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The lipid composition of some Antarctic Crustacea

ABSTRACT: A composition of lipids of some Antarctic Crustacea (*Euphausia superba*, *E. triacantha*, *Thysanoessa macrura* and Mysidacea gen. sp. indet.) caught in the Admiralty Bay (South Shetlands) was compared. Lipids of *E. superba* differed in low content of waxes that evidences for different management of lipids than in other examined Crustacea.

Key words: Antarctica, Crustacea, krill, lipids.

Introduction

In the catches of the Antarctic krill along with *Euphausia superba* small quantities of other species of Crustacea were generally found.

Along with a large number of papers concerning the content and composition of lipids in *E. superba* some information on *Euphausia crystallorophias* can be found (Bottino 1973, 1974, 1975, Ellingsen and Mohr 1981, Clarke 1984, Nachman 1985). In the majority of the above mentioned papers lipids of these two species were also compared. Few data can be found on the composition of lipids of other Crustacea found in the catches of krill. Clarke (1984) among many species of Antarctic macrozooplankton, besides the above mentioned *E. superba* and *E. crystallorophias*, has estimated the composition of lipids (classes) also in *E. frigida*, *E. triacantha* and in *Antarctomysis maxima* and in other nondetermined Mysidacea. The samples were taken during Antarctic summer, the majority of them from the South Georgia region. Reinhardt and Van Vleet (1984, 1986) have examined the lipids of *E. superba*, *Thysanoessa macrura* and *Antarctomysis ohlini* caught during the Antarctic summer in the region of Antarctic Peninsula.

The amount and composition of euphausiid lipids varied but most of them contained large quantities of waxes. *Thysanoessa* sp., caught in the region of

South Georgia at the end of January, contained some 15% of lipids and in March — only 3—5%. The composition of lipids was similar: about 20% were waxes, 50% phospholipids and there were only traces of triacylglycerols (Clarke 1984). On the other hand Reinhardt and Van Vleet (1984, 1986) for *T. macrura*, which had about 4% of lipids and which came from similar area (the authors did not give the date of fishing), obtained a different composition of lipids (50% of waxes, 11,7% of triacylglycerols and 37,5% of phospholipids). *E. triacantha*, caught from February to April around South Georgia, contained from 7,74 to 4,03% of lipids. Lipids consisted of a dozen or so percent of waxes and of similar amounts of triacylglycerols and phospholipids. Out of Mysidacea *Antarctomysis ohlini* contained 20% of waxes and as much as 56% of triacylglycerols whereas *Antarctomysis maxima* contained only 5,6% of waxes and 25% of triacylglycerols; the majority were phospholipids (Reinhardt and Van Vleet 1984, 1986).

It is evident from the above review that data concerning the composition of lipids of Crustacea which can be an admixture to Antarctic krill catches are divergent. It was probably caused by the different periods and regions of fishing, and also by the fact that the studies has been carried out on a frozen material. It was proved that both frozen storage and storage time before freezing (Kolakowska 1986, 1989) have serious influence on a composition of lipids.

The aim of this paper was the comparison of the composition of lipids in fresh material of *T. macrura* and *E. superba* coming from the same catch. Analysis was also made of lipids from a single sample of Mysidacea (gen. sp. indet.) from winter period and of *E. triacantha*, which were compared with *E. superba* from similar dates of fishing.

Material and methods

The Crustacea were caught in the Admiralty Bay (South Shetlands) using a pelagic trawl-net hauled behind a motor boat "Dziunia". *T. macrura* and *E. superba* both came from the same hauls performed on 6.05.1986 and 12.05.1986. *E. triacantha* was caught on 1.11.1986. *E. superba* was not caught in this period, so the comparative samples come from 6.11. and 20.11.1986. Mysidacea (gen. sp. indet.) were caught on 11.08.1986 under the ice cover. Living animals were examined. After dripping from sea water the sample was homogenized and taken for determination. Mysidacea, because of their small number (3 specimens), were crushed in the hand mortar before taking for analysis.

The extraction of lipids was done using a method of Bligh-Dyer (1959). The content of lipids was estimated gravimetrically by evaporation of a certain quantity of extract. The composition of lipids was estimated by a thin-layer

chromatography in a way described in the previous paper (Kołakowska 1986). Lipids were separated on a silica gel G-60-254 (Merck foil) in a combination with n-hexane — ethyl ether — acetic acid. Under UV lamp fractions of phospholipids and waxes were localized and were cut off and separated in the second direction: phospholipids in the mixture of chloroform — methanol — water (70:30:4), waxes in n-hexane. Chromatographs were visualized in iodine (15 min) and left for 24 hours. Single fractions were transferred to the flasks and heated with the sulphuric acid in the temperature of 125°C for 1 hour. Water was added to the samples in the proportion of 3:2, samples were cooled in snow. The absorbancy was measured at 345 nm with the Pay Unicam spectrophotometer. Standard curves of the respective glycerols were used for: mono, 1,2- and 1,3-di and triacylglycerols. For phospholipids the standard curve of the mixture of phosphatidylcholine and phosphatidylethanolamine with the rests of palmityl and stearyl acids were used, and for free fatty acids — of linolenic acid. The susceptibility of lipids to the oxydation was estimated as in the previous paper (Kołakowska 1988), i.e. by the exposition of lipids to the UV lamp for 180 min; on the average every 15 min their peroxide value was determined by the peroxide thiocyanate technique. Carotenoids were determined by measuring the absorbancy of the chloroform layer of the Bligh-Dyer extract in the range of 320—600 nm. The quantity of carotenoids was expressed as astaxanthine. Iodine value was determined according to Polska Norma PN-78/C-04281.

Results

The comparison of data for *T. macrura* and *E. superba* (Tab. 1) coming from the same catches shows that the quantity and composition of lipids, with the exception of reserve lipids, are similar in both species of Euphausiacea. A sum of triacylglycerols and waxes is almost the same, i.e. about 20% of all lipids. However, while in *E. superba* triacylglycerols clearly prevail (they are 3—4 times in *T. macrura* the quantities of the two fractions are similar. Therefore lipids of *T. macrura* contain twice as many waxes as lipids of *E. superba*. As it can be seen from the data in Fig. 1, the differences concern also the composition of this fraction. Lipids of *T. macrura* contain an additional fraction and more waxes. Lipids of *T. macrura* were less susceptible to oxydation catalized by the UV rays than the lipids of *E. superba* (Fig. 2). This is also reflected by the lower iodine value of *T. macrura* lipids (Tab. 1). The amount of carotenoids extracted with a chloroform-methanol mixture was slightly lower in *T. macrura* (Tab. 1, Fig. 5). The spectrum of absorbancy in examined wavelenght suggests the difference also in a composition of carotenoids.

A composition of lipids of *E. triacantha* was compared with that of *E. superba* caught in the similar period (Tab. 2), since it was not possible to catch

Table 1

The comparison of *E. superba* and *T. macrura* lipids

No.	Determination	6.05.1986		12.05.1986	
		<i>E. superba</i>	<i>T. macrura</i>	<i>E. superba</i>	<i>T. macrura</i>
1.	The content of lipids, % of wet weight	4.91	4.28	4.25	4.63
2.	Carotenoids µg/g of wet weight	41.02	37.20	44.54	31.99
3.	Carotenoids µg/g of lipids	834.68	892.0	1048.13	675.58
4.	Iodine value	136.84	116.47	131.38	87.10
Classes of lipids (% of lipids)		<i>E. superba</i>	<i>T. macrura</i>	<i>E. superba</i>	<i>T. macrura</i>
1.	Phospholipids	62.37	62.03	65.52	66.70
2.	Cholesterol + 1,2-diacylglycerols	2.10	1.66	3.17	2.24
3.	1,3-diacylglycerols	5.11	4.59	5.32	4.93
4.	Free fatty acids	3.28	4.19	2.52	2.54
5.	Triacylglycerols	18.03	11.36	17.80	11.98
6.	Waxes	5.29	12.23	4.68	8.46
	Phosphatidylcholine (% of phospholipids)	—	—	79.71	77.17
	Phosphatidylethanolamine (% of phospholipids)	—	—	20.29	21.92

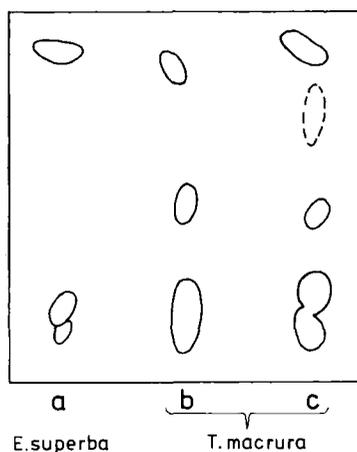


Fig. 1. Separation of the waxes fraction
Development: a, c — in H_2SO_4 ; b — in J_2 . *E. superba* — 40 µg wet weight, *T. macrura* — 30 µg wet weight

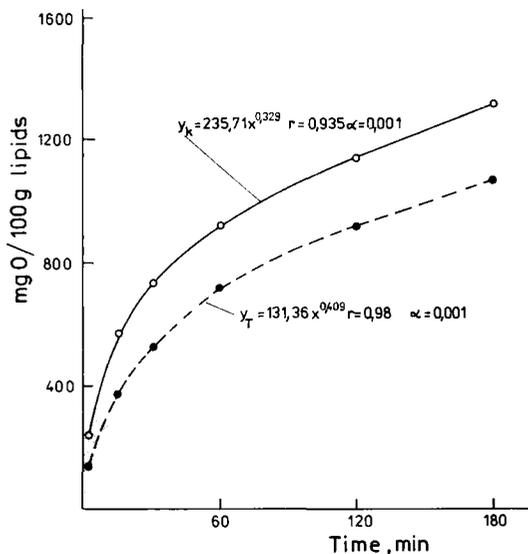


Fig. 2. Changes in the peroxide value of lipids under UV lamp: *E. superba* ○—○; *T. macrura* ●---●

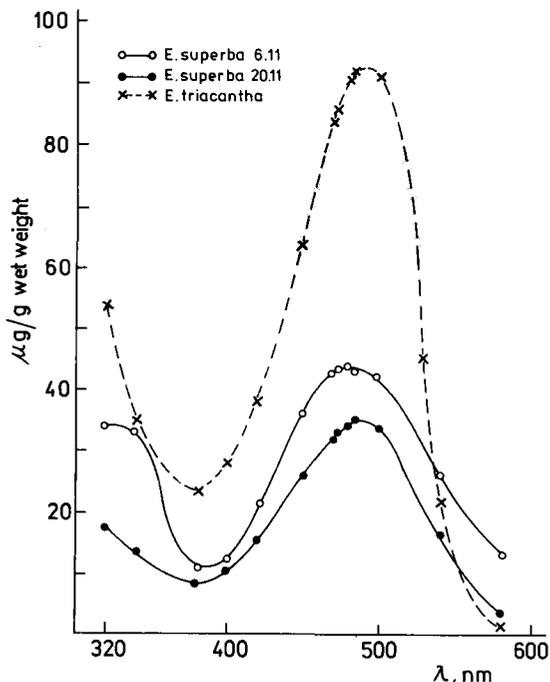


Fig. 3. Carotenoids of *E. triacantha* and *E. superba* extracted with lipids

sufficient number of specimens of both species exactly in the same time. As in the case of *T. macrura*, *E. triacantha* contained also more waxes than *E.*

Table 2

The composition of lipids in *E. superba*, *E. triacantha* and Mysidacea gen. sp. indet.

No.	Classes of lipids (% of lipids)	Mysidacea	<i>E. triacantha</i>	<i>E. superba</i>	
		gen.sp.indet. 11.08.1986	11.11.1986	6.11.86	20.11.86
1.	Phospholipids	58.26	67.89	75.05	71.85
2.	Cholesterol + 1,2-diacylglycerols	8.11	1.91	2.51	4.37
3.	1,3-diacylglycerols	—	—	2.44	4.55
4.	Free fatty acids	—	7.64	4.52	4.02
5.	Triacylglycerols	10.70	8.63	8.81	8.40
6.	Waxes	23.44	13.93	6.67	6.80
7.	Phosphatidylethanolamine	38.45	28.30	n	15.07
8.	Phosphatidylcholine	44.0	37.70	n	49.31
9.	Phosphatidylserine	—	13.80	n	15.06
10.	Lisophosphatidylcholine	—	12.60	n	17.12
11.	Diphosphatidylglycerol	17.09	7.54	n	3.42
12.	Other	—	trace	—	trace
13.	The total of lipids (% of wet weight)	—	1.96	1.35	2.54
14.	Carotenoids ($\mu\text{g/g}$ of wet weight)	—	91.15	44.54	35.43

n — not determined.

superba; waxes were dominant reserve lipids of *E. triacantha*. In the non polar lipids of *E. superba* triacylglycerols prevailed as previously.

The amount of carotenoids in *E. triacantha* was about 2,5 times higher than in *E. superba* (Tab. 2, Fig. 3). Spectrum of absorbancy was similar, suggesting the similarity of carotenoid composition.

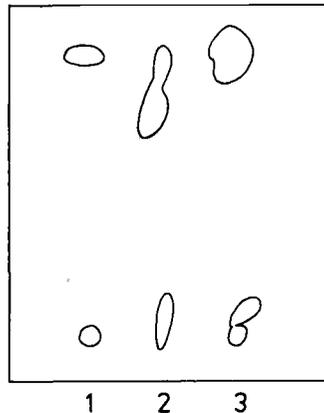


Fig. 4. Separation of the waxes fraction (developed in iodine): 1. Mysidacea gen. sp. indet. 10 μg wet weight 2. *Euphausia triacantha* 47 μg wet weight 3. *Euphausia superba* 34 μg wet weight

The sample of Mysidacea (gen. sp. indet.) was too small for all determinations. For this reason the determination of the amount of lipids gave divergent results and this information is omitted. The sample of Mysidacea, however, came from winter 1986 that was extremely severe. Animals were fished in the Admiralty Bay which was covered with ice of the thickness of about 2 m for over 2 months, but it was decided that this sample can still give an interesting information on the lipids of Mysidacea. In the composition of lipids of Mysidacea (Tab. 2) a high content of waxes and fraction of cholesterol + 1,2-diacylglycerols can be observed. The first was about twice as high as in lipids of *E. triacantha* and *T. macrura* and 4 times higher than in *E. superba*. The separation of this fraction (Fig. 4) evidences for its similarity to that of *E. superba*, i.e. that it consists mainly of cholesterol esters and hydrocarbons. However, it was not possible to separate clearly cholesterol esters and other waxes.

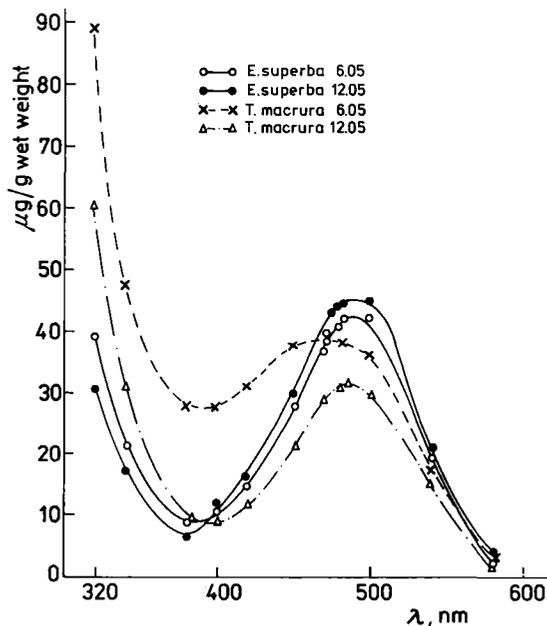


Fig. 5. Carotenoids extracted with lipids in *T. macrura* and *E. superba*

The general amount of phospholipids in the lipids of examined species was high: from 58,26% in Mysidacea to 75,05% in *E. superba*. The composition of phospholipids in all examined species was similar — with prevailing phosphatidylcholine and smaller amount of phosphatidylethanolamine (Tabs. 1 and 2).

Discussion

The results obtained in this paper, with the exception of general content of lipids, correspond neither with the results of Clarke (1984) nor Reinhardt and

Van Vleet (1984, 1986) obtained earlier for the same Crustacea. As it was mentioned in the introduction, also the results of Clarke (1984) and Reinhardt and Van Vleet (1984, 1986) were not convergent. It can be partly explained by different periods and fishing regions. It seems that higher content of phospholipids, several times lower quantities of free fatty acids, observed especially in the case of *E. superba*, and also the lack of monoglycerols found in the present work can be explained by the fresh state of the material used for analysis; animals in this study were examined immediately after fishing. It can be assumed that high quantities of wax esters (50% of lipids in *T. macrura*) and triglycerols (60% in *E. superba*) obtained by Reinhardt and Van Vleet (1984, 1986) were overestimated due to the fact that phospholipids, the largest fraction of total lipids could be considerably hydrolyzed during storage of frozen samples before analysis. In the present paper the detailed analysis of waxes was not possible, making the comparison with the results of the above mentioned authors difficult.

Despite the above differences, all Crustacea examined in this paper, in accordance with statements of Clarke (1984) and Reinhardt and Van Vleet (1984, 1986), contained more waxes than *E. superba*. The present results correspond also with earlier generalizations for Arctic organisms (Falk-Petersen et al. 1981).

E. triacantha and *T. macrura* contained a dozen or so percent of waxes (from 8.46 to 13.93) and if this whole fraction is to be treated as waxes only, that results are similar to those from Clarke's paper (1984) and much lower than those given by Reinhardt and Van Vleet (1984, 1985). The highest amount of that fraction was found in Mysidacea (as much as 23.44%). It could be supposed that in the middle of August (Antarctic winter) they really constituted an indispensable energy reserve. On the other hand *Antarctomysis ohlini* (Reinhardt and Van Vleet 1984, 1986) and *Antarctomysis maxima* (Clarke 1984) contained respectively 20.3 and 5.6% of waxes during the summer.

The comparison of the composition of phospholipids shows that *E. superba* contains the lowest amount of phosphatidylethanolamine and the highest amount of phosphatidylcholine among all examined Crustacea. The present and earlier author's researches (Kołakowska 1986) indicate that *E. superba* partly uses phospholipids and mainly phosphatidylcholine as reserve lipids. Fricke and Schreiber (1981) proved that along with the increased quantity of lipids in *E. superba* the amount of phosphatidylcholine increases as well, whereas the amount of phosphatidylethanolamine does not change. The observed differences in the composition of lipids between *E. superba* and *E. triacantha*, *T. macrura* and Mysidacea indirectly evidence also for the fact that except *E. superba*, all species prepare for winter by accumulating waxes. These data correspond well to the recent observations that *E. superba* feeds not only the phytoplankton and it feeds during the whole year.

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Streszczenie

Porównano skład lipidów *Thysanoessa macrura*, *Euphausia triacantha* i Mysidacea gen. sp. z lipidami *Euphausia superba*. Próby pochodziły z rejonu Polskiej Stacji Antarktycznej (Płd. Sztlandy) i poddane były analizie bezzwłocznie po odłowieniu.

Lipidy badanych gatunków różniły się od lipidów *E. superba* zdecydowanie większą zawartością frakcji wosków. U *E. triacantha* i *T. macrura* stanowiła ona 6.36—14.93% lipidów, tj.

około 2-krotnie więcej niż w *E. superba*. Najwięcej, bo 23.44% zawierały jej lipidy Mysidacea gen. sp. Skorupiaki te złowione zostały w zimie pod lodem. Zawartość karotenoidów w *T. macrura* i *E. superba* była zbliżona, natomiast w *E. triacantha* 2,5-krotnie wyższa. Stwierdzone różnice w składzie lipidów są pośrednim dowodem na to, że *Euphausia superba* gospodaruje lipidami odmiennie niż pozostałe badane gatunki.