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Astaxanthin dynamics in Baltic Sea mesozooplankton communities

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ABSTRACT

The red pigment astaxanthin is a powerful antioxidant, which occurs in eggs and body tissues of crustaceans and fish. It is produced by crustaceans from algal carotenoids. In a two-year field study we assessed natural concentrations and dynamics of astaxanthin in mesozooplankton communities in the brackish Baltic Sea area. Astaxanthin levels varied between 0.37 and 36 ng L^{-1}. They increased with salinity along the Baltic Sea gradient and were linked to zooplankton biomass and phytoplankton community composition. Astaxanthin concentrations showed typical seasonal patterns and varied from 0.2 to 5.1 ng ind^{-1}, 0.2 to 3.4 ng $(\mu g\ C)^{-1}$ and 6 to 100 ng mm^{-3}. These concentrations were inversely related to water temperature and strongly linked to zooplankton community composition. Communities dominated by the calanoid copepods *Temora longicornis*, *Pseudocalanus acuspes* and *Eurytemora* spp. generally held the highest concentrations. With increasing cladocerans:copepods biomass ratios community astaxanthin concentrations decreased and with higher relative biomass of *Acartia* spp. the proportion of astaxanthin diesters decreased. Diesters prevailed in the cold season and they are thought to improve the antioxidant protection of storage lipids during winter. Climate change causes higher temperature and lower salinity in the Baltic Sea proper. This modifies zooplankton community composition, but not necessarily into a community with lower concentrations of astaxanthin since *T. longicornis* (high concentrations) has been reported to increase with higher temperature. However, decreased astaxanthin production in the ecosystem is expected if a basin-wide increase in the cladocerans:copepods biomass ratios would occur with further climate change.

1. Introduction

The carotenoid astaxanthin is a major component of the antioxidant defence system in many aquatic animals (Miki, 1991). In aquatic systems, most astaxanthin is synthesised by crustaceans from carotenoid precursors, especially β-carotene and zeaxanthin, provided by algae (Matsuno, 2001). Fish cannot produce astaxanthin themselves and need to acquire it through their diet. Thus, crustaceans are the primary astaxanthin source for fish. The fat-soluble astaxanthin molecule has two hydroxyl groups, one at each end, which can bind to the carboxyl group of fatty acids by esterification. Therefore, astaxanthin may be present in its free form or as mono- or diesters of fatty acids (Breithaupt, 2004). Astaxanthin can also bind to proteins whereby blue-purple carotenoproteins are formed (Britton et al., 1997).

Besides its antioxidant role in cellular metabolism, astaxanthin is also known to act as a precursor for vitamin A (Liñán-Cabello et al., 2002) and as a photoprotector (Hairston, 1981; Hansson, 2000) in zooplankton. In Arctic freshwater lakes the astaxanthin body content, which makes the zooplankton more conspicuous while grazing in the upper water column, is a trade-off between photoprotection against ultraviolet radiation and the threat of predation (Hansson, 2000, 2004; Hays et al., 2001). However, photoprotection is probably not a major function in all environments. For example, in the temperate and more turbid North Sea, no correlations were found between the astaxanthin concentrations in *Calanus helgolandicus* and its vertical distribution in the water column or chlorophyll *a* concentrations (Sommer et al., 2006).

Increased incidence of yolk-sac fry mortality of Baltic populations of the Atlantic salmon (*Salmo salar* L.), known as the "M74 syndrome", has been observed since 1974 (Mikkonen et al., 2011). The M74 syndrome has been found to be linked with low astaxanthin levels, thiamine deficiency, imbalance in fatty acid composition and oxidative stress (Pettersson and Lignell, 1999; Pickova et al., 1999; Vuori and Nikinmaa, 2007). These observations have drawn attention to the role of the diet of the salmon, the two clupeids Baltic herring (*Clupea harengus membras*) and Baltic sprat (*Sprattus sprattus balticus*) (Keinänen et al., 2012; Nie et al., 2011). However, little is known about the astaxanthin availability in the zooplankton, which are key organisms for the flow of matter and energy in the pelagic zone (Kiørboe, 1997). This is especially so in the case of astaxanthin because it is the zooplankton that synthesises it and transfers it toplanktivorous fish (clupeids), from where it is transferred to the piscivorous fish (salmon).

The zooplankton in the pelagic zone of the off-shore Baltic Sea proper is dominated by the calanoid copepods *Acartia bifilosa*, *Acartia longiremis*, *Acartia tonsa*, *Centropages hamatus*, *Eurytemora affinis*,

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Pseudocalanus acuspes and *Temora longicornis*, and in summer also by the cladocerans *Eubosmina* (previously *Bosmina*) *corregoni maritima*, *Evadne nordmanni* and *Podon* spp. (Viitasalo et al., 1995). Smaller herring and all size classes of sprat are strictly zooplanktivorous. Seasonally the highest feeding activity of herring and sprat occurs in spring and summer, the main reproductive periods of calanoid copepods. The most important food item for both fish species in spring is *P. acuspes*, but in summer sprat switches to *T. longicornis*, *Acartia* spp. and cladocerans (Möllmann et al., 2004). In autumn and winter both herring and sprat seem to avoid *Acartia* spp. (Casini et al., 2004; Möllmann and Köster, 2002). Larger herring are essentially nektobenthos feeders, predating on mysids during the autumn and amphipods and polychaetes during the winter (Casini et al., 2004).

The carotenoid content and composition (the abundance of astaxanthin esters) is known to vary between zooplankton species (Foss et al., 1987; Łotocka et al., 2004), with ontogeny (Funk and Hobson, 1991; Łotocka and Styczyńska-Jurewicz, 2001; Łotocka et al., 2004) and with diet (Andersson et al., 2003; Rhodes, 2007; Van Nieuwerburgh et al., 2005). However, these previous studies were based on single copepod species, laboratory experiments or in zooplankton communities during short-term experiments. The ecologically significant natural concentrations and dynamics of astaxanthin availability and esterification in the marine environment are poorly known. Year-round astaxanthin concentrations in natural communities have not been studied before.

In this study we concentrate on the mesozooplankton size fraction (0.2–20 mm), which includes the preferred food items for herring and sprat. The objectives were (1) to assess possible regional and seasonal variation of astaxanthin concentrations in natural zooplankton communities in the Baltic Sea area and to identify the factors that would cause such variation, (2) to assess possible patterns in esterification of astaxanthin in the natural zooplankton communities and (3) to discuss implications of the results obtained in this study for higher trophic levels in the changing Baltic Sea ecosystem. To achieve these objectives, bulk measurements of phyto- and zooplankton communities from 94 off-shore stations, sampled in four seasons and two years, were analysed regarding biomass, stoichiometry, zooplankton taxonomic composition, as well as phyto- and zooplankton pigment composition.

2. Materials and methods

2.1. Field sampling

Eighty-four stations in the Baltic Sea and 10 stations in the transition zone between the Baltic and North Seas (Öresund-Kattegat area) were sampled during eight offshore cruises in 2004 and 2005 (Fig. 1). The Baltic Sea samples were taken in March, May, August and November and in the Öresund and Kattegat samples were taken in May and August. Water depth at the 94 offshore stations was 59 ± 25 m (average ± SD). At each station, surface water from 5 to 7 m of water depth was collected with submerged pumps for the measurement of salinity, temperature and inorganic nutrients, and to examine the plankton community.

Plankton samples were taken of two size fractions: <100 μm (dominated by phytoplankton, hereafter called "phytoplankton fraction") and >200 μm (dominated by mesozooplankton, hereafter called "zooplankton fraction"). To obtain the phytoplankton fraction, six replicate samples of 10 L seawater each were poured through 100 μm mesh plankton net. To obtain the zooplankton fraction, six replicate samples of 326–3809 L seawater each (volume depending on the biomass) were pumped over 200 μm mesh plankton net. Pumping took 15–45 min depending on the zooplankton biomass in the sea and during this time the samples were shaded from direct sunlight to avoid UV-destruction of the pigments. The continuous water flow during pumping rinsed the samples from algae and debris. The organisms

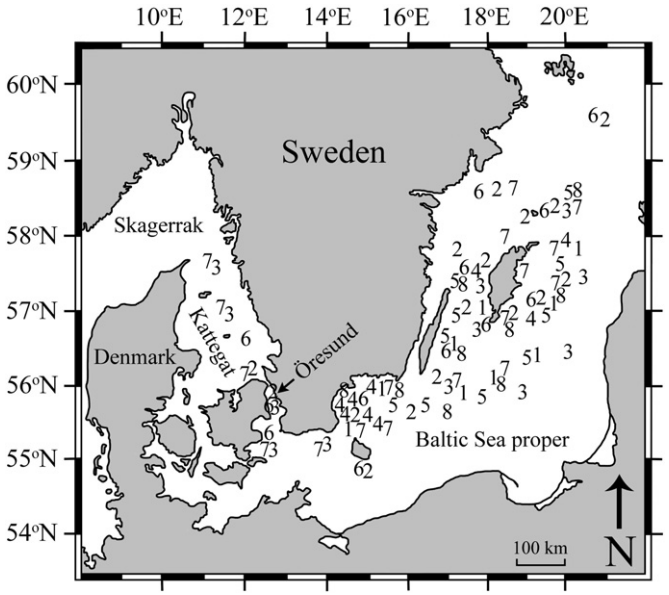

Fig. 1. Map of the Baltic Sea area, showing the 94 off-shore stations sampled during the eight expeditions, numbered 1–8 (1: 1–12 March 2004 with R/V Argos, 2: 7–16 May 2004 with R/V Fyrbyggaren, 3: 16–20 August 2004 with R/V Skagerak, 4: 15–25 November 2004 with R/V Argos, 5: 1–11 March 2005 with R/V Argos, 6: 15–25 May 2005 with R/V Fyrbyggaren, 7: 22–31 August 2005 with R/V Skagerak, and 8: 21 November–1 December 2005 with R/V Argos). Stations are indicated in the map by the number of the expedition (1–8).

caught on the net were mixed with ca. 600 mL of 0.7 μm-filtered seawater to obtain concentrated zooplankton samples. To be able to calculate the zooplankton density in the sea, the exact pumping time and speed, as well as the exact volume of the concentrated samples was noted.

For each of the six replicate samples of the phytoplankton fraction, six subsamples of 250–1500 mL (volume depending on the biomass) were collected on Whatman™ GF/F glass fibre filters. For each of the six replicate samples of the zooplankton fraction, four to six subsamples of the concentrated zooplankton samples, each containing organisms from 57 to 846 L (volume depending on the biomass) of the original seawater, were collected on Whatman™ GF/C glass fibre filters. Altogether, 36 filters with phytoplankton and 24–36 filters with zooplankton were collected for each of the 94 sampling stations. For each analysis reported in the present study three subsamples, each from a different replicate sample, were used. The rest of the filters were used for other analyses not reported here. All filters were packed in aluminium foil, quick-frozen in liquid nitrogen onboard and transferred to −80 °C storage upon return to land.

Six subsamples (50 mL) of the concentrated zooplankton samples, representing 6–85 L of the original seawater, were preserved with Lugol's acid iodine and kept in the dark at room temperature for taxonomic analysis of the zooplankton. For all stations, duplicate seawater samples (12 mL) for the analysis of inorganic nutrients were filtered through pre-washed 0.45 μm Millipore™ Millex-HA syringe filters and frozen at −20 °C.

2.2. Inorganic nutrients

Dissolved inorganic nitrogen (DIN: NO_2^- + NO_3^- + NH_4^+), dissolved inorganic phosphorus (DIP: PO_4^{3-}) and dissolved silica (DSi: SiO_2) in the 94 water samples were analysed in duplicate by segmented flow analysis using a Alpkem™ Flow Solution IV autoanalyser by the accredited nutrient laboratory at the Department of Ecology, Environment and Plant Sciences, Stockholm University.