

**Astaxanthin,
canthaxanthin and
astaxanthin esters in the
copepod *Acartia bifilosa*
(Copepoda, Calanoida)
during ontogenetic
development**

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KEYWORDS

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Abstract

The contents of astaxanthin, canthaxanthin and astaxanthin esters were studied in natural populations of the copepod *Acartia bifilosa* from the Pomeranian Bay and Gulf of Gdańsk in the southern Baltic Sea. Samples dominated by any one of three developmental groups: (1) nauplii, (2) copepodids I–III and (3) copepodids IV–V and adults of *Acartia bifilosa* were analysed by means of high performance liquid chromatography (HPLC). As ontogenetic development progressed, significant changes occurred in the proportion of particular pigments in the total pigment pool of the various developmental groups. Astaxanthin and canthaxanthin occurred in all the groups, the former being clearly dominant. However, an increasing percentage of astaxanthin esters was recorded in the copepodids I–III, and even more in the copepodids IV–V and adults group. Most probably, astaxanthin is the main pigment active in copepod lipid metabolism. Carotenoid pigments in copepods very likely act as efficient free-electron quenchers and may be involved as antioxidants in rapid lipid metabolism. The exogenously feeding stages (late nauplii and copepodids) transform plant carotenoids taken from food and are evidently

capable of metabolising astaxanthin by esterification and further degradation. It is emphasised that, according to literature data, astaxanthin esters may have an even higher quenching ability. It is suggested that crustacean carotenoid pigments, with their electron donor-acceptor abilities, may replace oxygen in peroxidation processes connected with lipid metabolism. The consequences of such a physiological role of astaxanthin for present-day estimations of energy balances in zooplankton communities are mentioned.

1. Introduction

Like all animals, the herbivorous Copepoda cannot synthesise the carotenoid pigments commonly found during biochemical assays of their tissues. They are only capable of metabolically transforming the β -carotene present in ingested plant food into their own principal keto-carotenoid – astaxanthin, via the intermediary pigments echinenone and canthaxanthin (Goodwin 1971, Simpson & Chichester 1981). The distinct coloration of most copepod species is due largely to astaxanthin, which occurs in their bodies in a free state, either dissolved in the lipid fraction, or bound to proteins to form carotenoproteins (Cheesman et al. 1967, Czczuga 1981, Bernhard 1989, Zagalsky et al. 1990). While the presence of these pigments has been recorded in many species of Crustacea, including most planktonic Copepoda, their importance in zooplankton ecology and physiology is still the subject of biochemical analysis and independent environmental and behavioural studies. Most published studies on carotenoid pigmentary composition in Crustacea have been performed on adult Decapoda: lobsters, shrimps and euphausiids (Goodwin 1971, Juhl et al. 1996).

Carotenoid contents and composition in herbivorous Copepoda have been investigated mainly with respect to the phytoplanktonic diet of copepods (Fronczak & Styczyńska-Jurewicz 1985, Meyer-Harms & von Bodungen 1997, Irigoien et al. 1998, Meyer-Harms et al. 1999) or in relation to vertical distribution (Herring 1972) and diel variation (Ringelberg & Hallegraeff 1976, Hairston 1980). Murugan et al. (1995) studied the occurrence of all cis- and trans- isomers of canthaxanthin in *Artemia salina* eggs and nauplii. As far as we know, no published data exist on carotenoid pigments in relation to the separate developmental stages of Copepoda, which differ not only in their morphology but also in their ecology and physiology. The aim of the present paper was therefore to determine qualitatively and quantitatively the composition of the principal carotenoid pool in the three developmental groups: nauplii, copepodids I–III and copepodids IV–V and adults of *Acartia bifilosa*.

2. Material and methods

The analyses of carotenoid contents in particular ontogenetic groups in the copepods were performed step by step from 1994 to 1999 by the successive processing of the zooplankton samples collected in various seasons from different depth layers at 4 sampling stations: 1 (54°00'N, 14°20'E) and 3 (54°10'N, 14°40'E) in the Pomeranian Bay, and 89 (54°30'N, 18°59'E) and 92 (54°29'N, 18°40'E) in the Gulf of Gdańsk.

The mesozooplankton of the Baltic Sea is never monospecific; the well-known succession allows for quite a precise separation of the dominant species and, if necessary, selected developmental stages, as well as a satisfactory description of their quantitative and qualitative pigmentary pattern.

The copepods were harvested by means of a standard 200 μm mesh WP2 closing net, after which they were stored for a few days in the dark at -20°C . Copepod samples in which a given developmental stage/group of *A. bifilosa* was distinctly prevalent ($> 80\%$ in a population) were then selected. Consisting only of about 10% of the total number of copepods harvested, these samples were then split into three sets: one with dominant naupliar stages I–VI (developmental group 1), another with prevalent copepodids I–III (group 2), and a third with dominant IV–V copepodids and adults. Samples in which only one naupliar or copepodid stage was dominant were rejected.

The HPLC procedure has been already applied in an earlier study on carotenoids occurring in marine bottom sediments (Łotocka 1998).

All data are reported as mean \pm SE. Differences between means have been analysed using Anova, Student's t-test.

3. Results

The total concentration of the main carotenoid pigments in successive developmental groups of *A. bifilosa* increased gradually during development (Fig. 1). The average concentrations were $563.1 \mu\text{g g}^{-1}$ dry wt. in the naupliar stages, $703.4 \mu\text{g g}^{-1}$ dry wt. in copepodids I–III, $780.8 \mu\text{g g}^{-1}$ dry wt. in copepodids IV–V and adults in the Gulf of Gdańsk; the respective figures for the Pomeranian Bay are: 610.1, 771 and $830.3 \mu\text{g g}^{-1}$ dry wt.

The copepods' own pigments found in this species were astaxanthin, astaxanthin mono- and di- esters, and canthaxanthin. The residual carotenoid pool, referred to here as 'remaining carotenoids', which has been excluded from present considerations, consisted of pigments originating from ingested food and occurring in copepod gut contents in the initial stages of plant food decomposition. The small amounts of echinenone and lutein, sometimes

recorded in the gut contents as well as in the copepod tissues, probably represented an initial stage of assimilation leading to further metabolic transformation of ingested algal carotenoids.

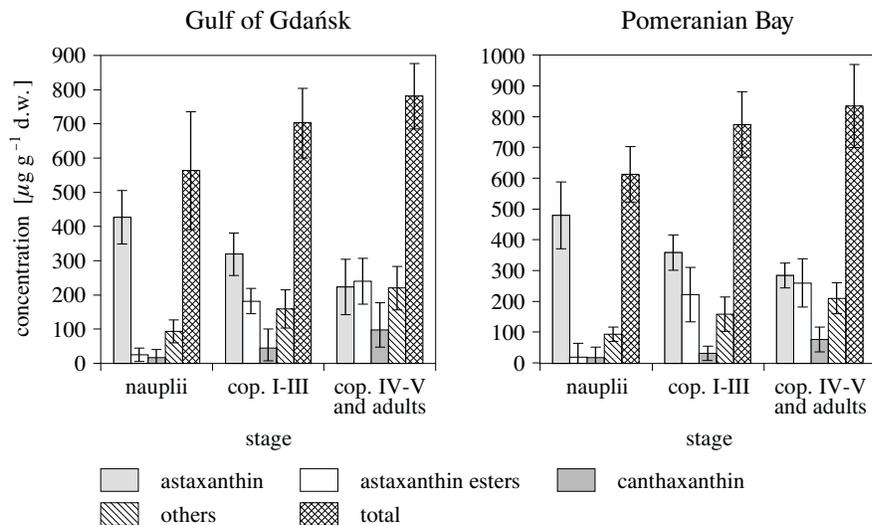


Fig. 1. Carotenoid concentrations of the same copepod stages of *Acartia bifilosa* from the Gulf of Gdańsk and Pomeranian Bay

In the naupliar group of *A. bifilosa* from the Gulf of Gdańsk the dominant pigment was astaxanthin with a mean concentration of $427.4 \mu\text{g g}^{-1}$ dry wt. (in the Pomeranian Bay – $477.4 \mu\text{g g}^{-1}$ dry wt.). The content of astaxanthin esters in the copepodid I–III group is already higher than in the younger stages. This tendency for the ester content to increase was also maintained in the copepodid IV–V and adult group from the Gulf of Gdańsk: the mean concentration of astaxanthin esters was $239.5 \mu\text{g g}^{-1}$ dry wt. ($259.4 \mu\text{g g}^{-1}$ dry wt. in the Pomeranian Bay).

The corresponding percentage of separate pigments found in samples from the Pomeranian Bay and Gulf of Gdańsk maintained the above tendency, which is shown in Fig. 2. It is also evident that concentrations of astaxanthin and astaxanthin esters, expressed as percentages of the total carotenoid pigment concentrations recorded in *A. bifilosa*, are very close in both basins. The percentage of astaxanthin in the naupliar pool from the Pomeranian Bay (78.2%) decreased significantly to 34.1% in the older, copepodid IV–V and adult group (Anova, $p < 0.05$). Simultaneously, the percentage of astaxanthin esters increased from 3.3% of the carotenoid pool present in the nauplii to as much as 31.2% in the IV–V copepodids and adults (Anova, $p < 0.05$).

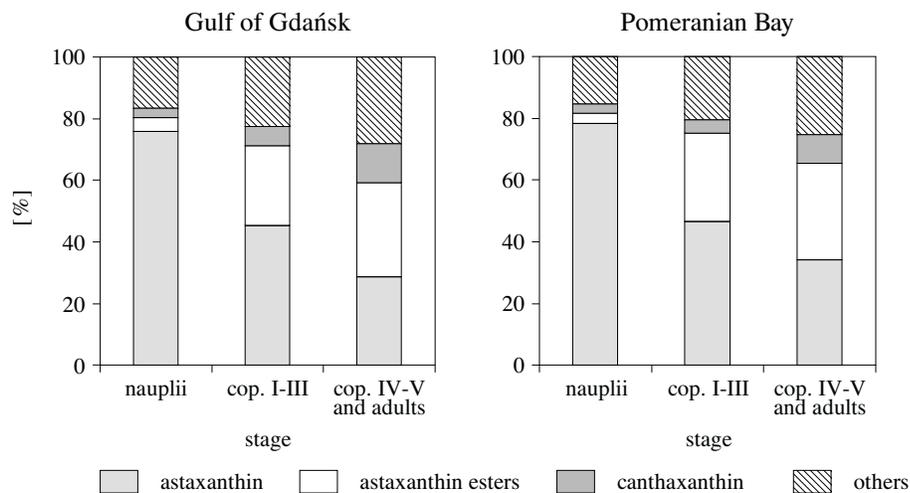


Fig. 2. Percentage of carotenoids during larval development of the *Acartia biflosa* in the Gulf of Gdańsk and Pomeranian Bay

Similarly, in the Gulf of Gdańsk, the decrease in astaxanthin concentration from 75.9% in nauplii to 28.6% in copepodids IV–V and adults correlated correspondingly with the significant increase in astaxanthin esters

Table 1. Comparisons between carotenoid concentrations of the same copepod stages of *Acartia biflosa* from samples taken at sites 1, 3 in the Pomeranian Bay and 89, 92 in the Gulf of Gdańsk

<i>Acartia biflosa</i>			Gulf of Gdańsk																
			Nauplii					Copepodids I-III					Copepodids IV-V and adults						
			ast.	a.e.	canth.	others	total	ast.	a.e.	canth.	others	total	ast.	a.e.	canth.	others	total		
Pomeranian Bay	Nauplii	ast.	ns																
		a.e.		ns															
		canth.			ns														
		others				*													
		total					ns												
	Copepodids I-III	ast.					ns												
		a.e.						ns											
		canth.							ns										
		others								ns									
		total									ns								
	Copepodids IV-V and adults	ast.										ns							
		a.e.											ns						
canth.													*						
others														ns					
total																ns			

* Anova, $p < 0.05$; ns = not significant – there are no difference between means; ast. – astaxanthin; a.e. – astaxanthin esters; canth. – canthaxanthin; others – other carotenoids; total – total carotenoids.

from 4.5% in nauplii to 30.6% in the IV–V copepodids and adults (Anova, $p < 0.05$) (Fig. 2).

The mean concentrations of these carotenoids in *A. bifilosa* do not significantly differ between the samples from the Gulf of Gdańsk and the Pomeranian Bay, except for canthaxanthin in copepodids IV–V and adults and ‘other carotenoids’ in nauplii (Anova, $p < 0.1$) (Table 1).

4. Discussion

The dominance of astaxanthin and its esterification in older developmental stages of copepods suggests that it plays an important part in lipid metabolism and oxidation. The proportion of carotenoid pigments in peroxidation processes (Simpson & Chichester 1981, Mobarhan et al. 1990, Bast et al. 1991), their well-known role as quenchers of oxygen singlets (Krinsky & Deneke 1982, Di Mascio et al. 1989, Terao 1989, Di Mascio et al. 1991, Krinsky 1991, Shimidzu et al. 1996) and as lipid antioxidants (Bast et al. 1991, Zhang et al. 1991), may be of key importance in understanding the common occurrence of astaxanthin in the Crustacea and its evident indispensability, not only for external coloration (functioning as camouflage and/or a photoprotective agent), but for vision, luminescence, sexual maturation and egg production. According to Miki (1991), astaxanthin, with its quenching rate constant of one hundred, is a more efficient quencher than α -tocopherol. Perhaps, because of this higher quenching efficiency, astaxanthin was favoured during the evolution of Crustacea. Since this compound, like canthaxanthin in lower Crustacea, is capable of readily forming a number of carotenoproteins, particularly pigment-protein complexes (Cheesman et al. 1967, Zagalsky et al. 1967, Czczuga 1981, Findlay et al. 1990) of a very sophisticated molecular structure (Zagalsky et al. 1990, Keen et al. 1991), it may have been extremely important for the metabolic pathways in that taxonomic group.

Astaxanthin and canthaxanthin may also serve as auxiliary compounds – as endogenous scavengers acting during rapid combustion of vitelline energetic material. The latter is the first source utilized to support naupliar mobility. Changes in energetic states of carotenoid molecules are structurally labile capable of entering into excitation states and to form a set of isomers differing in energy load, (Nash 1969): cis-trans structural isomers and R–S optical enantiomers (Renstrom et al. 1981, Matsuno et al. 1984). These data allowed us to hypothesise such a donor-acceptor (Platt 1959, Dingle & Lucy 1965) and singlet oxygen scavenging role (Foote et al. 1970, Bast et al. 1991, Hoegh-Guldberg & Jones 1999) to be played by carotenoids in crustacean energy metabolism. Antioxidation in lipid metabolism, trapping the oxygen singlet, accumulating and dissipating free electrons between

chemical structures of various copepod tissues is probably a major task of astaxanthin and its esters in crustaceans. Able to vary their energetic state and chemical configuration with ease, these pigments are permanently present in copepods throughout their ontogenetic development. Playing that role they are also subjected to a successive structural evolution by esterification. The carotenoid pool remains in proportion to body mass, which has to some degree been evidenced in the present paper. An increase in carotenoid content with increasing body size had been noted earlier in wild marine zooplankton (Herring 1972, 1973), and indicates indispensability of these pigments for life processes.

The rapid combustion of the lipid store in the energetic metabolism of Copepoda, especially distinct in extremely active migrants such as *Calanus helgolandicus* was recorded microscopically by Petipa & Ostrovskaya (1987). That approach led Petipa to the controversial conclusion that the 24-hour fat utilisation in *C. helgolandicus* migrants might be as much as 12–34 times higher than the standard level. However, such a drastic increase, even in extremely active copepods, obviously contradicts the physiological capacity of copepod respiration and existing data on oxygen consumption measured in migrating marine zooplankton. In general migrating animals increase their oxygen consumption by no more than 2–3 times in relation to the standard level (Torres et al. 1982, Klekowski & Sazhina 1985, Torres & Childress 1985). Nevertheless, Petipa's basically misleading conclusion on the one hand, but her correct data on lipid utilization in migrating copepods on the other is of importance for our own hypothesis. They yield another explanation: during the rapid combustion of lipid material in migrants there probably occurs a physiological 'replacement' of the oxygen molecule in the function it plays as electron acceptor. That role may be fulfilled in great part by carotenoid molecules of free astaxanthin, astaxanthin esters or astaxanthin-binding carotenoproteins. These, like oxygen, may serve as acceptors for free electrons released during constrained rapid peroxidation and the physiological utilisation of stored energy – the lipid material. According to existing analytical data, the material combusted by zooplankton organisms consists mostly of PUFA (polyunsaturated fatty acids), which may have originated directly from assimilated algal food rich in these compounds, or indirectly from the wax ester store. Wax esters are the dominant form of lipid accumulation in crustacean zooplankton (Sargent & Falk-Petersen 1988, Sargent 2000). As a result of the presence of carotenoid acceptors, which are structurally and energetically labile, the respired oxygen, which diffuses rather slowly into the copepod's haemolymph and tissues, could be used primarily for the conversion of wax esters back to PUFA. The astaxanthin acting as a trap for oxygen singlets

and/or harmful forms of energy (Conn et al. 1991), may thus be converted to esters, also highly antioxidant molecules, and later on – successively degraded. According to the experimental *in vitro* studies referred to in Kobayashi & Sakamoto (1999), astaxanthin esters function as powerful antioxidants under both hydrophobic and hydrophilic conditions. In any case, the close relationship of this pigment to the lipid store (that link is also spatially expressed – see the astaxanthin accumulation in the copepod fat organ) and lipid metabolism may explain its essential indispensability for the life of planktonic copepods. Our hypothesis may, however, evoke a new approach to all estimations of energy balances based exclusively on oxygen consumption data in zooplankton communities.

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