in planktonic hols in the Southern Ocean. To find out more about the feeding ecology as well as the trophic position of these organisms, zooplankton samples were taken during the SYSTCO expedition, and preserved in order to conduct biochemical analyses, more precisely estimating stable isotope ratios and identifying fatty acid signatures. This approach will here be presented on four pteropod species (*Clione limacina, Limacina helicina, Spongiobranchaea australis, Clio pyramidata sulcata*).

One aim of this study is to analyse and compare the fatty acid composition of these animals to discover possible adaptations to the polar environment as well as to identify the major food sources that are utilized. Furthermore, an approach is undertaken to define the trophic position of the studied animals. Preliminary results from stable isotope 13C and 15N analyses indicate a very basal position of all investigated pteropod species.

Keywords: Southern Ocean, pteropods, fatty acid signatures, stable isotope ratios

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Introduction

Among the (Sub-)Antarctic zooplankton, the molluscs play a considerable role beside the crustaceans as food source for either other zooplanktonic animals like chaetognaths, or for top

predators like diverse fish species, seals, whales, and seabirds (Newman 1998). In the present study we focused on the taxonomic most diverse and within the samples predominant group of molluscs, the commonly so called pteropods - holoplanktonic opisthobranch gastropods that are divided into the shelled Thecosomata and the naked or shell-less Gymnosomata.

The Thecosomata in our Antarctic samples are mainly represented by *Limacina helicina antarctica* (Woodward, 1854), *Clio pyramidata sulcata* (Pfeffer, 1879), and *Clio piatkowskii* (van der Spoel, Schalk & Bleeker, 1992), while the Gymnosomata comprise *Clione limacina antarctica* (Smith, 1902) and *Spongiobranchaea australis* (d'Orbigny, 1836), which displays a faunistic composition that is typical for the Southern Ocean pelagic ecosystem (van der Spoel & Dadon 1999, Hunt et al. 2008).

The general life history of pteropods has been subject of several studies (e.g., Lalli & Gilmer 1989, van der Spoel & Dadon 1999, Hunt et al. 2008; and references therein) and although this includes their feeding behaviour, information about the importance of pteropods within the food-web is still scarce. Attempts for analyzing food sources and food composition are mostly focused to selected species like the assumed to be bipolar *Clione limacina* (Phipps, 1774) (e.g., Falk-Petersen et al. 2001, Phleger et al. 2001, Böer et al. 2005) or to one of both polar regions. Comparable feeding data between the northern and southern populations are limited (e.g., Kattner et al. 1998) or difficult to extract from the brief summaries of van der Spoel & Dadon (1999) and Hunt et al. (2008).

Thecosomata are predominantly herbivorous with a tendency towards omnivory (van der Spoel & Dadon 1999, Hunt et al. 2008), using enormous free-floating mucous traps to gather their food. For instance, the diet of *Clio pyramidata sulcata* is made up of diatoms, tintinnids, copepods, silico- and dinoflagellates, foraminiferans, and polychaetes (Hopkins & Torres 1989). The gymnosomates show contrasting feeding habits by being specialized carnivores, actively hunting their prey with sucker-beset tentacles. According to van der Spoel & Dadon (1999) and (Hopkins & Torres 1989) adults of antarctic *Clione limacina antarctica* and *Spongiobranchaea australis*, monophagously feed *Limacina helicina antarctica* and *Clio pyramidata sulcata*, respectively.

The concept of using lipids as biomarkers in marine ecosystems has received considerable attention in the past few decades (e.g. Sargent & Falk-Petersen 1981, Sargent et al. 1988, Volkman et al. 1989, Falk-Petersen et al. 2002). Fatty acids are the primary constituent of most lipids and in marine organisms they are most commonly composed of chains of 14 to 24 carbon atoms of varying degrees of unsaturation (i.e. containing one or more double bonds).

These fatty acids generally remain intact through digestion, absorption and transport in the bloodstream, and are also taken up by tissues in their intact state. Thus, fatty acids can be deposited in animal tissue with minimal modification from diet and in a predictable way (Lee et al. 1971, Fraser et al. 1989, Graeve et al. 1994, Kirsch et al. 1998). Additionally, animals can biosynthesize a relatively limited number of fatty acids. These biochemical restrictions, coupled with the fact that fatty acids in the marine food web are exceptionally complex and diverse, provide the opportunity to use fatty acids to understand trophic interactions in marine ecosystems. Although a number of 'indicator' fatty acids exist which may be used as biological markers (e.g. Sargent et al. 1988, St. John & Lund 1996), it is likely that the quantitative pattern of all fatty acids in a species or individual will be most informative at higher trophic levels (e.g. Iverson et al. 2002). Some of the first evidence for a conservative transfer of marker fatty acids in neutral lipids up the food chain came from experiments on phytoplankton and copepods (Lee et al. 1971). Biomarkers can be traced through several trophic levels, and thus they provide information not only about potential prey but also about the base of the food web. It has been demonstrated that tissue fatty acids can be valuable in studying bottom-up trophic dynamics among and within fish and invertebrate species (e.g. Sargent et al. 1988, Kharlamenko et al. 1995, St. John & Lund 1996, Mayzaud et al. 1999). Fatty acid profiles in predators show an integration of prey fatty acids within periods of weeks to months; comparison of a fatty acid profile (or signature) of a certain prey with that of its potential predator will reveal dietary information beyond what is possible from stomach-content data alone. These biomarkers have been used to explore the feeding ecology of a number of marine organisms. Recent studies using lipid biomarkers have helped reveal aspects of Antarctic ecology not visible by conventional techniques (Phleger et al., 1998; Falk-Peterson et al., 1999; Nelson et al., 2000).

The main topic of this study is to identify differences in food preferences of co-existing pteropods in the Southern Ocean, and to elucidate their role in the food web and possible benthic-pelagic coupling.

Material and methods

The samples were taken on the Polarstern cruise ANT XXIV/2, taking place 28.11.2007 - 4.2.2008. Sampling was conducted by means of an RMT (Rectangular Midwater Trawl) which sampled the upper 200 m water layer, and a SUIT (Surface and Under Ice Trawl), which sampled directly under the ice cover (table 1). As soon as the catch was on board,

samples were sorted on ice and frozen at -80°C until they could be analysed shortly after the cruise.

Prior to the lipid analysis samples were freeze dried, weighed and allowed to extract in a 2:1 Dichlormethane-Methanol solution for at least 48 h. Lipid extraction was performed with minor modifications as described in Folch et al. (1957) using ultrasonic disruption in dichloromethane:methanol and a washing procedure with aqueous KCl solution. The analysis of fatty acid composition was performed with modifications as described in Kattner & Fricke (1986). Fatty acids are converted to their methyl ester derivatives (FAME) in sulphuric methanol and analysed using a gas chromatograph (HP 6890A) equipped with a programmable temperature vaporizer injector (Gerstel® CIS4plus) and a DB-FFAP column. The use of a large volume injector allows a measurement of very low lipid amounts. FAMEs and fatty alcohols are detected by flame ionisation and identified by comparing retention times with those derived from standards of known composition. For quantification of fatty acids an internal standard is used.

Prior to the stable isotope analyses samples were first freeze-dried and weighed, then dried at 40°C for at least 24 h and finally each animal was ground by hand with a mortar and pestle to obtain a homogenous powder. We always used the whole animal to prevent any bias by different C or N values in different tissues.

Table 1. Stationlist of antarctic pteropods, collected during the ANDEEP-SYSTCO expedition ANT XXIV-2. RMT – Rectangular Midwater Trawl; Suit – surface and under-ice trawl.

Station PS 71-	Date	Start coordinates	End coordinates	Gear	Day-time (night/day)
49	17.01.08	69°35.71'S, 00°01.89'W	69°35.41'S, 00°03.02'W	RMT	d
50	17.01.08	69°00.34'S, 00°00.60'W	69°00.73'S, 00°01.54'E	Suit	n
50	18.01.08	69°00.75'S, 00°01.43'E	69°00.74'S, 00°00.29'W	RMT	n
51	18.01.08	68°30.15'S, 00°00.43'E	68°29.38'S, 00°00.70'E	RMT	d
52	18.01.08	67°59.90'S, 00°01.53'W	67°59.65'S, 00°04.29'W	RMT	d
53	18.01.08	67°31.52'S,	67°30.66'S,	RMT	n

		00°00.07'E	00°00.07'W		
53	18.01.08	67°30.12'S, 00°00.45'E	67°29.34'S, 00°00.39'E	Suit	n
55	19.01.08	66°29.29'S, 00°00.74'E	66°28.46'S, 00°00.65'E	RMT	d
57	19.01.08	65°31.79'S, 00°00.12'E	65°31.06'S, 00°00.06'W	Suit	n
57	20.01.08	65°30.72'S, 00°00.01'E	65°30.40'S, 00°01.07'E	RMT	n
58	20.01.08	65°01.56'S, 00°00.71'W	65°00.77'S, 00°01.05'W	RMT	d
59	20.01.08	64°30.38'S, 00°03.30'W	64°30.31'S, 00°04.76'W	RMT	d
60	20.01.08	64°00.49'S, 00°01.93'W	64°00.51'S, 00°04.20'W	RMT	n
61	21.01.08	63°30.37'S, 00°01.77'W	63°30.53'S, 00°03.25'W	RMT	n
62	21.01.08	62°59.57'S, 00°02.37'E	62°59.81'S, 00°00.85'E	RMT	d
63	21.01.08	62°29.74'S, 00°01.52'E	62°30.05'S, 00°00.33'W	RMT	n
64	22.01.08	62°00.13'S, 00°00.07'W	62°00.45'S, 00°01.36'W	Suit	n
64	22.01.08	62°00.47'S, 00°01.17'W	62°00.66'S, 00°02.43'W	RMT	n
65	22.01.08	61°30.10'S, 00°00.11'W	61°30.56'S, 00°01.00'W	RMT	d
66	22.01.08	61°00.64'S, 00°01.71'W	61°01.17'S, 00°03.05'W	RMT	d
67	22.01.08	60°29.40'S, 00°00.09'E	60°28.41'S, 00°00.46'E	RMT	n
68	23.01.08	59°59.82'S, 00°01.32'W	59°59.77'S, 00°02.81'W	RMT	d

Results

All the pteropods that have been evaluated so far shows significant amounts of Eicosapentaenoic acid (EPA) 20:5(n-3), as well as Docosahexaenoic acid (DHA) 22:6(n-3) and 16:0, accompanied by other components.

For *Clio piatkowski*, the dominant FAs were DHA, followed by EPA, 16:0 and 18:1(n-9). *Clio pyramidata sulcata* showed the major FAs EPA, 16:0, DHA as well as 18:1(n-9) and 16:1(n-7).

Clione limacina had FA composition distinct from the other investigated pteropods. The dominant FAs were EPA, 16:1(n-7), DHA and 16:3(n-4), but in all analysed samples there were significant amounts of the odd chained, saturated FAs, typical for bacteria (15:0 and 17:0).

For *Spongiobranchaea australis*, the dominant FAs were again DHA, EPA and 16:0, followed by 18:1(n-7). In this family, the FAs 15:0 and 17:0 were also found, but in lower proportions than in *C. limacina*.

EPA, DHA and 16:0, followed by 20:1(n-7) were the major FA components of *Limacina helicina*.

Discussion

The Thecosomata (*Limacina helicina antarctica, Clio pyramidata sulcata* and *Clio piatkowskii*) are thought to be predominantly herbivorous with a tendency towards omnivory (van der Spoel & Dadon 1999, Hunt et al. 2008). The fatty acid patterns found in our study agree with this, as the polyunsaturated fatty acid (PUFA) 20:5(n-3) (EPA), being a major component in all analyzed samples is strongly detected in diatoms (Pond et al. 1998), and has been used as a diatom marker in marine environments (Volkman et al. 1980) and in the diet of invertebrates (Kharlamenko et al. 1995, Meziane et al. 1997). The other common PUFA 22:6 (n-3) (DHA) found in our samples has been reported to be dominant in dinoflagellates (Dalsgaard et al., 2003).

The gymnosomates (*Clione limacina antarctica* and *Spongiobranchaea australis*) are carnivores, the adults feeding on *Limacina helicina antarctica* and *Clio pyramidata sulcata*, respectively (van der Spoel & Dadon, 1999; Hopkins & Torres, 1989). The ratio between 18:1 (n-7) and 18:1 (n-9) is often used to distinguish carnivores from herbivores (e.g., Graeve et al., 1997), but it has to be treated with caution, as 18:1(n-9) is a major fatty acid in most marine animals (Dalsgaard et al., 2003). In our study, this ratio was not elevated in the carnivores compared to the species thought to be herbivorous. This question needs to be discussed in detail.

Among the fatty acids markers, the odd-branched fatty acids 15:0 and 17:0, iso and anteiso, as well as 18:1(n-7) are commonly considered as being predominantly synthesized by bacterial communities (Volkman et al. 1980) and they have been used as markers of bacteria in marine

invertebrates (Kharlamenko et al. 1995, Meziane et al. 1997). All of the three fatty acids have been found in considerable amounts in the gymnosomates *Clione limacina antarctica* and *Spongiobranchaea australis*. Both species are thought to feed monophagously on *Limacina helicina antarctica* and *Clio pyramidata sulcata*, respectively (van der Spoel & Dadon, 1999; Hopkins & Torres, 1989). However, these fatty acid markers were not detected in their prey, and their source needs to be subject to further investigations.

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