Dynamics of astaxanthin, tocopherol (Vitamin E) and thiamine (Vitamin B₁) in the Baltic Sea ecosystem: Bottom-up effects in an aquatic food web

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Abstract

The thesis combines laboratory experiments and field expeditions to study production, transfer and consumption of non-enzymatic antioxidants and thiamine in an aquatic food web. In particular, I (1) documented spatial and seasonal variation of tocopherols and carotenoids in the Baltic Sea pelagic food web, and (2) examined the effects of abiotic and biotic factors on tocopherol, carotenoid and thiamine concentrations in phytoplankton, zooplankton and fish.

Moderate differences in temperature and salinity affected α-tocopherol, β-carotene and thiamine production in microalgae. Furthermore, the results suggest that acute stress favors the expression of non-enzymatic antioxidants rather than enzymatic antioxidants. Because production of α-tocopherol, β-carotene and thiamine differ markedly between microalgae, the availability of non-enzymatic antioxidants and thiamine is likely to be highly variable in the Baltic Sea and is difficult to predict.

The transfer of non-enzymatic antioxidants from phytoplankton to zooplankton was biomass dependent. The field expeditions revealed that phytoplankton biomass was negatively associated with α-tocopherol concentration in mesozooplankton. Thus, increased eutrophication of the Baltic Sea followed by an increase in phytoplankton biomass could decrease the transfer of essential biochemicals to higher levels in the pelagic food web. This could lead to deficiency syndromes, of the kind already observed in the Baltic Sea. Astaxanthin is synthesized from precursors provided by the phytoplankton community. Thus biomass dependent transfer of astaxanthin precursors from phytoplankton to zooplankton could be responsible for astaxanthin deficiency in zooplanktivorous herring. Astaxanthin in herring consists mostly of all-Z-isomers, which are characterized by low bioavailability. Therefore, astaxanthin deficiency in salmon could be explained by the low concentration of this substance and its isomeric composition in herring.

Keywords: Baltic Sea, carotenoids, astaxanthin, tocopherols, Vitamin E, thiamine, Vitamin B1, pelagic food web, eutrophication, M74, phytoplankton, zooplankton, sprat, Sprattus sprattus balticus, herring, Clupea harengus, salmon, Salmo salar, cod, Gadus morhua, High Performance Liquid Chromatography (HPLC), electrochemical detection (ECD)

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The same thing that makes you live can kill you in the end.

Neil Young
List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.

I Häubner, N., Lewander, T. and P. Snoeijis. The analysis of vitamin E in phyto- and zooplankton samples. *Manuscript*

II Häubner N., Sylvander P., Vuori K. and P. Snoeijis. Response of alpha-tocopherol and beta-carotene production in microalgae to temperature, salinity and photon flux density *Submitted manuscript*

III Sylvander P., Häubner N. and P. Snoeijis. The thiamine (vitamin B₁) content of phytoplankton is affected by temperature, photon density and salinity. *Submitted manuscript*

IV Häubner N., Tallmark B. and P. Snoeijis. Phytoplankton biomass controls tocopherol concentrations in Baltic Sea zooplankton. *Submitted manuscript*

V Snoeijis P., Holeton C. and N. Häubner. Seasonal variation of astaxanthin production in a changing ecosystem. *Manuscript*

VI Nie X.P., Zie J., Häubner N., Tallmark B. and P. Snoeijis. Prey diversity and prey stomach contents affect astaxanthin levels in piscivorous fish. *Submitted manuscript*
My contribution to the papers

Paper I: I designed the method and laboratory experiments, was responsible for data capture, statistical analyses and compiled the manuscript.

Paper II: I designed the experiment together with the co-authors, was responsible for measurement of vitamin E, oxidative stress parameters (together with K. Vuori and P. Sylvander), data capture, statistical analyses and compiled the manuscript.

Paper III: I designed the experiment together with the co-authors and commented on the manuscript.

Paper IV: I participated in two sampling cruises, was responsible for Vitamin E measurement, data capture, statistical analyses and compiled the manuscript.

Paper V: I participated in two sampling cruises, was responsible for measurement of pigment composition in part of the phytoplankton samples and commented on the manuscript.

Paper VI: I participated in one sampling cruise, advised X. P. Nie and J. Zie on HPLC measurements and gave comments to the manuscript.
Contents

Introduction ................................................................................................................................. 9
  Organisms under oxidative stress ................................................................................................ 9
  Ecological implications of oxidative stress ................................................................................. 10
  Substances of interest .................................................................................................................. 11
  Aims of this thesis ..................................................................................................................... 13

Material & Methods .................................................................................................................... 14
  Experimental set up (paper II, III) .............................................................................................. 14
  Field sampling (paper IV, V and VI) .......................................................................................... 15
  Analysis .................................................................................................................................... 16

Results and Discussion ................................................................................................................ 19
  Vitamin and β-carotene production in microalgae (paper II & III) ............................................. 19
  Carotenoid and α-tocopherol concentration in the lower part of the pelagic food web (paper IV & V) ...................................................................................................................... 23
  Astaxathin concentrations in fish (paper VI) ........................................................................... 30
  Conclusions ............................................................................................................................... 31

Acknowledgements ..................................................................................................................... 33

Summary in Swedish .................................................................................................................... 35

Summary in German ..................................................................................................................... 38

References .................................................................................................................................. 41
Introduction

The flow of biochemicals within food webs in pelagic systems is founded on phytoplankton and its consumption by herbivorous zooplankton (Mueller-Navarra 2008, Reynolds 2008). Food-web interactions changed and/or are still changing dramatically due to climate change, eutrophication, contamination and overexploitation of fish stocks, which is especially true for the Baltic Sea (Cloern 2001, Elmgren 2001, Fitzmaurice 1993). Consequences for organisms inhabiting these ecosystems will be higher energetic costs and more frequent exposure to stress. This could lead to a shortage of essential nutrients in the pelagic food web, due to decreased production or increased consumption of these compounds (Lesser 2006). The overall aim of the thesis is to reveal patterns of production, transfer and consumption of essential biochemicals in the pelagic food web of the Baltic Sea.

Organisms under oxidative stress

Heterotrophs are not capable to produce many of the biochemicals required for their surviving and rely on the production from their feeding environment (Mueller-Navarra 2008). Antioxidants are among these required biochemicals and are needed to prevent organisms from oxidative stress.

The problem started at the very beginning of life. Oxygen is on one hand the base for all higher life on earth, but on the other a toxic and mutagenic gas (Halliwell & Gutteridge 2007). Every reaction where oxygen is involved gives rise to free radicals and non-radical oxidants (Asada 2006, Lesser 2006, Monaghan et al. 2009), i.e. reactive oxygen species (ROS), like superoxide, hydroxyl and nitric oxide species. ROS are essential in the control of cell function in biological systems (Halliwell & Gutteridge 2007), but if produced in excess of available antioxidants they induce oxidative stress and could harm macromolecules, like DNA and proteins, and membranes (Asada 2006, Halliwell 2006, Lesser 2006, Monaghan et al. 2009). This oxidative threat becomes lethal if antioxidants cannot fully neutralize the ROS that are produced, so that unquenched ROS remain for long enough to cause further reactions (Monaghan et al. 2009). Consequently aerobes had to evolve an efficient defense system against the hazardous effect of oxygen consumption for successful living. The
antioxidant defense could be roughly divided in endogenous, e.g. enzymes, and diet derived, e.g. vitamins A, C and E and carotenoids (Halliwell & Gutteridge 2007).


Ecological implications of oxidative stress

The non-enzymatic antioxidant defense of heterotrophs depends directly or indirectly, via precursors, on the production of antioxidants in phototrophs. This dependency could cause deficiencies in the food web, if the production or transfer of these biochemicals is decreased. This scenario is most likely in disturbed ecosystems. A good example is the Baltic Sea, which is regarded as one of the most polluted seas on earth (Fitzmaurice 1993, Elmgren 2001). Climate change, e.g. decreased surface salinity and increased surface temperature, in combination with eutrophication and pollution with toxins are predicted to increase the pressure on the ecosystem in the coming decades (Pawlak et al. 2007, Schiedek et al. 2007). Consequences for organisms inhabiting the Baltic Sea will be higher energetic costs and more frequent exposure to stress, which could lead to deficiencies, as mentioned earlier. Furthermore, a changed species composition of the phytoplankton could alter the production of antioxidants (Alheit et al. 2005, Van Nieuwerburgh et al. 2005). In the last decades, cases of predators suffering from symptoms associated with thiamine deficiency have been reported from the Baltic Sea, including salmonid fishes, crustaceans and birds (Amcoff et al. 1998, Balk et al. 2009, Bengtsson et al. 1999, Breitholz et al. 2001, Vuori & Nikinmaa 2007). The thiamine deficiency in salmon is associated with reduced levels of antioxidants in the fish, like astaxanthin, α-tocopherol and ubiquitone and was summarized as the M74 syndrome (Pettersson & Lignell 1999, Vuori & Nikinmaa 2007). However, it is not verified yet if thiamine deficiency causes a lack of non-enzymatic antioxidants or vice versa.

The M74 syndrome is accompanied by a regime shift in the Baltic Sea, which is related to climate change effects, eutrophication, overfishing and minimization of seal populations during the past century (Alheit et al. 2005, Österblom et al. 2007). The regime shift affects all levels in the pelagic food web of the Baltic Sea. The formerly diverse phytoplankton community is now characterized by low biodiversity, dominated by flagellates and cyanophytes (Wasmund & Uhlig 2003, Suikkanen et al. 2007). The same is true for the zooplankton, which has shifted towards a community thriving in lower salinity and more euphotic conditions (Möllmann et al. 2000, 2003).
This has been accompanied by a decreased population of the copepod *Pseudocalanus* spp., which is the main food source for Baltic herring (*Clupea harengus membras* L.) in spring (Möllmann 2003, 2005, Rönkkönen et al. 2004). This could be one explanation to why the main food source of the salmon, the Baltic herring has shown severe starvation symptoms (Cardinal & Arhennius 2000, Salimen et al. 2001). Finally, the Baltic sprat (*Sprattus sprattus balticus* Schneider) biomass has increased through overfishing and recruitment problems of its main predator, the Atlantic cod, *Gadus morhua* L. (Cardinale & Arrhenius 2000). The described regime shift in combination with major environmental changes and the deficiency syndromes observed in the Baltic Sea, leaves the question if the flow of essential biochemcials in the food web is still intact.

**Substances of interest**

Mainly vitamin E (tocopherols), carotenoids (astaxanthin and β-carotene) and vitamin B₁ were studied in the pelagic food web of the Baltic Sea and in the laboratory.

**Vitamin E (tocopherols)**

Vitamin E (tocopherols) is a group of closely related lipids and is one of the most important antioxidant in lipids (Carballo-Cardenas et al. 2003, Fryer 1992). Tocopherols can only be produced by photosynthetic organisms (DellaPenna & Pogson 2006, Munné-Bosch & Alegre 2002). α-Tocopherol is the most abundant form of the tocopherols in nature and has the highest antioxidant activity in vivo (Carballo-Cardenas et al. 2003). Mallet et al. (1994) showed a clear positive correlation between α-tocopherol content and antioxidant activity in a variety of vascular plant species. Furthermore, it was shown that *Arabidopsis thaliana* (L.) Heynh. mutants lacking tocopherols suffer extensively from oxidative stress (Semchuk et al. 2009). In animal tissues, the primary function of α-tocopherol is the stabilization of membranes by prevention of auto-oxidation of e.g. polyunsaturated fatty acids, up-regulation of antioxidant enzymes and improvement of the effectiveness of carotenoids (Lesser 2006, Mallick & Mohn 2000, Rønnestad et al. 1999, Vertuani et al. 2004). Furthermore, α-tocopherol ensures best utilization of food containing high quality lipids, like EPA and DHA, and maintaining body stores of essential fatty acids (Lewis-McCrea & Lall 2007). Direct effects of tocopherol deficiency could be experimentally observed in Atlantic salmon post-smolts, which suffered of extensive lipid liver degenerative lesion if tocopherol was deficient (Bell et al. 2000). Vitamin E deficiency in humans mostly effects the peripheral nervous
systems and causes lack of coordination and muscle movements (Traber & Sies 1996).

Carotenoids

Carotenoids are first and foremost pigments in the chloroplasts of all higher photosynthetic organisms, including algae, but at the same time serve as antioxidants in both autotrophic and heterotrophic organisms (Frank & Cogdell 1996, Matsuno 2001). Carotenoids are characterized by a long chromophore of conjugated double bonds, which enables the molecules to capture light and quench free radicals (Higuera-Ciapara et al. 2006).

β-carotene is a pigment in the antennae complex of photosynthetic organisms. It is involved in photooxidative protection in algae and higher plants and can additionally quench ROS directly (Frank & Brudvig 2004). Furthermore β-carotene is an important precursor for pigments of the xanthophylls cycle in plants (Demmig-Adams et al. 2002) and for Vitamin A and antioxidants in animals (Krinsky 1989, Matsuno 2001).

In aquatic animals, the fat-soluble red pigment astaxanthin is a widespread antioxidant, which also is used for camouflage and in spawning behavior (Matsuno 2001). Astaxanthin is mostly produced by crustaceans from precursors, as β-carotene and zeaxanthine (Matsuno 2001). In fish, astaxanthin protects fish roe and fry from oxidative stress (Izquierdo 2001). Furthermore, it was recently found, that astaxanthin could improve mitochondrial function through retaining mitochondria in the reduced state (Wolf et al. 2010).

Vitamin B1 (thiamine)

The water soluble vitamin thiamine (Vitamin B₁) has various functions in animals and plants. Thiamine functions as a co-enzyme for α-ketoacid dehydrogenase and transketolases in the Krebs cycle and the oxidative pentose phosphate pathway. Its pyrophosphate ester, thiamine diphosphate (TDP), is a cofactor in a number of metabolic reactions, including the synthesis of acetyl CoA (Lonsdale 2006). In humans, thiamine deficiency associated with an inadequate dietary intake has long been known to cause beriberi, a disease with a wide array of both neurological and cardiological symptoms (Haas 1988). In the marine food web, thiamine is mainly synthesized by phytoplankton and prokaryotes. Not all phytoplankton are able to synthesize thiamine and this ability differs within and between taxa (Croft et al. 2006).
Aims of this thesis

The aim of the thesis was to investigate the production, transport and consumption of non-enzymatic antioxidants, such as α-tocopherol (vitamin E), β-carotene and astaxanthin, and thiamine (Vitamin B₁) in the pelagic food web of the Baltic Sea.

To be able to measure vitamin E in plankton samples, it was necessary to improve existing methods in terms of efficiency and detection limit (Paper I).

Experiments in Paper II and III were conducted to reveal whether the effects of abiotic factors on production of α-tocopherol, β-carotene and thiamine differed between microalgae.

Paper IV and V were designed to answer the following questions: (1) do tocopherol and astaxanthin concentrations vary with time and location in the Baltic Sea proper? (2) Which abiotic / biotic factors influence the tocopherol and astaxanthin concentration in plankton of the Baltic Sea? (3) Are the tocopherol and astaxanthin concentrations sufficiently high to prevent possible deficiencies in top predators, like salmon?

Paper VI was aimed at evaluating why astaxanthin is limiting in the Baltic salmon through detailed analysis of astaxanthin dynamics in its two major planktivorous species herring and sprat, including seasonal and spatial variation in the Baltic Sea proper. A second aim was to examine if also cod, the major predator fish in the Baltic Sea suffers from astaxanthin shortage.
Material & Methods

The thesis combines data obtained at offshore expeditions in the Baltic Sea and laboratory experiments conducted at Uppsala University and Stockholm University. In the field expeditions, parts of the pelagic food web, such as phytoplankton, zooplankton, zooplanktivorous and piscivorous fish, were sampled to study spatial and temporal variation of tocopherol and pigments, including astaxanthin. Laboratory experiments aimed to simplify tocopherol extraction from plankton samples and to study the effects of abiotic factors on tocopherol, carotenoid and thiamine production in microalgae.

Experimental set up (paper II, III)

The effect of photon flux density, salinity and temperature on the production of α-tocopherol, β-carotene and thiamine were investigated in six different algae species (Table 1) in a 48 h incubation experiment.

Table 1. Overview of the microalgal cultures used in the incubation experiments

<table>
<thead>
<tr>
<th>species</th>
<th>class</th>
<th>strain identification</th>
<th>isolated</th>
<th>salinity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dunaliella tertiolecta</td>
<td>Chlorophyceae</td>
<td>SCCAP K-0591</td>
<td>unknown origin</td>
<td>7 PSU</td>
</tr>
<tr>
<td>Nodularia spumigena</td>
<td>Cyanophyceae</td>
<td>KAC 7</td>
<td>Kalmarsund, Baltic Sea</td>
<td>7 PSU</td>
</tr>
<tr>
<td>Prorocentrum minimum</td>
<td>Dinophyceae</td>
<td>KAC 72</td>
<td>Kalmarsund, Baltic Sea</td>
<td>7 PSU</td>
</tr>
<tr>
<td>Phaeodactylum tricornutum</td>
<td>Bacillariophyceae</td>
<td>KAC 37</td>
<td>Kattegat</td>
<td>26 PSU</td>
</tr>
<tr>
<td>Skeletonema costatum</td>
<td>Mediophyceae</td>
<td>KAC 44</td>
<td>Kattegat</td>
<td>26 PSU</td>
</tr>
<tr>
<td>Rhodomonas salina</td>
<td>Cryptophyceae</td>
<td>SCCAP K-0294</td>
<td>Öresund</td>
<td>7 PSU</td>
</tr>
</tbody>
</table>

The cultures were cultivated at 15°C, a photon flux density of 50 ± 10 μmol photons m⁻² s⁻¹ provided by daylight lamps (Osram L 36W/21-840 Lumilux Plus Eco) in a light:dark cycle of 16:8 hours. The cultures were stirred continuously, but not aired. Cultures were harvested in the late log phase, which was generally reached after 7-12 days and used to incubate for 48 h in two separate experiments. In a first experiment, the algae were exposed to low (50 ± 10 μmol photons m⁻² s⁻¹) and high photon flux intensity (240 ± 10 μmol photons m⁻² s⁻¹) at three different temperatures: 5°C; 15°C and 25°C. The second incubation was run with low salinity, i.e. 50 % of growth salinity and high salinity, i.e. 150 % growth salinity at the same temperatures as in
the first experiment and at 50 ± 10 μmol photons m\(^{-2}\) s\(^{-1}\). The algae cultures were collected on Whatman GFF filter in a light protected environment and immediately transferred to -80 °C.

Field sampling (paper IV, V and VI)

Eight offshore cruises were accomplished in 2004 and 2005. In total 96 stations were sampled in the Baltic Sea, Kattegat and Skagerrak (Fig. 1).

![Figure 1. Sampling stations of the eight offshore cruises in the Baltic Sea, Kattegat and Skagerrak conducted in 2004 and 2005 (paper IV and V).](image)

To reveal seasonal patterns expeditions were undertaken in March, May, August and November in both years. On all expeditions phyto- and zooplankton samples were taken. Zooplanktivorous and piscivorous fish were trawled on two expeditions in 2005. Plankton samples (paper IV, V) were collected by pumping surface water from 5-7 m depth. The same water was used to measure salinity, temperature and inorganic nutrients. Six replicate samples in the three size fractions <100 μm (dominated by phytoplankton), 100-200 μm (dominated by microzooplankton, but sometimes also containing considerable amounts of large phytoplankton) and >200 μm (dominated by mesozooplankton) were filtered. In March 2004, no samples were taken of the size fraction 100-200 μm. Mesozooplankton organisms of the >200 μm size fraction were identified to the genus level and counted. A pigment-based approach was chosen to
identify major algae groups within the size class $< 100 \, \mu m$. All samples for astaxanthin and tocopherol analysis were stored immediately in liquid nitrogen and transferred to -80 °C freezer at return on land.

Zooplanktivorous fish (Baltic sprat, *Sprattus sprattus balticus* and herring, *Caulerpa harengus*) and piscivorous fish (cod, *Gadus morhua*) were caught in the open Baltic Sea proper, between the Hanö Bight in the south and the northern Gotland area in the north (paper VI). Trawling was undertaken at 17 sampling stations, on 28 February - 10 March and 21 November - 1 December 2005 between 8:00 and 10:00 in the morning. Trawling time was ca. 45 min and average depth was 58 m (minimum 37 m, maximum 77 m). Salmon (*Salmo salar*), caught on 28 November 2005 at Station 99, from local fishermen at Kårehamn, Öland (paper VI). All fish were put on ice immediately after sampling and tissue samples from gonads, liver, muscle and stomach (including stomach contents) of female fish were taken within two hours from sampling, packed and frozen at -20°C until analysis. The length and weight of each specimen was noted before dissecting the fish.

**Analysis**

**Pigment and thiamine (paper III-VI)**

Phytoplankton samples were extracted after Wright et al. (1997). However samples from March 2004 (paper III) were analyzed following Andersson et al. 2003. Pigments were analyzed by reversed-phase high performance liquid chromatography (HPLC) with a modified solvent gradient as described by Pinto et al. (2003).

Zooplankton pigments were extracted as well after Wright et al. (1997). Astaxanthin analysis was conducted after Andersson et al. (2003), with slight modification.

The extraction protocol for fish followed Pettersson & Lignell (1998, 1999) with some minor modifications in the HPLC set up (paper VI). HPLC was carried out with an Agilent™ 1100 System or with a Shimadzu™, LC-10ADVP. Gradients of mobile phase A (80% methanol + 20% ammonium acetate buffer), B (90:10 acetonitrile:H$_2$O) and C (ethyl acetate) were programmed as follows: (time, A%, B%, C%), (0 min 100, 0, 0), (2 min 0, 100, 0), (19/17 min 0, 20, 80), (21 min 0, 100, 0), (24/23 min 100, 0, 0), (30/36 min 100, 0, 0). Chromatograms were integrated at 472 nm using the software ChromProcessor™ (ACD/Labs, Canada) or Clarity™ (Data Apex, Czech Republic).

Free thiamine (TF) and its phosphate esters, thiamine monophosphate (TMP) and thiamine diphosphate (TDP) were extracted and analyzed according to Pinto et al. (2002) with slight modifications.
Tocopherols (paper I, II, IV)

The protocol for tocopherol extraction was optimized as shown schematic in figure 2 (paper I). The final protocol is as follows (1) After placing the filter with the sample in a 15 mL test tube, 3 mL dimethylformamide (DMF) containing 0.05M sodium dodecyl sulfate (SDS) was added. (2) After vortexing for 30 s twice (3) 1.3 mL n-hexane and 0.5 mL ddH2O were added to the sample and vortexed again for 10 s. (4) The sample was then centrifuged at 4°C for 5 min at 4000 rpm. (5) 1 mL of the n-hexane was transferred to a new test tube. (6) The DMF phase was extracted twice with 1 mL n-hexane as described in (4) and (5). (7) The combined 3 mL of n-hexane were evaporated under a stream of N2 gas at room temperature. (8) The residue was redissolved in 500 μL EtOH/ddH2O (75:25 v/v) volume and filtered through 0.45 μm membrane. Analysis of tocopherols was carried out with a Coulochem III Detector (ESA Inc.) in combination with a two channel analytical cell. The potentials of the channels were set to 250 and 800 mV. Furthermore a guard cell (800 mV) was used. The samples were injected with a Midas™ Autosampler (Spark, The Netherlands) and Shimadzu™ Pump, Model LC-10ADVP (Shimadzu Cooperation, Japan). The column used was a ReproSil™ Gold C18 column (150 × 3 mm, particle size 3 μm) and the injection volume was 25 μL. The isocratic mobile phase used to separate tocopherols consisted of 75% methanol, 20% 1-propanol and 5% 25 mM ammonium acetate in ddH2O, pH 4.0. Peak identification was confirmed by co-elution and calibration with α- and γ-tocopherol.

Figure 2. Optimization of extraction protocol for tocopherol in phyto- and zooplankton samples

The protocol for tocopherol extraction was optimized as shown schematic in figure 2 (paper I). The final protocol is as follows (1) After placing the filter with the sample in a 15 mL test tube, 3 mL dimethylformamide (DMF) containing 0.05M sodium dodecyl sulfate (SDS) was added. (2) After vortexing for 30 s twice (3) 1.3 mL n-hexane and 0.5 mL ddH2O were added to the sample and vortexed again for 10 s. (4) The sample was then centrifuged at 4°C for 5 min at 4000 rpm. (5) 1 mL of the n-hexane was transferred to a new test tube. (6) The DMF phase was extracted twice with 1 mL n-hexane as described in (4) and (5). (7) The combined 3 mL of n-hexane were evaporated under a stream of N2 gas at room temperature. (8) The residue was redissolved in 500 μL EtOH/ddH2O (75:25 v/v) volume and filtered through 0.45 μm membrane. Analysis of tocopherols was carried out with a Coulochem III Detector (ESA Inc.) in combination with a two channel analytical cell. The potentials of the channels were set to 250 and 800 mV. Furthermore a guard cell (800 mV) was used. The samples were injected with a Midas™ Autosampler (Spark, The Netherlands) and Shimadzu™ Pump, Model LC-10ADVP (Shimadzu Cooperation, Japan). The column used was a ReproSil™ Gold C18 column (150 × 3 mm, particle size 3 μm) and the injection volume was 25 μL. The isocratic mobile phase used to separate tocopherols consisted of 75% methanol, 20% 1-propanol and 5% 25 mM ammonium acetate in ddH2O, pH 4.0. Peak identification was confirmed by co-elution and calibration with α- and γ-tocopherol.
Isotopic composition, enzyme activity and photosynthetic efficiency

Particulate carbon, nitrogen and phosphorus in papers IV and V were analyzed at the Department of Limnology, Uppsala University with a Carlo Erba NA1500 elemental analyser (Carlo Erba Strumentazione, Italy). In papers II and III a Leco CHN-analyzer was used for particulate carbon and nitrogen. Filters for particulate phosphorous were combusted and analyzed for molybdate-reactive orthophosphate with flow injection (Lachat Instruments, USA).

Photosynthetic efficiency was measured using a Phyto PAM (Walz, Germany) in 15 min dark-adapted microalgae cells.

Filter for super oxide dismutase (SOD) activity were extracted after Jangknekt et al. (2007): sonicated 2 × 30 sec (VibraCell, amplitude 92, 0.9s pulse) on ice in a buffer containing 50 mM KH$_2$PO$_4$ (pH 7.8), 0.1 mM EDTA and 1% Triton X-100. Protein concentration was measured using the RCDC Protein Assay (Bio-Rad, USA). SOD Assay Kit-WST (Fluka, Germany) in combination with 96well plate reader was used to measure SOD activity. Inhibition activity of SOD was determined kinetically at 450 nm.
Results and Discussion

Vitamin and β-carotene production in microalgae (paper II & III)

We found that the production of thiamine (vitamin B₁), α-tocopherol (vitamin E) and β-carotene is species specific. Salinity had strongest effect on thiamine synthesis, whereas temperature and salinity affected α-tocopherol and β-carotene most.

Initial concentrations

The initial α-tocopherol and β-carotene concentrations differed widely between species. *D. tertiolecta* and *N. spumigena* showed high α-tocopherol (Mean ± CI: 0.52 ± 0.07 mg g C⁻¹; 0.24 ± 0.07 mg g C⁻¹) and β-carotene concentrations (2.72 ± 0.21 mg g C⁻¹; 1.65 ± 0.0.35 mg g C⁻¹). *D. tertiolecta* showed only moderate initial concentrations of thiamine, whereas *N. spumigena* had the highest total thiamine concentrations (paper III). The differences in the concentrations are probably related to different strategies in oxidative stress defense, at least for α-tocopherol and partly β-carotene (enzymatic vs. non-enzymatic defense).

Effects of photon flux density and temperature

The response of thiamine, α-tocopherol and β-carotene to changed photon flux density, temperature and salinity varied among species. Three different categories of response to photon flux density and temperature could be identified: In *D. tertiolecta*, the α-tocopherol concentration was higher at high photon flux densities, but did not vary with temperature (Fig. 3 A). In *P. minimum*, *P. tricornutum*, *S. costatum* and *N. spumigena* it was affected by temperature, but not by photon flux density (Fig. 3 B-E). Finally in *R. salina* it varied with both (Fig. 3 F). However, it must be mentioned that in *P. tricornutum* and *S. costatum* cultures significant interaction effects of photon flux density and temperature were observed. These differences were interpreted as of minor biological importance, since the change of α-tocopherol in response to temperature is consistent in direction between the two photon flux density treatments.
Figure 3. α-tocopherol and β-carotene concentration in mg g C⁻¹ of Dunaliella tertiolecta (A), Nodularia spumigena (B), Prorocentrum minimum (C), Phaeodactylum tricornutum (D), Skeletonema costatum (E) and Rhodomonas salina (F) cultures after 48 h incubation at two different photon flux densities (50 and 240 μmol m⁻² s⁻¹) and three temperatures (5°C; 15°C and 25°C). Error bars represent 95% confidence intervals (n=3). Different letters above bars indicate significant differences at p<0.05.
β-carotene was positively associated with α-tocopherol, except in *D. tertiolecta* and *P. tricornutum* cultures, suggesting that the factors influencing the onset of the synthesis of these two substances are similar (Fig. 3). The variations of the α-tocopherol and β-carotene pool between the different temperature treatments reflect the ability of the species to cope a certain temperature range. For example *D. tertiolecta* showed earlier a high physiological plasticity towards temperature (Levasseur et al. 1990, Sosik & Mitchell 1994). *P. minimum*, *P. tricornutum* and *S. costatum* are species adapted to moderate temperatures as they occur in spring and autumn in the plankton of the Baltic Sea (Gasiunaite et al. 2005), which could result in a higher risk for oxidative stress in higher temperatures. *P. tricornutum*, *S. costatum* and *N. spumigena* were identified as thiamine producers. Thiamine synthesis was significantly affected by temperature, with highest values at 25°C for *P. tricornutum* and *N. spumigena* and at 15 °C for *S. costatum* (paper III). Photon flux density only affected thiamine production in *N. spumigena* with highest values at low photon densities and high temperatures (paper III). This suggests a higher need of thiamine in stressed cells.

**Effects of salinity and temperature**

*N. spumigena* and *P. tertiolecta* cultures reacted similar with increased α-tocopherol and β-carotene concentration with increasing salinity and temperature (Fig. 4 B, D). These drastic changes were rather unexpected, because the optimal growth salinity for *N. spumigena* is between 7 and 18 psu (Mazur-Marzec et al. 2005), for *P. tricornutum* a survival in up to 90 psu was observed (Kräbs & Buechel 2009). *D. tertiolecta* cultures responded with decreased α-tocopherol concentration in hyposaline conditions (Fig. 4 A). This conforms the findings of Jahnke & White (2003), who also observed increased α-tocopherol concentration in response to decreased salinity. In *P. minimum* and *S. costatum* cultures, salinity did not significantly influence α-tocopherol concentration (Fig. 4 C, E).

Salinity had the strongest effect on thiamine content in the microalgae. Thiamine content of *N. spumigena* cultures increased threefold with a 50 % increase in salinity. *P. tricornutum* increased thiamine di-phosphate sevenfold when salinity was decreased by 50 %. This indicates again the need of thiamine in stressed cells (paper III).

**Consequences for aquatic ecosystems**

The change of thiamine, α-tocopherol and β-carotene was already induced by moderate changes in abiotic factors and was not correlated to stress indicators, e.g. super oxide dismutase activity. Lack of correlation is
Figure 4. α-tocopherol and β-carotene concentration in mg g C\textsuperscript{-1} of Dunaliella tertiolecta (A), Nodularia spumigena (B), Prorocentrum minimum (C), Phaeodactylum tricornutum (D) and Skeletonema costatum (E) cultures after 48 h incubation at two different salinities (50\% and 150\% culture salinity) and three temperatures (5°C; 15°C and 25°C). White bars illustrate controls incubated in 100\% growth salinity at 15°C. Error bars represent 95\% confidence intervals (n=3). Different letters above bars indicate significant differences at p<0.05.

probably due to the higher reaction constants for O\textsubscript{2} and \textsuperscript{1}O\textsubscript{2} of carotenoids and α-tocopherol compared to SOD (Mallick & Mohn 2000). Alternatively it could be hypothesized that non-enzymatic antioxidants as α-tocopherol and β-carotene are synthesized faster and induced in response to relatively moderate changes in the abiotic parameters and or / acute stress (Okamota et al. 2001).
Our study shows that thiamine, α-tocopherol and β-carotene production in algae is affected by abiotic factors; temperature and salinity appear more important than photon flux density. Temperature and salinity are rather stable variables in aquatic ecosystems, at least seen in a time frame of days or weeks, but it will probably change drastically over the next decades (Pawlak et al. 2007, Walther et al. 2002). The present and predicted changes in salinity and temperature will, or have already begun, to affect phytoplankton species composition (Alheit et al. 2005). This should also change the availability of non-enzymatic antioxidants and thiamine, as it is strongly affected by the species composition. These species-dependent effects could be amplified by the influence of abiotic factors, as shown in our study. Consequently, the available antioxidant pool could fluctuate rapidly (within days) and will be difficult to predict.

**Alpha-tocopherol and carotenoid concentration in the lower part of the pelagic food web (paper IV & V)**

Alpha-tocopherol and carotenoid concentration varied with season but not sampling locations in the phytoplankton and zooplankton in the Baltic Sea. Furthermore, α-tocopherol concentration in zooplankton decreased in times of high phytoplankton biomass.

**Physicochemical parameters, biomass and particulate ratios**

Surface water temperature varied between 1.3 and 20.0 °C. Surface water salinity varied between 5.8 and 8.3 psu, following the north-south and east-west salinity gradient in the Baltic Sea. Inorganic nutrients, such as phosphate, nitrate and silicate, were negatively associated with surface water temperature.

The seasonal average of particulate carbon (POC) concentration of the phytoplankton-dominated fraction (< 100 μm) varied between 60 μg C L⁻¹ in March to over 300 μg C L⁻¹ in May-August (Table 2). The biomass was higher in March 2004 (303 μg C L⁻¹) compared to March 2005 (192 μg C L⁻¹), suggesting an earlier onset of the spring bloom in 2005. This is supported by the significant higher biomass of the mesozooplankton in 2005 compared to 2004. Generally the biomass of the fractions 100-200 μm and >200 μm together was 10fold lower than that of the <100 μm fraction.

Molar C:N ratios were relatively stable, with values from 7.4 to 9.5 (<100 μm), 4.7 to 6.3 (100-200 μm) and 4.9 to 6.3 (>200 μm), which indicates zooplankton dominance in the 100-200 μm size class. N:P ratios varied widely between 12.4 and 28.2 for the <100 μm fraction, between 11.0 and 33.1 for the 100-200 μm fraction and between 13.4 to 26.4 for the >200 μm fraction. C:P ratios varied between 102 and 225 for the <100 μm fraction,
Table 2. Seasonal averages (±CV) of particulate carbon (POC); (B) Baltic Sea area, (KS) Kattegat-Skagerrak area; (N) number of samples, (-) no samples taken

<table>
<thead>
<tr>
<th>Area</th>
<th>Month</th>
<th>Year</th>
<th>N</th>
<th>Fraction &lt;100 μm μg C L⁻¹</th>
<th>Fraction 100-200 μm μg C L⁻¹</th>
<th>Fraction &gt;200 μm μg C L⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>Mar</td>
<td>2004</td>
<td>9</td>
<td>77 ± 41%</td>
<td>-</td>
<td>2.2 ± 59%</td>
</tr>
<tr>
<td>B</td>
<td>Mar</td>
<td>2005</td>
<td>10</td>
<td>60 ± 56%</td>
<td>1.2 ± 80%</td>
<td>1.9 ± 52%</td>
</tr>
<tr>
<td>B</td>
<td>May</td>
<td>2004</td>
<td>14</td>
<td>303 ± 33%</td>
<td>5.7 ± 78%</td>
<td>3.6 ± 113%</td>
</tr>
<tr>
<td>B</td>
<td>May</td>
<td>2005</td>
<td>10</td>
<td>192 ± 28%</td>
<td>8.2 ± 53%</td>
<td>16.2 ± 83%</td>
</tr>
<tr>
<td>B</td>
<td>Aug</td>
<td>2004</td>
<td>9</td>
<td>269 ± 22%</td>
<td>4.4 ± 71%</td>
<td>15.3 ± 83%</td>
</tr>
<tr>
<td>B</td>
<td>Aug</td>
<td>2005</td>
<td>14</td>
<td>314 ± 21%</td>
<td>2.8 ± 40%</td>
<td>11.7 ± 71%</td>
</tr>
<tr>
<td>B</td>
<td>Nov</td>
<td>2004</td>
<td>9</td>
<td>123 ± 33%</td>
<td>4.5 ± 57%</td>
<td>7.3 ± 49%</td>
</tr>
<tr>
<td>B</td>
<td>Nov</td>
<td>2005</td>
<td>9</td>
<td>109 ± 21%</td>
<td>1.4 ± 32%</td>
<td>5.4 ± 36%</td>
</tr>
<tr>
<td>KS</td>
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<td>2004</td>
<td>2</td>
<td>148 ± 15%</td>
<td>9.1 ± 35%</td>
<td>14.6 ± 28%</td>
</tr>
<tr>
<td>KS</td>
<td>May</td>
<td>2005</td>
<td>2</td>
<td>188 ± 3%</td>
<td>3.8 ± 23%</td>
<td>17.7 ± 2%</td>
</tr>
<tr>
<td>KS</td>
<td>Aug</td>
<td>2004</td>
<td>3</td>
<td>152 ± 16%</td>
<td>5.2 ± 48%</td>
<td>8.9 ± 14%</td>
</tr>
<tr>
<td>KS</td>
<td>Aug</td>
<td>2005</td>
<td>3</td>
<td>147 ± 12%</td>
<td>6.8 ± 37%</td>
<td>11.9 ± 9%</td>
</tr>
</tbody>
</table>

Community composition

Most abundant species in the mesozooplankton community was *Acartia* spp., except for March 2004 (paper V). *Temora* spp. was abundant in the Baltic Sea proper in November. Cladocerans occurred in high abundances in May (*Evadne* spp.) and August (*Evadne* spp., *Bosmina* spp. and *Podon* spp.).

We chose to divide the phytoplankton community into classes based on marker pigments. Composition of the phytoplankton community varied between seasons. In March cryptophytes (alloxanthin) and dinoflagellates (peridinin) dominated. In August probably cyanobacteria dominated (echinone, zeaxanthin), together with prymnesiophytes (19'-hexanoyloxyfucoxanthin) and green algae (chlorophyll b, violaxanthin).

α-tocopherol in the phytoplankton and mesozooplankton (paper III)

α-tocopherol production in phytoplankton varied significantly between seasons. Peak concentration was observed in May in both years (0.10 ± 0.2 and 0.09 ± 0.3 ng μg C⁻¹) (Fig. 5). Antioxidant production could be affected by either intrinsic factors, like growth rate, or extrinsic factors, as temperature, light and UV intensity (Asada 2006, Donato et al. 2003, Rijstenbil 2003). Mostly extrinsic factors as temperature and nutrients affect α-tocopherol production in phytoplankton. In May temperature was
negatively associated with α-tocopherol production in phytoplankton (Fig. 6 A), which could be a response to elevated ROS concentration as a result of temperature stress. Nutritional status of the phytoplankton community was also correlated with α-tocopherol synthesis (Fig 6 A). The positive association of α-tocopherol with particulate phosphate is likely due to the fact that the α-tocopherol synthesis depends on by-products from the SHIKIMATE pathway (DellaPenna & Pogson 2006, Johansson et al. 2005).

Pigment composition of the phytoplankton community was used to identify algae classes related to α-tocopherol synthesis and was assumed to mirror the physiological state of algae cells. Especially pigments involved in the xanthophyll cycle, in combination with their precursors, can be used as marker pigments (Casper-Lindley et al. 1998, Demmig-Adams et al. 2002, Falkowski & Raven 1997). Positive association of α-tocopherol with β-carotene was observed throughout all seasons and both years.

**Figure 5.** Boxplot of α-tocopherol L seawater\(^{-1}\) and μg C\(^{-1}\) in November, May, August and March 2004 and 2005 in the Baltic Sea proper of size classes: <100 μm (dominated by phytoplankton) and >200 μm (dominated by calanoid copepods). Observe different scales of the y-axis.

β-carotene is known to increase in response to oxidative stress (Abd El-Baky et al. 2009, Malanga & Puntarulo 1995, Ye et al. 2008). Diadinoxanthin was
also positively associated with \( \alpha \)-tocopherol except in March (Fig. 6 A). The diadinoxanthin pool size depends on light intensity and rises with increasing radiation (Lavaud et al. 2002, Griffith et al. 2010), suggesting that even \( \alpha \)-tocopherol synthesis was dependent on radiation intensity. The pigments associated with \( \alpha \)-tocopherol in our study match well with the dominant species of the phytoplankton at that time of the year, except in summer. In summer there was a positive association between concentrations of \( \alpha \)-tocopherol and prasinoxanthin (Fig. 6 A). However, cyanobacteria clearly dominated the phytoplankton community in the summers of 2004 and 2005 (Svenskt HavsARKiv; www.smhi.se/klimatdata). Indicating \( \alpha \)-tocopherol production did not depend on the dominating algae class in summer. The lack of association between biomass (\( \mu \)g C) and \( \alpha \)-tocopherol L\(^{-1}\) and the positive association of nitrate in the water column with \( \alpha \)-tocopherol \( \mu \)g C\(^{-1}\) in summer support this conclusion. The results suggest that algae dependent on solved nitrate in the water column are mainly responsible for \( \alpha \)-tocopherol production in summer, which excludes nitrogen-fixing cyanobacteria.

No consistent seasonal pattern could be observed in the \( \alpha \)-tocopherol concentration in mesozooplankton in 2004 and 2005. Average concentrations varied between 0.6 \( \pm \) 0.2 and 1.3 \( \pm \) 0.3 ng \( \alpha \)-tocopherol \( \mu \)g C\(^{-1}\) (Fig. 5). The balance between steady state of ROS production and antioxidants in zooplankton can be disturbed by toxins, e.g. cyanobacteria (Karjalainen et al. 2007), ageing (Rodríguez-Graña et al. 2010) and increased UV-radiation (Yu et al. 2009, Souza et al. 2010). These factors are rather unlikely to explain the observed pattern in our study. Factors associated to \( \alpha \)-tocopherol concentration \( \mu \)g L\(^{-1}\) in mesozooplankton were highly variable (Fig. 6 B). But most important is the negative association of phytoplankton biomass with \( \alpha \)-tocopherol C\(^{-1}\) in mesozooplankton, which mirrors the functional relationship between food availability and astaxanthin production (Holeton et al. 2009). The authors found that above 150 \( \mu \)g food concentration the astaxanthin concentration decreased in *Acartia bifilosa*. This scenario can be explained with reduced gut residence time, due to increased ingestion rate, which decreases assimilation efficiency of essential compounds in the food (Kørboe & Tiselius 1987). The biomass in spring and summer 2004 / 2005 is far above 150 \( \mu \)g C L\(^{-1}\), which make this scenario even likely for \( \alpha \)-tocopherol assimilation in the Baltic Sea. However, phytoplankton biomass is positive associated to \( \alpha \)-tocopherol in mesozooplankton in August, which is contradictory to the scenario in May. This could possibly be explained by the different species composition of phytoplankton in these months. The phytoplankton community in August was dominated by cyanobacteria (Svenskt HavsARKiv; www.smhi.se/klimatdata). The presence of cyanobacteria can decrease ingestion rates of mesozooplankton (Kleppel et al. 1998, Meyer-Harms &
von Bodungen (1997) and thus overlay the effect of increased phytoplankton biomass on \(\alpha\)-tocopherol concentration in mesozooplankton.

**Figure 6.** Regression coefficients (Variables of Importance (VIP) > 1.0) of the factors associated with \(\alpha\)-tocopherol concentration (ng \(\mu\)g C\(^{-1}\)) in the Baltic Sea, extracted from the significant PLS components: (A) size class < 100 \(\mu\)m and (B) size class >200 \(\mu\)m in March (N=18), May (N=20), August (N=23) and November (N=18); Regression coefficient with largest VIP at the bottom of the respective graph.
Carotenoids in the mesozooplankton (paper V)

About 99% of the total carotenoids in mesozooplankton consisted of astaxanthin and only 1% of canthaxanthin (Table 3). Our measured concentrations were in the range of earlier published studies from northern Europe (Łotocka & Styczynska-Jurewicz 2001, Łotocka et al 2004, Sommer et al. 2006). Astaxanthin occurred to 65% in the free form and 35% in the esterified form. There was a clear seasonal trend in the >200 μm size fraction with three times higher astaxanthin concentration in the cold months of March and November compared to summer (August). The all-E-astaxanthin isomer was dominant in the mesozooplankton. The proportion of astaxanthin esters showed a seasonal pattern as well. In March mono-esters were more abundant than di-esters, in November both esters occurred in equal amounts.

Table 3. Seasonal averages (±CV) of astaxanthin in the mesozooplankton (> 200 μm); (B) Baltic Sea, (KS) Kattegat-Skagerak area; (N) number of samples

<table>
<thead>
<tr>
<th>Astaxanthin</th>
<th>Area</th>
<th>Month</th>
<th>Year</th>
<th>N</th>
<th>All-E</th>
<th>9Z</th>
<th>13Z</th>
<th>mono-esters</th>
<th>di-esters</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>Mar</td>
<td>2004</td>
<td>9</td>
<td>0.86 ± 26%</td>
<td>0.08 ± 26%</td>
<td>0.21 ± 26%</td>
<td>0.30 ± 51%</td>
<td>0.26 ± 38%</td>
<td>1.70 ± 26%</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>Mar</td>
<td>2005</td>
<td>10</td>
<td>0.80 ± 36%</td>
<td>0.07 ± 59%</td>
<td>0.20 ± 36%</td>
<td>0.32 ± 28%</td>
<td>0.20 ± 37%</td>
<td>1.59 ± 30%</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>May</td>
<td>2004</td>
<td>14</td>
<td>0.41 ± 29%</td>
<td>0.07 ± 64%</td>
<td>0.07 ± 50%</td>
<td>0.26 ± 43%</td>
<td>0.06 ± 65%</td>
<td>0.87 ± 33%</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>May</td>
<td>2005</td>
<td>10</td>
<td>0.59 ± 26%</td>
<td>0.02 ± 30%</td>
<td>0.07 ± 21%</td>
<td>0.26 ± 18%</td>
<td>0.08 ± 55%</td>
<td>1.02 ± 16%</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>Aug</td>
<td>2004</td>
<td>9</td>
<td>0.19 ± 38%</td>
<td>0.02 ± 74%</td>
<td>0.08 ± 92%</td>
<td>0.08 ± 60%</td>
<td>0.03 ± 135%</td>
<td>0.40 ± 54%</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>Aug</td>
<td>2005</td>
<td>14</td>
<td>0.35 ± 30%</td>
<td>0.02 ± 81%</td>
<td>0.07 ± 38%</td>
<td>0.20 ± 38%</td>
<td>0.07 ± 78%</td>
<td>0.72 ± 33%</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>Nov</td>
<td>2004</td>
<td>9</td>
<td>0.79 ± 34%</td>
<td>0.05 ± 32%</td>
<td>0.31 ± 40%</td>
<td>0.34 ± 46%</td>
<td>0.37 ± 56%</td>
<td>1.85 ± 38%</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>Nov</td>
<td>2005</td>
<td>9</td>
<td>1.06 ± 29%</td>
<td>0.06 ± 40%</td>
<td>0.12 ± 31%</td>
<td>0.41 ± 27%</td>
<td>0.38 ± 46%</td>
<td>2.04 ± 18%</td>
<td></td>
</tr>
<tr>
<td>KS</td>
<td>May</td>
<td>2004</td>
<td>2</td>
<td>0.28 ± 24%</td>
<td>0.02 ± 6%</td>
<td>0.05 ± 12%</td>
<td>0.14 ± 17%</td>
<td>0.08 ± 41%</td>
<td>0.58 ± 9%</td>
<td></td>
</tr>
<tr>
<td>KS</td>
<td>May</td>
<td>2005</td>
<td>2</td>
<td>0.44 ± 46%</td>
<td>0.02 ± 24%</td>
<td>0.07 ± 2%</td>
<td>0.33 ± 35%</td>
<td>0.13 ± 32%</td>
<td>0.99 ± 36%</td>
<td></td>
</tr>
<tr>
<td>KS</td>
<td>Aug</td>
<td>2004</td>
<td>3</td>
<td>0.51 ± 27%</td>
<td>0.03 ± 38%</td>
<td>0.17 ± 45%</td>
<td>0.23 ± 13%</td>
<td>0.09 ± 21%</td>
<td>1.04 ± 24%</td>
<td></td>
</tr>
<tr>
<td>KS</td>
<td>Aug</td>
<td>2005</td>
<td>3</td>
<td>0.45 ± 21%</td>
<td>0.02 ± 51%</td>
<td>0.10 ± 24%</td>
<td>0.20 ± 35%</td>
<td>0.11 ± 20%</td>
<td>0.88 ± 23%</td>
<td></td>
</tr>
</tbody>
</table>
Acartia spp. vary in copepods with ontogeny. Łotocka & Styczynska-Jurewicz (2001) reported that esters comprised 4-5 % in nauplii, 34-40 % in copepodid stages I-III and 48-54 % in adults and copepodid stages IV-V in Acartia bifilosa. Our samples were dominated by adults (>200 μm), which would exclude ontogenetic effects. More probably the seasonality of astaxanthin esters could be explained by the function of astaxanthin esters to protect storage lipids (Sommer et al. 2006).

Most variation of the total astaxanthin concentration in the mesozooplankton could be explained by the occurrence of either cladocerans (lower astaxanthin, higher canthaxanthin) or copepods (higher astaxanthin, lower canthaxanthin concentration) (Fig. 7). Acartia spp. is not grouped with the other copepods, suggesting a lower astaxanthin concentration in this genus, compared to other copepods. Acartia spp. always dominated in the mesozooplankton community. Thus seasonal variation in total astaxanthin concentration could not be attributed to changes in species composition. Instead, is it likely, that lipids are stored with astaxanthin protection in winter, while in warmer seasons lipids are used for growth. In addition physiological changes with season in copepods, such as egg production could explain astaxanthin variation. The peak in egg production of Pseudocalanus acuspes in the central Baltic has been reported to be in April.

Figure 7. PCA analysis with mesozooplankton genus composition of 94 sampling stations.
(Renz et al. 2007), which is consistent with higher astaxanthin concentrations in March compared to May in our study.

Astaxanthin concentrations in fish (paper VI)

With this field study we could show, that salmon and herring is suffering from astaxanthin deficiency, whereas cod and sprat not. We identified herring as non optimal food in terms of astaxanthin concentration, because of the high whole-body concentration of astaxanthin z-isomers, which have low bioavailability for salmon and cod.

Astaxanthin in different fish tissues

In the sampled fish species the isomeric composition of astaxanthin varied between different tissues, but not between areas and season (paper VI). The isomeric composition in cod and salmon was more similar to sprat muscle, liver and stomach, than to any of the herring tissues.

Astaxanthin concentrations in the gonads (per fresh weight) varied with seasons, but not with area in herring and sprat. Highest gonad concentrations were found in November, with averages of 8.9, 2.6 and 5.2 μg g⁻¹ for sprat, herring and cod, respectively (paper VI). Salmon gonads contained only 0.8 μg g⁻¹ astaxanthin. The liver and muscle concentrations were much lower than in gonads and no variation with season or area could be observed. The obtained salmon muscle concentrations confirm that Baltic Sea salmon suffer from astaxanthin deficiency. Muscle values varied between 0.02 and 0.06 μg g⁻¹, which are low compared to previously published data, e.g. 3-8 μg g⁻¹ for wild salmons caught in Europe and Canada (Schiedt et al. 1981) and 3-11 μg g⁻¹ in cultivated Atlantic salmon (Czeczuga et al. 2005).

Stomach concentrations of astaxanthin varied with

![Figure 5](image-url)
season only in herring, with lowest values in November. In all species sampled astaxanthin concentration per fresh weight in the different tissues was independent of fish body weight.

Astaxanthin model

Based on a simple model we estimated total body astaxanthin concentration in sprat and herring. Whole-body concentrations calculated for sprat were 0.150 μg g⁻¹ in March and 0.120 μg g⁻¹ in November and for herring 0.114 μg g⁻¹ and 0.090 μg g⁻¹, respectively. All-E-astaxanthin was estimated to about 0.065 μg g⁻¹ in sprat in both seasons, and to 0.037 μg g⁻¹ in March and 0.017 μg g⁻¹ in November in herring. We conclude that herring showed indications of astaxanthin deficiency, because of the large difference to its close relative sprat. Furthermore, sprat apparently represents a better food source for piscivorous fish in terms of total astaxanthin concentration and isomeric composition than herring does (Fig. 8).

The astaxanthin deficiency in herring could also be one explanation for the different astaxanthin concentration in salmon in the north Atlantic and the Baltic Sea. Atlantic salmon feeds only on two species in the Baltic Sea: herring and sprat (Salminen et al. 2001), and not on crustaceans as in the North Atlantic (Jacobsen & Hansen 1996). Cod feeds a much more varied diet than salmon in the Baltic Sea, including besides herring and sprat, isopods, mysids, polychaetes and other invertebrates (Uzars 1994). The feeding habits of salmon in combination with the high body concentration of astaxanthin Z-isomers, that have low bioavailability, can explain the astaxanthin deficiency of the salmon in the Baltic Sea.

Conclusions

Heterotrophs cannot survive without autotrophs, which produce a high diversity of essential biochemicals, such as ω3- polyunsaturated fatty acids (PUFAs), vitamins and pigments. So far non-enzymatic antioxidants are only sporadically studied in the context of food web ecology. In order to fill this gap we combined field expeditions with laboratory experiments to reveal patterns in production, consumption and transport of non-enzymatic antioxidants in the pelagic food web of the Baltic Sea. First, we developed a simplified and improved extraction protocol, which makes large-scale studies of tocopherol (Vitamin E) possible.

We could show that moderate alteration of temperature and salinity can increase production of α-tocopherol, β-carotene and thiamine in microalgae. Furthermore, the results indicate that the duration of the stress is important, as apparently only acute stress enhance non-enzymatic antioxidant production. The availability of these biochemicals depends also on the
composition of the phytoplankton community. This combination makes it difficult to predict future levels of non-enzymatic antioxidant production in aquatic ecosystems. However, it likely to be very variable, even within shorter time periods. It is therefore of interest to examine how zooplankton copes with variation in the available non-enzymatic antioxidant pool, whether zooplankton is the bottle neck in the transition of non-enzymatic antioxidants to higher levels in the food web, or if whether zooplankton functions as a buffer in balancing out possible fluctuations of the available non-enzymatic pool.

We could show that the transfer of α-tocopherol to zooplankton is negatively affected by phytoplankton biomass. Thus ingestion rate and assimilation efficiency of copepods determines the concentration of α-tocopherol in mesozooplankton, as previously shown for astaxanthin (Holeton et al. 2009). The high degree of eutrophication in the Baltic Sea is likely to decrease the availability of essential biochemicals for higher levels within the food web. Consequently eutrophication could be one explanation for the astaxanthin deficiency in Baltic Sea herring and its main predator salmon.
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Stress i näringsväven


Östersjön, mänsklig påverkan och tryck på ekosystemen

djurplankton i Östersjön. Antalet skarpsill har ökat, medan sill, torsk och lax har minskat i antal. Redan 1974 upptäckte forskare att laxen i Östersjön hade svårt att föröka sig. Man antog att fortplantningsstörningen var miljörelaterad och kallade den därför för M74. Senare fann forskare att brist av antixodianter och vitamin B1 orsakade M74 hos lax. I min avhandling har jag undersökt om det finns ett samband mellan de storskaliga förändringar som skett i Östersjön och bristen av essentiella ämnen i näringsväven. Eftersom näringsväven i öppet vatten är kort och okomplex utgör Östersjön ett utmärkt studiesystem för att undersöka flöden av olika ämnen i näringsväven. Djurplankton åter växtplankton, som i sin tur äts av mindre fiskar som skarpsill och sill som i sin tur äts av olika predatörer som torsk och lax.

För att undersöka ämnesflöden i den näringsväven i Östersjön koncentrerade jag mig på vitamin E (tocopheroler), som skyddar cellmembraner mot oxidativ stress, astaxanthin, en viktigt anitoxidant i djurplankton och vitmain B1 (tiamin). Jag ville veta hur olika miljöfaktorer kan påverka produktionen av dessa ämnen i växtplankton. Vad styr överföringen av antioxidanter från växtplankton till djurplankton? Och hur kan brist av antioxidanter uppstå hos fisk i Östersjön?

Antioxidantproduktion i växtplankton
Med hjälp av mina kolleger kunde jag visa att vitamin E, vitamin B1 och produktionen av karotenoider i växtplankton främst beror på temperatur och salthalt. Även mindre ändringar i de två faktorerna kan höja produktionen. Dessutom kan man fastslå att kortvariga avvikelser i temperatur och salthalt kan höja antioxidanthalten i växtplankton medan långvariga avvikelser kan höja enzymaktiviteten, något som inte gynnar konsumenten. Men det är svårt att förutse förändringar i produktionen av antioxidanter i Östersjön eftersom produktionen även beror på artsammansättningen av växtplankton.

Produktion, överföring och konsumtion av antioxidanter i Östersjön
Vi kunde hitta ett tydligt säsongbetonat mönster i halten av vitamin E i växtplankton och djurplankton. Produktionen av vitamin E och karotenoider i växtplankton var högst under maj månad i Östersjön. De högsta värdena i djurplankton kunde uppmätas under de kalla månaderna mars och november. Det fanns ett tydligt negativt samband mellan biomassen av växtplankton och halten av vitamin E i djurplankton. Om det finns mycket växtplankton ökar djurplanktonets födointag vilket ger ett sämre näringsupptag då födan går snabbare genom matsmältningssystemet. Därför kan eutrofieringen, vilket ger en ökad mängd växtplankton, ha negativa konsekvenser för halten.
antioxidanter i djurplankton och följdaktligen också i fisk. Så visade vi, att sill, liksom lax, lider av astaxanthinbrist. Astaxanthin är en viktig antioxidant som fisk kan inte producera själva utan måste få från djurplankton. Produktionen av astaxanthin i djurplankton i sin tur, beror på att förstadierna av ämnet syntetiserats i växtplankton.

Slutsatser
Stress im Nahrungsnetz


Die Ostsee, ein gefährdetes Ökosystem

Die Ostsee ist eines der am stärksten verschmutzten aquatischen Ökosysteme der Welt. Sie ist ein vergleichsweise junges Meer mit einer geringen Artenvielfalt und einem Salzgehaltsgradienten von Süden nach Norden. Im


Produktion von Antioxidantien im Phytoplankton
Produktion, Transfer und Konsum von Antioxidantien in der Ostsee


Fazit

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