

## **Carotenoids in *Niphargus casimiriensis* Skalski (Amphipoda) from Artesian Wells**

by

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Carotenoids are some of the commonest pigments found in nature. They are produced only by plants, both autotrophic and heterotrophic, whereas animals obtain them through the food they eat. These pigments may, however, in the latter undergo further conversion as a result, for example, of oxidation.

Various functions have been ascribed to carotenoids ranging from their participation in the photosynthetic process in plants to a role played by these pigments in protecting fruit and invertebrate animals, plankton crustaceans in particular, from the effect and photooxidation of light rays.

With the latter function in view, we were interested in the question as to whether carotenoids were present in crustaceans inhabiting subterranean waters where, naturally, the light factor does not play the important role it plays in water exposed to light. In order to study the problem, the carotenoid content of *Niphargus casimiriensis* Skalski, a species which is to be found in Poland in the artesian wells of Lower Kazimierz on Vistula, was investigated (Skalski, 1976).

### **MATERIAL AND METHODS**

The *Niphargus casimiriensis* specimens were collected from an artesian well in Lower Kazimierz on Vistula. On being removed from the well, the crustaceans were washed thoroughly in distilled water, dried on tissue paper, placed in bottles of dark glass and then covered with acetone. The material was then kept in a refrigerator until removed for the separation of the pigments. Columnar and thin-layer chromatographic methods were used for separation of the carotenoids.

Column and thin-layer chromatography were used in the investigations, the method employed was as described in a previous paper (Czeczuga, 1971).

Separation of the carotenoids was begun, after preliminary analysis, by

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means of columnar chromatography: activated aluminium oxide ( $\text{Al}_2\text{O}_3$ ) of Polish production (Gliwice), was used as absorbent.

For the experiments, a glass column, 1 cm in diameter and 15 to 20 cm in length, was used. The carotenoid extract was transferred to the column with  $\text{Al}_2\text{O}_3$ , moistened previously with pure petroleum ether at boiling point ( $60^\circ$  to  $80^\circ\text{C}$ ).

Hydrolysis of the ester compounds of the carotenoids was performed with 10% potassium hydroxide in methanol, under nitrogen in the dark, at room temperature ( $18^\circ\text{C}$ ) for 12 h.

Thin-layer chromatography on silica gel (6 plates) was also used to separate and identify the carotenoid pigments (Merck production) according to Stahl. The carotenoids were saponified with 10% KOH in methanol before separation. A Beckman spectrophotometer, model 2400 DU, and a Specol spectrophotometer were used for maximum absorption determinations.

The pigments were identified by the following methods:

- behaviour on column chromatography;
- absorption spectrum;
- comparison of  $R_f$  values by means of thin-layer chromatography;
- partition coefficient (Petracek and Zechmeister, 1956; Foppen, 1971).

Quantitative determinations of the various carotenoids were made by the method of Davies (1965).

## RESULTS

The results of the chromatographic analysis are presented in table 1 from which it can be seen that in *Niphargus casimiriensis*, derivatives of the carotenoids,  $\alpha$ -carotene (lutein-5,6-epoxide),  $\beta$ -carotene (phoenicoxanthin, isozeaxanthin, zeaxanthin, astaxanthin) and  $\gamma$ -carotene ( $\gamma$ -carotene-derivative celaxanthin-derivative) were found. The highest percentage of carotenoids consisted of  $\beta$ -carotene derivatives, zeaxanthin (35.8%) and astaxanthin (29.4%). For comparison, a chromatographic analysis of *Gammarus lacustris* G. O. Sars specimens was made (Table 2). In these specimens only  $\alpha$ -cryptoxanthin (4.2%) and lutein-5,6-epoxide (43.3%) as derivatives of  $\alpha$ -carotene were found and the derivatives of  $\beta$ -carotene, cryptoxanthin (40.8%) and astaxanthin (5.7%).

## DISCUSSION

The studies made in the nineteen-forties on the carotenoid pigments in invertebrates (Beatty, 1949) did not reveal the presence of carotenoids in crustaceans of the genus *Niphargus*. As a result, these findings, unconfirmed by other workers, have become part of the literature on the subject and are not infrequently cited in monographic works (Goodwin, 1960).

The carotenoids found by means of chromatographic analysis to be present in the *Niphargus casimiriensis* specimens have often been observed in other

Table 1. *Niphargus casimiriensis* Skalski, column chromatogram of carotenoids

No. of fraction	System of solvents	Maximum absorption in nm	Solvent	Partition ratio	Identification	Amount (in %)
I	2% acetone in petroleum ether	462	petroleum ether	29 : 71	phoenicoxanthin	9.7
II	10% acetone in petroleum ether	448, 472, 495	petroleum ether	20 : 80	$\beta$ -carotene-derivative	4.7
III	30% acetone in petroleum ether	451, 478	ethanol	22 : 78	isorenanthin	3.8
IV	50% acetone in petroleum ether	422, 471	hexane	20 : 80	lutein-5,6-epoxide	4.8
V	methanol (100%)	458, 490, 518	ethanol	15 : 85	zeaxanthin-derivative	11.8
VI	acetone-methanol-cold acetic acid (9:1:0.1)	426, 450, 478	hexane	11 : 89	zeaxanthin	35.8
VII	15% KOH in 90% methanol	485	benzene	10 : 90	astaxanthin	29.4

Table 2. *Gammarus lacustris* G. O. Sars, column chromatogram of carotenoids

No. of fraction	System of solvents	Maximum absorption in nm	Solvent	Partition ratio	Identification	Amount (in %)
I	2% acetone in petroleum ether	420, 448, 478	petroleum ether	73 : 27	$\alpha$ -cryptoxanthin	4.2
II	10% acetone in petroleum ether	425, 452, 480	hexane	86 : 14	cryptoxanthin	40.8
III	50% acetone in petroleum ether	422, 471	hexane	20 : 80	lutein-5,6-epoxide	43.3
IV	10% KOH in 90% methanol	485	benzene	10 : 90	astaxanthin	5.7

crustacean species. Phoenicoxanthin was first found in cirriped crustaceans by Herring (1971). This carotenoid was later detected in the crab *Talipes mutillii* (Fox, 1973), in the crayfish *Orconectes limosus* (Czczuga, 1976), and in the hermit crab *Eupagurus prideauxi*, from the fishing-grounds of West Africa (Czczuga and Klyszejko, 1976). An interesting finding was that of a  $\gamma$ -carotene derivative in *Niphargus casimiriensis*. More than once, we have been able to reveal the presence of  $\gamma$ -carotene in crustaceans (Czczuga, 1974) and the xanthophyll celaxanthin was found in *Niphargus tatrensis* and *Niphargus aquilex schellenbergi* collected from springs (Czczuga and Skalski, 1973). The remaining carotenoids lutein-5,6-epoxide, zeaxanthin, isozeaxanthin and astaxanthin belong to the so-called common carotenoids quite frequently found in crustaceans. Some of them were also found in the *Gammarus lacustris* specimens investigated.

Our previous investigations on carotenoids in species of the genus *Niphargus* (Czczuga and Skalski, 1973) were carried out on specimens which lived in springs whereas, as we know, Beatty (1949) obtained his data from studies on *Niphargus* species inhabiting underground water. For this reason, in our previous paper (Czczuga and Skalski, 1973) we suggested that the absence of carotenoids reported by Beatty in those species may be due to their subterranean habitat. As was stated in the description of Methods in the present paper, our *Niphargus casimiriensis* specimens were taken from a deep well where the ecological conditions were very similar to those of waters in underground caves. In view of the fact that these specimens were found to contain carotenoids, our previous suggestion would hardly seem tenable and it appears that the crustaceans of the genus *Niphargus* should be regarded as the same as all the other crustacean species which, despite taxonomic and environmental differences, contain some or other of the carotenoids. The results of the chromatographic analysis of the carotenoid content of various crustacean species from different ecological niches substantiates this. Carotenoids have been found in all the aquatic crustaceans, both fresh-water and marine species (Czczuga, 1975), studied to date. Investigations on the land crustacean, the *Oniscus asellus* (Czczuga, 1975), and the sand crab *Ocypoda cursor* (Czczuga, 1977) from the sandy African coast of the Mediterranean Sea revealed the presence of these pigments. The same results were obtained on analysing the carotenoid content of crustacean parasites of the fish *Argulus foliaceus* (Czczuga, 1971) or *Ergasilus sieboldi* (Czczuga, 1977). In our opinion the ecological niches inhabited by the various crustaceans affect only the composition of the various carotenoids and their quantitative relations.

In conclusion, it should be stated that in considering the carotenoid content of the crustaceans of the genus *Niphargus* the problem should be approached in the same way as for other crustacean species, in which only the composition and quantitative relations of carotenoids may be changed.

## SUMMARY

By means of columnar and thin-layer chromatography, the presence of carotenoids in *Niphargus casimiriensis* Skalski from an artesian well was studied.

There are qualitative and quantitative differences in the carotenoid contents of the *Niphargus casimiriensis* Skalski specimens.

## RÉSUMÉ

Les caroténoïdes présents chez *Niphargus casimiriensis* Skalski récolté dans un puits artésien ont été étudiés par chromatographie sur colonne et sur couche mince.

Des différences tant qualitatives que quantitatives apparaissent dans la teneur en caroténoïdes des individus de l'espèce *Niphargus casimiriensis* Skalski.

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