



Distribution of phytoplankton pigments in nine European estuaries and implications for an estuarine typology

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Abstract. Phytoplankton pigments were studied by Liquid Chromatography (HPLC) in nine West European estuaries. Three estuaries, i.e. the Rhine, Scheldt and the Gironde were sampled four times to cover the different seasons, whereas the other six estuaries were sampled once. Pigment distributions in estuaries reflect both riverine inputs as well as autochthonous blooms. Fucoxanthin was the most common accessory photosynthetic pigment showing that Diatoms were the most common group in the studied estuaries and were particularly dominant during autumn and winter. In the very turbid Gironde estuary, degradation processes were predominant between salinities 1 and 20, while Diatoms, Dinoflagellates and Cryptophytes bloomed above 20 salinity during spring and summer. This contrasted with the highly eutrophic but less turbid Scheldt, where phytoplanktonic blooms occurred at low salinities close to the city of Antwerp. In the Scheldt, we observed both a tenfold fluctuation of phytoplankton biomass and a fluctuating pigment diversity index. In contrast, chlorophyll *a* was always low in the Gironde, but we observed large variations of pigment diversity among samplings during different seasons. Distribution of pheopigments showed that the maximum turbidity zone (MTZ) was a highly reactive region for heterotrophic phytoplankton degradation. The Scheldt and the Thames were the most anthropogenic influenced estuaries contrasting with the Gironde estuary that has a less urbanised watershed. An estuarine typology is proposed based on three clusters emerging from a correspondence analysis of pigment variables and variables characterising the anthropogenic impact and physical forcing.

Introduction

Generally, in estuarine environments, phytoplankton is quantitatively the second source of particulate organic matter after terrestrial inputs from soil erosion (Abril et al. 2001). However, phytoplankton is the highest quality

food source and constitutes therefore the basis of the estuarine food web, which is of fundamental importance for the biology of these ecosystems (Heip et al. 1995; Gasparini et al. 1999; Burdloff et al. 2000). In addition, nutrient and CO₂ assimilation by phytoplankton considerably affects the estuarine water chemistry (Kromkamp et al. 1995; Rendell et al. 1997; Borges & Frankignoulle 1999). These processes result in rising the pH and sometimes creating conditions for spontaneous calcite precipitation (Manickam et al. 1985). Phytoplankton also act in the estuarine chemistry by producing biogas such as dimethyl sulfide (Sciare et al. 2002) and volatile selenium and iodine compounds by biomethylation (Tessier et al. 2002).

Liquid Chromatography (HPLC) of photosynthetic pigments have been used increasingly in oceanography for the quantification of the major taxonomic groups of phytoplankton (e.g., Wright et al. 1991; Letelier et al. 1993; Barlow et al. 1997). Therefore, several studies in marine system have searched for quantitative relationships between the biomass abundances of the different phytoplankton classes and their diagnostic biomarker pigment concentrations (Everit et al. 1990; Mackey et al. 1996; Roy et al. 1996; Bianchi & Argyrou 1997). The algorithms proposed are often powerful and satisfactory approximations in a defined ecological setting and biogeographic area, but can not reliably be extrapolated to other ecosystems. This is related to strong variations of specific pigment contents, which depend both on species and on environmental conditions as e.g. light limitation (Bianchi & Argyrou 1997; Schlüter et al. 2000; Llewelyn & Gibbs 2000). In estuaries, photosynthetic pigment patterns are further complicated by terrestrial contributions such as plant detritus and by the intensity of degradation processes. Therefore, Bianchi et al. (1993) have proposed to use pigment ratios, e.g., the chlorophyll *b*/lutein ratio, as an index to trace the sources of the organic matter. Degradation processes can also be traced by measuring coloured degradation products mainly arising from chlorophylls (Barlow et al. 1993; Head et al. 1994). So far, detailed pigment studies by HPLC are less common for estuaries and most published accounts concern North-American estuaries, which are temperate, macrotidal and submitted to a strong human impact like the European estuaries (Bianchi et al., 1993; Tester et al., 1995; Roy et al., 1996). These studies have shown the usefulness of pigment analysis for characterising estuarine phytoplankton community structures and tracing the sources of organic matter inputs.

Within the BIOGEST project, nine European estuaries were studied for biogas emissions and the underlying biological processes (Frankignoulle & Middelburg 2002). HPLC pigment analysis was the technique of choice for achieving an objective survey of particulate organic matter of photosynthetic origin, with the aim to understand their link with biogas emissions.

Here, we present data obtained from samplings during all the 18 Biogest cruises. Hence, we used pigment data to characterise the phytoplanktonic assemblages present during samplings in terms of the major taxonomic groups and address the importance of degradation processes and terrestrial inputs. In addition, pigment data have been combined with several basic physicochemical and geographical variables measured during the BIOGEST samplings to develop a typology for the Western European estuaries.

Materials and methods

Studied sites and sampling procedures

From 1996 to 1998, samples were collected from nine estuaries along the salinity transect of each estuary. Three estuaries, i.e. the Rhine, Scheldt and the Gironde were sampled during the four different seasons, while the other six estuaries were sampled once. Sampling dates and basic features of these estuaries are listed in Table 1. The sampling procedure was standardised for all the BIOGEST samples to facilitate comparisons between the different measurements. Thus, samples were taken from the subsurface water column (approx. 1m depth) with a 20-l Niskin bottle. Samples for pigments were filtered on board using GF/F glass fibre filters (Whatman), which were directly deep frozen in liquid nitrogen and kept frozen until analyses. Five to seven sampling stations were selected during the cruises according to salinity (approx. 5ppt salinity differences between adjacent stations) to cover the entire salinity gradient.

Methods

Frozen filters were cut into slices and placed into 5 to 10 ml of 100% acetone for extraction. This procedure prevents artifactual production of chlorophyllide (Jeffrey & Hallegraeff 1987). Pigment extracts were sonicated in cold acetone for 3×10 sec, allowed to stand in the dark at 4 °C for 1 hour, and then centrifuged for 10 min at 3000 rpm. The extracts were stored at -30 °C and the filters were re-extracted for 6 hours. This operation was repeated several time (6 to 12 hours extraction time) until we obtained a clear extract.

The Liquid Chromatography (H.P.L.C.) system used in this study consisted of a Perkin Elmer binary pump coupled to a ThermoSeparation Product (TSP) UV6000 diode-array spectrophotometer with an optical path of 5 cm. The free carboxylic groups in pigment molecules were methylated by addition of diazomethane to the pigment extracts. The extracts were dried under vacuum and shortly before injection dissolved in a calibrated volume

Table 1. Studied site description

	Sampling dates	Residence time of water (weeks)	Suspended matter (mg.l ⁻¹)	Riverine DIN load (mol.s ⁻¹)	Phytoplankton Biomass maximum (%)	Anthro- pogenic impact
Elbe (D)	April 97	2-4	55	126.0	22	+
Ems (NL)	July 97	4	240	18.3	1	+++
Rhine (NL)	October 96	<1	15	80.0	30	+
	July 97		10	78.0	25	
	November 97		10	143.0	1	
	April 98		15	113.6	35	
Scheldt (NL/B)	July 96	4-12	35	20.7	2	+++
	December 96		50	45.0	0	
	May 98		55	19.8	1	
	October 98		50	13.9	0	
Loire (F)	September 98	2-4	145	18.7	0	++
Gironde (F)	October 96	4-8	330	37.9	0	+
	June 97		200	50.4	20	
	September 97		315	70.5	25	
	February 98		180	76.7	0	
Douro (P)	September 97	<1	5	32.2	1	+
Sado (P)	September 97	4	75	0.8	25	++
Thames (UK)	February 99	4	60	119.3	5	+++

of solvent A. These extracts were filtered and injected using a TSP AS3000 programmable autosampler refrigerated at 4 °C. The background noise on this system was less than 5×10^{-5} AU over a wavelength range of 300 to 800 nanometer. The separation was performed using a reverse-phase column Lichrospher 100RP18 (250 × 4 mm; 5 μm particle size; 30000 to 60000 plates m⁻¹). We modified the method described by Wright et al. (1991) for use with a binary solvent pump. The eluents were programmed according to the gradient shown in the Table 2. Solvent A consisted of methanol, acetoni-

Table 2. Elution gradient in the HPLC analysis

Time (min)	Flow rate (ml.min ⁻¹)	%A	%B	Conditions
0	0.6	100	0	Injection
10	0.6	100	0	Isocratic phase
50	0.6	0	100	Linear gradient
55	0.6	0	100	Isocratic phase
60	0.6	100	0	Linear gradient
65	0.6	100	0	Equilibration

trile, 0.05 M ammonium acetate in distilled water (pH = 7.2) (50/45/5, v/v) and solvent B was ethyl acetate, methanol and acetonitrile (80/19/1, v/v).

Complete elution of pigments was achieved within 65 min. 50 μ l extract were injected in the column which was thermostated at 50 °C. Pigments were identified using authentic standards (International Agency for ¹⁴C Determination, VKI) by comparing their retention times and their UV-visible absorption spectra with those of the eluted fractions of the extracts. We checked the separation of lutein and zeaxanthin which present retention times of 9.5 and 9.8 minutes respectively. Pheopigments were prepared from acidified extracts of chlorophyll *a* rinsed in a SPE-cartridge (C18) (Touzard & Matignon), according to Bianchi (1991). Concentrations of standards were verified by UV-VIS spectrophotometry at the wavelength of maximal absorption using the literature values of extinction coefficients (Jeffrey 1997).

Pigment diversity can be appropriately described as a single value for each sample by the Shannon-Weaver index. This index has been drawn from information theory (Shannon & Weaver 1949). This index, H', was calculated according the following formula:

$$H' = -\sum a_i \log_2 a_i$$

With a_i equal to the weight fraction of pigment i of total pigment weight. The logarithm used was base two; accordingly, the units of the index were expressed in bits. In this case the index was calculated on 6 diagnostic pigments (see Table 3). Chlorophyll *b* and lutein were both chosen because they allowed to distinguish the Chlorophytes and the higher plants by their ratio (Bianchi et al. 1993); therefore the pigment diversity index ranged from 0 bits (only one diagnostic pigment present) to 2.58 (all pigments represented in equal amounts).

Table 3. List of diagnostic pigment biomarkers of phytoplanktonic groups

Pigments	Retention time (min)	Algal group
Fucoxanthin	5.2	Diatoms (1)
Lutein	9.5	Chlorophytes (1); Higher plants (1,2)
Chlorophyll b	26.5	Chlorophytes (1); Higher plants (1,2)
Alloxanthin	7.9	Cryptophytes (3)
Peridinin	4.5	Dinoflagellates (4)
19'-Hexanoyloxyfucoxanthin	5.5	Prymnesiophytes (5)

Sources: (1) Jeffrey (1974); (2) Bianchi & Findlay (1990); (3) Pennington et al. (1985); (4) Jeffrey et al. (1975); (5) Bjornland & Liaaen-Jensen (1989).

Results

Pigment distributions along the salinity gradients in the nine estuaries are shown in Figures 1a, 1b and 1c for the different sampling campaigns. Chlorophyll *a* concentrations varied by three orders of magnitude. We found the lowest concentrations of chlorophyll *a* ($0.2 \mu\text{g l}^{-1}$) in the Gironde and Rhine estuaries during autumn and winter samplings: the highest values observed during summertime samplings were 220 and $70 \mu\text{g l}^{-1}$ in the upstream part of the Scheldt and Loire estuaries, respectively. In most of the estuaries (Gironde, Rhine, Ems, Douro, Elbe, Sado and Thames) chlorophyll *a* ranged between 0.2 and $4 \mu\text{g l}^{-1}$.

Fucoxanthin was present in all samples and was most often the predominant carotenoid pigment. Fucoxanthin is the main accessory pigment in Diatoms (Bacillariophytes), but it also occurs in Chrysophytes and Prymnesiophytes. Prymnesiophytes were occasionally detected in low quantities in the downstream part of the Rhine estuary by the presence of 19'-hexanoyloxyfucoxanthin (Figure 1c). On the other hand, 19'-butanoyloxyfucoxanthin was always below the detection limit, indicating no contribution of marine Chrysophytes. In addition, several microscope studies on the riverine part of these estuaries have described that fresh-water Chrysophytes were not detected in significant amounts (Etcheber 1986; Muylaert & Sabbe 1999). Thus, the bulk of fucoxanthin in the estuaries was mainly attributed to Diatoms. Diatoms were present along the entire salinity transect in the nine West-European estuaries, and were persistent throughout the year. Fucoxanthin concentrations in the water column varied by more than two orders of magnitude, the lowest concentration was $0.1 \mu\text{g l}^{-1}$ in the Gironde and the Rhine and highest concentrations were 10–15 $\mu\text{g l}^{-1}$ in

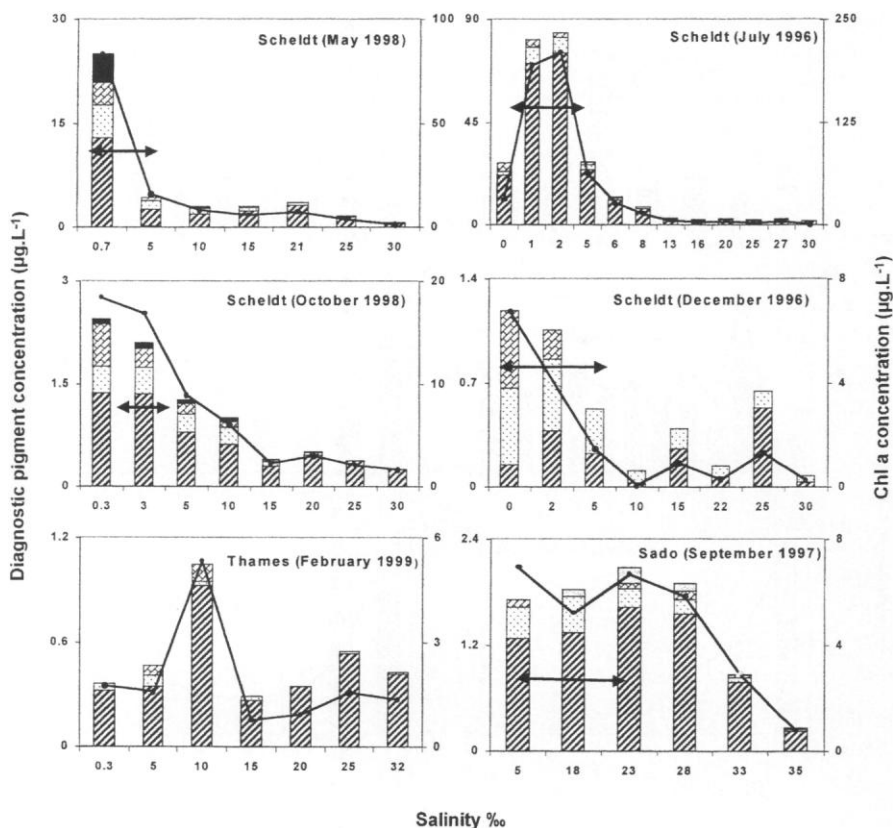


Figure 1a. Distribution of pigment contents in the surface waters along the salinity gradient, in 3 estuaries: Scheldt, Thames and Sado. Bars are the main diagnostic pigments concentrations ($\mu\text{g}\cdot\text{l}^{-1}$) and broken lines for the Chlorophyll *a* ($\mu\text{g}\cdot\text{l}^{-1}$).

Frames correspond to: █ fucoxanthin, ▨ lutein, ▩ chlorophyll *b*, ■ alloxanthin, ▤ peridinin, □ 19'-hexanoxyfucoxanthin. The double arrow shows the maximum turbidity zone (>100 NTU). Note different scaling and different seasons.

the upper regions of the Scheldt and Loire estuaries. The concentrations in the Gironde, Rhine, Ems, Douro, and Thames ranged between 0.1 and $1 \mu\text{g l}^{-1}$, which is in the lower range of values reported for the Hudson, Newport, Krka and St Lawrence estuaries (Bianchi et al. 1993; Tester et al. 1995; Ahel et al. 1996; Roy et al. 1996). Fucoxanthin distribution patterns were thus very similar to the chlorophyll *a* distribution patterns. Other diagnostic pigments (see Table 3, Figures 1a, 1b, 1c) included lutein and chlorophyll *b* (in the upstream part of most estuaries), alloxanthin (Rhine, Scheldt, Loire and Gironde), peridinin (Loire, Gironde, Douro and Sado) and 19'-hexanoxyfucoxanthin (Rhine in July downstream (salinity 30) and in the

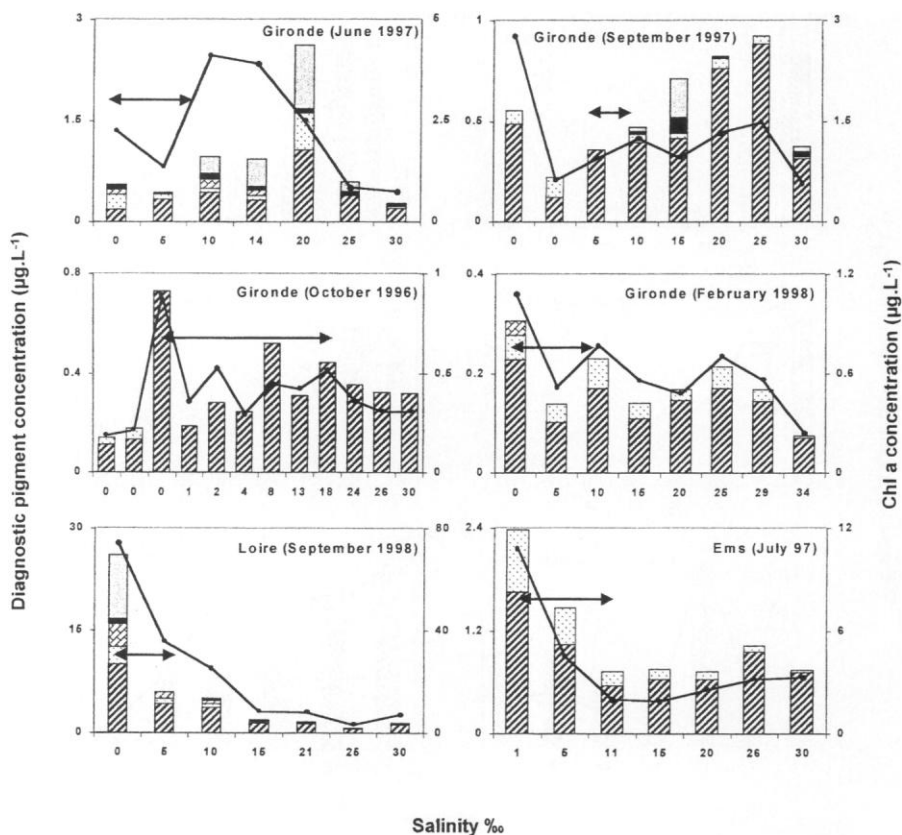


Figure 1b. Distribution of pigment contents in the surface waters along the salinity gradient in the Gironde estuary at each season and in the Loire and the Ems estuaries in summer. Graph type and frames as in Figure 1a.

Douro from salinity above 15). Hence, Chlorophytes, Cryptophytes and/or Dinoflagellates frequently constituted a significant part of the West European estuarine phytoplankton assemblages with occasionally minor contributions from Prymnesiophytes. However, zeaxanthin, echinenone, myxoxanthophyll and prasinoxanthin were always below the detection limits which means that no or very few Cyanobacteria and Prasinophytes (Jeffrey 1997) were present during our samplings in the estuaries.

For the Gironde, the Rhine and the Scheldt estuaries, we compared 4 samplings throughout the year, each during a different season. Three observations are highlighted. Firstly, the localisation of the chlorophyll *a* maximum moved throughout the year in the Rhine and the Gironde, where it was found upstream during the winter sampling and downstream during the summer

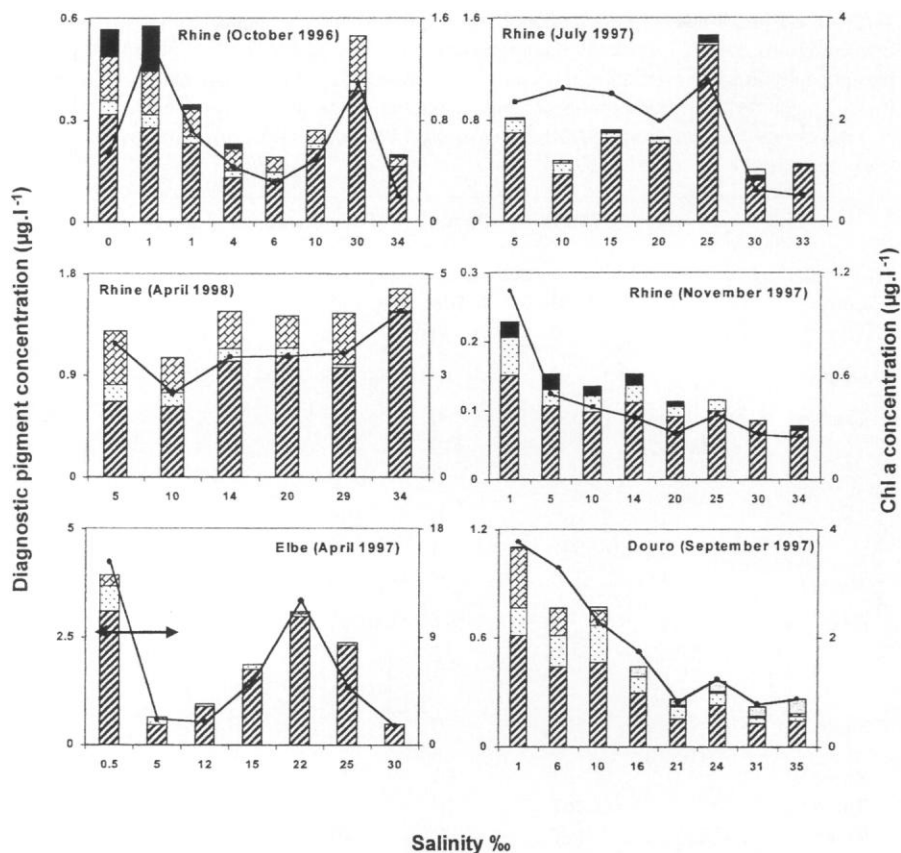


Figure 1c. Distribution of pigment contents in the surface waters along the salinity gradient, in the Rhine estuary at each season, the Elbe estuary in spring and the Douro estuary in summer. Graph type and frames as in Figure 1a.

sampling. Secondly, the pigment diversity was often minimal during the winter sampling and peaked during the summer sampling as exemplified by the Gironde. Thirdly, very strong variations of pigment concentrations were observed upstream in the Scheldt, where the chlorophyll *a* and fucoxanthin concentrations during the summertime sampling were 10 times higher than observed in winter.

The concentrations of chlorophyll *a* and its coloured degradation products (pheophorbide *a*, pheophytin *a* and pyropheophytin *a*) were averaged for three different zones, i.e. Upstream (U), Maximum Turbidity Zone (MTZ) and Downstream (D). These zones, which could be recognised in all estuaries except in the Rhine and the Douro, have been described to constitute

Table 4. Pheophorbide *a* (phide), pheophytin *a* (phytin) and pyropheophytin *a* (pyrophytin) concentrations ($\text{ng}\cdot\text{l}^{-1}$) and total pheopigment *a* (sum of pheophorbide *a*, pheophytin *a* and pyropheophytin *a*) to total chlorin *a* (sum of chlorophyll *a* and total pheopigment *a*) ratios (%) averaged for the different biogeochemical entities in the studied estuaries, i.e. *Upstream (U)*, *Maximum Turbidity Zone (MTZ)*, *Downstream (D)*, *Winter (W)*, *Springtime (Sp)*, *Summer (Su)*, *Autumn (A)*

		Phide	Phytin	Pyrophytin	Total pheo/ total chlorines (%)
Loire	MTZ	0	108	745	2
	D	0	0	89	1
Douro		0	16	23	2
Thames	U	0	0	17	1
	MTZ	0	45	153	4
	D	0	0	0	0
Ems	U	867	0	235	9
	MTZ	591	0	206	15
	D	220	0	46	9
Elbe	U	0	124	253	2
	MTZ	0	32	0	2
	D	0	3	51	1
Sado		579	0	167	14
Rhine W.		133	65	32	34
Rhine Sp.		267	110	46	12
Rhine Su.		111	197	49	16
Gironde W.	U	96	59	13	13
	MTZ	110	73	32	29
	D	78	110	53	30
Gironde Sp.	U	67	120	48	9
	MTZ	0	137	0	9
	D	36	87	25	6
Gironde Su.	U	190	168	68	20
	MTZ	402	614	133	55
	D	188	493	41	39
Scheldt A.	U	1270	1070	1220	16
	MTZ	1190	930	1510	18
	D	0	159	393	12
Scheldt W.	U	0	0	0	0
	MTZ	144	589	216	42
	D	364	310	132	58
Scheldt Sp.	MTZ	4570	10200	698	16
	D	223	367	65	9

different biogeochemical entities. The mean pheophorbide *a*, pheophytin *a* and pyropheophytin *a* concentrations and the total pheopigment *a* (sum of pheophorbide *a*, pheophytin *a* and pyropheophytin *a*) to total chlorin *a* (sum of chlorophyll *a* and total pheopigment *a*) ratios are listed in Table 4 for the different biogeochemical entities.

The pheopigment *a* concentrations ranged from 13 ng.l⁻¹ found in the Gironde during winter to 10,200 ng.l⁻¹ found in the Scheldt during summer. All the pheopigment *a* forms were not necessary present in each sampling. In the Loire, the Thames and the Elbe estuaries, we only observed the presence of pheophytins *a* (pyropheophytin *a* is a more degraded form of pheophytin *a* that has lost the carboxymethoxy group originally present in chlorophyll *a* at position 13-2'), whereas pheophorbide *a* predominated in the Rhine and Ems. Generally pyropheophytin *a* was lower than pheophorbide *a* and pheophytin *a*. The pheopigment *a* to total chlorin *a* product ratio (see above) ratio characterised three different situations. First, very low ratios of about 2% were observed in the Elbe, Douro and Loire. Second, intermediate ratios around 15% were observed in the Ems, Sado, and during occasional samplings in the Gironde (spring), Scheldt (autumn) and Rhine (spring and summer). Third, ratios exceeding 30% were observed in the Gironde (winter and summer), in the Rhine (winter) and in the Scheldt (winter and spring). Generally, the pheopigment *a* concentrations peaked in the MTZ except in the Elbe and the Ems where the maximum was located upstream.

Discussion

For the first time, nine Western European estuaries have been surveyed for photosynthetic pigments using both the same sampling procedure and same pigment analysis protocol. However, the sampling frequency used for this study did not allow to accurately describe the temporal phytoplankton distribution patterns in the very dynamic estuaries. Our data base provides basic features for describing phytoplankton assemblages during BIOGEST samplings and a first opportunity for a preliminary study comparing estuarine phytoplankton assemblages over a large range of latitudes, i.e. from 38.5° to 54.0° N for the nine studied estuaries.

Diatoms predominated the phytoplankton assemblages of all nine West European estuaries. These algae were present along the entire salinity transect, and appeared persistent throughout the year. This is in agreement with studies on estuaries in Northern America and in the Mediterranean (Bianchi et al. 1993; Tester et al. 1995; Roy et al. 1996; Ahel et al. 1996). In addition, Chlorophytes, Cryptophytes and/or Dinoflagellates frequently constituted a significant part of these West European estuarine phytoplankton assemblages

with occasionally minor contributions of Prymnesophytes. This is consistent with previous studies which have shown that Diatoms, Chlorophytes, Dinoflagellates constitute a characteristic temperate phytoplankton assemblage in macrotidal, temperate estuaries in North America and that Prymnesiophytes (Krka estuary: Ahel et al. 1996; Newport estuary: Tester et al. 1995), Cryptophytes (Newport estuary: Tester et al. 1995), Chrysophytes, Prasinophytes (St Lawrence estuary: Roy et al. 1996) and Cyanobacteria (Hudson estuary: Bianchi et al. 1993) have been found as temporary blooms.

Distribution of pigments along the estuarine salinity gradients

Generally, it has been described in the literature that two regions of phytoplankton production can be recognised in estuarine environments. Firstly, in the rivers and low salinity regions, freshwater species dominate that generally do not tolerate salinities exceeding 5 (Ahel et al. 1996). Secondly, at salinities exceeding 15, marine species develop. In contrast, between 5 and 15 of salinity, phytoplankton production is generally low despite the existence of species with a broad salinity tolerance (Mc Lusky 1989). Most often, this salinity range corresponds to the MTZ, where phytoplankton productivity is inhibited due to low light penetration (Kromkamp et al. 1995; Irigoien & Castel 1997). In the nine estuaries presented in this study, the pigment distributions along the salinity transects can be described according to different patterns. A first pattern showed an upstream maximum followed by a continuous decrease of the pigment content along the salinity gradient to the sea. A clear example was the Douro for salinities between 1 and 21, while above 21 the pigment composition and concentrations were rather constant. Similar patterns were observed in the Ems and the Loire and occasionally in the Scheldt (October) and in the Rhine (November). A second pattern showed an autochthonous estuarine bloom characterised by a local increase of pigment content downstream the MTZ, above salinity 15. This pattern was observed very clearly in the Elbe sampled in spring, where Diatoms showed a peak at salinity 22 (Figure 1c). This also occurred in the Gironde during spring and summer samplings, between salinities 15 and 25, where the phytoplankton biomass comprised Dinoflagellates (peridinin) and Diatoms with a minor contribution from Chlorophytes and Cryptophytes. Also in the Scheldt, a phytoplankton productivity peak principally composed of Diatoms was observed in May 1998 at salinity 21 (Wollast et al., pers. comm.). The Sado estuary was strongly influenced by its geomorphology. Between salinities 18 and 28 the broadening of the estuary combined with a low riverine input made this environment more similar to a lagoon with large tidal flats, which may contribute to the total pelagic photosynthetic biomass through resuspension of microphytobenthos. This part of the estuary was characterised by

maximum pigment concentrations corresponding to Diatoms, Dinoflagellates and Chlorophytes. Thirdly, we observed a maximum of pigment concentration at salinities 10 and at 0 to 5 in the Thames and in the Scheldt (May and July), respectively. In both cases, these regions corresponded to the MTZs, where concentrations of solid particles exceeded 100 mg.l^{-1} . Because no primary production was clearly observed in the MTZ of the Thames (Wollast *et al.* this issue), we suggest that riverine phytoplanktonic particles were probably retained and accumulated in the MTZ of the Thames. The Scheldt also showed a peak of biomass (mainly chlorophyll *a* and fucoxanthin) in its MTZ. But in contrast to the Thames, in the Scheldt it was associated with a maximum of primary production (Kromkamp *et al.* 1995; Wollast *et al.*, this issue). This phenomenon was surprising because light penetration was rather low and the productivity was therefore limited despite high nutrient concentrations. Kromkamp *et al.* (1995) and Muylaert and Sabbe (1999) have suggested that in the MTZ the low specific growth rates of phytoplankton are compensated by an important reduction of grazing due to oxygen stress in this region ($< 15\%$ of saturation). In other words, reduced grazing losses might allow accumulation of phytoplankton biomass in the MTZ, and the low specific photosynthesis rates were compensated by a high biomass, which thus explained the peak of the primary productivity.

Due to accumulation of algal biomass, the pheopigment content in the MTZ is generally higher than in other parts of the estuary. Moreover, the ratio of total pheopigments *a* to total chlorin *a* products was also high in the MTZ reflecting the importance of degradation processes in this zone. Generally, the pheophytin forms were prevalent in the MTZ, excepted in the Ems. This indicates that heterotrophic bacterial activity was the major pathway for phytoplankton degradation (Bianchi 1988; Goosen 1999).

Pigment diversity and variability among seasonal samplings

In the Scheldt estuary, pigment concentrations were minimal ($0.2 \mu\text{g l}^{-1}$ fucoxanthin and $0.5 \mu\text{g l}^{-1}$ chlorophyll *a*) during the winter sampling and peaked in summer ($70 \mu\text{g l}^{-1}$ fucoxanthin and $220 \mu\text{g l}^{-1}$ chlorophyll *a*). Relatively high levels were observed in spring, while much lower levels were found in autumn. Lutein and most often chlorophyll *b* were present in addition to fucoxanthin in the upstream part of the estuary. These two pigments are present in all Chlorophytes and higher plants. The decrease of chlorophyll *b* and lutein along the salinity gradient showed that terrestrial higher plants and freshwater chlorophytes inputs were subjected to degradation in the estuary (Bianchi *et al.* 1993). This pattern was persistently observed in the Scheldt and also in the winter sampling of the Gironde. In the Scheldt, the source of chlorophyll *b* and lutein was the Chlorophytes

bloom upstream around the city of Antwerp, whereas the winter observation in the Gironde was more likely related to terrestrial inputs. The Gironde showed much smaller variations of total pigments. The communities in this estuary were dominated by Diatoms in autumn and winter samplings, while the autochthonous estuarine phytoplankton bloom downstream the maximum turbidity zone also comprised significant contributions from Dinoflagellates and Chlorophytes in spring and summer samplings. This was also found by Muylaert and Sabbe (1999) in their microscope study of phytoplankton species in spring 1993 in the Gironde. The Rhine also showed relatively small fluctuations of total pigment concentrations during the studied periods. The community structure varied significantly between sampling series. This was probably related to the very short residence times of water masses (one week), which might have limited the blooming of autochthonous estuarine populations.

We used the Shannon-weaver index (see methods) to describe the pigment diversity of each sampling. In ecology, the Shannon-Weaver index has been most often applied on species distributions (e.g., Muylart & Sabbe 1999), but recently this approach has been applied on HPLC pigment distribution patterns (Nübel et al. 1999). Generally, it was found that pigment diversity was extremely low during winter and autumn samplings (around 0.5 bits), whereas the highest values were observed during spring and summer samplings (around 2 bits). This pigment diversity index associated with the averaged chlorophyll *a* concentrations allowed us to distinguish two different patterns in the Gironde, Rhine and Scheldt estuaries. The first pattern was found for the Scheldt where we observed strong variations of chlorophyll *a* among samplings with a much higher pigment diversity during spring and autumn samplings, compared to summer and winter samplings. The second pattern was found in the Gironde and the Rhine, and characterised by minor variations of chlorophyll *a* and high fluctuations in the pigment diversity index among samplings.

The pheopigment *a* to total chlorin *a* product ratio allows to appreciate the health state of phytoplankton and the importance of degradation processes. The highest ratios were observed in the Gironde, Rhine and the Scheldt showing the importance of degradation processes in these estuaries. In the Rhine, this ratio peaked during winter and in the Gironde it peaked both in winter and summer, while springtime appeared to be the healthy period for phytoplankton growth characterised by a low ratio. The importance of degradation processes during the summer sampling in the Gironde as reflected by the high ratio is probably related to the increasing retention time and increasing temperature during summertime. In the Loire, Douro, Thames and Elbe,

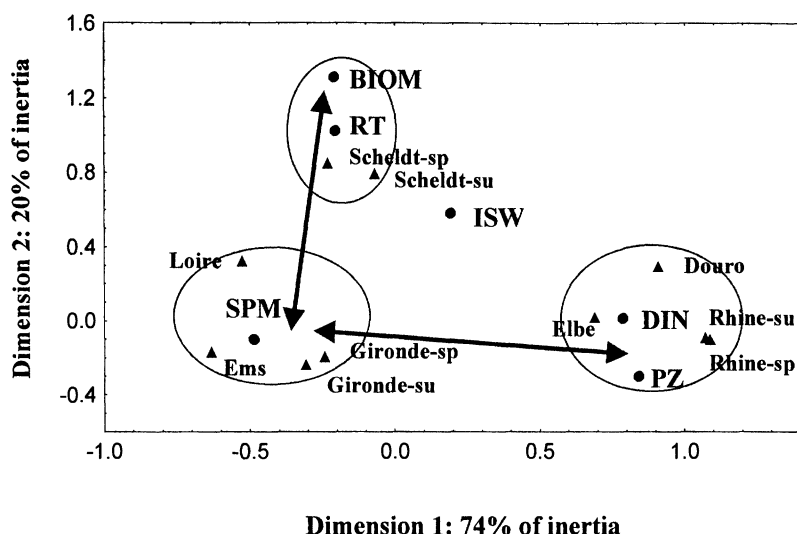


Figure 2. Biplot of the Scheldt, the Douro, the Gironde, the Loire, the Rhine, the Elbe and the Ems data of the correspondence analysis on the phytoplanktonic variables (biomass-BIOM, zone of production maximum-PZ and pigment diversity-ISW) and physical and anthropogenic variables (residence time of water-RT, nitrogen load-DIN and suspended matter concentration-SPM, see Table 1). The two dimensions chosen explain 74% and 20% of the inertia, respectively.

pheophorbides were not detected and the low to intermediate ratios attributed to pheophytins indicated some effect of bacterial degradation (Bianchi 1988; Goosen 1999). In contrast, in the Sado and Ems pheophorbides prevailed with respect to pheophytins, which indicated that grazing activity was a major phytoplankton degradation pathway (Barlow 1993). In the Rhine, Scheldt and Gironde the mixtures of pheophytins and pheophorbides indicated that both phytoplankton degradation pathways were important.

Comparison of the pigment distribution based on estuarine typology

We have combined phytoplankton pigment data with several characteristic indices of anthropogenic influence and physical forcing on the estuaries in order to elaborate a typology. The indices of physical forcing were the residence time of the water masses (RT) and the suspended matter average concentrations (SPM). We have chosen the dissolved inorganic nitrogen load (DIN) of riverine inputs as a measure of the anthropogenic influence. These variables were combined with the chlorophyll *a* concentrations (BIOM), the location of the maximal production across the estuary (PZ, see Table 1)

and the Shannon-Weaver index (ISW) were calculated from pigment data. Samplings during the warm periods (spring and summer) were selected for the comparisons (Thames data were excluded). The Sado was also excluded, because it has very different features due to its hydrographic form (very small river flow and lagoonal morphology of the estuary). Thus our data base comprises 6 variables (DIN, RT, SPM, PZ, BIOM and ISW) and 10 data points representing averaged values (Douro, Elbe, Ems, Loire, and 2 sampling in the Gironde, Rhine and Scheldt to be able to compare the phytoplankton characteristic in terms of estuarine typology for different periods). The typology was based on a correspondence analyses and its results are shown in Figure 2. The two first axes explained respectively 20 and 74% of the variation, thus the two-dimensional representation in Figure 2 covered more than 94% of the total variability.

This figure is read in terms of proximity and clustering of the estuaries. In addition, the variables have been projected in the same plane to visualise their weight in the inertia of the two axes. Three clusters emerged from the analyses. The Elbe, the Rhine and the Douro had similar features, characterised by a high DIN load, short residence times and low chlorophyll concentrations. The Ems, the Loire and the Gironde formed a second group, characterised by a high SPM concentration. The Scheldt was separated from this second group along the second axis and was clearly individualised by a high biomass and a long retention time. These three variables (SPM, DIN and BIOM) explained the maximum of variability of the estuarine features. This correspondence analyses showed that the Gironde, the Scheldt and the Rhine each belonged to a different cluster of estuaries, which justifies the focus of detailed studies on these three estuaries within the frame of the BIOGEST project. The only factor which seemed to have a minor influence was the Shannon Weaver pigment diversity index: this variable was not useful when comparing all estuaries during the warm periods, although these periods represented here the highest biomass and number of phytoplankton classes. Moreover, this index reflects diversity of phytoplanktonic taxa and not species, so the differences among estuaries were smoothed.

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