POLISH POLAR RESEARCH (POL. POLAR RES.) POLSKIE BADANIA POLARNE	1	2—3	139—145	1980

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Qualitative analysis of carotenoids in particular parts of body of males and females of *Euphausia superba* Dana *(Crustacea)* *)

ABSTRACT: In adults of both sexes of *Euphausia superba* analysed in total without alimentary tract there were identified with help of chromatographic and spectrophotometric methods: β-carotene, free and ester astaxanthine, cantaxanthine, β-cryptoxanthine, lutein and dihydroxy-ζ-carotene, and additionally in females zeaxanthine and flavoxanthine. However, particular parts of body (with an exception of eyes, legs and trunk) and especially the exosceleton cotain less carotenoids, mainly carotene and astaxanthine.

Key words: Antarctic, *Euphausia superba* (*Crustacea*), carotenoids

1. Introduction

It is already known that females of *Euphausia superba* have more carotenoids, qualitatively and quantitatively, when compared with juvenile forms and with adult males (Jackowska, Czerpak and Mical 1980). The composition of krill carotenoids depends mainly on age structure, sex, kind of food and physico-chemical conditions of the environment (Czeczuga and Kłyszejko 1978, Jackowska, Czerpak and Mical 1980). It can be assumed that the majority of identified about 10 carotenoids of studied crustaceans are the precursors of vitamin A for antarctic vertebrates (Fisher, Kon and Thompson 1955, Herring 1972, Czeczuga and Kłyszejko 1978, Jackowska, Czerpak and Mical 1980). The distribution of carotenoids in particular organs and parts of krill body as dependent on sex is also an important problem and this was the aim of present paper.

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*) Research carried out during the Third Antarctic Expedition of PAS to Arctowski Station in 1978/1979 within the Project MR-II-16.

2. Materials and methods

Material for studies was collected during the antarctic summer 1978/1979 (from 5 Dec. 1978 to 15 Feb. 1979) in Admiralty Bay, close to Arctowski Station.

The collected material consisted of adult individuals of both sexes of *E. superba* without alimentary tracts, the trunks without alimentary tracts, eyes, legs and exosceletons, and eyes, legs and exosceletons by themselves.

An analysis of the qualitative composition of carotenoids in the distinguished parts of material was done with the help of extraction, adsorption thin layer and column chromatography and spectrophotometric methods (Gilchrist and Green 1960, Czerpak 1969, Czerpak and Czeczuga 1969).

While identifying carotenoids their absorption maxima in respective organic solvents were applied (Table I) and R_f values in particular combinations of solvents (Table II), described in detail in following papers: Gilchrist and Green (1960), Czerpak (1969), Czerpak and Czeczuga (1969), Foppen (1971), Herring (1972), Goodwin (1976), Czeczuga and Kłyszejko (1978) and Jackowska, Czerpak and Mical (1980). Details of methods of carotenoids analysis are to be found mainly in Czerpak (1969) and Jackowska, Czerpak and Mical (1980).

3. Results and discussion

The following carotenoids were found in studied parts of *E. superba* body (Tables I and II):

The adult individuals of both sexes without alimentary tract, studied as a whole, contained the most of carotenoids: β-carotene, free and ester astaxanthine, cantaxanthine, β-cryptoxanthine, lutein and not determined precisely, probably dihydroxy-ζ-carotene, and females additionally flavoxanthine.

The trunks without alimentary tracts, eyes, legs and exosceletons of both sexes contained also large selection of carotenoids: β-carotene, β--cryptoxanthine, astaxanthine ester and dihydroxy-ζ-carotene, the females additionally lutein.

The eyes of both sexes also contain carotenoids: β-carotene, cryptoxanthine, lutein, free astaxanthine and dihydroxy-ζ-carotene, of females additionally flavoxanthine.

Legs of both sexes are characterised by the presence of large selection of carotenoids, mainly of β-carotene, lutein, flavoxanthine, free astaxanthine and dihydroxy-ζ-carotene. In legs of females cantaxanthine and zeaxanthine were also identified.

The exosceleton of krill is, on the other hand, qualitatively poor in carotenoids, as it contains only β-carotene, free and ester astaxanthine, and the one of females additionally cantaxanthine.

The comparison of the obtained data on the occurrence of carotenoids in some particular parts of body of *E. superba* with earlier own data (Jackowska, Czerpak and Mical 1980), and data of other authors

Table I.

The separation and identification of carotenoids from particular parts of krill body done by adsorption column chromatography and spectrophotometry

Numbers of consequent fractions	Applied elunts	Absorption maxima of identified carotenoids (nm)	Kind of solvent	Identification*)	Kind of analysed material									
					whole individuals**)		trunks***)		eyes		legs		exosceletons	
					females	males	females	males	females	males	females	males	females	males
1	petroleum ether with boiling point 40—50°C	426; 450; 478	petroleum ether	β-carotene	+	+	+	+	+	+	+	+	+	+
2	2—5% acetone in petroleum ether	425; 452; 483	petroleum ether	β-cryptoxanthine	+	+	+	+	+	+	−	−	−	−
3	8—10% acetone in petroleum ether	468	petroleum ether	astaxanthine-ester	+	+	+	+	−	−	−	−	+	+
4	15% acetone in petroleum ether	377; 398; 421	petroleum ether	dihydroxy-ζ-carotene (?)	+	+	+	+	+	+	+	+	−	−
5	25—30% acetone in petroleum ether	465	petroleum ether	cantaxanthine	+	+	−	−	−	+ ?	+	−	+	−
6	45—50% acetone in petroleum ether	421; 445; 476	petroleum ether	lutein	+	+	+	−	+	+	+	+	−	−
7	100% acetone	423; 451; 481	acetone	zeaxanthine	−	−	−	−	−	−	+	−	−	−
8	10% n-propanol in acetone	421; 447—448	ethanol	flavoxanthine	+	−	−	−	+	−	+	+	−	−
9	5—10% KOH in 90% acetone	470—471	acetone	free astaxanthine	+	+	−	−	+	+	+	+	+	+

*) According to: Fisher, Kon and Thompson (1955), Gilchrist and Green (1960), Czerpak (1969), Czerpak and Czeczuga (1969), Foppen (1971), Herring (1972), Goodwin (1976), Czeczuga and Kłyszejko (1978),

**) without alimentary tracts,

***) without alimentary tracts, eyes, legs and exosceletons

Table II.

Carotenoids in particular parts of krill body, identified by thim layer chromatography in solvents combinations I—II

Solvent combinations	Numbers of consequent bends	R_f values (average from 3—5 measurements)										Carotenoids	Ranges of R_f values***)
		whole individuals*)		trunks**)		eyes		legs		exosceletons			
		females	males	females	males	females	males	females	males	females	males		
I: benzene-petroleum ether-acetone (10:2.5:2)								0.98	0.97	0.96	0.97	β-carotene	0.94—0.98
	1	0.97	0.98	0.98	0.96	0.98	0.96	0.86	0.90	—	—	dihydroxy-ζ-carotene (?)	0.85—0.95
	2	0.93	0.89	0.92	?	0.88	?	—	—	0.82	0.76	astaxanthine ester	0.77—0.83
	3	0.81	0.83	0.79	0.80	—	—	0.71	—	0.72?	—	cantaxanthine	0.70—0.76
	4	0.75	0.71	0.76?	—	—	0.72?	—	—	—	—	β-cryptoxanthine	0.62—0.70
	5	0.62	?	0.64	0.65	0.62	0.65	0.55	0.54	—	—	lutein	0.45—0.55
	6	0.52	0.55	0.50	—	0.50	0.45	0.43	—	—	—	zeaxanthine	0.43—0.47
	7	—	—	—	—	—	—	0.35	0.40	—	—	flavoxanthine	0.33—0.41
	8	0.36	—	—	—	0.37	—	0.28	0.26	0.29	0.33	free astaxanthine	0.25—0.35
	9	0.29	0.25	—	—	0.27	0.25						
II: benzene-ethyl ether-methanol (17:2:1)								0.96	0.98	0.95	0.94	β-carotene	0.93—0.99
	1	0.98	0.97	0.96	0.95	0.95	0.94	0.80	0.82	—	—	dihydroxy-ζ-carotene (?)	0.78—0.84
	2	0.84	0.80	0.81	?	0.82	?	0.68	—	?	—	cantaxanthine	0.67—0.75
	3	0.68	0.72	0.75?	—	—	0.70?	—	—	0.61	0.60	astaxanthine ester	0.60—0.66
	4	0.60	0.59	0.62	0.63	—	—	—	—	—	—	β-cryptoxanthine	0.57—0.61
	5	0.57	?	0.60	0.59	0.61	0.60	0.45	0.48	—	—	lutein	0.43—0.49
	6	0.45	0.46	0.44	—	0.47	0.44	0.41	—	—	—	zeaxanthine	0.40—0.44
	7	—	—	—	—	—	—	0.38	0.39	—	—	flavoxanthine	0.36—0.40
	8	0.37	—	—	—	0.38	—	0.33	0.30	0.31	0.34	free astaxanthine	0.28—0.36
	9	0.28	0.32	—	—	0.30	0.31						

*) without alimentary tract,

**) without alimentary tract, eyes, legs and exosceletons,

***) According to: Czerpak (1969), Czerpak and Czeczuga (1969), Foppen (1971), Jackowska, Czerpak and Mical (1980).

$$R_f = \frac{\text{distance from the origin to the middle of the band of particular carotenoid in mm}}{\text{distance from the origin to the front of solvent combination spot in mm}}$$

(Fisher, Kon and Thompson 1955, Herring 1972, Czeczuga and Kłyszejko 1978) showed that adults of both sexes and juvenile forms of *E. superba* and *E. crystallorophias* and adult *E. crystallorophias* contain a large selection of carotenoids indentified previously in other crustaceans, especially in crabs, shrimps and crayfishes.

The most common carotenoids occurring in juvenile forms of *E. superba* and *E. crystallorophias* and in adults of these species are the free and ester astaxanthine, β-carotene, β-cryptoxanthine and dihydroxy-ζ-carotene. The lutein, cantaxanthine, flavoxanthine and zeaxanthine occur rarely and only in some parts of body of *E. superba*.

The adult female individuals studied in total, and some parts of their bodies, are much richer in number of carotenoids, especially xanthophylls, as compared with male individuals. It is known from the literature data (Rakusa-Suszczewski and Opaliński 1978) that female individuals consume more oxygen while breathing than males, and this can result in their larger amount of oxidized forms of carotenoids (xanthophylls).

The obtained results allow to state that the whole individuals of both sexes of *E. superba*, and eyes and legs of especially females contain larger selection of carotenoids than exosceleton and the trunk.

Astaxanthine and β-carotene are the most common carotenoids of studied parts of body of juvenile and adult forms of both sexes of *E. superba*. The whole adults, and especially the females and their eyes are the most rich in carotenoids, which are the provitamines of vitamin A.

The whole adult individuals of both sexes of *E. superba*, as well as their eyes and legs are much richer, as compared with the trunks and exosceletons, in various forms of intensively oxygenised xanthophylls. Also, the studied parts of body of adults of *E. superba* (except of eyes and legs) of mainly male individuals, contain much smaller assortment of carotenoids as compared with adult individuals studied in total, especially females, and with previously studied by the authors juvenile forms of *E. superba* and *E. crystallorophias* and adult *E. crystallorophias*.

The authors are grateful to R. Stępnik, M.Sc., member of the Third Antarctic Expedition of PAS to the Arctowski Station, for collecting the material for this study.

4. Summary

The occurrence of cartenoids was studied with help of adsorption column and thin layer chromatography and spectrophotometry in whole adults of both sexes of *Euphausia superba* and in their eyes, exosceletons, corps and legs, separately. The following were identified: β-carotene, β-cryptoxanthine, free and ester astaxanthine, cantaxanthine, lutein, flavoxanthine, zeaxanthine and not determined precisely, probably dihydroxy-ζ-carotene (Table I and II).

It was found on the basis of the obtained results that adult females analysed in total and some of their parts, especially eyes and legs are much richer in assortment of carotenoids than male individuals, expecially sexually mature.

It was found that eyes and legs of adult individuals of both sexes are much richer in assortment of carotenoids than exosceletons and trunks.

5. Резюме

Исследуя с помощью метода адсорбционной колонной и тонкослоевой хроматографии а также спектрофотометрии содержание каротиноидов у целых взрослых особей обоих полов *Euphausia superba* а также некоторых частях тела как глаза, панцирь, корпус и конечности идентифицировано следующие каротиноиды: β — каротин, криптоксантин, свободный и эфирный астаксантин, кантаксантин, лютеин, флавоксантин, зеаксантин и неопределённый вероятно двугидрокси-ζ-каротин (таблица I и II).

На оснований полученных результатов исследований констатировано, что взрослые особи женского пола исследованные в целом а также некоторые их части тела, особенно глаза и конечности содержат значительно больше каротиноидов в сравнении с мужскими особями, особенно зрелыми.

Среди исследованных частей тела зрелых особей обоих полов крыля доказано, что глаза и конечности содержат значительно больше каротиноидов в сравнении с корпусом и панцирем.

6. Streszczenie

Badając za pomocą metod adsorpcyjnej chromatografii kolumnowej i cienkowarstwowej oraz spektrofotometrii zawartość karotenoidów w całych osobnikach dorosłych obu płci *Euphausia superba* oraz w niektórych częściach ciała jak oczy, pancerz, korpus i odnóża, zidentyfikowano następujące karotenoidy: β-karoten, β-kryptoksantynę, wolną i estrową astaksantynę, kantaksantynę, luteinę, flawoksantynę, zeaksantynę i dokładnie nieokreślony, prawdopodobnie dwuhydroksy-ζ-karoten (tabela I i II).

Na podstawie uzyskanych wyników badań stwierdzono, że osobniki dorosłe płci żeńskiej badane w całości oraz niektóre ich części ciała, zwłaszcza oczy i odnóża są znacznie bogatsze w karotenoidy w porównaniu z osobnikami męskimi, zwłaszcza dojrzałymi płciowo.

Spośród badanych części ciała u osobników dorosłych obu płci kryla wykazano, że oczy i odnóża są znacznie bogatsze w karotenoidy w porównaniu z pancerzem i korpusem.

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Paper received 23 October 1979

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