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Astaxanthin production in marine pelagic copepods grazing on two different phytoplankton diets

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Abstract

The red carotenoid astaxanthin is a powerful natural antioxidant of great importance in aquatic food webs where it is abundant in eggs and body tissues of fish and crustaceans. Little is known about the impact of the phytoplankton diet on astaxanthin production in copepods, its major pelagic producers. We followed the transfer of carotenoids from phytoplankton to copepods in a mesocosm experiment on the northern Atlantic coast (Norway) and recorded the astaxanthin production in copepods. Wild copepods grazed on nutrient-manipulated phytoplankton blooms, which differed in community composition and nutrient status (nitrogen or silicate limitation). The copepod pigments consisted mainly of free astaxanthin and mono- and diesters of astaxanthin. We found no significant difference in astaxanthin production per copepod individual or per unit C depending on the phytoplankton community. However, in the mesocosms astaxanthin per unit C decreased compared with natural levels, probably through a lower demand for photoprotection by the copepods in the dense phytoplankton blooms. The total astaxanthin production per litre was higher in the silicate-limited mesocosms through increased copepod density. Pigment ratio comparisons suggested that the copepod diet here consisted more of diatoms than in the nitrogen-limited mesocosms. Silicate-saturated diatoms were less grazed, possibly because they could invest more in defence mechanisms against their predators. Our study suggests that the production of astaxanthin in aquatic systems can be affected by changes in nutrient dynamics mediated by phytoplankton community composition and copepod population growth. This bottom-up force may have implications for antioxidant protection at higher trophic levels in the food web.

Keywords: Food web; Copepod diet; Carotenoid; Antioxidant; Eutrophication; Mesocosm experiment

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1. Introduction

Shifts in nutrient availability can cause fundamental changes in community structure and composition of primary producers in marine environments. This has been reported for many aquatic systems worldwide (Smith et al., 1999). Such changes are expected

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to have profound bottom-up effects on trophic relationships, which may go beyond the primary producer level and affect higher trophic levels such as fish and mammals. Trophic relationships include the transfer of important trace compounds such as vitamins and antioxidants. For example, thiamine (vitamin B_1) and astaxanthin deficiencies are involved in the M74 syndrome (Amcoff et al., 1998; Petterson and Lignell, 1998), a reproductive disorder in Baltic populations of the Atlantic salmon (*Salmo salar* L.), which is thought to be related to large-scale changes in the ecosystem, including eutrophication (Bengtsson et al., 1999; Hansson et al., 2001). In the present study we focus on the effects of nitrogen and silicate limitation on the production of the carotenoid astaxanthin in the food web.

The red pigment astaxanthin is one of the strongest antioxidants in nature (Edge et al., 1997) and abundant in many marine animals such as salmonids and crustaceans (Matsuno, 1989). β-carotene obtained from phytoplankton is considered to be the main precursor for astaxanthin synthesis in zooplankton, via other carotenoids (Kleppel et al., 1985; Matsuno, 2001). Recently, two pathways of synthesis from β-carotene to astaxanthin were suggested to occur in the green microalga *Haematococcus* sp.: (1) via echinenone, canthaxanthin and adonirubin, (2) via cryptoxanthin, zeaxanthin and adonixanthin (Harker and Young, 1995; Boussiba, 2000). In marine pelagic food webs, copepods are the major producers of astaxanthin. The most important function of astaxanthin in copepods is that of an antioxidant for protecting lipids from peroxidation (Bandaranayake and Gentien, 1982; Terao, 1989). Secondly, pigmentation and thereby photoprotection against PAR (Hairston, 1979, 1980) and UV light (Hansson, 2000) has been suggested. According to Ringelberg (1981), a third function could be that astaxanthin esters, since they are lipids, serve as sources of metabolic energy, even if they contribute to only ca. 2% of the total lipid content of a copepod body (Hairston, 1980).

The aim of our study was to examine effects of phytoplankton diet on the production of astaxanthin in pelagic copepods on the northern Atlantic coast (Norway). A mesocosm experiment was carried out, in which wild copepods grazed on nutrient-manipulated phytoplankton communities which differed in community composition and nutrient status (nitrogen and silicate limitation). The present paper focuses on pigment dynamics and astaxanthin production, whereas species composition and carbon and nitrogen dynamics of the same experiment are presented in an earlier paper (Van Nieuwerburgh et al., 2004).

2. Material and methods

2.1. Experimental set-up

A mesocosm experiment was performed in a natural lagoon (Hopavågen, 63° 40′ N, 09° 39′ E) close to the mouth of the Trondhjem fjord (Norwegian Sea) from 29 August to 9 September 1999. The six mesocosms consisted of white polyethylene enclosures with a diameter of 1.0 m, a water-filled depth of 2.5 m and a volume of ca. 2000 l. The enclosures were, with random placement, submerged in the water and attached to rubber rings on a floating raft. Natural seawater was pumped into the enclosures through a 90 μm-mesh plankton net, thus excluding larger plankton.

The six mesocosms received two different nutrient treatments in triplicate: three mesocosms were enriched with N and P (NP treatment) and three mesocosms were enriched with N, P and Si (NPSi treatment). The nutrient additions followed the Redfield ratio of N:P (16:1 on a molar basis) and three times the optimal ratio (Si:N = 3:1) for diatoms to ensure excess Si (Redfield et al., 1963). Every morning of Days 1–8, we added 4.00 μM $NaNO_3$ and 0.25 μM NaH_2PO_4 for the NP treatment and 12.00 μM Na_2SiO_3, 4.00 μM $NaNO_3$ and 0.25 μM NaH_2PO_4 for the NPSi treatment. Bicarbonate (to avoid carbon limitation), in the form of 28 μM $NaHCO_3$ following the Redfield ratio C:N:P = 106:16:1, as well as trace metals, chelators and vitamins [in 0.5% of f/2 medium concentrations (Guillard and Ryther, 1962), but excluding thiamine] were added to all mesocosms on Days 1–8. No nutrients were added on Days 9–12 to study possible effects of decaying algal blooms on copepod pigmentation. The nutrient treatments resulted in complete Si-limitation in the NP treatment after Day 2 and complete N-limitation in the NPSi treatment after Day 4, while P was not limiting throughout the experiment in any of the treatments (Van Nieuwerburgh et al., 2004).