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Sediment pigments as biomarkers of environmental change

PhD Thesis

Nina Reuss

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COGCI

Freshwater Biological
Laboratory
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Data sheet

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Abstract:	This thesis demonstrates the usefulness of sedimentary pigments as biomarkers of environmental change in estuarine systems and as biomarkers in lakes in relation to climate change. These are two fields where sediment pigment records as measured by high-performance liquid chromatography (HPLC) are emerging areas of study. A detailed study was undertaken to examine the effects of different storage of sediment samples on pigment concentrations and resulted in recommendations to obtain the best quality data with storage of samples. In estuarine systems, pigments in the sediment record were found to reflect the phototrophic community of different systems as well as major changes in eutrophication conditions. However, it was also recognized that the quality of the pigment record is highly dependent on the preservation regime in the sediment. Therefore, selection of an appropriate investigation site is of utmost importance in determining the impacts of environmental change. The pigment record reliably identified major differences in Arctic lake response to climate change, and emphasized the importance of in-lake processes and location in mediating the response. Strongly stratified lakes showed the largest variability while lakes dominated by benthic algae were more stable. Pigment biomarkers are particularly valuable in multi-proxy studies where they can provide a more complete picture of the phototrophic community and where they are often the only fossil remains of non-siliceous algae and phototrophic bacteria.
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Publications from this thesis:

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Preface

This thesis is written as part of the fulfillment of a Ph.D. from the Faculty of Science, University of Copenhagen, Denmark. During the project I was based at the Department of Marine Ecology, National Environmental Research Institute, Roskilde, Denmark and registered under the COGCI (Copenhagen Global Change Initiative) Ph.D. School. The thesis was supported by the European Union 5th Framework MOLTEN project (EVK3-CT-2000-00031).

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Nina Reuss

Copenhagen, one snowy day in March, 2005

Abstract

This thesis demonstrates the usefulness of sedimentary pigments as biomarkers of environmental change in estuarine systems and as biomarkers in lakes in relation to climate change. These are two fields where sediment pigment records as measured by high-performance liquid chromatography (HPLC) are emerging areas of study. A detailed study was undertaken to examine the effects of different storage of sediment samples on pigment concentrations and resulted in recommendations to obtain the best quality data with storage of samples. In estuarine systems, pigments in the sediment record were found to reflect the phototrophic community of different systems as well as major changes in eutrophication conditions. However, it was also recognized that the quality of the pigment record is highly dependent on the preservation regime in the sediment. Therefore, selection of an appropriate investigation site is of utmost importance in determining the impacts of environmental change. The pigment record reliably identified major differences in Arctic lake response to climate change, and emphasized the importance of in-lake processes and location in mediating the response. Strongly stratified lakes showed the largest variability while lakes dominated by benthic algae were more stable. Pigment biomarkers are particularly valuable in multi-proxy studies where they can provide a more complete picture of the phototrophic community and where they are often the only fossil remains of non-siliceous algae and phototrophic bacteria.

Sammenfatning (Danish summary)

Formålet med dette ph.d.-projekt var at undersøge sedimentpigmenter som indikator for miljøændringer i estuarine områder og i søer i relation til klima, to områder hvor sedimentpigmenter ikke traditionelt har været brugt. I forbindelse med den aktuelle debat omkring klimaændringer og effekten af udledning af næringsstoffer til vandmiljøet er det helt afgørende at have viden om langtidsændringer og naturlig variation. De fleste overvågningsprogrammer går dog sjældent længere tilbage end nogle få årtier, og andre metoder må tages i brug. Paleoøkologiske metoder kan give den baggrundsviden, som er nødvendig for at vurdere ændringer i miljøet i dag samt forudse effekten af fremtidig global opvarmning.

Undersøgelsesområdet i dette projekt inkluderede fire estuarier i Nordvesteuropa over en periode på omkring 100 år i relation til næringsstofbelastning, samt søsystemer i Sydvestgrønland gennem de sidste ca. 2.000 år i relation til klimaændringer. Derudover blev der udført et eksperiment for at vurdere effekten af forskellige opbevarings metoder for sediment til pigmentanalyser. Analyserne blev udført ved hjælp af HPLC (High Performance Liquid Chromatography).

Vi fandt, at sedimentpigmenter reflekterede algesammensætningen i forskellige estuarine systemer. I to af de undersøgte områder reflekterede pigmenterne desuden udviklingen i næringsstofkoncentrationer gennem de sidste 100 år i god overensstemmelse med historiske optegnelser og rekonstruktioner baseret på sammensætningen af kiselalger. Dog viste undersøgelserne også, at der er begrænsninger i brugbarheden af sedimentpigmenter til at bestemme udviklingen i estuarier, da pigmenterne er meget afhængige af bevaringsforholdene i sedimentet. Det er derfor af stor betydning at udvælge undersøgelsesområder, der ikke er under stor fysisk påvirkning fra fx strøm eller bunddyr samt gerne har anoxiske forhold for at få bedst mulige resultater af pigmentanalyserne.

I de grønlandske søer identificerede sedimentpigmenterne store forskelle i reaktionen på klimaændringer. De største variationer blev fundet i søer med stærk lagdeling, mens søer domineret af bentiske samfund var forholdsvis stabile. Disse resultater peger på, at ændringer i sammensætningen af alger og fototrofe bakterier er styret af en kombination af regionale klimapåvirkninger og interne forhold i søerne (fx lagdeling) samt søens udformning, oplandsforhold og lokale klimatiske forhold.

På baggrund af resultaterne i dette projekt blev sedimentpigmenter vurderet til at være gode indikatorer for miljøændringer både i søer og estuarier og over forskellige tidsskalaer. Derudover giver pigmenter et godt billede af hele det fototrofe samfund, som ofte ikke efterlader andre fossile indikatorer end pigmenter.

1 Introduction

Background

Two major external factors are influencing the aquatic environment today: climate change and anthropogenic eutrophication. Global climate models predict that we are facing pronounced climate warming, which will have consequences for both terrestrial and aquatic ecosystems (Overpeck et al. 1997; IPCC 2001). Globally, the coastal zone has experienced increased anthropogenic impacts through the last centuries (Conley 1999; Rabalais & Nixon 2002). In Europe, the EU has begun to implement the Water Framework Directive, which is focused on assessing water quality in all water bodies spanning from ground water to coastal marine waters. The directive requires that *undisturbed* or *reference* conditions should be defined for these aquatic environments. Monitoring records rarely go back more than a few decades and in order to define reference conditions other approaches are needed. Paleoecological assessments can provide the long-term records of ecological status and natural variability that are needed to interpret changes observed in the environment today and predict future changes.

Paleoecology

Analysis of fossil sediment records has become a widely used method for estimating changes in nutrient status and climate change, in both freshwater and marine systems (Cornwell et al. 1996; Battarbee 2000; Hodgson et al. 2003). Changes in chemical and biological indicators preserved in the sediment can provide information on the physical environment at the time of sedimentation as well as information on climate change through changes imposed on the aquatic environment (Smol & Cumming 2000). Some of the most widely used biological indicators in paleoecological studies are diatoms, chironomids, foraminifera and pollen. In addition, statistical methods combining modern distributions of a biomarker in terms of their optima and tolerances to hydrochemical gradients have been developed, which can in turn be used to infer historical changes from fossil assemblages. This so called *transfer function* methodology has primarily been applied to diatoms and chironomids for lakes and coastal areas, and has been used for quantitative reconstruction of changes in nutrient status, temperature and salinity (Hall et al. 1997; Walker et al. 1997; McGowan et al. 2003; Weckstrom et al. 2004).

Sedimentary pigments

Pigments represent the phototrophic community as they are produced by algae and other photosynthesising organisms and are specific to particular groups (Jeffrey et al. 1997). The sedimentary pigment records can therefore be used to reconstruct past phototrophic communities and production, and have been shown to reflect changes due to a wide variety of forcing factors such as increased nutrient load, changes in grazing pressure and acidification (Leavitt & Hodgson 2001). To date, the most extensive sediment pigment analyses have been conducted on lake systems and the focus has been on changes in the phytoplankton structure as a response to increased nutrient load.

Multi-proxy and dating

Each paleoecological technique has advantages and disadvantages. Studies including several biological indicators in combination with geochemical markers have proven to be the most effective and reliable method to determine lake status through time and interpret past changes in climate (Fritz 1996; Battarbee 2000). Multi-proxy studies have a long history in paleolimnological investigations (e.g. Engstrom et al. 1985) but are also becoming increasingly common in paleoclimatological analyses (Pienitz et al. 2000; Korhola et al. 2002), and in marine and esturaine areas (Zimmerman & Canuel 2000; Hodgson et al. 2003; Paper III, IV, VI).

An important aspect in paleoecology is getting a reliable chronology of the sedimentary record. Radioisotope dating by ^{210}Pb are appropriate for dating up to approximately 120 years, while peaks of ^{137}Cs can help constrain the most recent dates (early 1960s nuclear testing, 1974-77 Sellafield and 1986 Chernobyl). The primary method used for sediments more than a few hundred years old is radiocarbon dating (^{14}C). Both long and short-term dating are inherently coupled with uncertainty ranging from a few years to several decades. Therefore, comparison of the sediment records with monitoring data or ice-core temperature records should be done with care.

Objectives

This Ph.D. project focused on the use of sedimentary pigments as a biomarker of long-term changes in phototrophic community structure. The aim was to obtain fossil pigment records from different environments at different time scales, and evaluate its use as an indicator for anthropogenic and climate induced changes. Pigment analysis was carried out by high-performance liquid chromatography (HPLC) and the interpretation of the pigment data was conducted in a multi-proxy perspective. There were two major areas and timescales of this study; i) Northern European estuaries during the past ~100 years in relation to anthropogenic eutrophication, and ii) different lake systems in Southwest Greenland during the late Holocene (~2000 years) in relation to climate and in-lake processes. Additionally, as a consequence of the experience working with sediment pigments, a laboratory experiment was designed to estimate the effect of storage of sediment samples for pigment analysis.

2 Pigments as biomarkers

Why pigments?

Pigments are present in all photosynthetic organisms and function primarily as light harvesting agents for photosynthesis and photo protection (Porra et al. 1997). In addition, pigments are useful as biomarkers due to their taxonomic specificity (Jeffrey et al. 1997) and hold the potential of representing the entire phototrophic community and overall primary production. Pigment analyses have been extensively used to determine the phytoplankton community structure in water samples as a supplement or alternative to microscopical counts (Millie et al. 1993). Preserved pigments in the sediment record have been used to investigate past phototrophic production and communities (Leavitt & Hodgson 2001). Some of the most commonly encountered pigments in the water column and sediments of marine and freshwater environments are shown in Table 1.

Table 1 Summary of pigments recovered in the water column and in sediments and their taxonomic affinities. Compiled for freshwater and marine ecosystems from Leavitt & Hodgson (2001) and Jeffrey et al. (1997), respectively. The relative degree of chemical stability is ranked from most (1) to least (4) stable, from Leavitt & Hodgson (2001). Pigments with least stability are rarely found in the sediment.

Pigment	Affinity (major groups or process)	Stability
Chlorophylls		
Chl a	All photosynthetic algae, higher plants	3
Chl b	Green algae, euglenophytes, higher plants	2
Chl c $_{family}$	Dinoflagellates, Diatoms, Chrysophytes	4
Carotenoids		
β-carotene	Most algae and plants	1
α-carotene	Cryptophytes, prochlorophytes, rhodophytes	2
Alloxanthin	Cryptophytes	1
Fucoxanthin	Diatoms, prymnesiophytes, chrysophytes, raphidophytes, several dinoflagellates	2
Diadinoxanthin	Diatoms, dinoflagellates, prymnesiophytes, chrysophytes, raphidophytes, euglenophytes, cryptophytes	3
Diatoxanthin	Diatoms, dinoflagellates, chrysophytes	2
Peridinin	Dinoflagellates	4
Zeaxanthin	Cyanobacteria, prochlorophytes, rhodophytes, chlorophytes	1
Canthaxanthin	colonial cyanobacteria	1
Myxoxanthophyll	colonial cyanobacteria	
Echinenone	Cyanobacteria	1
Lutein	Green algae, euglenophytes, higher plants	1
Neoxanthin	Green algae, Euglenophytes, higher plants	4
Violaxanthin	Green algae, Euglenophytes, higher plants	4
Okenone	Purple sulphur bacteria	1
Isorenieratene	Green sulphur bacteria	1
Chlorophyll degradation products		
Pheophytin a	Chl a derivative (general)	1
Pheophytin b	Chl b derivative (general)	2
Pheophorbide a	Grazing, senescent diatoms	3
Pyro-pheo(pigments)	derivatives of a and b-phorbins	2

Chemical structure

Pigments are bound in pigment-protein complexes as part of the light harvesting complexes of reaction centers in photosynthetic organisms (Porra et al. 1997). Chlorophylls are magnesium coordination complexes of cyclic tetrapyrroles containing a fifth isocyclic ring, often referred to as the phorbin, with a long-chain isoprenoid alcohol ester group, except in most of the chlorophyll c pigments (Fig.1). The carotenoids are hydrocarbons (carotenes) and oxygenated derivatives of carotenes (xanthophylls). The chromophore, the part of the molecule that absorbs light, of carotenoids consists of a system of regularly alternating single and double bonds.

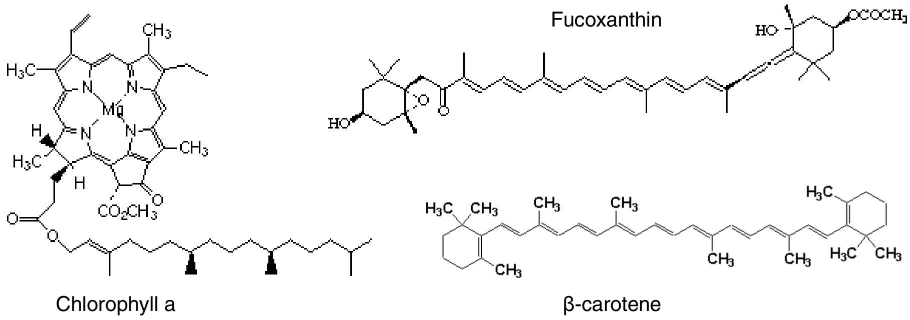


Figure 1 Chemical structures of chlorophyll a (consisting of a Mg-phorbin and a phytol group), and the carotenoids fucoxanthin (xanthophyll) and β-carotene (carotene).

Pigments are labile compounds

In comparison to other organic material, pigments are labile compounds and the individual stability of the pigments varies (Table 1). In a study of marine organic matter from the central equatorial Pacific, Wakeham et al. (1997) assigned overall reactivity of different biochemical classes and found pigments (chlorophylls) to be the most labile, followed by lipids, amino acids and finally carbohydrates as the most refractory group. Pigments are degraded in the aquatic environment by chemical, photochemical, and biological processes. Chlorophylls contain nitrogen and are therefore more prone to being salvaged during senescence and biological breakdown than the carotenoids. Chlorophyll degradation pathways include allomerization (oxidation), demetallation (loss of the Mg), and dephytylation (loss of phytyl chain), with the five-ring phorbin being relatively stable (Porra et al. 1997). Most of these breakdown products are detectable by regular pigment analysis. Carotenoids are found mostly in trans-form and are inherently more stabile than chlorophylls. However, unlike chorophylls they are often broken down to colorless compounds, by destruction of the long chain of alternating double bonds, that can not be detected by regular pigment analysis methods (Leavitt 1993; Leavitt & Hodgson 2001).

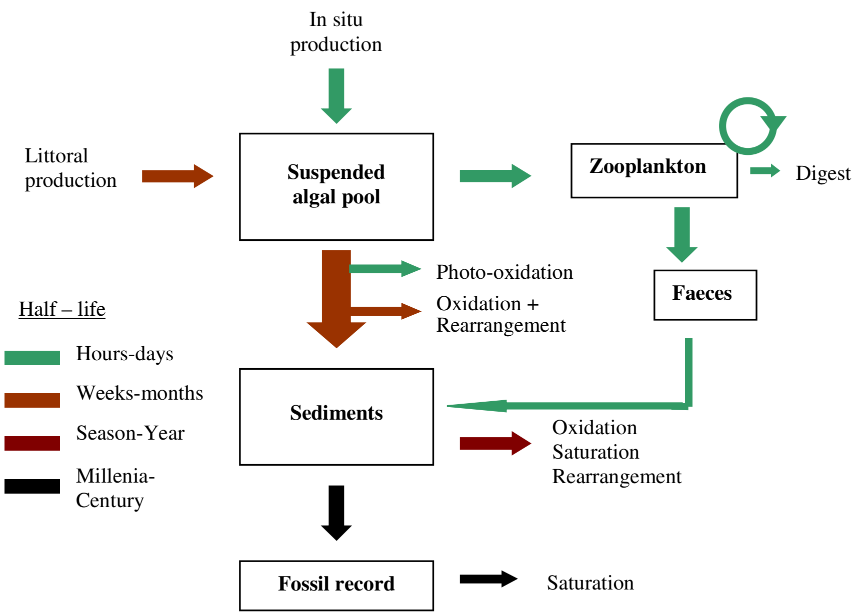
Pigments as quantitative biomarkers

For pigments in the water column a mathematical algorithm has been developed, CHEMTAX (Mackey et al. 1996), for calculation of the abundance of individual algal classes based on the pigment concentrations in natural samples. The algorithm is based on a matrix of pigment ratios and assumes a typical pigment composition for all members in individual algal classes. This constitutes a major source of error in the determination of abundance as pigment composition can vary considerably within algal classes and may also be influenced by light and nutrient conditions (Schluter et al. 2000; Henriksen et al. 2002). Despite these uncertainties, the method has proven successful in various aquatic systems. However, this technique is not appropriate for sediment pigment assemblages due to the selective degradation of indicator pigments.

Flux and degradation of pigments

Only a fraction of the phototrophic production from the water column eventually ends up at the sediment surface and is incorporated into the fossil record. The most extensive degradation of pigments takes place during deposition through the water column and in the surface sediment especially if light and oxygen are available (Fig. 2; Leavitt 1993; Cuddington & Leavitt 1999). Knowledge about degradation, deposition and herbivore digestive processes that affect the fossil pigment record is therefore very important when using pigments in paleoecological investigations.

Figure 2 Major fluxes of autochthonous pigments in fresh water lakes with indications of approximate decay coefficients (for specific references on decay coefficients see Leavitt 1993). Most degradation of pigments occurs during deposition through the water column and in the surface sediments. Redrawn from Leavitt (1993). Benthic fauna is not included but can have large influence on pigment preservation, particularly in the marine environment.



Due to the high degradation of pigments during deposition through the water column, sinking rate is of great importance to the fraction of pigments from the water column reaching the sediment. Differential sinking rates between algae groups can affect the pigment composition in the sediment favoring pigments from fast-sinking organisms. Several mechanisms can promote faster sedimentation such as aggregation and coagulation, which increase with increased biomass, and scavenging from larger sinking particles (Kiørboe et al. 1994). Zooplankton grazing can also alter the flux and relative abundance of pigments. Degradation of pigments occur during passage of the gut, however, faster downward transport in faecal pellets may enhance pigment preservation by escaping degradation in the water column (Leavitt 1993). In addition, sub-surface blooms and benthic production that have not experienced extensive degradation in the water column can bias the sediment record by contributing with a larger proportion of the pigments in the sediment than their proportion of the total phototrophic biomass in the system.

Preservation conditions and choice of sampling site

Oxygen concentrations at the sediment-water interface greatly influences pigment preservation in sediments (Sanger 1988; Leavitt 1993; Sun et al. 1993) and preservation of organic material in general (Canfield 1994). Increased anoxia will not only increase preservation of pigments but also exclude the benthic animal community, thereby reducing ingestion and bioturbation, which has a significant effect on the degradation of sedimentary pigments (Bianchi et al. 2000b; Ingalls et al. 2000). However, it has been shown that ingestion by some deposit-feeding macrobenthos does not affect pigments of live diatoms (Hansen & Josefson 2004), and may rather reduce the time the algae spend in the water-sediment interface by burial, possibly reducing degradation of the pigments. Oxygen conditions at the sediment surface are often influenced or even controlled by the primary production and the amount of organic material reaching the sediment because oxygen is depleted as the organic matter is degraded. It can therefore be difficult to distinguish if increased pigment concentrations are an indication of increased production or increased preservation conditions in the sediment and this should be taken into account when interpreting the sediment pigment record (Leavitt 1993).

The bathymetry of the depositional area and the deposition rate are other important factors influencing pigment preservation. Slumping and focusing of sediment material from shallow, oxygenated sediments into the deep depositional sites can dilute the sediment pigment record (Sanger 1988). Conversely, high depositional rate has been shown to increase effective burial of pigments into the fossil record thereby decreasing degradation at the sediment surface (Leavitt 1993). Furthermore, large variations in accumulation rate of biological indicators may occur over short distances within a lake (Anderson 1989) or estuary (Westman & Sohlenius 1999), as well as microstructures created by macrofauna may be important for the distribution of sediment pigments (JLS. Hansen, pers. comm.).

The fact that there are so many different physical, chemical and biological factors influencing sediment pigment preservation, makes the choice of sampling site critically important. A site with minimal physical disturbance and anoxic conditions will provide the best conditions for pigment preservation. In addition, the most reliable record of long-term development in the phototrophic community is found in areas where no significant changes have occurred in basin morphology and preservation conditions.

Pigments as paleoecological biomarkers

Despite problems with degradation of sedimentary pigments they are still valuable as paleoecological indicators and are often the only fossil remains of non-siliceous algae. For more than 30 years the sedimentary pigment record has been extensively used by paleoecologists in lake sediments while its use is still expanding in estuarine and marine sediment studies. The pigment records reflect the phototrophic community and can be used to track long-term changes in algae and bacterial populations (Sanger 1988; Leavitt & Hodgson 2001). Stable fossil pigments indicating total algal abundance and several specific pigments have been shown to correlate with total algal biomass and biomass of individual algal groups, respectively (Leavitt & Findlay 1994). In addition, specific chlorophyll degradation products can be used as indicators of the presence of grazing activity (Bianchi et al. 1988; Chen et al. 2003), while certain carotenoid pigments can document historical changes in UV radiation conditions in lakes (Leavitt et al. 1997). Bacteriochlorophyll and carotenoids from anoxygenic phototrophic bacteria have successfully been used as biomarkers for anoxic events in coastal waters and state changes in lake systems (Repeta 1993; Chen et al. 2001; Squier et al. 2002). In addition, analysis of sedimentary pigments is relatively simple and less time consuming than other biological paleoindicators. Thus, this approach presents a valuable tool for evaluating ecological changes in the aquatic environment, such as the impact of eutrophication and climate change on ecosystem functioning. However, combining the sedimentary record with other biological and geochemical indicators in multi-proxy studies still constitutes the most robust method for interpreting long-term changes in ecosystem structure.

Use of pigment concentrations

Carotenoids hold the largest potential for identifying the phototrophic community contributing to the sediment record due to their taxonomical specificity and high stability relative to other pigments. However, as mentioned above, many factors influence the relative abundance of carotenoid pigments. Examining pigment concentrations individually with each pigment scaled against its historical maximum has therefore been suggested as the most reliable method of interpreting the sediment pigment record (Leavitt 1993). Normalizing pigment concentrations to the organic carbon pool can also partly compensate for bias from pigment degradation at different preservation conditions (Leavitt 1993) and exclude artefacts due to water content and salt effects. The sediment pigment inventory (concentration integrated over a specific depth) can be valuable when comparing sites with different sedimentation rates (Sun et al. 1994). Concentration data is generally recommended for paleoecological reconstruction, however, accumulation rate that takes sedimentation rate into account can also be valuable for comparisons between sites (Leavitt & Findlay 1994). Creating accumulation profiles of the sedimentary record depends on a good chronology of the sediment record, which can be difficult to obtain, and therefore accumulation profiles should be interpreted with caution.

3 Sampling and analysis of sediment pigments

Sampling

Sediment samples for pigment analyses can be collected by various coring methods including box cores, Haps cores, Russian cores, and freeze cores, followed by a subsequent sectioning of the cores that determines the maximal resolution of sediment analyses. Due to the labile nature of pigments certain precautions have to be taken. Keeping the collected cores as dark and cold as possible, preferably frozen, both before and after sub-sampling is important to prevent pigment degradation. It is recognized, however, that logistical constrains, e.g. small sampling vessels with no storage space and sampling in remote places, may hinder optimal storage. Use of freeze cores has proven excellent for coring surface sediments that often have a high water content. Freezing the sediment *in situ* also provides good preservation conditions for pigments. However, the disadvantage of freeze cores is the relatively small sample size obtainable for each level and the limited length of the core (up to ca. 1 m). Sediment analyses of less labile indicators are not affected by the choice of coring method except for the ability to conduct high-resolution sampling.

Effect of storage

Even though sediment pigments have been investigated for several decades there has been no consensus on storage practice. Various storage practices have been used over time without exact knowledge of the effect it can have on the results. Only recently, proper recommendations for the storage and handling of sediments for pigment analysis have been put forward by Leavitt & Hodgson (2001). It is generally accepted that sediment pigments are labile compounds and therefore should be protected from light, heat and oxygen. Constraints on storage practice are now becoming an issue as the focus on multi-proxy studies increases and individual analyses have different requirements. Some approaches such as germination experiments of diatoms, require that the sediment has not been frozen prior to analysis (Hansen & Josefson 2003), while analyses of general geochemical biomarkers such as carbon, nitrogen, biogenic silica and metals as well as various isotopes require dried or preferably freeze-dried sediment. In order to investigate in greater detail the effect of storage on the sedimentary pigment results a storage experiment was set up using natural sediment samples collected from an oxic and an anoxic depositional site in an eutrophic Danish estuary (Paper I).

The storage study included both current and past methods of storage, e.g. raw and freeze-dried material were stored at various temperatures for 6 months (+20, +6, -20 and -80°C). Little difference was found between most of the storage practices, except for freeze-dried sediment at room temperature (+20°C), which showed significant degradation of pigments. No additional preservation was found for samples stored at extreme cold (-80°C) or flushed with inert gas, which has become common practice in many pigment laboratories and was recommended by Leavitt & Hodgson (2001). The recommendation for optimal preservation of pigments from the present study is to keep samples frozen at <-20°C as raw samples until just before analysis. However, refrigerated raw samples can also be kept for an extended period without significant degradation. Freeze-dried samples, which are often most practical for transport over longer distances, should always be stored frozen. Previous investigations of storage effects on pelagic samples collected on filters showed much more variability in degradation between different types of storage and recommended that filters should be stored at <-80°C and never freeze-dried (Mantoura et al. 1997). This difference in degradation of pigments on filters compared to sediment samples is probably due to the pigments being highly exposed on filters as opposed to being protected in the sediment matrix.

Extraction of sedimentary pigments

Chlorophylls and carotenoids are water-insoluble in contrast to other pigments found in the living cells such as anthocyanins and phycobilins that are usually broken down before getting incorporated into the sediment (Sanger 1988). Various methods have been used for extraction of pigments and involves extraction by soaking in organic solvents with or without disruption (sonication, grinding) or freeze-drying. The most common extraction solvents include acetone, methanol, ethanol or a combination. No single method will be optimal for all pigments or all sediment types. A review of extraction methods and solvents used for water samples are given by Wright et al. (1997), while studies of extraction efficiency have also been performed on marine sediment samples (Buffan-Dubau & Carman 2000; Louda et al. 2000). Leavitt & Hodgson (2001) describe a successful method for freshwater sediments using a combination of acetone, methanol and water for extraction of freeze-dried samples. Freeze-drying has been shown to improve pigment extraction and acetone to be an effective extraction solvent for marine sediment samples (Buffan-Dubau & Carman 2000). This method has been extensively used in studies covering various marine sediment types and was chosen for all analyses in this thesis project.

Separation and identification

Identification of sediment pigments has traditionally been conducted using spectrophotometric and fluorometric techniques, primarily for quantifying chlorophyll a. However, these techniques are limited by not being able to quantify and identify the multiple pigment assemblages often found in natural samples (Millie et al. 1993). High performance liquid chromoatography (HPLC) has now become the method of choice for fast and reliable quantification and identification of multiple pigments in a single run, but as for extraction procedures, no single method is optimal for all analyses (Jeffrey et al. 1999). Two main techniques, detailed by Mantoura & Llewellyn (1983) and Wright et al. (1991), and modifications thereof have been successfully used for sediment pigment analyses in freshwater and marine systems. Both techniques rely on reversed phase HPLC systems but use two and three solvent gradients for separation, respectively. For the sedimentary pigment work of this thesis, a modification of Wright et al. (1991) as described by Chen et al. (2001) was used as it has been shown to effectively separate the main indicator carotenoids and a large number of chlorophyll degradation products.

Identification of pigments in natural samples is primarily conducted by a combination of retention time and absorbance spectra compared to authentic standards of individual pigments (Fig. 3). However, compared to water column samples, chromatograms of sedimentary pigment samples contain a large number of peaks including unknown pigments and pigment degradation products and co-elution may occur. Lakes with good preservation conditions show relatively few unidentified peaks (Fig. 4) compared to estuarine systems where extensive amounts of unknown peaks and chlorophyll degradation products can be found. Pigment identification in sediment samples based on these two criteria (retention time and absorbance spectra) is, therefore, only tentative. However, limited amount of sample in field experiments and large number of samples often precludes further chemical analysis for accurate identification. Mass spectrometry (MS), a new in-line technique that does not require extra material, is now suggested as a valuable supplement for secure identification of sediment pigments (Hodgson et al. 1997; Leavitt & Hodgson 2001). However, this technique requires expensive equipment, which is often not available in many laboratories. Detailed descriptions of separation and identification techniques of pigments can be found in Jeffrey et al. (1997) and Leavitt & Hodgson (2001). These references also provide descriptions of quantification of pigments, which is conducted by determining a response factor or standard curve linking the area of a peak to concentration for individual pigments based on standards. Some of these standards are now commercially available from many different companies, e.g. DHI Water and Environment, Denmark, or they can be prepared from cultures (see Jeffrey et al. 1997). In addition, it is highly recommended to include an internal standard during extraction in order to normalize pigment concentrations as it significantly reduces variability of analyses (Paper I).

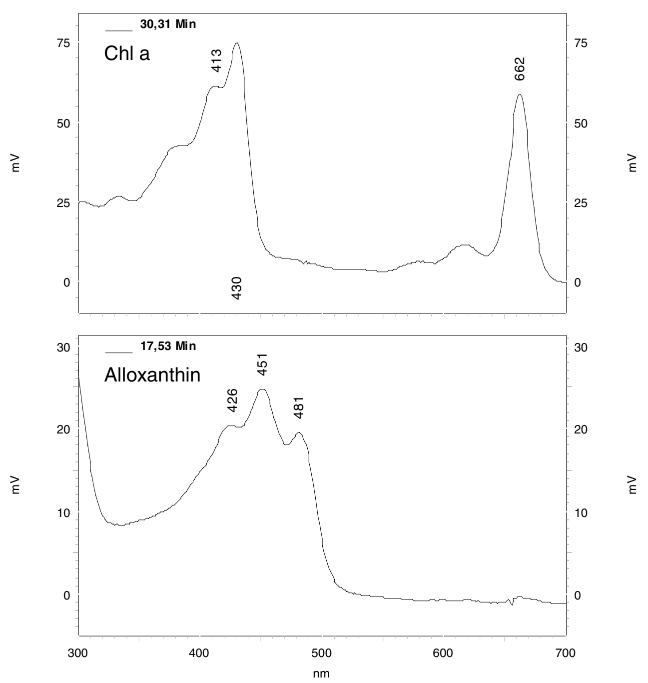


Figure 3 Absorbance spectra of chlorophyll a and alloxanthin obtained by an on-line photodiode array (PDA) detector from a sediment sample of Mariager Fjord, Denmark. Chlorophyll a has peak absorbance at 430 nm and 662 nm while alloxanthin has three peaked absorbance around 451 nm. Also note the difference in retention time (in top left corner, Min = minutes). A fluorescence detector was used for additional identification of the fluorescent chlorophyll compounds.

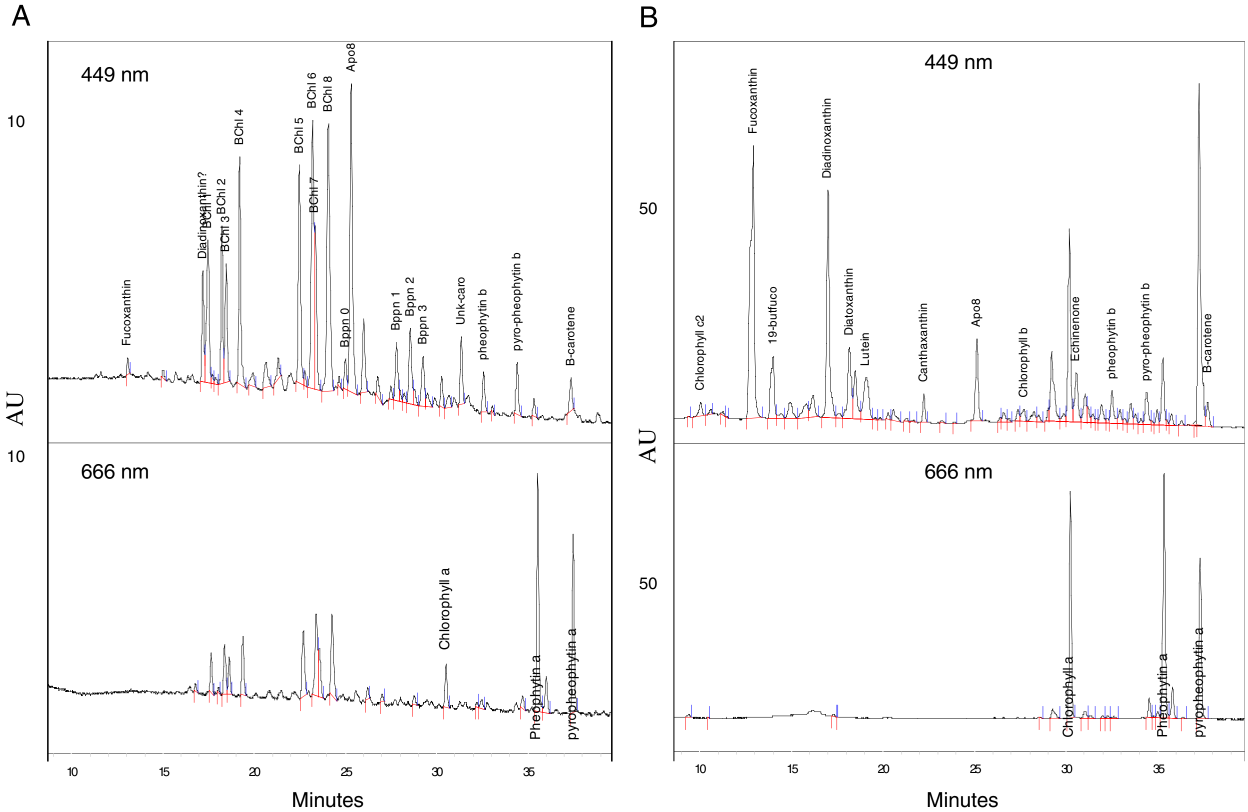


Figure 4 An example of chromatograms obtained at 449 nm and 666 nm for two Greenland lakes dominated by green sulphur bacteria (A), indicated by bacteriochlorophyll e isomeres (BChl), and an algal phototrophic community (B), mainly consisting of diatoms as indicated by high concentrations of fucoxanthin and diadinoxanthin. Quantification of carotenoids, chlorophyll b and c and bacteriochlorophyll were conducted at 449 nm and for chlorophyll a compounds at 666 nm. The full runtime was 49 minutes.

4 Estuarine systems

Many estuaries around the world have been heavily affected by anthropogenic disturbance and eutrophication for centuries (Conley 1999; Rabalais & Nixon 2002). Unlike lakes, estuaries are open systems and are unlikely to contain unaffected areas to be used to define reference conditions as required by the EU Water Framework Directive. Therefore, other methods have to be considered such as paleoecological investigations.

Complex systems

Estuaries are complex systems compared to lakes because of the influence from both fresh and marine water masses and often also by extensive currents and benthic macrofauna. Enclosed bays or estuaries where a sill restricts the water exchange, as well as sites with temporal or permanent bottom water anoxia, are likely to provide the best continuous sedimentary records. This has most recently been confirmed by the EU funded MOLTEN project, which conducted paleoecological investigations of four different estuarine systems in Northern Europe (http://craticula.ncl.ac.uk/Molten/jsp/index.jsp). The project focused on using diatom-based transfer functions to reconstruct historical nutrient concentrations over the last ca. 100 years from paleoecological records. A further goal was to develop a tool for coastal managers to assess the necessary nutrient reductions to reach conditions of minimal anthropogenic impact as defined by the Water Framework Directive. The best records were obtained from relatively enclosed estuarine systems while it was impossible to establish a chronology for the sediment record from a mudflat that showed signs of hiatuses (missing layer of sediment) down core.

Pigments in estuarine sediments

As an integrated part of the MOLTEN project an intensive pigment study was conducted. This showed that the choice of sampling site and physical setting of the estuaries are of utmost importance for the usefulness of sedimentary pigment analyses (Paper II). The dominating pigments at all four sites reflected the algae community known from monitoring records, while the down core records showed very different trends. The pigments showed reliable records of past productivity and dominating phototrophic communities in an almost permanently anoxic estuary (Mariager Fjord, Denmark) and a relatively shallow but highly eutrophic enclosed bay (Laajalahti, Finland). However, a mudflat (Ems-Dollard, The Netherlands) and a deep, steep sided, estuary (Himmerfjärden, Sweden) had heavily degraded pigment records which showed no correlation to known changes in nutrient conditions. These differences are a result of the very different preservation conditions at the four sites (Fig. 5). Dark and anoxic conditions at the Danish site and almost lake-like conditions in the shallow Finish bay provided stable and relatively good preservation conditions for the pigments. The daily exposure of the sediment to air and light at the mudflat caused heavy degradation of pigments except for the pigments contained within the live benthic algal community. The result was an almost exponential decline in the pigments and very low concentrations below 5-10 cm. In spite of temporal anoxia, the preservation of pigments at the steep sided estuary was low due to focusing of re-suspended material from the sides to the deep depositional site. This dilutes the pigment signal with heavily degraded material, which has been exposed to oxygen and light higher up in the water column. Thus, while the sediment pigment record can provide a good picture of the dominating phytoplankton community in an estuary, the value of sediment pigments as indicators of environmental change in estuarine sediments depends on environmental constraints and preservation conditions in the sediment, i.e. the availability of oxygen, resuspension and focusing processes.

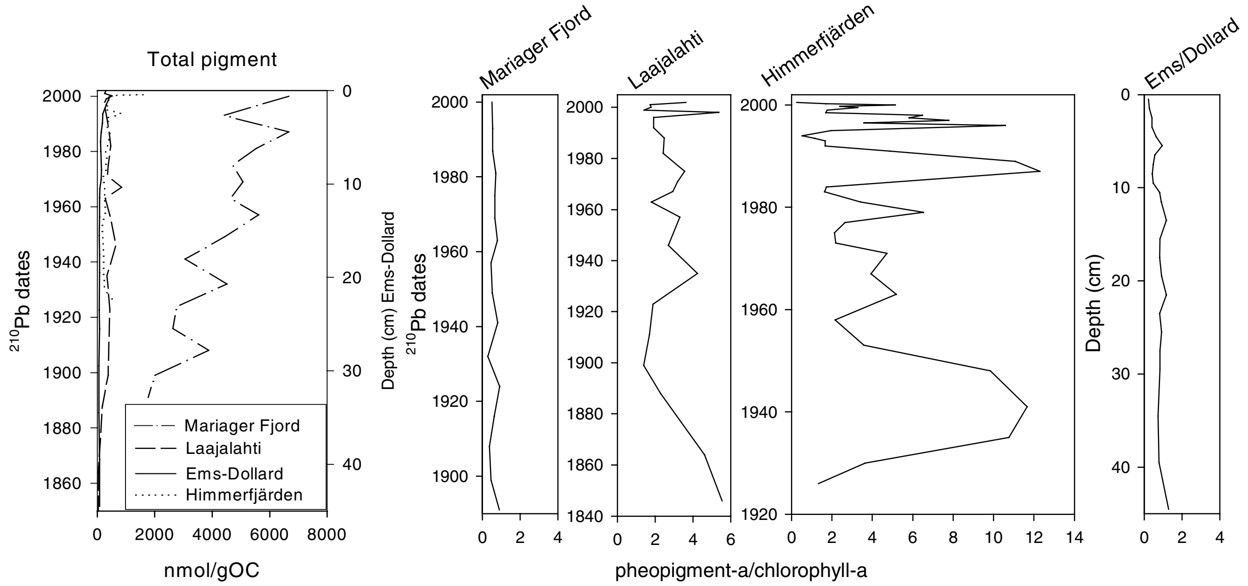


Figure 5 Total pigment concentration from four estuaries in NW Europe (left panel) and the ratio of pheopigment a to chlorophyll a, a measure of preservation conditions (right panels). A low ratio indicates good preservation conditions with the best preservation at Mariager Fjord, with almost permanent anoxic bottom water conditions, and the worst at Himmerfjärden, a steep sided estuary influenced by resuspension and focusing of material. Pulses of diatom production reaching the sediment without extensive prior degradation may cause the large variation in the ratio. The low ratio found at Ems-Dollard, a tidal mudflat, is probably a result of a live benthic algal community in combination with degradation of the more stable degradation products of chlorophyll a at this exposed site. (Modified from Paper II)

Estuarine pigment studies

Resent studies focusing on the sediment pigment record from estuarine sites have revealed changes in eutrophication and dominating flora and have provided knowledge of long-term system functioning (e.g. Chen et al. 2001; Hodgson et al. 2003; Rabalais et al. 2004). In addition, the pigment record in the Baltic Sea has demonstrated the occurrence of cyanobacterial blooms over thousands of years (Bianchi et al. 2000a) while changes in chlorophyll derivatives have been linked to climatic phenomena over the last ~8000 years (Kowalewska 2001). More short-term studies have correlated monitoring records of phytoplankton and sedimentary pigments over a 30-year eutrophication period in Himmerfjärden, a deep temporary anoxic estuary (Bianchi et al. 2002). Studies focusing on the surface sediments have revealed spatial and seasonal distributions of microphytobenthos communities (Brotas & Plante-Cuny 2003), benthic processes (Sun et al. 1994), and distribution of the spring bloom sedimentation (Hansen & Josefson 2003).

Multi-proxy studies

In light of the complex nature of estuarine sites, multi-proxy studies involving both geochemical and biological indicators are becoming the method of choice for paleoecological studies in these areas. The multi-proxy approach has been used in various estuarine systems (e.g. Cornwell et al. 1996; Zimmerman & Canuel 2000; Hodgson et al. 2003; Smittenberg et al. 2004; Paper III, IV, VI). However, only the most recent of these multi-proxy studies include sedimentary pigments and recognize their value as a supplementary biomarker of environmental change.

Development in Northern European estuaries

Compiled multi-proxy evidence from estuaries in Northern Europe over the past 150 years has revealed previous conditions characterized by lower nutrient status followed by a considerable eutrophication resulting in increased primary production (Paper III, IV, VI). Significant changes were observed within algal classes, e.g. diatoms and dinoflagellates. In contrast, the pigment record showed no change in the overall dominance in the phototrophic community, which is probably controlled by the salinity and physical setting of the individual estuaries, but showed significant variation in concentrations. Quantitative estimates of the nutrient conditions in the estuaries have been provided by development of diatom-based transfer functions (Clarke et al. 2003; Weckstrom et al. 2004), which have been extensively used in freshwater systems. The diatom-inferred reconstruction of nitrogen concentrations in the estuaries suggested major increases over the last ca. 100 years, but there was also evidence of some recovery of one of the estuaries after years of changed managing practices that have reduced the nutrient flow to the estuary. The combination of these results showed that the pigment record was the indicator that most closely followed the recovery trend (Paper IV, Fig. 6). No quantitative estimation of nutrient concentrations could be resolved for the deep anoxic estuary in Denmark, however, several geochemical indicators including pigments indicated increased eutrophication over the last 100 years (Paper III). Base line nutrient conditions for coastal waters can therefore be defined by the use of quantitative paleocecological methods, while multi-proxy evidence can provide valuable knowledge about the ecological functioning of the system before anthropogenic eutrophication.

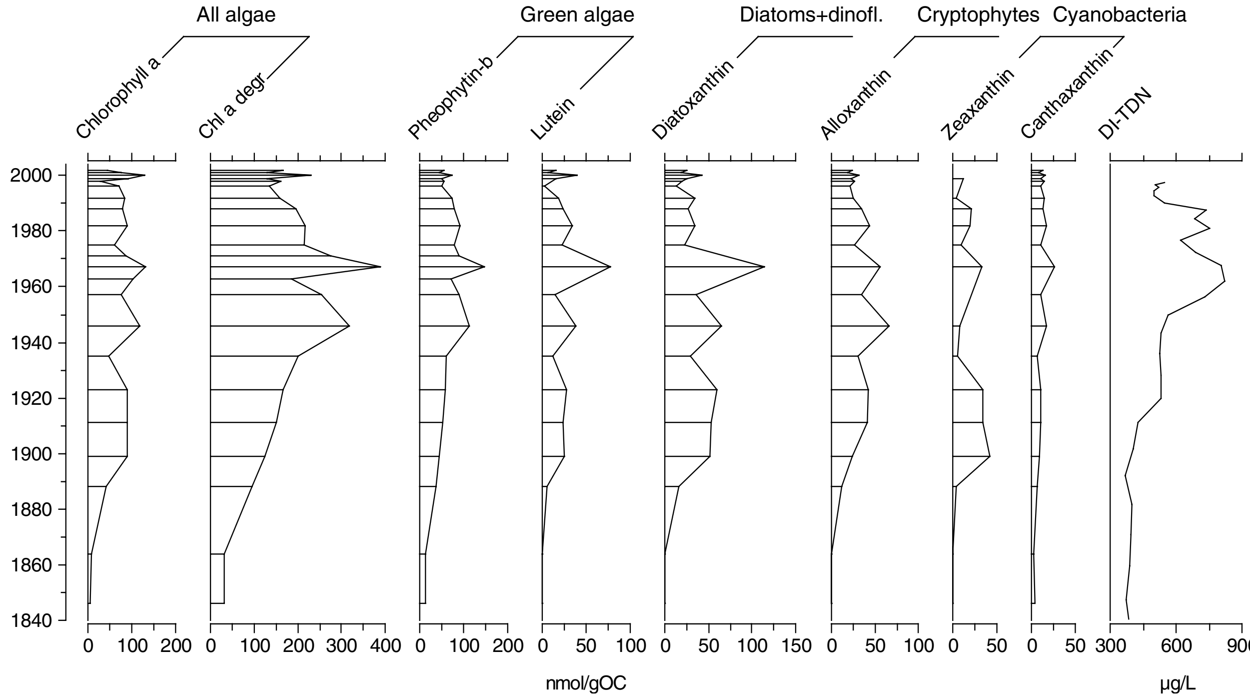


Figure 6 Sediment pigments and diatom-inferred total dissolved nitrogen concentration (DI-TDN) from Laajalahti, Finland. All dominating pigments show a characteristic peak around 1965. This corresponds to the time period of the most intensive eutrophication known from historic records and from nutrient reconstruction based on diatom abundance. The pigments indicate dominance of green algae and higher plants, diatoms and dinoflagellates as well as cryptophytes. This community composition was in good agreement with the monitoring data, although higher amounts of the cyanobacteria indicators were expected. Despite the large variation in concentrations over time no major changes in the phototrophic community structure were observed. (Modified from Paper II and IV).

5 Lakes and climate

Climate signals in Arctic lakes

Concerns about global warming have lead to increased scientific focus on lake sediments as a record for long-term changes in climate. Lakes in the Arctic experience minimal direct anthropogenic influence and are sensitive systems with high potential for paleoecological studies of long-term climate changes that can provide the basis for interpreting changes in the environment today (Smol & Cumming 2000; Battarbee 2000; Fritz 2003). Lakes close to climatically sensitive thresholds, e.g. the treeline, that amplify the signal by feedback mechanisms register changes particularly well compared to more buffered systems (Battarbee 2000). However, most paleoclimatological approaches are indirect and reflect changes in lake status (e.g. pH, salinity, DOC, nutrients, thermal balance), which is controlled by climate. For example, increased temperature and reduced ice cover can have substantial effects on primary production as a result of changes in the length of the growth season, while changes in effective precipitation can affect the conductivity of the lakes, which is important for circulation patterns. Therefore, detailed knowledge of the relationship between lake status, water column processes and sediments are required to interpret fossil records.

Climate changes have been inferred from paleoclimate records such as tree ring sequences and ice-cores (Overpeck et al. 1997; Dahl-Jensen et al. 1998). In addition, several paleolimnological techniques including chironomids, diatoms, pollen, and stable isotopes have been used to infer changes in salinity, nutrient status and vegetation, in relation to changes in temperature and precipitation (Fritz 1996; Battarbee 2000). While previous analyses have focused primarily on reconstructing changes in climate, the present challenge is to assess the effects of such changes on ecosystem functioning. Few studies have used the sedimentary pigment record for reconstructing climate changes (Vinebrooke et al. 1998; Lami et al. 1998; Pienitz et al. 2000; Korhola et al. 2002). However, since pigments represent a wider range of the phototrophic community than more traditional paleotechniques, by including phototrophic bacteria and several algal flagellates that do not leave any other fossil evidence, they are valuable in multi-proxy studies by giving a more complete picture of the ecological system. Recent work confirms the usefulness of the sedimentary pigment record in providing knowledge about long-term climate effects on the biological functioning of lakes (Lami et al. 1998; Leavitt et al. 2003; McGowan unpublished; Paper V).

Climate change in the Arctic

Global climate models predict that the Arctic will be the area of most pronounced warming in the future. It is therefore essential to have specific knowledge about the inherent temporal variability and long-term development in these systems to evaluate the potential effects of future climate change on the biological systems. Climate reconstructions for the North Atlantic region based on Greenland ice-cores show that major changes in temperature have occurred during the Holocene (last ~10000 years) and particularly during the past 2000 years (Fig. 7, Dahl-Jensen et al. 1998). A cooling period, extending from the end of the Climate Optimum (8000-5000 BP) reached a minimum around 2000 BP and was followed by the Medieval Warm Period and the Little Ice Age (LIA). The LIA has in Greenland been followed by a warming period peaking around 1930 AD with subsequent cooling towards the present (Dahl-Jensen et al. 1998; Hanna & Cappelen 2003). Diatom-inferred conductivity profiles from two closed-basin oligosaline lakes in Southwest Greenland suggest that marked aridity prevailed between ca. 7000-5600 BP followed by positive precipitation conditions ca. 5600-4000 BP. In contrast to these long stable periods, an oscillating precipitation balance characterized the period from 4000 BP to the present (McGowan et al. 2003).

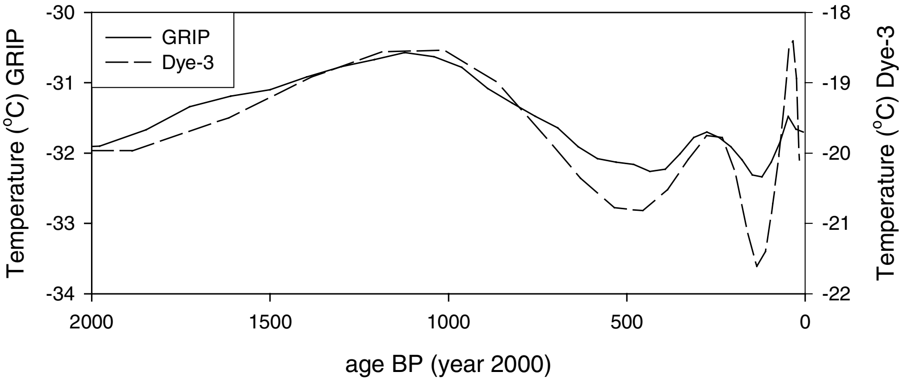


Figure 7 Temperature reconstruction for the last 2000 years from the Greenland inland ice cores, GRIP and Dye-3 (Dahl-Jensen et al. 1998). The time period includes the Medieval Warm Period (MWP, 1400-800 BP), the Little Ice Age (LIA, 700-150 BP) and the following warming and cooling trends.

Southwest Greenland

Lakes in the Kangerlussuaq area, SW Greenland, are good sensors of climate change as they are close to climatically sensitive thresholds situated along a regional climatic gradient between the inland continental climate and the more maritime environment at the coast. Paleolimnological studies of pollen and macrofossils in the area have shown distinct responses in the limnic and terrestrial biological communities to changes in climate (e.g. Bennike 2000). However, a study of the sedimentary pigment records indicated that different lake types react very differently to climate change (Paper V). This suggests strong dependence on in-lake processes and local conditions in shaping the phototrophic community in response to changes in temperature and precipitation, making direct interpretations of climate change much more complicated.

Climate vs. in-lake processes

The study of sedimentary pigments as indicators of ecological structure in lakes from SW Greenland included both freshwater and oligosaline systems with marked differences in phototrophic communities and temporal variability (Paper V). The lake most sensitive to climate was a recently closed lake, which is presently intermediate between a dimictic, freshwater and an oligosaline, meromictic state (Fig. 8). The transition from a dimictic, freshwater system to a more stratified state with light penetration into an anoxic hypolimnion was indicated by a change in the phototrophic community from an algal to a bacterial dominated community. Lake level lowering and loss of outflow likely caused the state change of this lake approximately 1000 years BP coinciding with the Medieval Warm Period. In an oligosaline, meromictic lake, large short-term variation was observed, likely due to the tight coupling between the phototrophic community and the physical structure of the water column. However, no major directional trends were observed indicating that in-lake processes are probably the primarily controlling factor in this lake as opposed to climate change. Two freshwater lakes dominated by benthic algae showed very little response to climate change due to the stable nature of these benthic systems. The results demonstrate that changes in the ecological structure and phototrophic community in these lakes are driven by a combination of factors including climate forcing mediated by in-lake processes but also by local climate, and lake basin and catchment morphology.

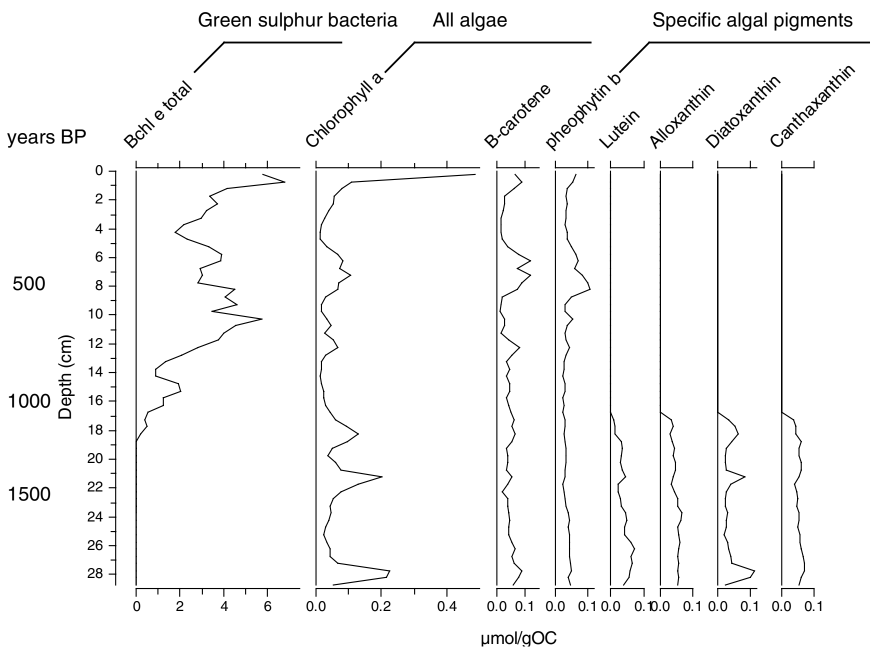


Figure 8 Sediment pigments from a recently closed lake in SW Greenland (SS86). The pigments show large changes around 16-18 cm (~1000 years BP) indicating a shift from an algal to a bacterial dominated community as a result of a shift in stratification conditions in the lake from a mixed to a stratified system. This was concurrent with the culmination of a prolonged warming period about 1000 years ago causing loss of outflow and increasing concentration of ions and nutrients in the lake. Bchl e = bacteriochlorophyll e. (Modified from Paper V)

6 Conclusions and perspectives

The ongoing debate about climate change and anthropogenic impacts on aquatic systems and the resulting demand for management plans for aquatic environments has increased the need for knowledge about long-term changes and natural variability. Paleoecological studies are often the only means to obtain this information. This study has contributed to the knowledge about the forces and limitations of sedimentary pigments as a biomarker of environmental change in estuarine environments and in lakes in relation to climate change.

Anthropogenic eutrophication

Sedimentary pigments in estuarine systems were shown to reflect changes in plankton community structure as well as the overall changes in nutrient loading inferred from historical records and diatom-based nutrient reconstructions. However, the results from the work presented in this thesis also recognized that the preservation regime is very important for the usefulness of sediment pigments (Paper II). Therefore, to be able to exploit the full potential of the labile pigment biomarker record careful site selection is required.

The pigment record provided good supplementary evidence of the phototrophic community in multi-proxy studies (Paper III, IV). Multi-proxy studies are particularly important for reliable characterization of ecological changes in complex systems like estuaries. Pigments are useful for qualitative assessments of dominating plankton community and potentially for relative production estimates over time. However, in compliance with the EU Water Framework Directive quantitative estimates are needed and this can be provided by diatom transfer functions. Further investigations are required in estuarine systems to evaluate the full potential of pigments in multi-proxy studies in these complex systems.

Analysis of sedimentary pigments is a relatively fast method and, therefore, holds future prospects of incorporation into routine investigations such as large monitoring campaigns where traditional time consuming algal counts are not feasible, or to extend an investigation area by combining a few microscopic counts with an extended pigment investigation. However, the labile nature of pigments requires that precautions to reduce light and heat during handling and storage of the samples be taken to gain good results (Paper I).

Climate

Predicting effects of global climate change is of international importance, as climate does not recognize borders. The work presented in this thesis contributes to the understanding of ecological impacts of climate change and the importance of in-lake processes and location in mediating the lake response (Paper V). However, knowledge about the link between water column processes and the sedimentary record in these areas, e.g. sedimentation and degradation processes, deserves further attention.

Sediment pigments proved to be good indicators of lake-ecosystem response to climate change and long-term variability in the phototrophic community, which is needed for predicting possible effects of future climate change. Large differences in the response to climate of different lake types from SW Greenland revealed that in-lake processes have a significant influence on mediating the individual lake response. Strongly stratified lakes showed the largest variability while benthic dominated systems were more stable. In addition, the pigments provided a more complete picture of the phototrophic community than other biomarkers commonly used to study long-term variability in lake ecosystems. However, the sedimentary pigment record primarily indicates ecological changes while supplementary proxy evidence is needed for full interpretation of climate variability in the studied area.

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