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Trace metal/phytoplankton interactions in the Skagerrak

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Abstract

Algal community species composition, as estimated by high performance liquid chromatography (HPLC) pigments and microscopy analysis, and trace metal speciation (Cu and Co) and distributions (Fe, Zn, Co and Cu) were measured along a summer transect across the Skagerrak. In waters of Baltic origin, with elevated trace metals levels, but very low macronutrients, a mix of dinoflagellates and haptophytes dominated the low biomass. In the Jutland current, which had high dissolved iron concentrations, a mixed bloom (4–6 μg/l chl *a*) of diatoms (major species—*Leptocylindricus danica*) and dinoflagellates (*Ceratium* sp.) was present. In the waters of the central Skagerrak derived from the North Sea, below the low salinity Baltic water, a large diatom (major species—*L. danica*) bloom (7.7 μg/l) was present at 35 m. This bloom formed below the pycnocline, and was located at the nutricline for silicate. The lowest concentrations of trace metals were found in the water of North Sea origin. *Synechococcus*-like cyanobacteria were observed in the upper waters across the survey area, as were strong binding ligands for Cu, but no clear numerical relationship existed between them, as had been observed by Moffett [Deep-Sea Res. 42 (1995) 1273]in the Sargasso Sea. The [Co]/[Zn] hypothesis of Sunda and Huntsman [Limnol. Oceanogr. 40 (1995) 1404] for coccolithophorids and diatoms was examined using the field data collected.

Keywords: Skagerrak; Trace metals; Phytoplankton

1. Introduction

The Skagerrak, along with the Kattegat, forms the outer part of the estuary of the Baltic Sea system. The Skagerrak is a deep basin (maximum depth 700 m) with a mean depth of 200 m and a sill to the south at 270 m, through the Norwegian Trench, giving it a fjord-like character. The basic circulation is of a counter-clockwise gyre (surface current speeds 10–20 cm s^{-1}), which is dominated by out-flowing Baltic water at the surface with a salinity of 25 to 30 (Rodhe, 1996). Below this surface layer is a layer of North Sea water with salinity 33–35. Atlantic water (North Sea) with salinities exceeding 35 enters the Skagerrak from the northwest and forms the intermediate and deep waters of the Skagerrak. A further feature of the Skagerrak is a mixture of various North Sea waters entering the region from the west and southwest, predominantly as surface water. This water has slightly lower salinities (31–35) and indicates either returning Skagerrak water or polluted water from the southern North Sea, supplied by the Jutland Current. Occasionally during high river flows, mainly from the Elbe, elevated levels of nutrients (Rydberg et al., 1996) and

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suspended particulate matter (SPM) (Rodhe and Holt, 1996) are found in the Jutland Current.

The average distributions of the macronutrients (silicate, phosphate and nitrate) in the Skagerrak have been found to show a general similarity (Rydberg et al., 1996). The maximum nitrate concentrations in the Skagerrak 9–10 μM are found in the Atlantic deep-water inflow ($S>35$) (Rydberg et al., 1996). Lowest values, 2–3 μM, are found in the surface waters of the central Skagerrak. Higher values are found close to the Danish coast, where influx of nutrient-rich waters from the southern North Sea and continental rivers can be important. Silicate concentrations are typically 2–3 μM in surface waters, increasing to 4–5 μM at 100-m depth. A strong seasonal influence is also seen on the levels of these macronutrients found in the Skagerrak, with the lowest values found during the summer, when all three macronutrients can be strongly depleted from surface waters.

The Skagerrak has a long history of plankton investigations, and these studies reflect the development of methods to examine phytoplankton species distribution. The first studies were carried out by Cleve (1897) using net hauls, Gran (1915) with the centrifugation method and Braarud et al. (1953) employing sedimentation chambers. Later studies have also employed epifluorescence microscopy for picoplankton (Karlson, 1995; Karlson and Nilsson, 1991) and pigment analysis by high performance liquid chromatography (HPLC) (Karlson et al., 1996) to further identify the phytoplankton species present in the Skagerrak. Typically, the diatom-dominated spring bloom starts during the period from February to the beginning of April, depending on the stratification, often lasting up to 3 weeks (Lindahl and Hernroth, 1983). During the summer period, surface waters are depleted of nutrients, resulting in oligotrophic conditions and an ecosystem that is dominated by the microbial loop (Kuylenstierna and Karlson, 1994). The Skagerrak has in recent years also seen a number of toxic or nuisance blooms of phytoplankton (i.e. *Gyrodinium aureolum*: Lindahl, 1985; *Chrysochromulina polylepis*: Lindahl and Dahl, 1990; Nielsen et al., 1990).

There have been few studies on the trace metal distribution in the Skagerrak. In general, these studies have found elevated levels of Cu, Fe, Zn and Co in the water flowing out of the Baltic, with concentrations similar to the open North Sea at depth in the central Skagerrak ocean (Magnusson and Westerlund, 1983; Westerlund and Magnusson, 1982). High concentrations of these metals have also been found close to the Danish coast and in the Jutland current. There have been no studies on trace metal speciation in the Skagerrak published to our knowledge. The present work seeks to examine in detail the interactions between copper speciation and phytoplankton community structure in the Skagerrak.

The picoplanktonic cyanobacteria *Synechococcus* has a distinct seasonal distribution in the Skagerrak, appearing only in the summer when water temperatures exceed 10 °C (Karlson and Nilsson, 1991; Kuylenstierna and Karlson, 1994). Works by Moffett and colleagues in the Sargasso Sea (Moffett, 1995) and coastal waters of Massachusetts (Moffett et al., 1997) have shown a strong positive relationship between the distribution of *Synechococcus* and the presence of strong copper binding ligands (denoted L1, with log $K' >13$). Laboratory studies have subsequently shown that only *Synechococcus*, and possibly *Prochlorococcus*, produce these strong copper binding ligands under copper stress (Croot et al., 2000; Moffett and Brand, 1996). Studies in Gullmars fjord, Sweden, adjacent to the Skagerrak have shown a strong seasonal correlation in both the distribution of the L1 ligand and the abundance of *Synechococcus* (Johansson et al., 2000). A central aspect of the present work was to examine the relationship between *Synechococcus* and the presence of L1 in the central Skagerrak.

Other trace metals have also been shown to influence community species composition. Graneli and coworkers showed that the prymnesiophyte, *C. polylepis*, which was responsible for a major toxic bloom in the Skagerrak and Kattegat in 1988, showed increases in both biomass and growth rate when grown with elevated cobalt concentrations (Granéli and Haraldsson, 1993; Granéli and Risinger, 1994; Segatto and Granéli, 1995). Indeed, Granéli and Haraldsson (1993) suggested that cobalt availability in the Kattegat may act as a structuring force for phytoplankton biomass and/or species composition. Similarly Sunda and Huntsman (1995a) proposed that variations in the ratio of Co to Zn could influence the relative growth of diatoms and coccolithophores. They suggested that high $[Co^{2+}]/[Zn^{2+}]$ ratios should favour the growth of coccolithophores such as *Emi-*

liania huxleyi, while high $[Zn^{2+}]/[Co^{2+}]$ ratios could inhibit *E. huxleyi* and instead favour the growth of diatoms. In the present study, we also sought to examine the influence of the $[Co^{2+}]/[Zn^{2+}]$ ratio on the abundance of both diatoms and coccolithophores in the Skagerrak.

2. Materials and methods

2.1. Water sampling

Sampling was performed from *R.V. Skagerak* on July 29, 1997 at eight stations in the central Skagerrak on a transect between Hirtshals, Denmark and Torungen, Norway (see Fig. 1). Station positions and bottom depths are displayed in Table 1. At each station, the CTD (ADM-mini), equipped with a Dr. Haardt mini backscat fluorometer, was lowered down through the water column to obtain vertical profiles of salinity, temperature and chlorophyll fluorescence. Seawater samples were taken at every second station (see Table 1) with pre-cleaned GoFlo samplers (8 l) mounted on a 6-mm Kevlar hydrowire. At each of these stations, samples were collected from the Kevlar wire at various depths in the upper 50 m of the water column.

Immediately upon recovery, the GoFlo sampler was wrapped in plastic bags and mounted on bottle racks. All handling of the GoFlo samplers was performed while wearing plastic gloves. Seawater samples were then drawn into 500 ml acid-cleaned PE bottles, double bagged (Ziplok) and placed in the dark at 4 °C. Seawater was filtered over acid-cleaned Nuclepore membrane filters (47 mm diameter, 0.4 μm pore size), mounted in all-Teflon filter holders (Savillex), in a class-100 laminar airflow bench.

Samples for total dissolved trace metals were acidified with 1 ml quartz distilled HCl per liter of sample, and stored for at least 1 week prior to analysis. Samples for competitive ligand exchange–cathodic stripping voltammetry (CLE–CSV) were run at natural pH, within 24 h of collection.

2.2. Reagents

All plasticware used in this work was extensively acid cleaned before use. All solutions were prepared using 18 MΩ Milli-Q water (Millipore system). Q-HCl (6 M) and Q-acetic acid (17.4 M) were made by redistillation of Merck trace-metal grade acids in a quartz sub-boiling still. Ammonium hydroxide was purchased from J.T. Baker.

2.3. Nutrients

Dissolved macronutrients (nitrate, phosphate and silicate) were analysed in duplicate at each station according to the procedures outlined in Parsons et al. (1984). Samples were frozen in liquid nitrogen until immediately prior to analysis at KMF using an automatic four-channel nutrient analyser (TRAACS 800 system, Braun and Luebe, Germany).

2.4. Trace metal analysis

2.4.1. Cu speciation by CLE–CSV

Instrument settings and protocols with salicylaldoxime were identical to those described by Campos and van den Berg (1994). CLE/CSV measurements were made with an Ecochemie μAutolab connected to a Metrohm VA 663 voltammeter used in the static mercury drop electrode mode. Cu titrations were performed as follows. Each sample filtrate was divided into 20-ml aliquots in 125-ml Teflon bottles. These were spiked with different concentrations of Cu (0–80 nM) and salicylaldoxime ($5–10 \times 10^{-6}$ M). The solutions were generally allowed to equilibrate for 3–6 h before analysis, by which time steady-state values were obtained. For analysis, 20 ml of solution was transferred to a Teflon sample cup and installed on the electrode, which was set to hanging drop mode. Instrument settings were: depositional potential -0.08 V (vs. Ag/AgCl electrode); deposition time, $t_d = 1–2$ min; scan range -0.08 to -0.75 V; scan rate, 25 mV s^{-1}; modulation time, 0.01 s; interval time, 0.1 s; pulse height, 25 mV. Reduction of the copper salicylaldoxime complex produces a well-defined peak at approximately -0.33 V.

Salicylaldoxime (Aldrich) required purification before use. This was accomplished by recrystallization in aqueous EDTA solution (10^{-3} M) followed by double recrystallization in Milli-Q to remove the EDTA. A solution containing 1×10^{-2} M salicylaldoxime (hereafter referred to as SA) in methanol was used as a stock solution. Side reaction coefficients for

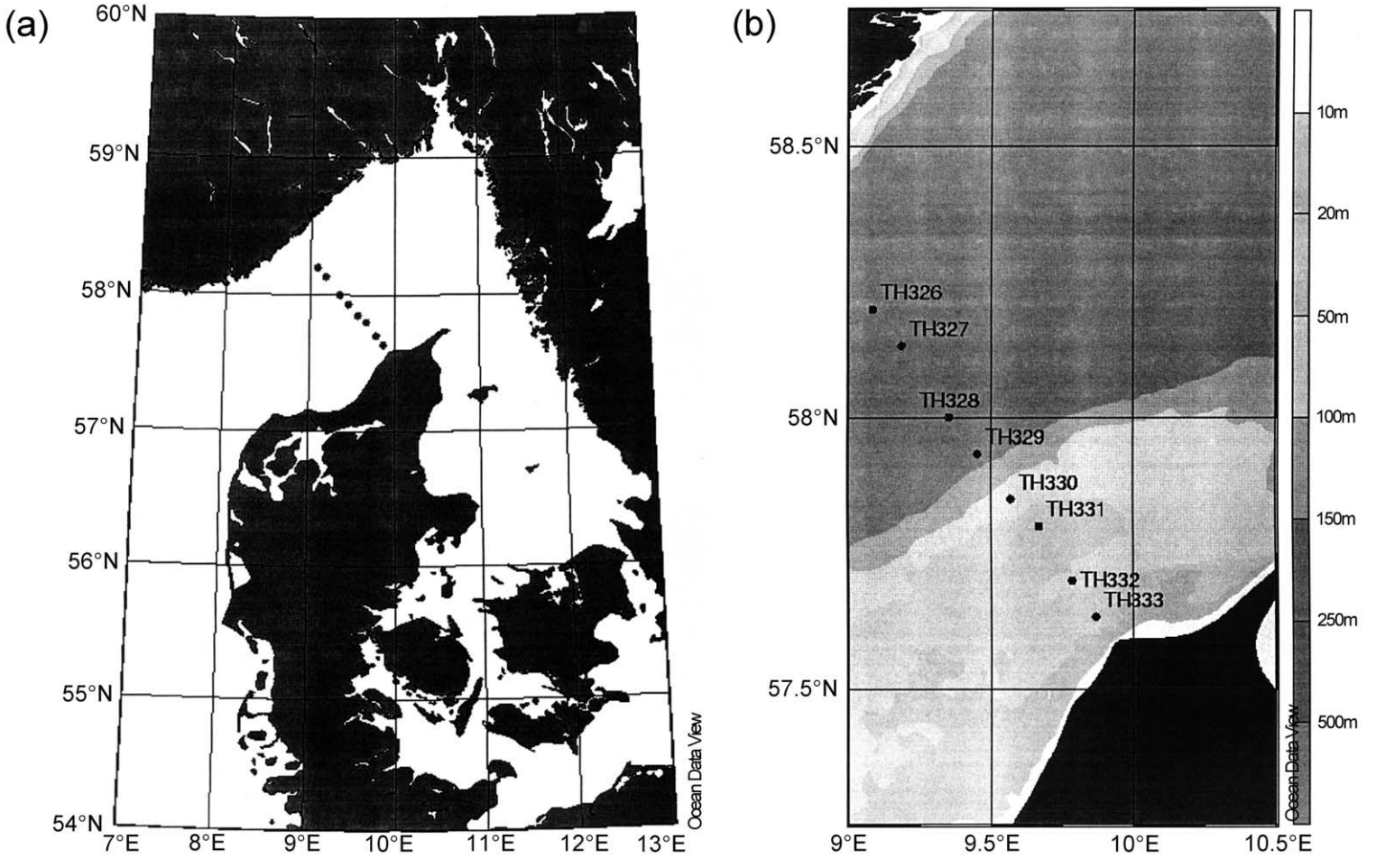

Fig. 1. (a) Station Positions. (b) Local bathymetry and station positions for the Skagerrak cruise of the 29th of July 1997.

Table 1
Station locations

Station	Latitude	Longitude	Depth[a] (m)	Transect[b] (km)	Comment[c]
TH326	58°12′ N	09°05′ E	415	78.7	CTD
TH327	58°08′ N	09°11′ E	640	68.4	
TH328	58°00′ N	09°21′ E	425	50.7	CTD
TH329	57°56′ N	09°27′ E	165	41.0	
TH330	57°51′ N	09°34′ E	72	29.5	CTD
TH331	57°48′ N	09°40′ E	34	21.1	
TH332	57°42′ N	09°47′ E	64	8.0	CTD
TH333	57°38′ N	09°52′ E	25	0.0	

[a] Depth as measured by the echosounder onboard the *R.V. Skagerrak*.

[b] Distance in kilometres along the Hirtshals–Torungen transect line; the origin is station TH333.

[c] CTD denotes only CTD measurements performed at this station.

Cu complexes with SA were taken from Campos and van den Berg (1994).

To determine ligand concentration and conditional stability constant data from Cu titrations, the fraction of Cu present as the $Cu(SA)_2$ complex at each point on the titration curve must be known. Therefore, the system must be calibrated accurately so that $[Cu(SA)_2]$ can be calculated from the peak current signal generated by the cathodic scan.

The peak current i_p is related to the concentration of $Cu(SA)_2$ in solution by the equation

$$i_p = S[Cu(SA)_2] \quad (1)$$

where S is the sensitivity. S is readily determined in UV-oxidized samples by standard additions of Cu. However, in natural samples, S must be determined from the linear portion of the titration curve when all complexing ligands are saturated to distinguish the effects of ligand competition which does not affect S from surfactant interferences which do (van den Berg, 1984).

For the present work, we were only interested in the detection of strong copper binding ligands, which workers in the field normally denote as L1, and these typically possess log K'>12. Thus, we restricted our investigations to detection windows (van den Berg and Donat, 1992; van den Berg et al., 1990) suitable for determining this level of copper complexation (5 and 10 μM SA). The overall process is related to the conditional stability constants and ligand concentrations of all ligands in the sample by the relationship

$$\frac{[Cu(SA)_2]}{[Cu_T]} = \frac{\beta_2[SA]^2}{1 + \Sigma K_i L_i + \beta_2[SA]^2} \quad (2)$$

K_i is the conditional stability constant, L_i the concentration of the ith natural ligand; K_iL_i the side reaction coefficient for the naturally occurring ligands, and $\beta_2[SA]^2$ the side reaction coefficient for SA complexes, which was determined against model ligands (EDTA, DTPA) and at the different salinities encountered in this study. The side reaction coefficient for all naturally occurring ligands (including inorganic ligands) is related to free cupric ion concentration by the relationship

$$\frac{[Cu_f^{2+}]}{[Cu_T] - [Cu(SA)_2]} = \frac{1}{1 + \Sigma K_i L_i} \quad (3)$$

Data in this study were analyzed with a single ligand model that was a nonlinear fit to a Langmuir adsorption isotherm, this model has been described previously by Gerringa et al. (1995). These workers made a convincing case from a statistical perspective for selecting a nonlinear fit over linearization plots, such as van den Berg/Ruzic or Scatchard plots. The single ligand model is derived from

$$K = \frac{[CuL]}{[Cu_f^{2+}][L_f]} \quad (4)$$

where

$$[L] = [L_f] + [CuL] \quad (5)$$

Rearranging Eqs. (4) and (5) yields a reciprocal Langmuir isotherm:

$$\frac{[CuL]}{[Cu_f^{2+}]} = \frac{K[L]}{1 + K[Cu_f^{2+}]} \quad (6)$$

We used the program Origin (Microcal Software) to solve Eq. (6) for K and [L] by nonlinear regression analysis with Cu_f^{2+} as the independent variable and, $\Sigma K_iL_i/[Cu_f^{2+}]$ as the dependent variable. In reality, because weaker ligands are present in the media (such

as carbonate and weak, naturally occurring ligands), a more correct form of the equation would be

$$\frac{\Sigma[\mathrm{CuL}_i]}{[\mathrm{Cu}_f^{2+}]} = \Sigma K_i L_{i(i>1)} + \frac{[\mathrm{L}_1]K_1}{1 + K_1[\mathrm{Cu}_f^{2+}]} \quad (7)$$

$\Sigma K_i L_{i(i>1)}$ is the side reaction coefficient for the weaker ligands, and K_1 and L_1 represent K and L in Eq. (6). Data was rejected if more than 90% of the copper was present as $Cu(SA)_2$ as error analysis has shown that inclusion of this data can lead to erroneously high ligand stabilities.

2.4.2. Total copper and zinc

The total dissolved Cu (Cu_T) and Zn (Zn_T) concentrations in each sample was determined after 4-h UV oxidation (1200 W medium pressure Hg lamp—Ace Glass) of a 100 ml aliquot of seawater, acidified to pH 2 with Ultrex HCl, in a quartz tube. The sample pH was adjusted to 7.7 with isothermally distilled ammonia and HEPES buffer. Cu_T was analysed by standard additions using CSV with 5×10^{-6} SA (as described above). Zn_T was analysed by standard additions using CSV with APDC (van den Berg, 1985).

2.4.3. Labile and total cobalt

For the determination of labile cobalt by adsorptive cathodic stripping voltammetry (ACSV), 20 ml of sample were put into the voltammetric cell, the pH was adjusted by addition of 400 μl of a pH 9.1 ammonia buffer (1 M) and 40 μl of 10 mM nioxime. After an hour, the oxidant was added (4 ml of 5 M $NaNO_2$) and the sample was purged for 4 min using dry nitrogen, prior to the deposition step. This method was devised from early work on Nioxime by other workers with the inclusion of the sensitivity enhancement using nitrite (Bobrowski, 1990; Bobrowski and Bond, 1992; Donat and Bruland, 1988; Gao et al., 1996; Herrera-Melian et al., 1994; Vega and van den Berg, 1997). The deposition potential was set to -0.9 V for 60 s. After the initial measurement, subsequent additions of 40 μl of 50 nM Co were made in order to determine the labile cobalt concentration by standard additions.

The stock 1 M ammonia buffer solution was prepared by adding 16 ml of concentrated NH_4OH to 174 ml of Milli-Q water, 10 ml of HCl_{conc} was also added. A 5 M nitrite solution was prepared by adding 34.5 g of NO_2 to 100 ml of Milli-Q water. A 10 mM nioxime (cyclohexane-1,2-dione dioxime) solution was used for the formation of a Co(II)–nioxime complex.

Attempts to measure Co speciation by competitive ligand exchange were complicated by two factors: (1) Possible redox effects from the use of high concentrations of nitrite. In samples from Gullmars fjord, Sweden we observed significant differences between speciation results using the nitrite method and without, suggesting that there was some effect. We are currently carrying out further work on the possible influence of nitrite on speciation results. (2) Linear response to Co additions, showing no curvature, indicating no excess of Co binding ligand. This occurred for all samples measured during this cruise with (pH 9.1) or without (pH 8.0) added nitrite. For the present study, we concentrate on the short-term 'labile' cobalt that was recoverable from the samples after incubating with Nioxime for 1 h at seawater pH. It is currently unclear exactly what forms of cobalt will be labile; it probably includes Co(II) inorganic and weak organic complexes, and may also include some Co(III) complexes. Total cobalt was calculated with the same procedure as labile, except that the samples were UV-radiated to break any organic complexes (system described above as for total copper and zinc).

2.4.4. Measurement of dissolved iron

During this cruise and in the land-based laboratory, Fe measurements were made using a luminol-based chemiluminescent flow injection technique modified from that used by Powell et al. (1995). This method is based on the catalytic oxidation of luminol by Fe(II), emitting blue light ($\lambda_{max} \sim 440$ nm); see Bowie et al. (1998) and references therein for more details. All the iron is first reduced to Fe(II), using the reducing agent, sodium sulfite. The Fe(II) in the sample is then measured using flow injection analysis with a photomultiplier tube measuring the light produced from the luminol oxidation. For the present work, iron concentrations were significantly high enough, to permit the use of direct injection of the Fe(II) sample, and so no preconcentration was needed. Further details of this method can be found in Powell et al. (1995).

5-Amino-2,3-dihydro-1,4-phthalazinedione (luminol) was purchased from Sigma. Sodium sulfite, SigmaUltra grade, was purchased from Sigma. All other

chemicals were reagent grade quality or higher and used without purification. HCl carrier (0.012 M) was prepared in a clean hood using Q-HCl. The Fe(III) reducing reagent was 1.0 mM $NaHSO_3$ in 2.0 M ammonium acetate buffer (buffer pH 5, final sample pH 4.5). The reducing reagent was cleaned, by passing the solution through an 8-hydroxyquinoline column (prepared according to the method outlined in Landing et al., 1986) immediately prior to use. Luminol reagent (1.0 mM) was prepared in 0.2 M borate buffer and adjusted to pH 12.6 with sodium hydroxide. This reagent was prepared at least 24 h in advance to allow for removal of metals in the reagent by adsorption to the walls of the bottle. Stock iron solutions (10 mM) were prepared from dissolution of either ferrous ammonium sulfate (Fe(II) stock) or ferric chloride (Fe(III) stock) in 0.2 M HCl.

Samples were left acidified for at least 24 h prior to analysis, at which time the reducing reagent and buffer (40 × dilution) were added to the acidified samples and allowed to react for at least 60 min. The sample was then analysed by flow injection, in triplicate, using the technique of standard additions. Analysis of the certified reference materials NASS 4 (our value: 1.87 ± 0.08, certified value: 1.88 ± 0.29) and NASS 5 (our value: 3.65 ± 0.27, certified value: 3.71 ± 0.63 nM) were undertaken as an internal check with good results. The system blank was determined to be 0.04 ± 0.02 (3sd).

2.5. Phytoplankton pigments—HPLC analysis

Seawater (1070 ml) was gently vacuum filtrated (< 5 mm Hg) onto GF/F filters (i.d. 20 mm). The filters were immediately frozen in liquid nitrogen (− 196 °C) and analysed within 24 h. For extraction, 3 ml 100% methanol was added and the samples were sonicated (50 W) for 30 s and filtered through a 0.2 μm PFTE syringe filter into brown glass vials. The vials were kept on a cooled autosampler (< 0 °C) until analysis, which occurred within 10 h. Pigments were analyzed by HPLC according to Wright et al. (1991) with a modification of the solvent protocol according to Kraay et al. (1992). Solvent A was 85% methanol + 15% ammonium acetate buffer (0.5 M in H2O) as described by Kraay et al. (1992), B = 90% acetonitrile and C = 100% ethylacetate. Flow rate was 1 ml min^{-1}. The column used was 250 × 4.6 mm packed with 5 μm Spherisorb ODS2 (Jones Chromatography). The sample was diluted with water to 80% immediately before injection onto a 100 μL sample loop. Absorbance was detected at 436 nm and fluorescence at 668 nm with excitation at 436 nm. Pigments were identified through comparison with known pigments from several unialgal cultures. The identities of the pigments were confirmed by on-line recording of absorption spectra (400–750 nm) using a Linear 206 detector. A mixture of known pigments was run every day and every 15th sample. Pigments measured were chl *a*, chl *c*1 + *c*2/Mg2.4D, chl *c*3, peridinin, 19'-butanolyoxyfucoxanthin (19′-but), 19′-hexanoyloxyfucoxnathin (19'-hex), *cis*-fucoxanthin, dinoxanthin/violaxanthin, fucoxanthin, diadinoxanthin, alloxanthin, diatoxanthin, zeaxanthin, chl *b*, beta-carotenes and the degradation products chlorophyllide *a*, pheophytin *a* and pheophorbide *a*. Pigment compositions are expressed in μg/l. The detection limit was approximately 0.1 μg pigment/l seawater.

The taxonomic composition of the algal community was estimated from the HPLC pigment data using the program CHEMTAX (Mackey et al., 1996) and from direct application of linear equations for the pigment ratios for selected taxa (Letelier et al., 1993; Peeken, 1997). Pigment ratios for the algal classes were constructed using known pigment ratios established from laboratory experiments using phytoplankton from the representative taxa (Mackey et al., 1996).

2.6. Nano- and microplankton cell counts

Samples for nanoplankton abundance were preserved in 50 ml glass bottles with cold glutaraldehyde to a final concentration of 1% and stored at 4 °C. Samples for microplankton abundance were collected in 200 ml amber glass bottles and fixed with 1 ml acidic Lugol's iodine solution. Sedimentation chambers (10 ml Utermohl) were used for the phytoplankton cell counts. The sedimentation chambers were left to settle for 24 h after they were initially set, and then the cells were counted on a Zeiss Axiovert 135 inverted microscope using a 20 × /0.40 objective. The counting included an initial general assessment of the chamber (at 19 × magnification) in order to identify most of the species contained within the funnel. A subsequent linear transect was performed to count the number of cells that

fell within the field of view. A Graticules LTD measuring slide was used to measure the field of view, with the 20 × objective used for each transect. The funnel diameter of the chamber was measured with a regular ruler. Once the number of cells in the transect were counted, the total number of cells per liter was calculated using the number of cells, the total volume (ml), Lugol volume (ml), filtrated volume (ml), transect length (mm), funnel diameter (mm) and field of view (mm).

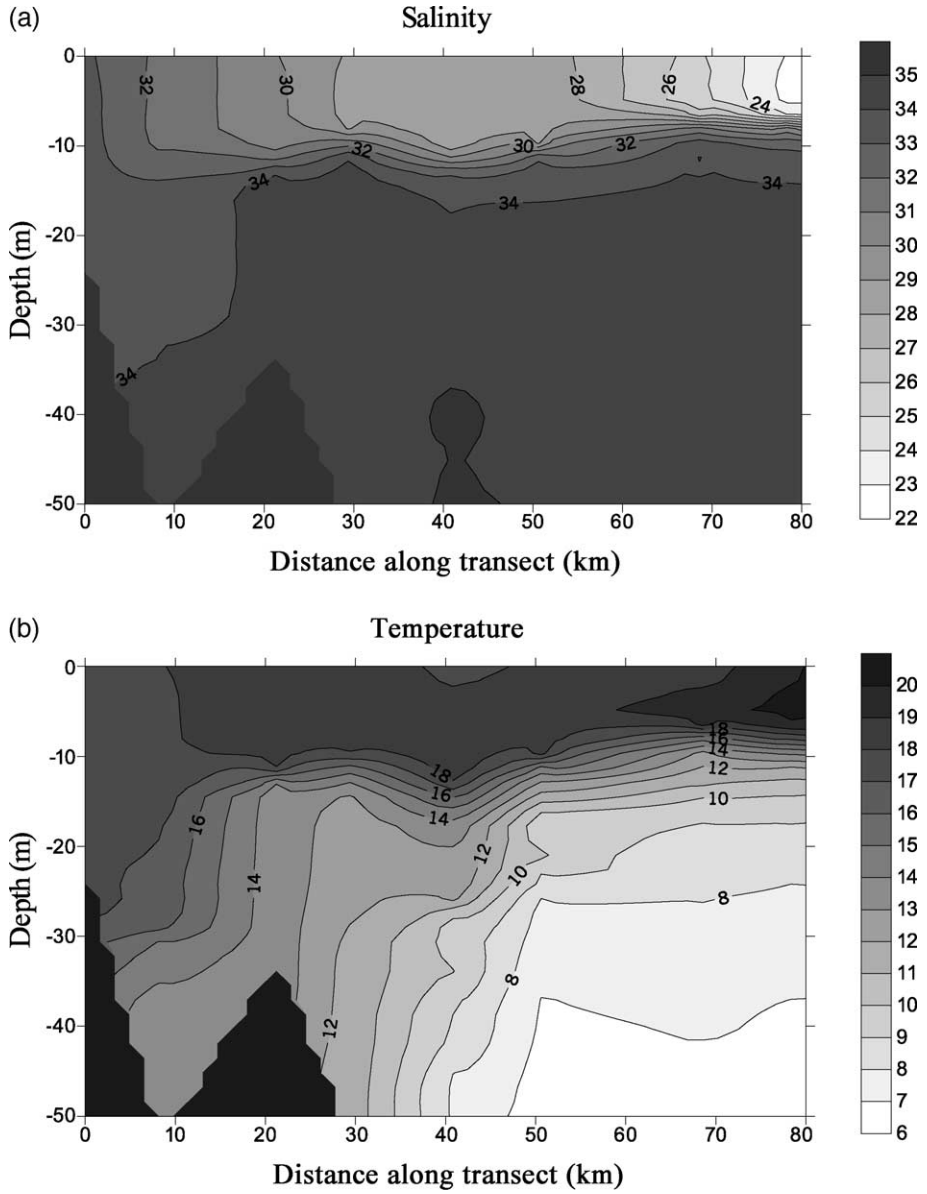

Fig. 2. (a) Salinity contour plot, (b) temperature contour plot and (c) contour plot of CTD fluorescence (arbitrary units). All constructed from CTD data along a transect from Hirtshals to Torungen, July 27, 1997. Note the darkened area in the lower left corner of the plots, which depicts the local bathymetry.

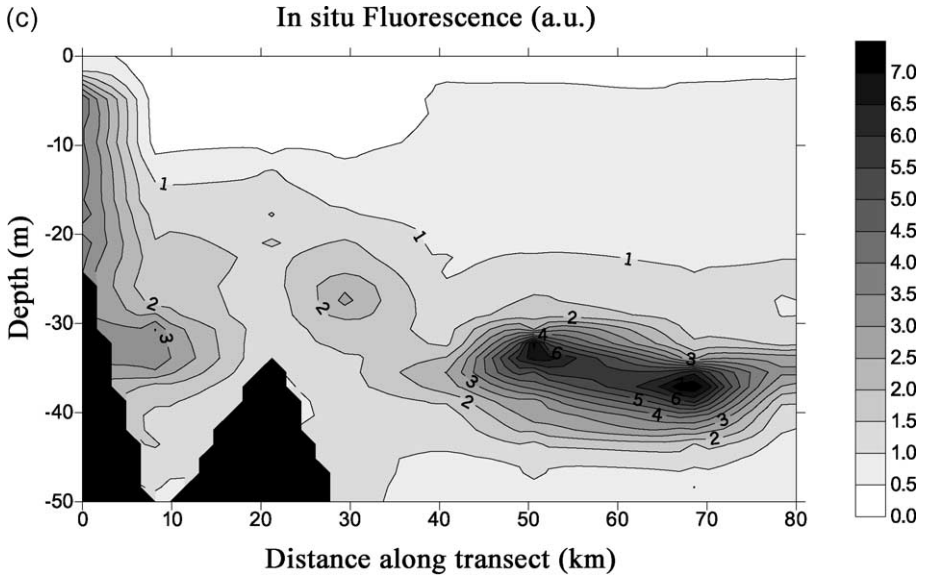

Fig. 2 (*continued*).

2.7. Picoplankton abundance

Water samples for picoplankton abundance were obtained after prefiltration through a 2 μm (Nuclepore) filter and fixed with cold glutaraldehyde to a final concentration of 1% and stored at 4 °C. Upon return to the laboratory, the sample was filtered through a black stained polycarbonate filter (Nuclepore) with a pore size of 0.2 μm using vacuum < 100 mm Hg. Filters were mounted in fluorescence-free immersion oil and were counted immediately. Organisms were observed at 1000 × magnification using a Leitz Dialux epifluorescence microscope, equipped with a 50 W mercury lamp and filter sets for UV-blue and green excitation. Eukaryotic picoplankton were counted using blue excitation light; *Synechococcus* were counted in green light (Kuylenstierna and Karlson, 1994).

3. Results and discussion

3.1. Hydrography

Surface salinity decreased along the transect from Hirtshals to Torungen (Fig. 2a) and showed the presence of Baltic water ($S < 28$) at the surface in the central Skagerrak (stations TH327 and TH326). Subsurface salinities (below 10 m) were relatively stable at $S > 34$, consistent with water originating from the North Sea. At the southern end of the transect (TH333), close to the Danish Coast, the water column was well mixed with no strong pycnocline present. Away from the Danish coast, a strong halocline was present throughout the survey area at approximately 10-m depth. Surface water temperatures increased slightly along the survey transect (Fig. 2b), ranging from 17 °C at the southern end of the transect (TH333) to 20 °C at the northern end (TH326). A strong vertical temperature gradient was present at the northern end of the transect (TH329–TH326), where the warm Baltic water (17–20 °C) overlay the colder central Skagerrak water (6–8 °C), leading to a strong thermocline at around 10-m depth.

Close to the Danish coast, the waters were highly turbid, with Secchi depths of only 5 m. These waters were also characterised by large amounts of household flotsam, consistent with entrainment from European rivers and transport with the Jutland current into the Skagerrak (Rodhe and Holt, 1996). Water clarity increased towards the central Skagerrak with Secchi depths increasing to over 12 m for stations TH329 and TH327. The CTD-fluorometer showed the presence of two regions of high chlorophyll fluorescence over the transect survey (Fig. 2c). The first region was close to

the Danish coast at station TH333 in the well-mixed waters of the Jutland current. The second region of high fluoresence was a strong subsurface (35 m) area in the central Skagerrak (TH329–TH327), well below the pycnocline.

3.2. Nutrients

The macronutrients (phosphate, silicate and nitrate—Table 2) were at low levels throughout the survey region, as would be expected for a summer time survey. In the central Skagerrak, reactive silicate concentrations (Fig. 3) were depleted to below 0.2 μM; similarly, nitrate levels were below 0.05 μM in the region coincident with the high fluorescence signal. Macronutrients in the upper water were strongly depleted, but increased below 35 m at the northern end of the transect, consistent with the influx of the more nutrient rich deep water from the North Sea.

3.3. Trace metal distributions

Data for total dissolved metals are presented in Table 2. Iron concentrations were very high (42.3–43.1 nM) close to the Danish coast at station TH333, and these high values were probably due to resuspended material (small colloids that could pass through the 0.4-μm filter) in the Jutland current. Iron concentrations (Fig. 4) decreased away from the coast and were found to be 0.7–2.4 nM in the central Skagerrak, with elevated concentrations (4.3–6.0 nM) in the low salinity Baltic water. The high concentrations at 30-m depth at Station TH331 are probably due to the close proximity to the sediments, while the upper water column at this station is intermediate between the high iron waters of the Danish coast and the low iron waters of the Central Skagerrak. This distribution pattern for dissolved iron is consistent with an earlier study by Westerlund and Magnusson (1982), who also found elevated concentrations of iron close to the Danish coast and in the low salinity Baltic water.

Total dissolved copper concentrations showed a similar distribution to iron, with the exception that the highest values (3.6–9.8 nM) were found in the water of Baltic origin. Copper concentrations were elevated close to the Danish coast, perhaps reflecting an anthropogenic or landmass influence. The lowest concentrations (0.7–2.3 nM) were again found in the central Skagerrak water as for iron. Total dissolved Zinc concentrations were more uniform (4–5 nM) across the survey area, with only slightly elevated

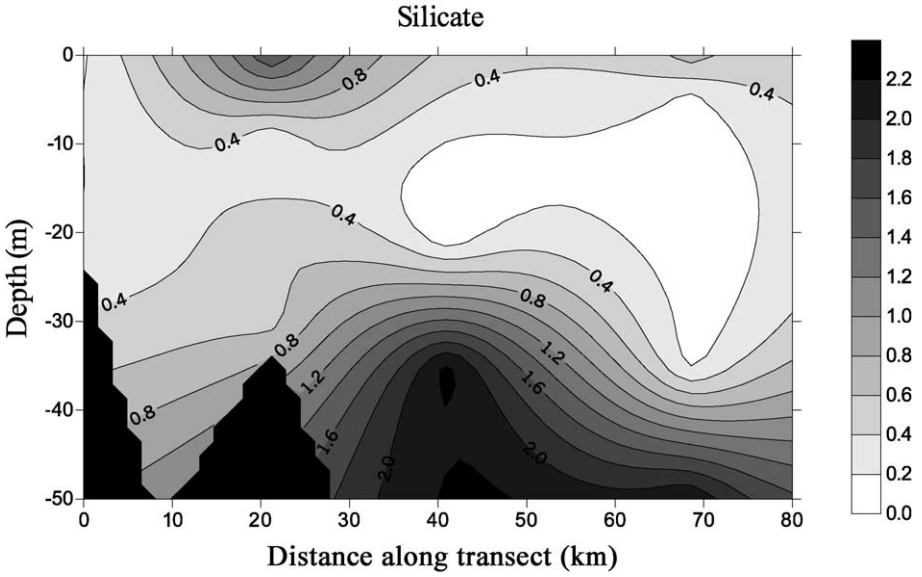

Fig. 3. Contour plot of reactive Silicate concentrations along a transect from Hirtshals to Torungen, July 27th, 1997.

Table 2
Nutrient and trace metal concentrations in the Skagerrak

Station	Depth (m)	$NO_3 + NO_2$	PO_4	Si	$[Fe]_{tot}$	$[Cu]_{tot}$	$[Zn]_{tot}$	$[Co]_{lab}$	$[Co]_{tot}$	Co_{lab}/Co_{tot}
TH327	0	0.07	0.12	0.71	4.3	9.8	9.6	192	257	0.75
	5	0.50	0.03	0.12	6.0	3.6	5.1	184	198	0.93
	20	0.02	0.08	0.13	1.1	0.9	3.6	96	164	0.59
	35	2.41	0.24	0.15	1.3	2.3	4.0	23	139	0.17
	50	6.78	0.45	2.20	0.7	–	6.8	49	100	0.49
TH329	0	0.77	0.10	0.55	3.5	6.0	4.1	197	234	0.84
	10	0.07	0.04	0.19	4.9	3.8	4.0	158	279	0.57
	20	0.03	0.07	n.d.	0.8	1.8	5.1	50	123	0.41
	35	0.01	0.14	2.26	2.1	0.7	–	60	97	0.62
	50	2.21	0.29	2.22	2.4	1.3	5.5	31	86	0.36
TH331	0	0.13	0.05	1.61	7.0	3.6	4.9	207	212	0.98
	10	0.12	0.04	0.16	4.7	2.9	4.1	170	272	0.63
	20	0.13	0.06	0.61	5.7	4.0	3.2	232	312	0.74
	30	0.10	0.07	0.53	26.7	2.0	6.8	189	265	0.71
TH333	0	0.07	0.07	0.18	43.1	5.1	6.4	224	266	0.84
	10	0.25	0.08	0.21	43.1	4.4	7.0	316	425	0.74
	20	0.12	0.05	0.53	42.3	4.8	–	247	440	0.56

(–) Denotes no sample; n.d. denotes not detectable. Nutrient concentrations are in μM, trace metal concentrations are in nM, except for Co (pM). Estimated errors (3σ) are approximately ± 0.01 μM for the nutrients, ± 0.1 nM for Fe, Cu and Zn, and ± 7 pM for Co. See text for full experimental details.

concentrations found in the (4.0–9.6 nM) Baltic water and close to the (6.4–7.0 nM) Danish coast. The results for Cu and Zn are consistent with the early work of Westerlund and Magnusson (1982), where they found dissolved Cu concentrations of 1.9–7.0 nM and Zn concentrations of 5.6–26 nM, with both

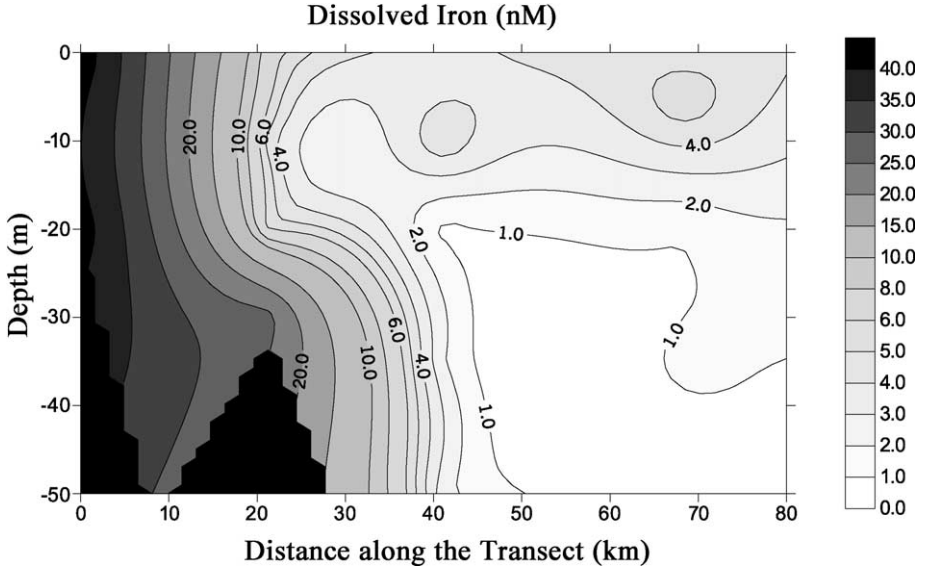

Fig. 4. Contour plot of dissolved Iron distribution along a transect from Hirtshals to Torungen, July 27th 1997.

elements having their maximum concentrations in waters close to the Danish coast.

Total dissolved cobalt concentrations (Table 2) were also highest (266–550 pM) close to the Danish coast, with the lowest values in the central Skagerrak (86–164 pM) waters. Waters of Baltic origin had slightly elevated cobalt concentrations (198–279 pM) over central Skagerrak waters. Early results in the same region by Westerlund and Magnusson (1982) also found a similar pattern for Co distribution in these waters with total dissolved concentrations from 50 (central Skagerrak) to 662 pM in Danish coastal waters. Labile cobalt measurements showed a similar distribution overall to the total cobalt results. Interestingly, the ratio of labile cobalt to total cobalt (Table 2) was found to be below 0.5 in the vicinity of the high CTD fluorescence region, indicating some possible changes in Co speciation in this region.

3.4. Copper speciation

Results from the CLE–CSV copper speciation measurements are shown in Table 3. Strong Cu chelators (log K' >12) were found in all the samples tested, similar to those found in the Sargasso Sea (Moffett, 1995; Moffett et al., 1990). There was a slight tendency towards higher conditional stability constants near the surface, while ligand concentrations increased with depth and with proximity to the coast. The estimated free Cu concentrations are also shown in Table 3, and indicate that it was highly unlikely that any phytoplankton were under significant stress from toxic levels of Cu at this time, based on comparisons with laboratory experiments on algal cultures (Brand et al., 1986; Sunda and Huntsman, 1995b). An early less extensive survey in the Skagerrak, in late autumn (October 1996), found no evidence for strong Cu chelators, suggesting the influence of seasonality in these results (Croot, unpublished results). A companion study in Gullmars fjord, Sweden, also found that the strong Cu chelator was apparently only present during the summer months, when the surface water temperature was above 10 °C (Johansson et al., 2000).

Table 3
Copper speciation results in the Skagerrak, July 29, 1997

Station	Depth (m)	Log K'	[L] (nM)	[Cu] (nM)	pCu
TH327	20	n.d.	1.9	0.9	~ 13.6
	35	12.9	10.2	2.3	13.4
TH329	20	13.1	6.1	1.8	13.5
	35	12.6	17.9	0.7	14.0
TH331	10	12.9	8.6	2.9	13.2
TH333	~ 1	13.0	14.2	5.1	13.3

n.d.—denotes that the conditional stability constant was not determinable using the 5 μM SA detection window. This implies that the conditional stability constant was at least log K' >13.6. The dissolved Cu concentrations can also be found in Table 2. The estimated free copper concentration is also shown, $p\mathrm{Cu} = -\log[\mathrm{Cu}^{2+}]$, and is calculated from the CLE–CSV data. Error estimates for the ligand concentrations are on the order of ± 0.3 nM, and ± 0.1 for log K' and pCu. It is assumed that the other weaker (L2 class) Cu ligands do not significantly influence the Cu speciation at these locations.

At station TH333, CSV scans also revealed the presence of a peak associated with compounds containing the thiol moiety (Leal et al., 1999). Thiols containing compounds such as glutathione may be released from the cells during processes such as grazing, senescence, nutrient stress or metal stress caused by high concentrations of Cd, Cu, Zn or Hg. It was not possible to determine the thiols responsible for this peak or their source. Thiol type peaks were not observed at any of the other stations occupied.

3.5. Algal pigments

Algal pigment data are presented in Table 4. There is good general agreement between the chlorophyll *a* data and the CTD fluorescence measurements, indicating that there was a strong bloom present below the pycnocline in the central Skagerrak, particularly at station TH327 (max chl *a* = 7.7 μg/l). There were also high chlorophyll *a* levels (4–6 μg/l) encountered along the Danish coast at station TH333. Chlorophyll *a* levels were low in the water of Baltic origin (0.25–0.32 μg/l). Concentrations of chlorophyll *b* were low, mostly at the southern end of the transect (max chl *b* = 0.13 μg/l), or undetectable throughout, indicating that there was very little contribution to the biomass from Prasinophyceae, Chlorophyceae and Euglenophyceae. Peridinin was present throughout the survey region, indicating the presence of dinoflagellates. Maximum concentrations were found in Danish coastal waters and at 35-m depth at TH329. The accessory pigment 19′-butanoyloxyfucoxanthin was found throughout the study area at low levels and indicates the presence of pelagophytes and haptophytes (prymnesiophytes). Fucoxanthin is a maker pigment for diatoms, but is also found to a lesser

Table 4
HPLC algal pigments — July 29, 1997 in the central Skagerrak

Station	Depth (m)	Chl *a* (μg/l)	Chl *b* (μg/l)	Peridinin (μg/l)	19′-but (μg/l)	Fucoxanthin (μg/l)	19′-hex (μg/l)	Diadinoxanthin (μg/l)	Alloxanthin (μg/l)	Zeaxanthin (μg/l)
TH327	0	0.246	n.d.	0.021	0.013	0.041	0.067	0.042	n.d.	0.011
	5	0.290	n.d.	0.039	0.012	0.040	0.072	0.040	n.d.	0.011
	20	0.556	n.d.	0.169	0.020	0.085	0.095	0.053	0.005	0.030
	35	7.710	0.108	0.634	0.044	3.848	0.207	0.221	0.038	0.025
	50	0.262	n.d.	n.d.	n.d.	0.139	0.012	0.008	n.d.	n.d.
TH329	0	0.292	n.d.	0.028	0.015	0.046	0.070	0.041	n.d.	0.020
	10	0.322	n.d.	0.038	0.016	0.048	0.080	0.044	n.d.	0.024
	20	0.699	0.033	0.204	0.018	0.092	0.130	0.053	0.013	0.027
	35	2.346	0.127	1.588	0.041	0.457	0.178	0.139	0.026	0.028
	50	0.453	0.050	0.068	0.012	0.139	0.075	0.017	0.004	n.d.
TH331	0	0.373	n.d.	0.102	0.017	0.037	0.075	0.073	n.d.	0.025
	10	0.675	0.033	0.216	0.022	0.079	0.131	0.043	0.008	0.027
	20	1.997	0.057	0.273	0.030	0.724	0.169	0.079	0.017	0.019
	30	1.634	0.058	0.214	0.025	0.565	0.147	0.061	0.013	0.017
TH333	0	4.075	0.057	0.996	0.038	1.144	0.173	0.273	0.098	0.024
	10	6.073	0.058	1.497	0.055	1.835	0.190	0.357	0.165	0.024
	20	5.250	0.063	1.531	0.045	1.722	0.193	0.313	0.153	0.028

19′ hex (19′-hexanoyloxyfucoxanthin); 19′ but (19′-butanoyloxyfucoxanthin).

extent in haptophytes, Raphidophyceae and some dinoflagellates (Mackey et al., 1996). High concentrations of fucoxanthin were found at station TH333 close to the Danish coast and also at 35-m depth at station TH327, coincident with the maximum in chlorophyll *a*. 19′-Hexanoylfucoxanthin is a marker pigment for haptophytes, but has also been found in some dinoflagellates from the Skagerrak (Tangen and Björnland, 1981). Diadinoxanthin is considered a light protective pigment and is found in diatoms, dinoflagellates and haptophytes (Mackey et al., 1996). Fig. 5 shows the ratio diadinoxanthin/chlorophyll *a* vs. depth for all the stations, it can be clearly seen that this ratio was highest near the surface, consistent with this pigments role as a photo-protector (see below). This result had also been seen in an early study in the Skagerrak (Karlson et al., 1996). At station TH333, where the water column was known to be mixed to the bottom, this ratio stays relatively constant, indicating the algae were also being rapidly mixed. Alloxanthin, marker pigment for cryptophytes, was not detectable in waters of Baltic origin but small amounts were found in the central Skagerrak and higher concentrations (0.15 μg/l) at station TH333. Zeaxanthin is found mostly in cyanobacteria, but is also present in the Chlorophyceae and in prasinophytes. Detectable zeaxanthin concentrations were only found above 35 m, and were at reasonably constant values throughout.

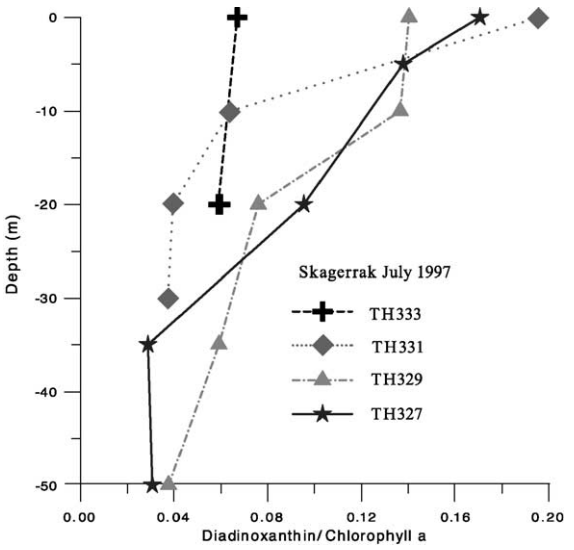

Fig. 5. Plot of the ratio of Diadinoxanthin/Chlorophyll *a* against depth for all stations.

Table 5
Phytoplankton community speciation as identified, to species level where possible, by light microscopy (cells/ml) and by epifluorescence microscopy for cyanobacteria (1×10^6 cells/ml)

Organism	TH333			TH331				TH329					TH327				
	0 m	10 m	20 m	0 m	10 m	20 m	30 m	0 m	10 m	20 m	35 m	50 m	0 m	5 m	20 m	35 m	50 m
Autotrophic dinoflagellates																	
Prorocentrum micans	2111	2111	4222	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Gymnodinium sp.	6334	–	–	–	6334	14,780	6334	2111	8445	6334	19,003	–	2112	21,114	19,002	34,111	3248
Ceratium furca	2111	23,223	16,889	2112	–	–	–	–	–	4223	4223	–	–	–	4223	–	–
Ceratium fusus	8445	4222	2111	–	–	–	–	–	–	–	10,557	–	–	–	–	4873	–
Ceratium horridum	–	2111	–	–	–	–	2111	–	–	–	–	–	–	–	–	1624	–
Ceratium macroceros	–	–	–	–	–	–	–	–	2111	–	14,780	–	–	–	–	–	–
Autotrophic dinoflagellate A	–	–	–	–	–	–	21,114	8446	12,667	71,788	23,226	4223	12,670	40,116	54,896	81,217	1624
Autotrophic dinoflagellate B	–	–	–	–	–	25,337	2111	–	–	–	–	–	–	–	2111	–	–
Heterotrophic dinoflagellates																	
Dinophysis sp.	8445	8445	2111	–	–	2111	2111	–	–	2111	4223	–	–	–	–	–	–
Protoperidinium sp.	–	2111	–	–	–	–	–	–	–	–	–	–	–	–	2111	–	–
Heterotrophic dinoflagellate	–	–	–	6335	14,778	8446	4223	31,672	35,891	14,780	2111	4223	19,004	50,673	6334	6497	–
Centric diatoms																	
Chaetoceros sp.	4223	–	4222	–	–	6334	–	–	–	2111	10,557	2111	–	–	38,005	9746	–
Leptocylindrus danicus	559,513	969,037	971,108	6335	–	190,027	181,582	–	–	–	2111	–	–	–	128,794	1,192,270	8121
Proboscia alata	16,891	19,001	8444	23,227	12,667	10,557	4223	52,787	31,669	27,448	124,576	33,783	69,683	44,339	25,336	1624	3248
Pennate diatoms																	
Cylindrotheca closterium	–	16,890	6333	–	–	12,668	–	–	–	4223	–	–	–	–	2111	1624	–
Pseudonitzchia sp.	–	–	–	–	–	–	–	–	–	2111	2111	–	–	–	4223	22,741	–
Thalassionema nitzchioides	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	105,583	–
Unknown pennate diatoms	6334	2111	–	–	6334	–	2111	–	–	33372	16,892	–	–	–	–	–	–
Cryptophyta																	
Cryptomonas sp.	44,339	90,781	99,222	8446	–	16,891	23,226	10,557	–	10,557	–	14,780	–	2111	23,225	27,614	–
Cyanobacteria																	
Synechococcus sp.	20	20	20	29	32	9	9	29	40	24	20	3	25	32	36	18	2
Unidentified marine flagellates																	
CF: small flagellate (2)	12,668	–	8444	6335	6334	–	–	–	–	–	–	–	–	–	–	–	–
CF: small flagellate (long)	–	–	–		2111	–	–	–	–	–	–	–	–	–	–	–	–

The ratio of diatoxanthin (data not presented) to chlorophyll *a* increased at 10 m depth (vs. surface) at TH327. Usually, diatoxanthin is thought to be a 'light protector' through the xantophyll cycle but it has been previously reported to increase in senescent cells (Arsalane et al., 1994; Klein, 1988). It is possible that the algae in the surface waters at TH327 were using diatoxanthin as a light protective pigment, as the pigment diadinoxanthin also shows the same trend, but more work is needed on this to fully elucidate their occurrence in phytoplankton. There were very small traces of chlorophyllide *a* and pheophytin *a* present; however, it is too small to be accurately quantified.

3.6. Phytoplankton direct cell counts

The direct cell counts from microscopy are presented in Table 5. The main feature to notice is the large numbers of the centric diatom *Leptocylindrus danicus* at stations TH333 and TH327, with a maximum cell density of 1.19 million cells l^{-1} at 35 m at TH327. It would appear that there were two separate blooms at station TH327 and TH333, but both with a relatively similar diatom community. Another centric diatom *Proboscia alata* was also present in high numbers throughout the region. Pennate diatoms were not as common as centric diatoms, with the exception of the bloom at 35 m at TH327, where over 100,000 cells l^{-1} of the pennate diatom *Thalassionema nitzchioides* was present.

Autotrophic dinoflagellate concentrations were also high throughout the survey region. *Ceratium* sp. were found mostly at TH333 in the Danish coastal waters, although significant numbers were found at other locations throughout the survey area. The distribution of *Gymnodinium* sp. was in direct contrast to that of the *Ceratium* sp. with high cell concentrations of this species in the central Skagerrak sector of the survey. An unknown autotrophic dinoflagellate species was also present at significant concentrations (max 80,000 cells l^{-1}) over the central Skagerrak region. Similarly, an unknown heterotrophic dinoflagellate was present in waters away from the Danish coast, while *Dinophysis* sp. was the most abundant heterotrophic dinoflagellate identified at TH333.

There are limited data on the presence of haptophytes, as many of these species dissolve in Lugol's. Direct visual identification from video of live specimens at the time of sample collection indicated that there were some *E. huxleyi* present at the southern end of the survey. Samples from TH333 showed the presence of both *E. huxleyi* and *Corisphaera gracilis*, but many of the *E. huxleyi* were dead, particularly at 20-m depth. At station TH331, *Umbellosphaera corolla* was present in addition to the same species as at TH333. For waters in the central Skagerrak, no coccolithophorids were reported from station TH329, though at TH327 several species were identified.

Cryptomonas sp. was found in high numbers (90,000 cells l^{-1}) at station TH333, consistent with the pigment data for alloxanthin described previously. They were also present throughout the survey region but at lower concentrations, with up to 20,000 cells l^{-1} present in the bloom at station TH327. There was also a good correlation between alloxanthin concentrations and *Cryptomonas* sp. cell numbers ($R = 0.954$), which differs from early work in the Skagerrak where no correlation was found (Karlson et al., 1996).

The cyanobacteria *Synechococcus* sp. were present throughout the survey area, but were mostly confined to the upper 35 m of the water column, cell concentrations ranged from 2 million cells l^{-1} at 50-m depth at TH327 to a maximum of 40 million cells l^{-1} at 10-m depth at TH329. There was no direct correlation between zeaxanthin and *Synechococcus* cell numbers. However, if the zeaxanthin per cell was calculated (assuming zeaxanthin only from *Synechococcus*), there was a trend towards increasing zeaxanthin per cell with depth (data not shown), perhaps indicating a photopackaging effect. Zeaxanthin per cell yields in *Synechococcus* have been found to be sensitive in culture to changes in light intensity and quality (Bidigare et al., 1989; Kana et al., 1988).

3.7. Class abundances by HPLC pigments

Estimates of the percentage of the total chlorophyll *a* represented by each phytoplankton taxon were calculated using the program CHEMTAX (Mackey et al., 1996). Recently (Schlüter et al., 2000) have shown that the influence of light and nutrients can have significant influences on the pigment ratios found in the algae, and they suggested that where possible the pigment ratios used in CHEMTAX should reflect the dominant phytoplankton species in the region investigated. For our work, we used as our initial pigment

ratios values derived from the literature for identical or similar species to those identified by microscopy (Mackey et al., 1996; Schlüter et al., 2000). As our data set was small, we ran several different runs, in which the algal taxa present were varied, to optimise the fitting parameters and examine the robustness of the fit. Fig. 6 displays the results from the final optimised CHEMTAX routine. In no runs were there significant ($x < 5\%$) contributions from Euglenophyceae or Prochlorophyceae (as estimated by chlorophyll *b*). Prasinophytes may have been present, and one or two cells were seen in the live video film, but prasinoxanthin was not detected in the HPLC data, CHEMTAX runs with prasinophytes included, only estimated them to be significant (7.6%) at one station, TH329, 50 m. Chlorophyceae were only present at very low levels throughout the survey region, with the highest estimates (11–13%) below 10 m at station TH329. *Synechococ-*

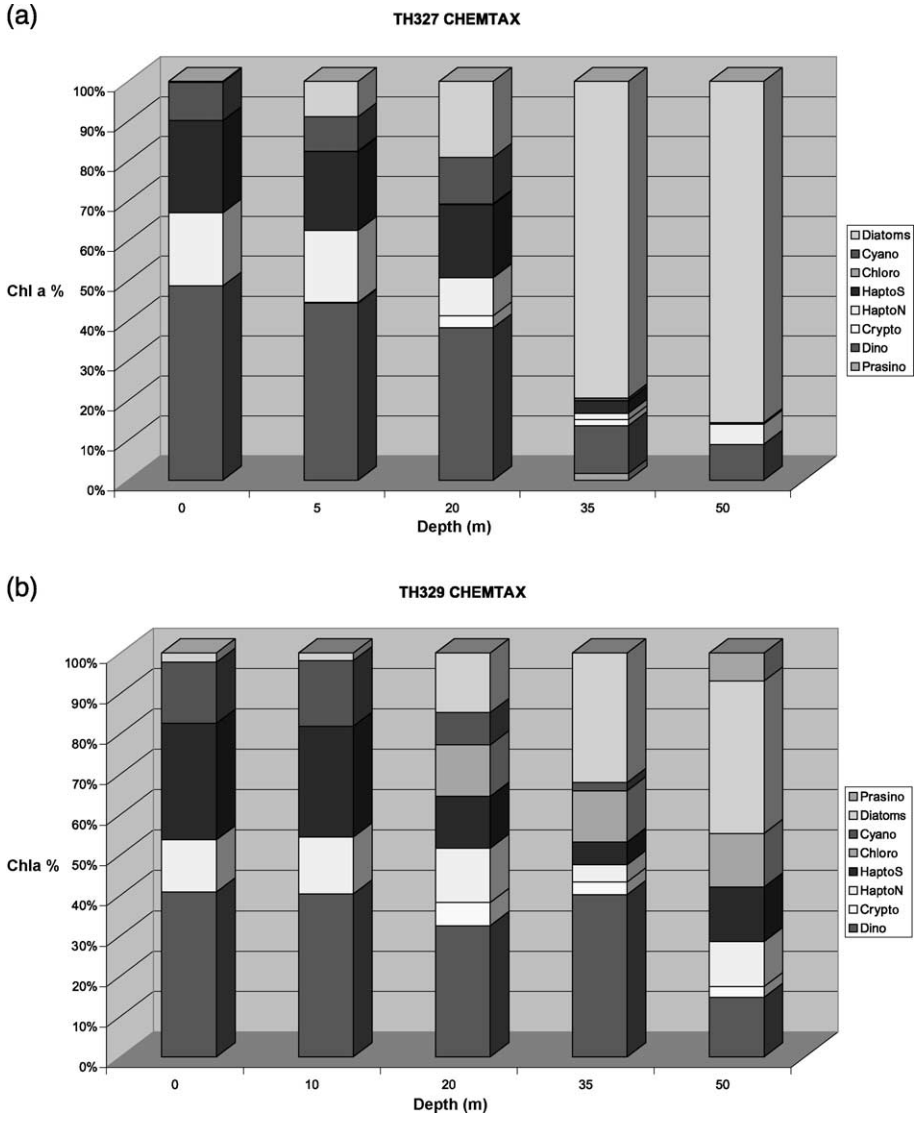

Fig. 6. Bar graphs of percentage contribution to the total chlorophyll *a* by each taxa, estimated using CHEMTAX. (a) Station TH327, (b) Station TH329, (c) Station TH331 and (d) Station TH333.

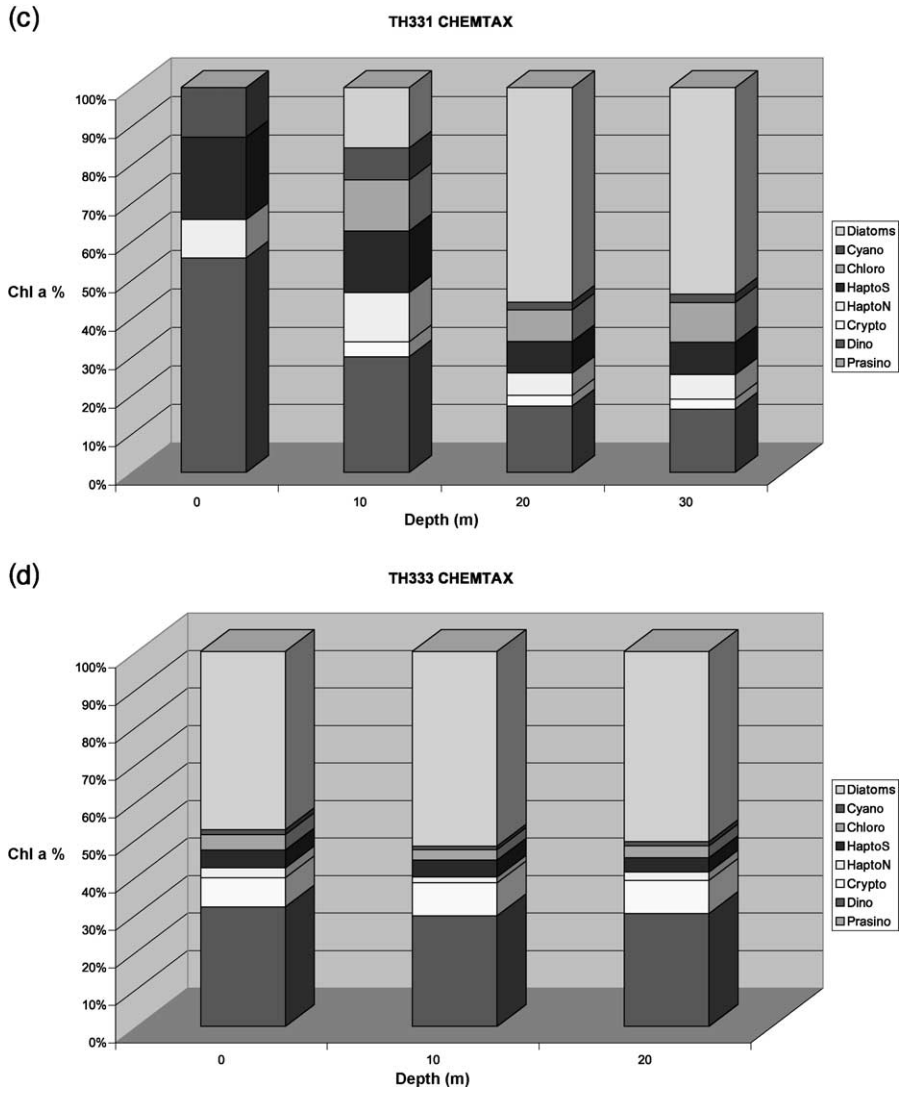

Fig. 6 (*continued*).

cus-like cyanobacteria made up to 10–16% of the chlorophyll *a* in water of Baltic origin, but in central Skagerrak water, their contribution was much less. Estimates of Cryptophyceae contribution to total chlorophyll *a* were low throughout (<5%), except at station TH333, where they reached a maximum of 9%, in qualitative agreement with the direct cell counts.

Dinoflagellates contributed (CHEMTAX using the marker pigment peridinin) around 40–50% of the chlorophyll *a* signal in the Baltic waters, but only 10% in the waters of North Sea origin at TH327, where the diatom bloom was observed. In the coastal waters close to Denmark, approximately 30% of the chlorophyll *a* signal was from dinoflagellates (Fig. 6d).

For the CHEMTAX algorithm, we chose to use two estimates of haptophytes (Wright et al., 1996), one containing 19′-hex, fucoxanthin and 19′-but (denoted here as HaptoS, possibly similar to *Phaeocystis* sp.), the other with no 19′-but (denoted here as HaptoN, possibly similar to *E. huxleyi*). The CHEMTAX results suggested that HaptoS were found mostly

in the Baltic waters where they contributed around 20% of the chlorophyll *a* signal at station TH329. HaptoN had a similar distribution, but made a smaller contribution to the total chlorophyll than HaptoS. Overall, the haptophytes (HaptoS + HaptoN) contributed up to 40% of the chlorophyll signal in the surface Baltic waters (Fig. 7a) but less than 20% in the rest of the transect. The CHEMTAX data indicate that the haptophytes were apparently underestimated in the cell counts from the preserved samples and in the live video samples. As some dinoflagellates are also known to contain 19′-hex (Tangen and Björnland, 1981), it is also that the chlorophyll *a* contribution of the haptophytes may be overestimated.

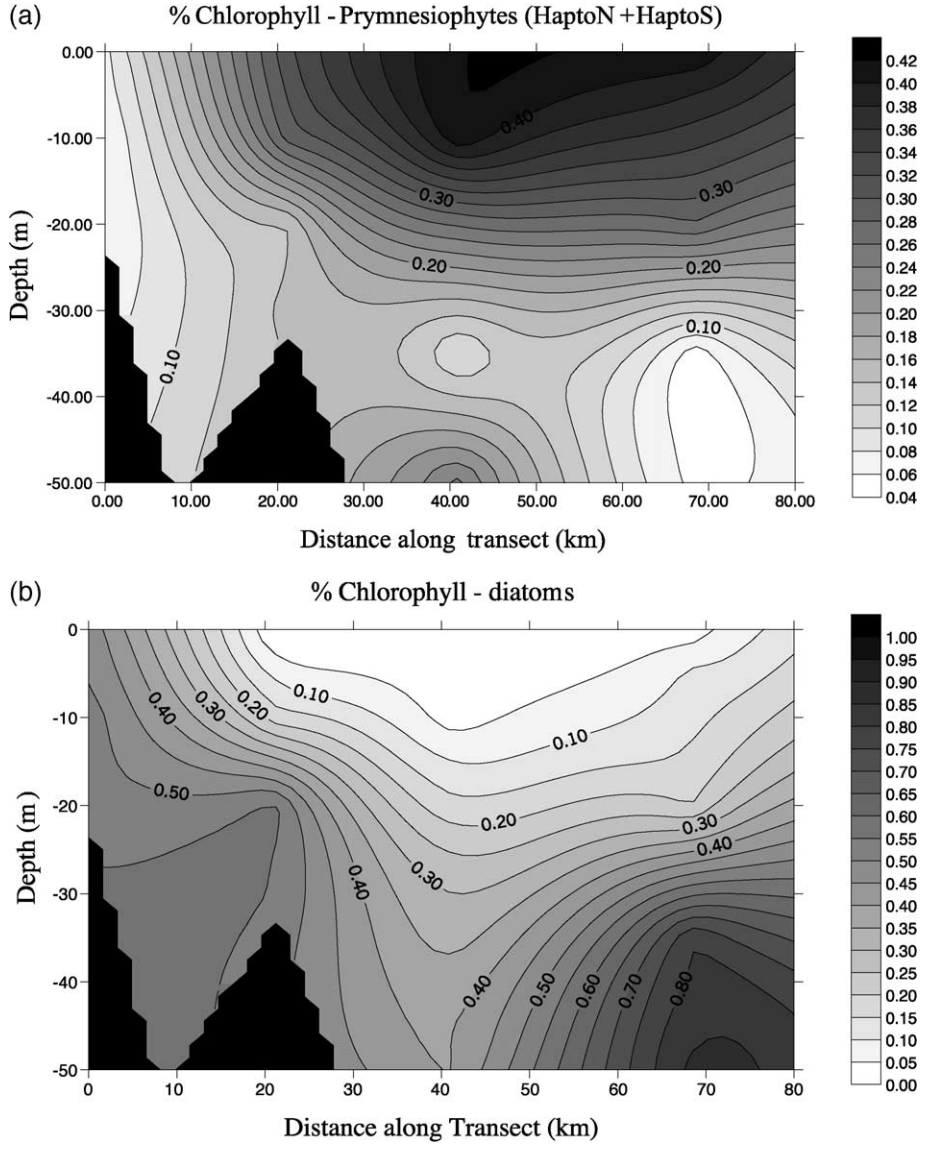

Fig. 7. Cross section along transect for the contribution of the total chlorophyll *a* from (a) prymnesiophytes (HaptoS + HaptoN) and (b) diatoms as estimated from HPLC pigments using CHEMTAX.

The diatoms (Fig. 7b) were the most dominant algal taxa found in the North Sea waters, where up to 80% of the chlorophyll *a* was estimated by CHEMTAX to be derived from diatoms. In the coastal waters close to the Danish coast, diatoms again dominated the chlorophyll signal (47–52%). Overall, the diatom contribution to chlorophyll *a* as estimated by CHEMTAX was qualitatively similar to the direct cell counts (Table 5).

Attempts to compare biomass estimates from the pigment data with those from direct cell counts found poor quantitative correlation's (data not shown). Previous studies (Karlson et al., 1996; Schlüter et al., 2000) have also found poor correlation's between pigments and cell counts, which they have ascribed to the differences in volumes of water filtered for analysis and the subjectivity of microscopic analysis, especially when small phytoplankton dominate.

The distribution of the phytoplankton taxa overall indicates that Baltic waters were dominated by dinoflagellates and haptophytes, while the central Skagerrak waters and the coastal waters were dominated by diatoms. This distribution of the diatoms is, as expected, strongly under silicate control at this time (compare Fig. 3 with Fig. 7b) and the large diatom blooms found at TH333 and TH327 are apparently supplied by upwelling of elevated Si concentrations in the deep waters.

3.8. Algal community/trace metal speciation

There was no apparent correlation between the concentration of the strong copper ligand and *Synechococcus* cell numbers, although *Synechococcus* was present throughout the survey area. As the ligand concentrations increased with depth, it is possible that photochemical processes were destroying these ligands in near surface waters. Sunlight is known to degrade the ligands produced at high Cu concentrations in culture by the *Synechococcus* strain DC2 (Croot et al., 1999). We cannot rule out other algae as sources for the ligands produced by *Synechococcus*, as culture experiments typically show a close 1:1 relationship with the dissolved Cu concentrations (Moffett and Brand, 1996); in this study, we have an excess of ligand at all stations sampled. A few other phytoplankton species produce copper complexing ligands (Croot et al., 2000) that could have been measurable by the detection window employed in our study, and this may also in part explain the high ligand concentrations found. Future work needs to concentrate on directly isolating the copper binding ligands and obtaining structural information that could link them to individual algal species.

The ratio of $[Co]_T/[Zn]_T$ across the transect and its effect on the distribution of coccolithophorids and diatoms is complicated by the influence of other factors—notably silicate abundance. In general, our study indicates that during the time we sampled, Si had a greater control on the presence or absence of diatoms than the $[Co]_T/[Zn]_T$ ratio. Though when Si was present, diatoms dominated the biomass in waters that were relatively low in both total dissolved zinc and cobalt. $[Co]_T/[Zn]_T$ ratios varied from 0.01 to 0.10 (mol/mol) across the transect, with the lowest values being found in the central Skaggerak waters. This $[Co]_T/[Zn]_T$ ratio is much lower than the 0.4 found in the North Pacific by Martin et al. (1989). At this high ratio (Sunda and Huntsman, 1995a), predicted *E. huxleyi* should be favoured over diatom growth. Using this same hypothesis, the Skagerrak is then classified as a region where diatoms should dominate over coccolithophorids when silicate is not limiting. In this study, we did not obtain direct measurements (see above for details) of either $[Co^{2+}]$ or $[Zn^{2+}]$, but we can make some assumptions about their speciation. In work performed in Gullmars fjord, adjacent to the Skagerrak, Zn^{2+} speciation measurements obtained by ASV (Croot, unpublished data) indicated that in these waters, there was relatively little complexation by organic ligands. The data for Co^{2+} from Gullmars fjord and during this cruise is less clear, as explained earlier. In general, though the Skagerrak appears to be a region of high $[Zn^{2+}]$ and low $[Co^{2+}]$, it should also be remembered that the Co/Zn hypothesis applies to growth rates, and our present study focuses on a single snapshot of the algal community. The hypothesis also does not include other important phytoplankton taxa such as the dinoflagellates and cyanobacteria, which can also produce substantial blooms.

The work of Sunda and Huntsman (1995a) indicates that under the trace metal conditions we encountered in the Skagerrak during our survey, diatom growth could be favoured over that of coccolithophorids. Obviously, there are other factors that are important, as indicated by the effects of silicate concentrations in this study.

Perhaps of more interest to this region is whether the effects of Zn and Co have the same effect on other haptophytes such as *Phaeocystis* sp., which are known to form blooms in the spring particularly in the waters of the Jutland current (Karlson et al., 1996). If this Zn/Co effect was also seen in the haptophyte *C. polylepis*, responsible for the Skagerrak wide bloom in 1988, it may help explain reasons for the bloom formation along the lines postulated by Granéli and coworkers (Granéli and Risinger, 1994; Segatto and Granéli, 1995) by which an increase in Co availability may have seeded the bloom. How Zn^{2+} effects the growth of *C. polylepis* and *Phaeocystis* sp. is currently unknown and may have a major influence on spring bloom formation in these waters.

The iron concentrations measured in the Skagerrak during this survey do not indicate any iron limitation of algal growth, nor is there any physiological evidence for this from the algae themselves, as might be expected for a coastal region. Instead, the macronutrients appear to be the major controlling factor on algal biomass at this time. What phytoplankton species are dominant at a particular time and why are questions we are slowly starting to unravel, and will need to do so if we are to understand the formation and impact of harmful algal blooms. Laboratory studies have demonstrated that trace metal speciation may play an important role by which some phytoplankton species may be favoured over others, but we are only beginning to examine this in the natural environment. A short snap-shot study as described here opens slightly the window to understanding these processes, but time series data and investigations into rate processes will no doubt reveal more information about the interactions between phytoplankton community structure and trace metal speciation in the Skagerrak and other seas.

4. Conclusions

The Skagerrak is a region of contrasts and as such provides an interesting natural study region to examine the interactions between trace metal speciation and phytoplankton community structure. Our study is a first attempt to unravel these complex interactions and allows us to begin to understand how changes in metal fluxes to this region may affect phytoplankton production and species composition. Extension of this work to examine a range of temporal scales would allow a greater understanding of the key processes involved; yet, even from our short study, we can see how certain taxa may be favoured and this knowledge could put us a step closer to predicting future outbreaks of harmful algal blooms, similar to the *C. polylepis* bloom of 1988.

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