Decreased astaxanthin at high feeding rates in the calanoid copepod Acartia bifilosa

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In marine food webs, copepods are the major producers of a carotenoid pigment astaxanthin, which is an important antioxidant. The availability of astaxanthin for higher trophic levels can be affected by changes in phytoplankton stocks and copepod feeding; however, the functional relationship between food availability and astaxanthin production is poorly understood. We hypothesized that with a given food type and quality, astaxanthin content in copepods is positively related to feeding and egg production rates. The hypothesis was tested by measuring astaxanthin accumulation in concert with ingestion and egg production rates in the copepod Acartia bifilosa exposed to different algal concentrations (Tetraselmis suecica; 0 to 1200 μg C L−1). Egg production and ingestion rates increased with increasing food availability and reached a plateau at ≥400–600 μg C L−1. In contrast, increasing accumulation of astaxanthin with increasing food availability was observed only at concentrations ≤150 μg C L−1. Contrary to our hypothesis, at 600–1200 μg C L−1 copepods had maximal ingestion and egg production rates, but low astaxanthin contents. It is suggested that this low accumulation of astaxanthin at high food concentrations results from a food-dependent decrease in assimilation efficiency. These findings are important for the understanding of astaxanthin dynamics within marine food webs, where increases in phytoplankton biomass may translate to a trade-off between zooplankton quantity and its nutritional quality for zooplanktivores.

INTRODUCTION

There is increasing interest in carotenoids as an important aspect of food quality in aquatic food webs (Pettersson and Lingell, 1999; Liñán-Cabello et al., 2002; Vuori and Nikinmaa, 2007). Carotenoids are important antioxidants and often exhibit other biological functions, such as regulatory effects on intra- and intercellular signalling and gene expression (Sies and Stahl, 2005). Astaxanthin is an abundant carotenoid in many marine organisms, notably salmonoids and invertebrates (Cheeseman et al., 1967; Higuera-Ciapara et al., 2006). Besides its role in body pigmentation, astaxanthin is a potent antioxidant and has also been suggested to function as a vitamin-A precursor (Schiedt et al., 1985; Liñán-Cabello et al., 2002).

With the exception of a few species rarely occurring in nature, astaxanthin is not produced by aquatic autotrophs. Instead, carotenoids produced by autotrophs (primarily β-carotene and to some extent zeaxanthin) are used by animals as precursors for astaxanthin synthesis (Katayama et al., 1973; Foss et al., 1987). Animals have a dietary requirement for carotenoids as they are not capable of carotenoid synthesis de novo. In shrimp and copepods, astaxanthin synthesis occurs soon after absorption of precursors from the gut, with little accumulation
of astaxanthin precursors in body tissues (Dall et al., 1995; Juhl et al., 1996). Once synthesized, the free astaxanthin can be esterified by attachment of one or two fatty acids into mono- or di-esters, respectively. In its free and esterified form, astaxanthin constitutes the majority of the carotenoid pigment in most crustacean zooplankton (Foss et al., 1987; Llínán-Cabello et al., 2002) and is the main pigment occurring in the vitellus of copepod eggs (Harrison, 1990). The consumption of precursors and subsequent synthesis of astaxanthin by herbivorous zooplankton thus represents an important entry point for astaxanthin within marine food webs. Herbivorous zooplankton are therefore an important link in the transfer of carotenoids to higher trophic levels.

A better understanding of how changes in the phytoplankton community might affect food webs and fisheries requires knowledge of changes in the quantity of food available for trophic transfer as well as its quality and consequences for fish physiology. For example, in the Baltic Sea, a decline in Baltic salmon stocks (Anonymous, 2004) has been suggested to be at least partially related to M74, an early mortality syndrome of maternal origin in which the fry experience extremely high mortality rates. Its occurrence is associated with low thiamine (Amcoff et al., 1998) and astaxanthin content (Pettersson and Lingell, 1999) in the eggs and fry of affected females. Recent studies of Baltic salmon have shown that thiamine deficiency is an indicator of prior oxidative stress, and suggest that the symptoms of M74 are the result of a long-term disturbance to the redox state of the fish (Vuori and Nikinmaa, 2007). Interestingly, a diet enriched with astaxanthin has been shown to increase defenses against oxidative stress in rainbow trout (Nakano et al., 1995), highlighting the importance of antioxidants, such as astaxanthin, in the fish diet. As herbivorous zooplankton are the major astaxanthin producers in the pelagic food web, a decrease in the astaxanthin content of zooplankton would represent a potential decrease in astaxanthin availability for fish.

Previous studies of calanoid copepods have demonstrated that tissue pigments are highly dynamic. Large decreases (20–30% in 4 h) and increases (∼50% in 24 h) in total astaxanthin content have been found in starving individuals and with the re-introduction of food to starved individuals, respectively (Juhl et al., 1996). Considering the dietary origin of astaxanthin precursors, it does not seem surprising that feeding copepods might contain more astaxanthin than starved copepods. The carotenoid composition of the diet has also been shown to be an important predictor of astaxanthin accumulation in one species of marine harpacticoid copepod (Rhodes, 2007). The interpretation of studies in which food concentration is present in varying amounts, however, is complicated by the lack of knowledge about the functional relationship between food availability and astaxanthin production. On the basis of the existing knowledge, we put forward a simple hypothesis that, with a given food type and quality, astaxanthin content in copepods is positively related to feeding, metabolic activity and egg production rates. Using Acartia bifilosa, a common Baltic calanoid copepod as our model species, we conducted a feeding experiment to test the hypothesized functional responses of astaxanthin. A gradient in ingestion and growth rates was created using different food concentrations, across which we measured the response in individual astaxanthin content concurrently with ingestion, respiration and egg production rates.

METHOD

Experimental organisms

Copepods were collected on 18 June 2007 in the Himmerfjärden Bay, Trosa archipelago, in the northwestern Baltic proper (58°59’N 17°44’E) using a 200 µm WP2 net. The contents of the tow were diluted with surface water, brought to the lab within a few hours and kept at 16°C with gentle aeration until sorting commenced. Adult Acartia bifilosa were sorted in Petri dishes filled with GF/F filtered seawater using wide-mouthed pipettes. The algal culture, Tetraselmis suecica (Prasinophyceae; CCMP908; The National Center for Culture of Marine Phytoplankton, USA) was grown exponentially in f/2 growth medium under constant illumination (90 µmol photon PAR m⁻² s⁻¹) at 16°C and 7‰ salinity in artificial seawater (ASW; Instant Ocean™, Aquarium Systems). Cultures were maintained in extended exponential growth through a semi-continuous harvesting regime (∼30% exchange every other day).

Experimental setup

In feeding experiments, 1.2 L bottles were prepared in triplicate with dilutions of algal culture to nominal concentrations of 0, 30, 150, 600 and 1200 µg C L⁻¹. Concentrated algal growth medium was added to each bottle, sufficient to prevent nutrient limitation of algal growth during incubations. Groups of ∼30 adult females and 1–5 males (to ensure fertilization) of A. bifilosa were placed in each bottle. In parallel, to determine individual dry weight (DW), three pooled samples of about 100 individuals were prepared in tin
capsules, dried at 60°C for 24 h and weighed using a Sartorius M3P microbalance to the nearest microgram. The copepods were acclimated to the experimental conditions and respective food concentrations for 22–24 h. All incubations were carried out on a rotating plankton wheel turning at a rate of 0.5 rpm in an environmental chamber at 16°C. Following acclimation, the animals were gently sieved through a submerged 200 µm sieve, a few dead individuals were removed and the live ones were transferred to the experimental bottles with freshly prepared algal suspension at the respective concentrations. For each food concentration, two bottles without copepods served as controls. All experimental bottles were then incubated for another 24 h, during which the concentration of algae in treatments with copepods decreased by as much as 20%.

**Ingestion, egg production, egg viability and respiration rates**

Upon termination of the feeding experiment, the contents of the bottles were gently poured through a submerged 200 µm sieve and the number and activity of the copepods were recorded. Only a few dead individuals were noted and removed. Active, healthy females (10 individuals from each of two replicates per treatment) were then retained for subsequent measurement of respiration rates in incubations without food, and the remaining live females were used for pigment measurements. The final algal concentrations (cells mL⁻¹) in the filtrate from the feeding incubations were determined using a laser particle counter Spectrex PC-2000 (Spectrex Corp., CA, USA) and converted to carbon equivalents using the regression log10C = 0.76 × log10V – 0.29 (Mullin et al., 1966), where C is carbon content (in pg cell⁻¹) and V is cell volume (in µm³) obtained from the particle counter. The ingestion rate (I, in µg C ind⁻¹ day⁻¹) was calculated according to Frost (Frost, 1972). Eggs were collected from each replicate on a pre-wetted 35 µm sieve, rinsed with GF/F filtered seawater into Petri dishes. Microscopes (Wild, Heerbrugg, ×50, or Leica DM, IRB, ×100) were used to count the eggs released in each replicate over the 24 h period of feeding. No cannibalism was assumed to occur as no empty eggshells were ever observed. The egg production rate (EPR, egg female⁻¹ day⁻¹) was calculated in each replicate as the number of eggs divided by the number of females. To assay egg viability, eggs were stained using fluorescence probes following the method of Buttino et al. (Buttino et al., 2004). In replicates with less than 40 eggs, all eggs were used for staining, whereas for replicates with more than 40 eggs, a random batch (40 ± 16 eggs) was used. The stained eggs were counted using an epifluorescent microscope with a blue filter (Leica DM, IRB, ×100 magnification).

Copepod respiration rates were measured immediately after the feeding experiment in vials filled with filtered seawater, as algae-free media was required to maintain stable conditions in the closed vials. Copepods (10 females from each of two replicates per treatment) were incubated in 5 mL enclosed vials immersed in a water bath (15°C). Controls (5 replicates) contained only filtered seawater and were monitored for 24 h just prior to treatments containing copepods to record the baseline. Oxygen concentration in the treatment vials was measured continuously over a 24 h period with point measurements every 5 min from each of 10 channels using fibre-optic sensors (PreSens). Respiration rate (R, µL O₂ ind⁻¹ day⁻¹) was determined by regression analysis as the rate of change in oxygen concentration over 1 h of measurements, beginning 1 h after the transfer to the respiration vials. Values from the first hour were disregarded as they were likely to have been affected by handling stress and temperature acclimation. Measurements from the second hour were used as these values were assumed to give the best representation of the respiration rates of copepods feeding at the respective food concentrations.

**Pigment analysis**

Live females were collected for pigment analysis from each replicate at two time periods: (i) immediately following the food treatments (48 h total duration) and (ii) on conclusion of subsequent respiration measurements (24 h without food). The difference in pigment between the two measurements was considered to be representative of the response to starvation. At each time period, live copepods were pooled by replicate and collected on small (0.3 × 0.3 cm) sections of GF/F filters (Whatman™, 2.3 µm) and placed immediately in −80°C storage. Replicate samples contained 17 ± 5.6 (mean ± SD) individuals after the first time period and 10 individuals in each replicate after the second. To confirm that the algae did not contain astaxanthin, the feeding suspension from a random subsample of replicates was filtered onto GF/F filters after removal of experimental animals. Extraction and analysis of the pigments by high-performance liquid chromatography (HPLC) was completed within 2 months of sampling. Pigments were extracted by sonication on ice in 100% methanol (Vibra Cell, pulse 0.5 s, amplitude 92, 1 min), then stored in the dark overnight at 4°C. After centrifugation at 8000g for 8 min at 4°C, the supernatant was collected. A second aliquot of methanol was used to rinse the remaining pigment from the pellet. The combined extract was then evaporated on ice under a nitrogen atmosphere. Pigments were re-suspended in a 400 µL solution of
100% methanol with a low concentration of β-apo-8-carotenal (Sigma™) as an internal standard. Pigment extract was filtered through 0.45 µm PTFE/PP filters (Titan™) and diluted to 80% with 0.5 mol L⁻¹ ammonium acetate buffer just prior to injection.

HPLC was carried out with an Agilent 1100 system, equipped with fluorescence and scanning (UV) diode array detectors. A method for separation of astaxanthin and its esters (Andersson et al., 2003) was modified to increase the sensitivity of the analysis. Aliquots of 100 µL extract/buffer mixture were injected onto a Gemini C18 (Phenomenex™) column (3 µm particle size, 30 × 3 mm, 110 A˚) at 0.3 mL min⁻¹ with the following solvent gradient timing [(time, A%, B%, C%), (0 min, 100, 0, 0), (2 min, 0, 100, 0), (19 min, 0, 20, 80), (21.5 min, 0, 20, 80), (24 min, 0, 100, 0), (26 min, 100, 0, 0)]. Chromatograms were integrated at 490 nm using Agilent TM ChemStation software (Rev. B.01).

Identification of canthaxanthin and unesterified astaxanthin peaks was confirmed by absorption spectra and calibration with synthetic canthaxanthin (Roth™) and astaxanthin (Sigma™), respectively. Extracts of Haematococcus pluvialis, a natural source of astaxanthin, were used to confirm the elution timing of astaxanthin esters; mono- and di-esters of astaxanthin were quantified using the response factor for unesterified astaxanthin. All reagents used were of HPLC grade.

Statistics and calculations
Non-parametric statistical methods were used, as sample sizes were inadequate to determine adherence of the data to normality. Comparisons among treatments were made using Mann–Whitney U and Kruskal–Wallis tests. The change in copepod astaxanthin content between incubations with and without food was assessed with a Wilcoxon signed-ranks test. Jonckheere–Terpstra tests were used to test for trends in respiration, pigment content and egg production rate with increasing food concentration. To determine if there were differences in egg viability, Fisher’s exact test was carried out using Stata Statistical Software (v.8, StatCorp 2003). With the exception of the Fisher’s test, statistics and figures were completed with SPSS (r15.0 SPSS Inc.).

RESULTS

Ingestion, egg production and viability, and respiration
Ingestion and egg production increased with increasing food availability (Fig. 1A,B). To estimate maximal

Fig. 1. Acartia bifilosa: (A) ingestion \(I = 4.55 \times (1 - e^{-0.008 \times \text{algal concentration}}); r = 0.91\), (B) egg production rate \(\text{EPR} = 7.87 \times (1 - e^{-0.005 \times \text{algal concentration}}); r = 0.90\) and egg viability (crosses) as a function of food availability; individual astaxanthin (free, circles; esterified, triangles) content after incubation in (C) food treatments and (D) subsequent seawater-only conditions. The average free astaxanthin in all treatments after the seawater-only incubations (dashed line) is shown on both plots of astaxanthin to provide a point of reference. Points indicate treatment means of triplicate measurements (unless the number of replicates is specified at the top of the figure); bars indicate the standard error of the mean.
ingestion, the data were fitted to the Holling Type II Model (Fig. 1A). Maximal ingestion was estimated as 4.4 μg C ind⁻¹ day⁻¹, which is equivalent to 3.44% of body C day⁻¹, calculated according to our measurements of individual DW (2.56 ± 0.24 μg DW ind⁻¹; mean ± SD) and assuming carbon was 50% of the DW.

Differences in egg production were highly significant when ordered according to food availability (P = 0.001, n = 13; Jonckheere–Terpstra test) and egg viability remained high in all treatments (85–100%; Fig. 1B), with no detectable difference among the treatments (P > 0.05, n = 15; Fisher’s exact test). Respiration rates in starved individuals were lower (1.80–1.97 μL O₂ ind⁻¹ day⁻¹) than in fed individuals (3.86–7.97 μL O₂ ind⁻¹ day⁻¹), with the difference between average R at 30 and 600 μg C L⁻¹ being ~25%. However, the increase in R with food concentration was not significant (P = 0.1, n = 10; Jonckheere–Terpstra test).

**Pigments**

On conclusion of the feeding experiment, there was a large range in total (i.e. free and esterified) astaxanthin content between the treatments (0.1–1.1 ng ind⁻¹). Individual astaxanthin content (Fig. 1C) increased with food availability in a trend that was significant in the lower range of food concentrations (0–150 μg C L⁻¹) for both free (P = 0.011, n = 9; Jonckheere–Terpstra test) and esterified (P = 0.005, n = 9) fractions. In contrast, the patterns of ingestion, egg production and respiration, however, astaxanthin accumulation decreased in the highest food treatments >2-fold relative to copepods at the next lowest food concentrations. Comparisons using Mann–Whitney U-tests indicated that within “high” (600 and 1200 μg C L⁻¹) and “low” (30 and 150 μg C L⁻¹) food treatments, concentrations of free astaxanthin were statistically indistinguishable (P < 0.028, n = 11). Similarly, astaxanthin mono-esters were significantly different between “high” and “low” food treatments (P = 0.011, n = 11), although concentrations in the “high” food group were relatively variable (P = 0.083, n = 5).

Following the incubations without food, there was an overall decline in the astaxanthin content of the copepods (Fig. 1D). Measurements taken after the algae-free incubations showed a significant overall decrease in free astaxanthin (P = 0.028, n = 6; Wilcoxon signed-ranks test) compared with measurements taken the previous day, after the feeding experiment (Fig. 1C). The largest decreases occurred in the treatments with initially low food availability (30 or 150 μg C L⁻¹), although the astaxanthin contents of these treatments were still slightly elevated relative to the average (dotted line, Fig. 1D). Copepods which were without algae during the feeding experiment showed a slight decrease in both free and esterified astaxanthin after an additional 24 h without food (Fig. 1D). In all treatments, except those initially incubated in 600 μg C L⁻¹ algae, astaxanthin mono-esters decreased in a similar pattern as free astaxanthin, maintaining proportions of mono-esters close to 45% of total astaxanthin. In contrast, the proportion of mono-esters in copepods initially incubated in 600 μg C L⁻¹ algae increased from 30% following food incubations to 90% during the subsequent incubation without food.

None of the algal samples contained detectable levels of astaxanthin or pigments that co-eluted with astaxanthin. Astaxanthin di-esters were not detected in any of the copepod samples; if di-esters were present below detection limits (0.01 ng ind⁻¹), they would have accounted for <5% total astaxanthin on average. The carotenoid canthaxanthin is known to occur in low levels in some copepods (Kleppel et al., 1988; Łotocka et al., 2004), but was negligible (<5% of total animal carotenoids, on average) or below detection limits (0.02 ng ind⁻¹) in our samples. Algal carotenoids (e.g. zeaxanthin) were detected in recently feeding copepods, but were not included in the analysis as they represented gut contents, an assumption supported by their absence in starved individuals.

**DISCUSSION**

Our findings are not consistent with the hypothesized simple, positive relationship between food availability and astaxanthin stores. Instead, the data suggest that this type of relationship occurs only when food availability is below a certain threshold. At algal concentrations ≤150 μg C L⁻¹, we observed increases in concentrations of astaxanthin (all forms) and increasing ingestion and egg production in concert with increased food availability. In contrast, at algal concentrations ≥600 μg C L⁻¹, where ingestion rates were maximal, astaxanthin levels in copepods were low and independent of either algal concentrations or feeding activity.

In contrast, all growth-related parameters showed the expected functional responses to food availability: ingestion and egg production varied in concert and reached a plateau at algal concentrations of 600 μg C L⁻¹. This suggests that at <600 μg C L⁻¹, copepod growth was limited by food availability, whereas above these algal concentrations, it became food saturated. The lack of...
apparent correlation between respiration and food availability was likely due to the limited number of measurements, although the observations of lower respiration in starving individuals indicate decreased metabolic activity (Thor, 2002).

The increase in copepod astaxanthin levels with increasing algal concentrations suggests that over a certain range of food availability, copepods are able to accumulate and transform astaxanthin precursors obtained from their diet into astaxanthin stores in their bodies. Our observations of decreased astaxanthin at high or saturating food concentrations suggest, however, that increased intake of food is not a sufficient condition for accumulation of astaxanthin. The decoupling of feeding and growth rates from astaxanthin accumulation when food availability is high is consistent with two possible scenarios, neither of which is exclusive of the other: (i) astaxanthin accumulation in the body is reduced when pigments are allocated to elevated egg production and (ii) concentration-dependent variations in food assimilation alter the potential for astaxanthin accumulation.

To evaluate whether the decreased astaxanthin observed in feeding treatments with algae ≥600 µg C L⁻¹ could be attributed to the elevated egg production as more pigment is allocated to the eggs (the first scenario), we attempted to calculate the total astaxanthin pool for an egg producing female. As no suitable measurements of pigments in copepod eggs were found in the literature, the estimate was based on the average astaxanthin content of Baltic Acartia spp. nauplii, 510 µg astaxanthin g DW⁻¹ (Lotocka et al., 2004). The estimate derived from the nauplii pigment content should represent a lower bound to the possible range of egg pigments as in early development, non-feeding naupliar stages are likely to have a negative pigment budget. This corresponds to 49 µg astaxanthin g⁻¹ after normalizing to egg dry mass (0.072 µg, based on the egg diameter and a ratio of 0.28 × 10⁻⁶ µg DW µm⁻³, Huntley and Lopez, 1992). According to this estimate and the observed EPR values, in treatments with 600 µg C L⁻¹, clutches contained 0.38 ng of astaxanthin, equivalent to 66% of the total astaxanthin budget (i.e. in the female body and the released eggs). Correspondingly, in the treatments with 30 and 150 µg C L⁻¹, the average estimated astaxanthin in the egg clutches was less due to relatively low EPR: 0.04 and 0.18 ng female⁻¹, respectively, corresponding to 9 and 25% of the total astaxanthin, respectively, and assuming equal pigment content per egg in all treatments. These estimates of the pigments allocated to egg production are very close to the reported one-half of the total female astaxanthin channelled to egg sacs by a harpacticoid copepod Tigriopus falco (cf. Goodwin, 1960) and suggest that the first scenario alone cannot account for the low female astaxanthin content in high food treatments. Even if the potential loss of pigments with released eggs is included in the net pigment budget, total astaxanthin is still about 20% higher in copepods feeding at 150 µg C L⁻¹ compared with those feeding at the higher food concentrations.

The second scenario represents a more likely explanation, as gut evacuation rate has been shown to increase and gut residence time to decrease with increased food concentration in different copepod species (Kiorboe and Tiselius, 1987; Besiktepe and Dam, 2002). A reduction in gut residence time may lead to decreased assimilation efficiency, as there is less time for ingested material to be absorbed through the gut wall. Using average values for I, EPR and R, carbon assimilation efficiency (AE) was calculated as AE = (EPRC + RC)/I, where EPRC + RC are carbon equivalents for EPR and R, respectively; EPRC was calculated based on measured egg diameter (79 µm) and the ratio of 0.14 × 10⁻⁶ µg C µm⁻³ (Kiorboe et al., 1985), while for calculating RC, a respiratory quotient of 0.9 for ammonotelic crustaceans was applied (Omori and Ikeda, 1984). The calculated carbon AE values were >1 at the lowest food levels, moderately high (0.82) at 150 µg C L⁻¹ and dropped to 0.66–0.68 at the highest food concentrations. Thus, the decreased values of carbon AE in ≥600 µg C L⁻¹ compared with those in 150 µg C L⁻¹ lend further support to the significance of concentration-dependent variations in food assimilation for astaxanthin accumulation. While at higher feeding rates and low assimilation efficiencies, the total amount of the assimilated material can remain high, very little is known about differential uptake of micronutrients in copepods (Båmstedt et al., 2000). Carotenoids are digested to a much lesser degree than chlorophylls during copepod feeding incubations, with a dramatic decline (down to 0%) in carotenoid destruction at saturating food conditions (McLeroy-Etheridge and McManus, 1999). This might result in even greater differences in carotenoid AE between the treatments than those observed for carbon. Consequently, very high availability of food may be associated with maximal ingestion rates but inefficient processing due to decreased assimilation and insufficient uptake of astaxanthin precursors.

The average astaxanthin content in feeding A. bifilosa females was 201 µg g⁻¹ DW. This value is just below the values previously reported for adult Acartia spp. (293 µg g⁻¹ DW) and Pseudocalanus acuspes (239 µg g⁻¹ DW) from the southern Baltic Sea (Lotocka et al., 2004), but is similar to total astaxanthin measurements of 185 µg g⁻¹ DW in the harpacticoid copepod Nitokma
*low astaxanthin at high feeding rates in copepods*

When food is withdrawn, astaxanthin is reduced rapidly to near-uniform levels (seen by comparison of values relative to the reference line in Fig. 1C,D). The similar astaxanthin concentrations in all treatments after incubations without food (i.e. 24 h of starvation) suggest that starving copepods can maintain a baseline of astaxanthin stores. This conclusion is supported by the stable pigment content of copepods which were without food for the total duration of all incubations (i.e. starved for a total of 72 h). The relatively low R and EPR of copepods in this treatment suggest that copepods may be able to maintain astaxanthin concentrations because of both reductions in metabolism and decreased pigment allocation to eggs.

Our results have ecological relevance particularly in the context of eutrophication, a well-known problem in many coastal areas, including the Baltic Sea (Elmgren, 2001). In coastal areas of the northern Baltic proper, phytoplankton biomass is typically below 300 μg C L⁻¹ (Lahm and Stahl, 2005) and 2001). In coastal areas, including the Baltic Sea (Elmgren, 2001) and oxidative stress (Vuori and Nikinmaa, 2007). This mechanism could, at least in part, explain the occurrence of M74 in Baltic salmon, which has earlier been related to astaxanthin deficiency (Pettersson and Lingell, 1999) and oxidative stress (Vuori and Nikinmaa, 2007).

In conclusion, our work has shown that the copepod *A. bifilosa* can accumulate astaxanthin when feeding, but high feeding rates may be associated with decreased astaxanthin accumulation. The mechanisms of this decrease require further investigation and more algal and zooplankton species should be tested. Nevertheless, these findings make an important contribution to the understanding of astaxanthin dynamics as an aspect of food quality within marine food webs, where increases in phytoplankton biomass may translate to a trade-off between the quantity of zooplankton and its nutritional quality for zooplanktivores. At food concentrations below saturation level, copepod growth is food limited, which controls the population abundance. An increase in phytoplankton biomass could release the food-related constraints on copepod growth, but could also lead to lowered astaxanthin content in copepod stocks. Although it is possible that decreases in astaxanthin content have no detrimental effect on the copepods themselves, decreased astaxanthin may represent a reduction in the nutritional quality of copepods as prey for zooplanktivores. Consequently, in systems affected by eutrophication copepods may represent an abundant but poor-quality food source for fish.

Zooplankton feeding behaviour varies between species and food type; therefore, quantitative interpretations should be made with caution and further investigations of astaxanthin dynamics in copepods and other zooplankters should take into account these factors. Species-specific variations in functional responses and digestive physiology, superimposed on variations in the quantity and digestibility of phytoplankton species, may lead to large differences in the astaxanthin content of zooplankton stocks, both seasonally and spatially. Moreover, it is possible that other biochemical compounds essential for the physiology of both zooplankters and zooplanktivores (e.g. other carotenoids, fatty acids, etc.; Liñán-Cabello et al., 2002; Kainz et al., 2004; Sies and Stahl, 2005) behave in similar fashion and their uptake could be less efficient at saturating food levels. This implies that a better understanding of the physiology and regulation of the fluxes of these essential compounds will significantly inform studies at the interface of ecology, biochemistry and molecular physiology.

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