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Lipids of some Antarctic animals of the Admiralty Bay (King George Island, South Shetland Islands).

ABSTRACT: The amount and composition of lipids in some Antarctic animals were studied. The material consisted of crustaceans (*Euphausia superba*, *Paramoera* sp., *Orchomene* sp.), tunicates (*Salpa thompsoni*) and vertebrates (*Notothenia rossi marmorata* and *Hydrurga leptonyx*). The author's data are discussed on the background of available literature information.

Key words: Antarctica, animal lipids, krill.

1. Introduction

The composition of lipids in animal bodies depends mainly on their diet and this is one of the factors which is said to account for differences between lipids of animals inhabiting various regions (Ackman et al. 1970, Bottino 1974, Morris and Culkin 1976, Clarke 1977, 1980). This is the reason why an analysis of lipids may supply us with valuable information on the food chain in a given ecosystem. The composition of fatty acids is most frequently used for this purpose but hydrocarbons and sterols are also studied (Lee, Nevenzel and Paffenhofer 1971, Yamada 1972, Bottino 1974, Bishop, James and Olley 1976, Morris and Culkin 1976, 1977, Tinoco 1982).

Some aquatic invertebrates contain also lipids which are absent in their diet (Lee, Nevenzel and Paffenhofer 1971, Yamada 1972, Morris and Sargent 1973, Mankura, Kayama and Iijima 1986). The lipids of Antarctic animals have been poorly investigated. Most of the works on the subject deal

with the Antarctic krill but the majority of these works on krill concerned the frozen material, the fact that must have exerted strong influence on the lipid composition (Kołakowska 1986).

Rakusa-Suszczewski and Dominas (1974) investigated the contents and qualitative lipid composition of the amphipod species *Paramoera walkeri*. Reinhardt and Van Vleet (1984) have studied the classes of lipids and hydrocarbon composition in some Antarctic amphipods, in krill (*E. superba*) and in a salp (*Salpa thompsoni*).

The lipolytic activity and lipid oxidation in the frozen Antarctic krill are mentioned by Galas et al. (1979), Krawczuk-Krogulecka et al. (1979), Nagayama et al. (1979), Warzecha, Sawicka and Jakubowska (1979) and Kosačkina and Lobanova (1981).

Lipid composition and their oxidation are very important for the technology of exploiting the marine biological resources; they decide about their resistance and nutritional value.

The aim of the present paper was to compare the composition and oxidation of lipids of various Antarctic animals from the Admiralty Bay.

2. Material and methods

Investigations were carried out at the "H. Arctowski" Station (King George Island) during the VIIIth Antarctic Expedition of the Polish Academy of Sciences (December 1983 — December 1984). Material for the study was obtained in the Admiralty Bay (62°09′51″S; 58°27′45″W). The krill (*Euphausia superba*), amphipods *Paramoera* sp. and *Orchomene* sp., a salp (*Salpa thompsoni*), sea leopard (*Hydrurga leptonyx*) and fish (*Notothenia rossi marmorata*) were investigated.

The study material consisted of the following elements: whole krill body, contents of the krill stomach, whole amphipods (alive) whole salps, salp's stomach filled with digested food, salp's body without the stomach and alimentary tract, fish fillet without skin, sea leopard muscles from the dorsal part and its subcutaneous adipose tissue.

The research methods employed were following:

a) Lipids were extracted with the Bligh-Dyer method (1959). The lipid content was quantitated gravimetrically by evaporating a given volume of the extract. The lipids were separated applying thin-layer chromatography in the following way: a part of the chloroform layer of the Bligh-Dyer extract was condensed in a vacuum evaporator and then separated on plates covered with silica gel 60G (Merck) of a size of 20 × 20 cm with a gel layer 0.22 mm thick, employing as a solvent a mixture: hexane — ethyl ether — acetic acid (60:40:1.5). Stains were

localized in iodine vapours (15 minutes) and after 24 hours lipid classes were determined quantitatively by heating with sulfuric acid for an hour (Kabara and Chen 1976). The samples were cooled in snow and their absorbance was measured with a Pye Unicam spectrophotometer at a 345 nm wavelength. Pattern curves were prepared for phospholipids basing on the phospholipid mixture KIT SCHWARZ/MANN (Dickinson and Co.); for glicerides: on trioleate 1-3 and 1-2 dioleate 1-monoleate, for unsaturated fatty acids on the basis of linolenic acid (Fluka AG).

b) Iodine value was determined according to the Polish Standard 78/C-04281.

c) Carotenoid contents (recalculated for astaxanthin) was calculated on the basis of the maximal absorbance of the chloroform layer of Bligh-Dyer extract.

d) Oxidation rate was measured in the following way: 50 cm^3 of the chloroform layer of Bligh-Dyer extract was evaporated in a vacuum evaporator at up to 300°K and samples in the chloroform-methanol (2:1) solution were applied to strips (2.5 × 7 cm) of filter paper. The lipid samples were of 0.01 g each. The strips were exposed for 8 hours to light from an UV lamp of 344 nm wavelength, from a distance of 15 cm. Samples were taken at 0.5—1 hour intervals, the strips were eluted with chloroform and the peroxide value determined with the sulphur-cyanide method (Standard 74/820-007) and the absorption of the eluate at a 470 nm wavelength.

e) The enzymatic activity was determined according to Galas et al. (1979), using as a substrate: soybean oil, trioleate and lecithin. The crude enzyme solution was obtained by extracting the material with a 45% ethanol at a proportion of 3:10. The ratio of the substrate to the enzyme extract was: triglyceride and soybean oil 2:0.6—1.8 g of wet material weight (2000:11—33.5 mg of protein); lecithin 0.15:0.3—1.5 g of wet weight (150:0.5—1.5 mg of protein). The amount of free acid groups was determined after incubation at 305°K by titration of the samples with the 0.1 n solution of potassium hydroxide. The protein content in the extract was measured using the method of Lowry et al. (1951).

3. Results

The amount of lipids in whole krill bodies, in amphipods and in salps recalculated for dry weight was similar and amounted to 17.25% (mean for samples collected over a year); 15.25% and 15.47%, respectively. The lipids constituted only about 0.3% of wet salp weight and on the average about 3% of wet krill and wet amphipod weights (Table 1). In the case of each of the above taxa the amount of lipids varied depending

Table 1

Lipid and carotenoid contents of some animals from the Admiralty Bay

Species	Sampling date	Lipid content in % of wet weight	Iodine number	Carotenoids	
				ug/g of the wet weight	ug/g of the lipids
Euphausia superba	annual mean	3,180	161,67	31,08	1327
E. superba	10.11.84	1,290	–	46,68	3604
E. superba (stomach contents)	10.11.84	2,830	–	14,93	5268
Orchomene sp.	7.03.84	4,580	128,64	54,77	1241
Paramoera sp.	12.07.84	1,760	150,88	101,82	5785
Orchomene sp.	13.07.84	3,510	102,79	45,45	1295
Salpa thompsoni	30.12.83	0,333	–		
S. thompsoni	27.03.84	0,293	34,77		
S. thompsoni (stomach)	27.03.84	0,755	226,80	62,27	8248
S. thompsoni	13.03.84	0,619	33,64	21,93	430,46
S. thompsoni (without stomach)	13.03.84	0,376	–	12,50	388,56
Notothenia rossi marmorata (muscles)	22.08.84	1,077	–	0,818	75,96
Hydrurga leptonyx (muscles)	29.10.84	1,310	–	33,95	2947

on the collecting date of the sample. The lipids of filled stomachs constituted a considerable part of the lipids of the whole salp. The muscles of the sea leopard and of the notothenid fish contained about 1% of lipids.

The quantitative lipid composition (Table 2) of the investigated species varied. Phospholipids dominated among the krill lipids. In general the lipid composition of krill reflects the lipid composition of its food. The presence of phosphatic acids in these lipids evidences the process of the digestion of phospholipids in the krill stomach. The lipid composition of Amphipoda depended on species and data of the catch; however, in each of the samples triglycerides either dominated or constituted almost a half of the total lipid contents. The percentage of cholesterol was also higher than in krill.

The lipids of the salp body clearly differed in their composition from the lipids of their filled stomach. Phospholipides and diglycerides + cholesterol composed almost half of the body lipids and cholesterol esters + waxes constituted a considerable part (17%), whereas triglycerides constituted only 5% of them. In contrast, the lipids of a filled salp stomach had the composition similar to the lipids of the krill stomachs, except triglycerides which were a bit more abundant in the salp. The lipid composition of

Table 2

Lipid composition in some animals from the Admiralty Bay

Species	Sampling date	phospho-lipids	tri-glycerides	1,2 diacyl-glycerol + cholesterol	1,3 diacyl -glycerol	monoacyl -glycerol	free fatty acids	cholester esthers and waxes
Euphausia superba	annual mean	72,79 ±2,12	13,26 ±0,84	5,73 ±0,12	2,01 ±0,11	–	3,72 ±0,04	1,29 ±0,08
Orchomene sp.	7.03.84	50,02 ±1,23	40,63 ±1,51	2,45 ±0,41	1,33 ±0,20	–	4,12 ±0,02	1,42 ±0,10
Paramoera sp.	12.07.84	30,03 ±1,06	38,92 ±0,12	16,17 ±0,55	trace	trace	12,00 ±0,10	1,89 ±0,06
Orchomene sp.	13.07.84	11,80 ±0,67	68,71 ±1,10	10,86 ±0,36	2,49 ±0,22	–	5,18 ±0,69	0,96 ±0,02
Salpa thompsoni	13.03.84	39,66 ±1,30	46,27 ±0,90	4,51 ±0,71	1,40 ±0,17	–	1,55 ±0,02	6,61 ±0,03
S. thompsoni (without stomach)		24,34 ±0,21	5,24 ±0,09	29,50 ±0,93	5,24 ±0,02	–	18,72 ±0,16	16,85 ±0,01
S. thompsoni (stomach)	27.03.84	59,20 ±1,65	24,10 ±0,86	4,66 ±0,02	1,59 ±0,32	–	5,54 ±0,024	0,60 ±0,12
Notothenia rossi marmorata (muscles)	22.08.84	77,24 ±1,31	8,63 ±0,14	7,64 ±0,07	0	–	5,08 ±0,024	1,39 ±0,03
Hydrurga leptonyx (muscles)	29.10.84	69,61 ±0,09	15,64 ±0,20	4,75 ±0,03	1,66 ±0,01	–	6,67 ±0,018	1,65 ±0,03
H. leptonyx (subcutaneous adipose tissue)	10.11.84	4,42 ±0,03	91,35 ±0,10	0,88 ±0,06	0,87 ±0,012	–	2,41 ±0,001	0
E. superba	10.11.84	73,45 ±1,11	9,50 ±0,94	6,18 ±0,68	0,52 ±0,036	–	9,32 ±0,12	0,85 ±0,001
E. superba (stomach)	10.11.84	70,98 ±1,73	5,15 ±0,87	7,80 ±0,68	–	–	9,52 ±0,02	1,23 ±0,06

the whole salp is a resultant of the body lipids and of the lipids of its filled stomach.

The lipids of the subcutaneous adipose tissue of the sea leopard are composed almost entirely of triglycerides. The lipids of its muscles are mostly phospholipids and other classes of lipids occurring in proportions similar to those of krill. The lipid composition of nototheniid fish muscles is similar. In general the lipid composition of krill and of muscles of sea leopard and nototheniid fish are similar, the share of triglycerides being slightly higher in the last ones.

Changes in the peroxide value under the influence of ultraviolet radiation were of logarithmic character (Fig. 1). Lipids of the salp and of the

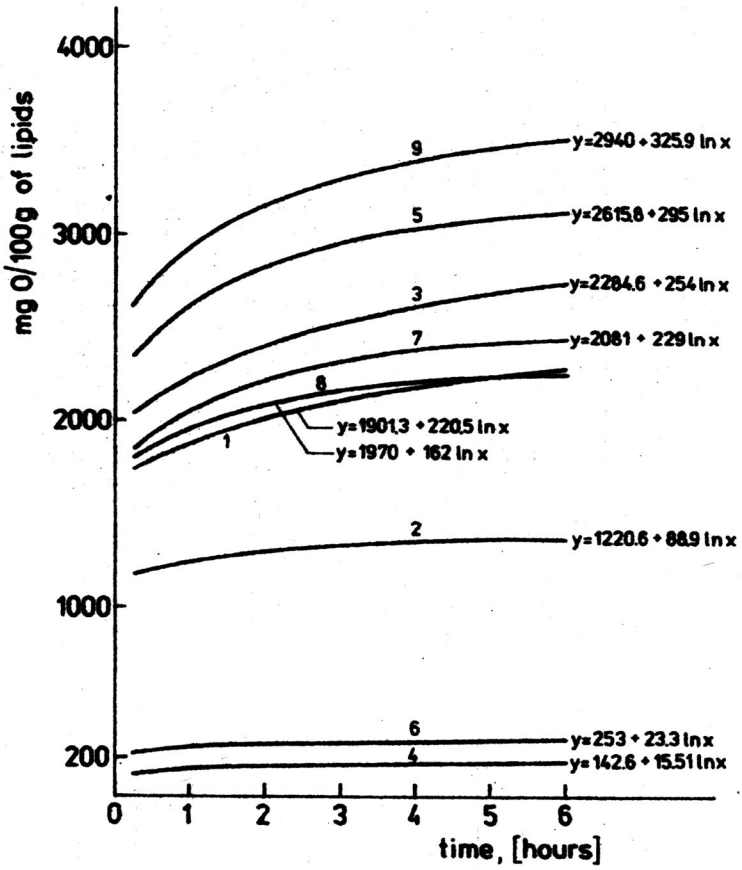

Fig. 1. Peroxide number of lipids during the UV radiation

1. Lipids of *Euphausia superba* (caught on 10.11.84), 2. Lipids of the stomach contents of *Euphausia superba* (caught on 10.11.84), 3. Lipids of *Orchomene* sp. (caught on 7.03.84), 4. Lipids of *Paramoera* sp. (caught on 12.07.84), 5. Lipids of *Orchomene* sp. (caught on 19.07.84), 6. Lipids of *Salpa thompsoni*, 7. Lipids of *Notothenia rossi marmorata*, 8. Lipids of *Hydrurga leptonyx*, 9. Lipids of the subcutaneous adipose tissue of *Hydrurga leptonyx*

amphipod *Paramoera* sp. were distinguished by their low susceptibility to oxidation (Fig. 1, Tab. 3). From Table 1 it follows that salp lipids were characterized by a very low iodine value, which is not the case with the amphipod lipids.

The degree of oxidation (Fig. 1) under the influence of ultraviolet radiation was similar in the lipids of krill and of nototheniid and sea leopard muscles; it was higher in the lipids of the amphipods *Orchomene* sp. The rate of oxidation (Tab. 3) was similar in all the above mentioned

Tabela 3

Oxidation rate of lipids of some animals from the Admiralty Bay

Species	Sampling date	Oxidation rate mg0/100 g of lipids/min. after UV irradiation		
		1h	2h	3h
Euphausia superba	10.11.84	28,16	3,31	8,41
E.superba (stomach contents)	10.11.84	14,57	2,96	−5,69
Orchomene sp.	7.03.84	24,42	16,11	5,15
Orchomene sp	13.07.84	19,57	30,07	7,88
Paramoera sp.	12.07.84	1,71	0,53	0,32
Salpa thompsoni	13.03.84	4,90	−0,98	−0,80
Notothenia rossi marmorata (muscles)	22.08.84	25,85	20,72	−3,38
Hydrurga leptonyx (muscles)	29.10.84	10,60	18,06	1,88
H. leptonyx (subcutaneous adipose tissue)	29.10.84	21,17	20,91	21,70

species. The lipids of the subcutaneous adipose tissue of the sea leopard were the most susceptible for oxidation. The rate of reaction was twice higher than in muscle lipids.

In the same oxidation conditions the rate of lipid oxidation was lower in the stomach contents than in the whole body of krill. Increase in the peroxide value in the 3 hours period of oxidation was in the krill lipids about 2 times higher than in the lipids of its food. The iodine value was determined only in some chosen samples. Data included in Tab. 1, except those of the salp, do not indicate any direct correlation between oxidability and iodine value or the carotenoid contents.

The lipolytic activity was investigated haphazardly. Out of three species (Tab. 4) krill contained the most active lipases, the salp less active ones (recalculated for dry weight) and *Orchomene* sp. the least active ones. The activity of the crude enzymatic extract was about 10 times higher when the lecithine was the substrate than in the case of triglicerides and a soybean oil.

Table 4

Lipolytic activity of some animals from the Admiralty Bay

Species	Sampling date	Substrate								
		Triglyceride			Phospholipid			Soybean oil		
		umol of acid groups per 1 g of:								
		wet weight	dry weight	protein	wet weight	dry weight	protein	wet weight	dry weight	protein
Euphausia superba	27.03.84	58,33	226,08	3176,04	150,00	1162,79	8024,25	–	–	–
E. superba	6.09.84	–	–	–	–	–	–	52,50	290,50	1296,29
Orchomene sp.	22.08.84	39,16	145,61	2851,94	–	–	–	34,16	131,38	2487,86
Salpa thompsoni	27.03.84	8,33	173,54	9823,18	83,33	1736,04	98231,80	–	–	–

4. Discussion

Out of six investigated species of marine animals collected in the Admiralty Bay in 1984 krill, *Orchomene* sp. and salps had similar lipid contents in their dry weight, i.e. about 15—17%. These lipids were mainly structural and stock lipids as well as the lipids of the food filling the alimentary tract of the animals.

The contents of lipids in the muscles of the nototheniid fish and of the sea leopard amounted only to about 5% of their dry weight. These lipids are probably mostly structural ones. Stock lipids are stored in the sea leopard in the form of abundant subcutaneous tissue and in the nototheniid fish mainly in the liver (Clarke et al. 1984).

In the case of krill the present data on the lipid contents are concordant with those from the available literature (Mauchline and Fisher 1969, Grantham 1977, Clark 1980, 1983, Kołakowska 1985, 1986). The present results of amphipod lipids studies were similar to those obtained by Rakusa-Suszczewski and Dominas (1974) for *Paramoera walkeri*, whereas the amount of lipids in salps and amphipods recorded by the present author were rather lower than those obtained by Reinhardt and Van Vleet (1984).

On the basis of their fatty acids composition Yamada (1972), after Shorland, divides the lipids of water animals into homolipids and heterolipids. The lipids of animals from the Admiralty Bay might be ascribed to these two groups on the basis of quantitative composition of lipid classes. Accordingly, in Antarctic summer the krill lipids are homolipids because the quantitative composition of their lipid classes is almost identical with the lipid composition of krill's food. Already Bottino (1984) proved that the composition of fatty acids in *Euphausia superba* Dana reflects the fatty acid composition of phytoplabkton. The lipids of *Notothenia* muscles might be also classed among homolipids because the quantitative composition of particular classes of its lipids reflects the lipid composition of krill and phytoplankton lipids.

According to Yamada (1972) and Bishop, James and Olley (1976) fishes can assimilate lipids of their diets but it influences mainly the composition of their stock lipids.

The lipids of krill and phytoplankton (i.e., contents of krill stomachs) and of nototheniid muscles are similar in respect to their high (over 70%) contents of phospholipids. The recurrence of this composition in the food chain is probably the result of animals' physiological requirements, which, according to Morris and Culkin (1976) decide about the lipid (fatty acids) composition of Crustacea as much or even more than the diet.

The amount of phospholipids in the muscles of nototheniid fish and of the sea leopard amounts to about 0.9% of their tissue weight and this

is probably the amount indispensable for their structural and biologically active functioning (Hanahan and Nelson 1984); phospholipids are generally more abundant (especially in summer) in krill. Presumably, they play also a role of stock lipids, which is supported by their varying content over a year and by the decrease of their amount in the starving period (Kołakowska 1985); this stock might be related also to the reproductive function.

The lipids of the salp might be classed among heterolipids. The lipid composition of the whole salp, and particularly of its body, significantly differs from the lipid composition of the salp's diet. The salp lipids are rich in waxes and sterols. Their content in the whole salp exceeds 10 times and in its body 20 times the content of these compounds in the diet of salps. Voogt and Van Rheenen (1975) observed that tunicates may synthetize the sterols. Salps, probably synthetize waxes and hydrocarbons, which is indirectly proved by the very low iodine value of their lipids and their resistance to oxidation. Reinhardt and Van Vleet (1984) have found that hydrocarbons of the salp lipids were mainly the saturated hydrocarbons. The high phospholipase activity of the salps probably serves for rapid decomposition of their diet rich in phospholipids, which, in turn, enables the synthesis of their own necessary lipids. The salp lipid composition presented in the present paper is concordant with the results of Morris, Mc Cartney and Bone (1984) obtained for three salp species of the Western Mediterranean but differs from Reinhardt and Van Vleet's (1984) data for *Salpa thompsoni*. The proper comparison of these results is difficult due to the lack of information on the methods applied in the last of the above mentioned publications.

Amphipod lipids are relatively rich in typical stock lipids, i.e. triglycerides, which is probably caused by their higher energetic requirements (Rakusa-Suszczewski and Klekowski 1973) but can also reflect the lipid composition of their diet.

The lipids of salps and of the amphipod *Paramoera* sp. are unique in their high resistance to oxidation. In the case of salps it may be caused by the low content of unsaturated fatty acids and a higher activity of phospholipases. Judging from their high iodine value the lipids of amphipod *Paramoera* sp. contain, however, a lot of unsaturated fatty acids. May be that the carotenoids, which are abundant in *Paramoera* sp. (twice as much an in *Orchomene* sp.), have an anti-oxidation effect. According to Burton and Ingold (1984) carotenoids may play a role of antioxidants.

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6. Streszczenie

Badano lipidy niektórych zwierząt antarktycznych: kryla (*Euphausia superba*), sprzągli (*Salpa thompsoni*), obunogów: *Orchomene* sp. i *Paramoera* sp., lamparta morskiego (*Hydrurga leptonyx*) i ryby (*Notothenia rossi marmorata*) z Zatoki Admiralicji (Wyspa Króla Jerzego). Oznaczano skład lipidów (klasy), podatność na utlenianie UV, w kilku próbach aktywność lipolityczną. Sucha masa *E. superba*, *Orchomene* sp. i *S. thompsoni* zawierała 15—17% lipidów (całe zwierzę), zaś sucha masa mięśni *H. leptonyx* i *N. marmorata* 5% lipidów o różnym składzie. W lipidach kryla, lamparta morskiego i ryby dominowały fosfolipidy (ok. 70% lipidów), podatność na utlenianie tych lipidów była podobna. W lipidach sprzągli dominowały sterole i woski w ilościach 10—20-krotnie większych niż w ich diecie. Lipidy *S. thompsoni* i *Paramoera* sp. wyróżniały się opornością na utlenianie; pierwsze prawdopodobnie ze względu na niską liczbę jodową i wysoką aktywność fosfolipaz, natomiast lipidy *Paramoera* sp. zawierały co najmniej dwukrotnie więcej karotenoidów (5785 μg/g lipidów) niż w przypadku pozostałych badanych zwierząt.