Sources and composition of particulate organic carbon in the Baltic Sea: the use of plant pigments and lignin-phenols as biomarkers

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ABSTRACT. Water samples were collected in the Baltic Sea, along a north to south transect that extended from Bothnian Bay to the Baltic proper, in July 1994—just after the period of peak freshwater discharge. Plant pigments and lignin-phenols were used as biomarkers to determine the dominant sources of phytoplankton and terrestrial inputs along the transect. A distinct gradient in chlorophyll a concentrations that increased from north (2.1 µg L⁻¹) to south (18.9 µg L⁻¹) indicated that the dominant blooms occurred from the southern Bothnian Sea to the Baltic proper. High fucoxanthin (3.9 µg L⁻¹) and peridinin (1.9 µg L⁻¹) concentrations in the Bothnian Bay and Sea reflected late stages of diatom and dinoflagellate blooms, respectively, that typically occur in summer months in the northern Baltic (Andersson et al. 1996). High zeaxanthin (8 µg L⁻¹) concentrations in the southern Bothnian Sea and Baltic proper indicated dominance of cyanobacterial blooms in these regions. Decreasing concentrations of total lignin-phenols (µ) from north (0.22) to south (0.02) indicated that terrestrial inputs of organic carbon were associated with high freshwater inflow into the northern Baltic. Further examination of lignin-phenol ratios indicated that the dominant source of terrestrial material into the Baltic was from woody gymnosperms.

KEY WORDS: Biomarkers · Baltic Sea · Carbon cycling · Plant pigments · Lignin-phenols

INTRODUCTION

Few marine areas in the world have received more research attention than the Baltic Sea. Almost completely land-locked, the Baltic is largely influenced by the characteristics of its drainage basin. The surface area of the drainage basin is more than 4 times as large as the sea itself (Ehlin 1981). The Baltic Sea is a non-tidal, brackish, estuarine water body consisting of the following 5 major basins: Bothnian Bay (BB); Bothnian Sea (BS); Gulf of Finland (GF); Gulf of Riga (GR); Baltic proper (BP). Surface salinities in the Baltic range from ~2% in the northernmost BB to ~10% at the Danish sounds. The basins differ substantially in the percentage of freshwater input relative to the basin volume. Terrestrial inputs are considered to be most pronounced in the GF, BB and GR, and less important in BS and BP (Elmgren 1984, Wulf & Stigenbrandt 1989, Zweifel et al. 1995). However, the characteristics and fate of this allochthonous material in the basins are still largely unknown.

Information on the balance of organic matter inputs in the basins of the Baltic was first reviewed and synthesized by Elmgren (1984). There has been considerable attention given to the importance of the microheterotrophic pathway of carbon utilization (Larsson & Hagström 1979, 1982, Zweifel et al. 1993, 1995). In a system like the Baltic this potential energy source (via microheterotrophs) could be of considerable importance for secondary production and, if utilized, impose quite different characteristics on the trophic structure of the different basins. The first attempt to estimate bacterial production was made by Larsson & Hagström (1982)—the subject has been further discussed in 2 recent articles by Zweifel et al. (1993, 1995). Estimating the actual availability of allochthonous materials...
(quantitatively) in a field situation remains difficult in most systems. A qualitative description of the character and origin of allochthonous materials provides a basis and useful prerequisite for investigations of its availability.

Plant pigments and lignin-phenols have been shown to be useful biomarkers for the characterization of organic matter and its sources in land-margin aquatic ecosystems (Hedges & Ertel 1982, Ertel & Hedges 1984, Hedges et al. 1988, Bianchi et al. 1993a, b). In this paper we present to our knowledge the first data set of these biomarkers in the Baltic Sea. Samples were taken on 1 cruise during June 1994 following a gradient of decreasing terrestrial influence from the northern BB via the Bothnian Sea north (BSN) and south (BSS) to the BP (Fig. 1). Two of the major rivers flowing into the BB and BS were also sampled for comparative purposes and source evaluation. Thus, the primary objectives of this study were as follows: (1) to characterize the dominant changes in phytoplankton composition (and blooms) using class-specific plant pigments (carotenoids); (2) to characterize differences in terrestrial inputs of particulate organic carbon (POC) using lignin-phenols.

METHODS

Sampling. Water samples were collected in July 1994 during a 5 d cruise (July 14 to 18) aboard the RV 'Fyrvägaren' along a north to south transect in the Baltic. Whole water GF/F filtrates of surface water were collected from 2 river stations and 4 offshore stations. Water depths of the offshore stations varied between 85 and 110 m (Fig. 1). The 4 stations represented at the sampling time a salinity gradient from BB (3%), BSN (5%), BSS (6%), to BP (7%) at a sampling depth of 6 m. The 2 rivers, Luleälven (Lu) and Ångermanälven (Án), empty into the BB and BS, respectively, and are the main Swedish rivers emptying into their respective basin, second only to the Kemijoki River (in Finland) which has a mean annual flow rate of 580 m$^3$ s$^{-1}$. The drainage basins for both rivers are largely dominated by managed coniferous forests. Both Lu and Án are hydropower regulated, and both have mean annual flow rates of ~550 m$^3$ s$^{-1}$. Samples taken for this study were obtained shortly after the annual peak flow of freshwater into the Baltic. The within year fluctuations in flow rates are however considerably less pronounced in these rivers than in unregulated rivers.

Water samples at river stations were taken at the river mouths and were collected by immersing a 10 l polyethylene container 3 dm below the surface and opening it. At offshore stations, 10 l of water was collected from 6 m depth by vacuum suction through a stiff polyethylene tube into a polypropylene vacuum container; this method provided valve-free transportation with minimal friction forces acting on the organisms during transport to the container. Microscopical analyses revealed that the fine structures (e.g. bristles, antennae, and flagellae) of organisms sampled by this method were intact. Samples were vacuum filtered through 47 mm Whatman GF/F filters (nominal pore size 0.7 μm) until water flow ceased (6 to 8 l). The filters were wrapped in aluminum foil and immediately frozen (-22°C). All samples were taken in triplicate.

Plant pigments. Plant pigments were extracted from GF/F filters using 100% acetone (see Bianchi et al. 1995 for further details on extraction procedures). Canthaxanthin was placed in all acetone extracts as an internal standard. Reversed-phase high performance liquid chromatography (RP-HPLC) was used for plant pigment analyses according to the method of Wright et
al. (1991) as modified by Bianchi et al. (1995). High purity standards for chlorophylls \(a\) and \(b\) (chl \(a\) and \(b\)) were obtained from Sigma Co. Standards of fucoxanthin, lutein, peridinin, and zeaxanthin were kindly provided by Hoffman LaRoche Co., Basel, Switzerland. Standard precision for plant pigments ranged between 3 and 4%, based on 3 replicates.

**Microscopic analyses.** Plankton for microscopic analysis were collected in a 6 \(\mu m\) net at each sampling occasion by vacuum suction from a depth of 6 m through a stiff polyethylene tube into a polpropylene vacuum container. Plankton within the container were continuously separated into 3 size fractions (6–20, 20–50, and 50–100 \(m\)) to avoid clogging; samples were examined microscopically, and no samples were taken in the 2 rivers (Table 1). Dominant species were identified, and their total abundance in all size classes was estimated subjectively on a 3 level relative scale (sporadic occurrence, common, dominant). No attempt was made to convert this to a measure of biomass or volume.

**Lignin-phenols.** Lignin-phenols were extracted using the method of Hedges \& Ertel (1982) as modified by Opsahl \& Benner (1993). Capillary column gas chromatography was used to analyze lignin-phenols in particulates according to the procedure of Bianchi et al. (1997). Phenolic standards were obtained from Sigma Chemical Co. Calculated response factors for phenolic standards and peak areas were used to determine actual phenol concentrations based on the recovery of the internal standard (ethyl vanillin). Peak identities were confirmed using gas chromatography-mass spectrometry (GC-MS). Standard precision for lignin-phenols ranged between 5 and 15\%, based on 2 replicates.

Lignin-phenol concentrations are presented using the lambda indices that have typically been used to estimate the relative contribution of total lignin-phenols to total organic carbon. For example, lambda (A) is defined as the total weight in milligrams of the sum of vanillyl (V) and syringyl (S) index phenols produced from the oxidation of 100 mg organic carbon (OC), and \(\text{LAMBDA} \ (A)\) is the total weight in milligrams of the sum of V, S, and cinnamyl (C) index phenols produced from the oxidation of 100 mg OC (Hedges \& Parker 1976, Hedges \& Mann 1979). The p-hydroxy phenols were not included in our discussion because these phenols can also be produced by non-lignin constituents (Wilson et al. 1985).

**Carbon analysis.** Due to the use of the remainder of the filter for lignin-phenol analysis, OC content was determined on the particles of the permeate from the sampling for microscopical analysis (i.e. particulate matter from 0.7 to 6 \(\mu m\)). After prefiltering through a 6 \(\mu m\) mesh filter the water was vacuum filtered through 47 mm Whatman GF/F filters until water flow ceased (6 to 8 \(l\)). There is a risk that this may cause an underestimate in the carbon content of whole water filters, but the 0.7 to 6 \(\mu m\) fraction was the limiting factor for clogging of the GF/F filters. The carbon content of the filters was determined by a Carlo-Erba CHN-analyzer.

**Statistics.** Analysis of variance (ANOVA) was used to test for significant differences in pigment and lignin-phenol concentrations among stations. Homogeneity of variances was tested with a Cochran C-test. All variances of plant pigment and total lignin-phenol data were homogenous, except for the S/V and the C/V ratios. To correct for non-homogeneity of variances, all ratios were treated with an arcsine transformation prior to ANOVA. A Fisher's least significant differences (LSD) procedure was used to determine specific differences in mean concentrations of pigments and lignin-phenols between stations. A Pearson correlation

### Table 1. Relative estimates of phytoplankton species composition and abundance in open Baltic Sea samples. Estimates of abundance were made on a 3 level relative scale (+ sporadic, ++ common, +++ dominating) and were not based on actual cell counts. Plant pigment biomarkers characteristic of different classes of phytoplankton are provided for comparison (Rowan 1989)

<table>
<thead>
<tr>
<th>Class</th>
<th>Chl (a)</th>
<th>Chl (b)</th>
<th>Fucoxanthin</th>
<th>Lutein</th>
<th>Peridinin</th>
<th>Zeaxanthin</th>
<th>Genus or species</th>
<th>Strn</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacillariophyceae</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Chaetoceros spp.</td>
<td>+++</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Thalassiosira spp.</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Dinophysalis acuminata</td>
<td>+++</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Dinophysis nageyica</td>
<td>+</td>
</tr>
<tr>
<td>Dinophyceae</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>Dinobryon spp.</td>
<td>+</td>
</tr>
<tr>
<td>Chrysophyceae</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>Nodularia spumigens</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Aphanizomenon flos-aquae</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Cryptomonas and Rhizomonas</td>
<td>+</td>
</tr>
<tr>
<td>Cryptophyceae</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Pollen of Scots pine</td>
<td>+</td>
</tr>
<tr>
<td>Other</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Pinus sylvestris</td>
<td>+</td>
</tr>
</tbody>
</table>
Chlorophyll concentrations were also significantly different among stations (ANOVA, $F = 279, p < 0.001$) and ranged from 0.8 ± 0.1 to 2.6 ± 0.2 μg l$^{-1}$. Chlorophyll b/a ratios generally decreased in the north to south gradient and ranged from 0.14 to 0.46 (Fig. 2A). Concentrations of lutein (a marker for chlorophytes) were significantly different among stations (ANOVA, $F = 396, p < 0.001$) (Fig. 2B). The highest concentration of lutein (3.2 ± 0.5 μg l$^{-1}$) occurred at Stn BP. Lutein concentrations were highly correlated with chlorophyll a and b concentrations ($r^2 = 0.98, n = 18, p < 0.001$) and ($r^2 = 0.98, n = 18, p < 0.001$), respectively (Fig. 2B). Zeaxanthin concentrations (a marker for cyanobacteria) differed significantly among stations (ANOVA, $F = 324, p < 0.001$, respectively) (Fig. 2B). Zeaxanthin and chlorophyll a concentrations were significantly correlated among stations ($r^2 = 0.99, n = 18, p < 0.001$), with the highest concentrations occurring at Stn BP (Fig. 2B). Concentrations of fucoxanthin (a marker for diatoms) and peridinin (a marker for dinoflagellates) were significantly different among stations (ANOVA, $F = 450, p < 0.001$ and ANOVA, $F = 324, p < 0.001$, respectively) (Fig. 2B). Unlike zeaxanthin, fucoxanthin and peridinin were not correlated with chlorophyll a concentrations, ($r^2 = 0.17, n = 18, p > 0.05$; and $r^2 = 0.10, n = 18, p > 0.05$). Conversely, the highest concentrations of fucoxanthin (3.9 ± 0.2 μg l$^{-1}$) and peridinin (1.9 ± 0.1 μg l$^{-1}$) were found at Stn BSN (Fig. 2B).

Microscopical analysis of phytoplankton samples (>6 μm) generally agreed with pigment data (Table 1). At Stns BB, BSN, and BSS phytoplankton were dominated by diatoms mainly from the genera Chaetoceros and Thalassiosira. Starting at Stn BSN and going south, there was an increase in the occurrence of dinoflagellates from the genus Dinophysis. At Stn BP dinoflagellates and cyanobacteria dominated the phytoplankton (Table 1). The presence of Aphani-zomenon flos-aquae in the cyanobacterial community increased from Stns BSN to BP. At the time of sampling, Stn BP was characterized by a substantial cyanobacterial bloom consisting of A. flos-aquae and Nodularia spumigena. Mid-summer cyanobacterial blooms, consisting mainly of these 2 species, are a typical feature of the Baltic proper. At Stns BSN and BSS the 2 cryptomonad genera Cryptomonas and Rhodomonas were present in large numbers; there was also sporadic occurrence of different species of the chrysophycean genus Dinobryon.
Lignin-phenols

Total lignin-phenols (A) and (λ) across all stations ranged from 0.015 to 0.22 and 0.017 to 0.21, respectively (Fig. 3A). Both A (ANOVA, F = 26, p < 0.05) and λ (ANOVA, F = 18, p < 0.05) values differed significantly among stations. The highest values of A (mean = 0.22, SD = ±0.03 mg V+S+C/100 mg OC) and λ (mean = 0.21, SD = ±0.04 mg V+S/100 mg OC) were found at Stn Lu which was located in the Luleälven River. Conversely, the lowest values for A and λ were found at the southern end of the transect at Stns BSS and BP, respectively (Fig. 3A). S:V ratios ranged from 0.3 to 0.5 with the highest values occurring at Stn An (0.5 ± 0.1) (Fig. 3B). C:V ratios ranged from 0.09 to 0.36 with the highest values at Stns BSN (0.28 ± 0.1) and BSS (0.36 ± 0.03) (Fig. 3B). Based on microscopical analysis, the 2 northernmost Stns BB and BSN had significant amounts of pollen derived from Scots pine.

DISCUSSION

Phytoplankton gradient

Changes in plant pigment concentrations along our sampling transect generally reflected the current temporal and spatial dynamics of blooms in the Baltic—from a north to south gradient. Annual phytoplankton production estimates reported by Elmgren (1984)—across the same north-south gradient (25 g C m⁻² yr⁻¹ in the BB to 160 g C m⁻² yr⁻¹ in the BP)—are consistent with the trends that we observed in our chl a concentrations. The cyanobacterial bloom at Stn BP was most likely responsible for the high chl a concentration (18.9 μg l⁻¹) at this station. Chl b concentrations generally agree with those of chl a. However, there were no identified chlorophytes (which contain chl b and lutein) in the species composition to corroborate the observed pattern in either chl b or lutein concentrations. Small species of chlorophytes, e.g. Microcystis pusilla (3 to 5 μm) and Pseudoscutocellidida marina (2 to 3 μm), are common in the Baltic (Tikkanen & Willen 1992) and would easily pass a 6 μm mesh. It is likely that chlorophytes smaller than 6 μm were present on the GF/F filters used for pigment analyses. Chlorophytes did not appear to represent a significant component of the phytoplankton in the Baltic at this time of year, but they are present based on work by Andersson et al. (1996). The most significant contribution of chlorophyte pigment occurred at Stn BP, where lutein and chl b concentrations were highest. The low abundance of chlorophytes in this region (for the period of July) supports the findings from a recent study in this region (Andersson et al. 1996).

At the time of our sampling, the spring bloom of diatoms and dinoflagellates (Skeletonema, Thalassiosira, Chaetoceros, Peridinella, and Peridiniurn) had completely died out in the BP and BSS; however, blooms were still in the last stages of decline in the BSN and BB. Late diatom and dinoflagellate spring blooms (Skeletonema, Thalassiosira, and Dinophysis) at northern stations (BB to BSS) were reflected in higher levels of fucoxanthin (~2 to 4 μg l⁻¹) and peri-
significant compared to other regions of the Baltic and concentrations of fucoxanthin and peridinin in bothterigenous inputs to sediments in the Bothnian Bay were est phytoplankton abundance occurring from the the greatest freshwater inputs also occur (Elmgren north (-2.1 pg l⁻¹) to south (-18.9 pg l⁻¹) with the high-allochthonous material in the northern Baltic whereous suggestions that there are higher inputs of the Baltic during peak flow period (July 1994), we have centrations in POC across a north to south gradient in lignin-phenol data, suggesting a decreasing gradient from north to south in terrestrially derived inputs to the Baltic (C. Roliff unpubl.). These results support previu suggestions that there are higher inputs of allochthonous material in the northern Baltic where the greatest freshwater inputs also occur (Elmgren 1984). In an earlier study it was also concluded that t-igenous inputs to sediments in the Bothnian Bay were significant compared to other regions of the Baltic and

Terrestrially derived inputs

Lignin-phenols indicated that inputs of terrestrially derived particulate organic matter are greater in the north than in the south of the Baltic Sea. The samples richest in terrestrially derived organic matter were those from the Luleälven River, which discharges into the BB. It should be noted that lignin-phenol values (λ and Λ) may have been overestimated since they were normalized to a particulate size fraction of carbon (0.7 to 6 μm)—not the full spectrum of POC particles. However, stable carbon isotope analyses on water particulates in the size interval of 0.7 to 6 μm support the lignin-phenol data, suggesting a decreasing gradient from north to south in terrestrially derived inputs to the Baltic (C. Roliff unpubl.). These results support previous suggestions that there are higher inputs of allochthonous material in the northern Baltic where the greatest freshwater inputs also occur (Elmgren 1984). In an earlier study it was also concluded that terrigenous inputs to sediments in the Bothnian Bay were significant compared to other regions of the Baltic and primarily consisted of land humus (Gripenberg 1934). Total lignin-phenols at Stn Lu (Luleälven River) were significantly higher than those found at Stn Än (Ängermanälven River). In fact, total lignin-phenol concentrations at Stn Än were not significantly different from concentrations found in the BB and BSN. Stn Än is situated at the mouth of the Ängermanälven River. The depth of the river at this station is considerable (19 m), and the water mass is affected by wind-transported seawater that displaces river water. It should also be noted that while we have only discussed POC inputs, much of the terrigenous inputs from rivers in the northern Baltic may be in the form of dissolved organic matter (DOM) (Zweifel et al. 1995). It has also been shown that this refractory DOM may provide the carbon demands for a significant fraction of the bacterial production (22 to 99%) depending on the availability of nutrients (Zweifel et al. 1995).

The low S:V and C:V ratios at all stations in the Baltic indicated that the dominant sources of lignin were derived from woody gymnosperm tissues (Hedges & Parker 1976, Hedges & Mann 1979). While S:V ratios are slightly higher (0.3 to 0.5) than observed in other regions where gymnosperm inputs dominate (0.1 to 0.3), low C:V ratios (0.1 to 0.4) confirm that these inputs are within the range typically found for woody gymnosperm tissues (Hedges & Mann 1979). Basically, vanillyl phenols are lignin products in both woody and herbaceous tissues, while syringyl phenols are present in angiosperm tissues but very low in gymnosperm tissues (Hedges & Mann 1979). Lignin-derived cinnamyl phenols are almost exclusively from the oxidation of herbaceous tissues (Hedges & Mann 1979). While it has been shown that selective degradation of certain lignin-phenols can lead to misinterpretations of source materials, recent lab studies have confirmed that S:V and C:V ratios of senescent and highly degraded tissues are similar (Haddad et al. 1992, Opsahl & Benner 1993, 1995). The dominant signatures of gymnosperm tissues in the Baltic, as indicated by the low S:V ratios, are not surprising since most of the drainage basins into the Baltic are dominated by managed coniferous forests.

CONCLUSIONS

On the basis of plant pigment and lignin-phenol concentrations in POC across a north to south gradient in the Baltic during peak flow period (July 1994), we have concluded the following: (1) There was a strong gradient in the concentration of chl a that increases from north (~2.1 μg l⁻¹) to south (~18.9 μg l⁻¹) with the highest phytoplankton abundance occurring from the southern Bothnian Sea to the Baltic proper. (2) High concentrations of fucoxanthin and peridinin in Both-
nian Bay and Bothnian Sea in June reflected late stages of diatom and dinoflagellate blooms that typically occur in these northern regions of the Baltic. (3) High concentrations of zeaxanthin in the southern Bothnian Sea and Baltic proper reflected the dominance of cyanobacterial blooms in these regions during summer months. (4) A decreasing concentration gradient of lignin-phenols from north to south indicated that the highest inputs of terrestrially derived POC are from the northern Baltic, where the highest freshwater discharge occurs. (5) Based on lignin-phenol biomarkers, the dominant source of terrestrially derived vascular plant materials to the Baltic is from woody gymnosperms.

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