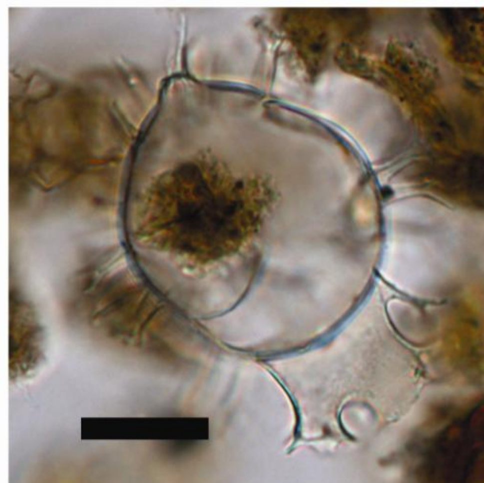
Palynological and organic geochemical indications of recent eutrophication and anthropogenic disturbances in Charlotte Harbor, Florida, USA.



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ABSTRACT

One of the large and increasingly important global problems is coastal eutrophication. Human disturbances includes large-scale fertilizer use which increases the nutrient availability in many coastal areas and can lead to toxic algal blooms or ecosystem turnover. Detecting past human disturbances in an area can improve the understanding of the nature and scope of the change induced and can be used in unraveling the differences between human induced change and natural variability. Charlotte Harbor (CH) is an until recently relatively undisturbed coastal-plain estuarine system in south-western Florida, U.S.A. Therefore, it is very useful in detecting anthropogenic and natural changes. A high-resolution multi-proxy study is conducted on a ^{210}Pb dated sediment push core from CH, comprised of the last 140 years. Dinocyst and geochemical analyses provided consistent evidence for environmental changes induced by cultural eutrophication and human disturbance over the last 50 years. There is increased disturbance of the CH catchment and coastal area by deforestation (lower tree pollen, increased early succession species as *Ambrosia*), urbanization (population rise, large-scale waterfront development), pollution (waste water, P mining and slime spills) and agriculture (fertilizer usage, irrigation, water withdrawals). Changes in both biomarkers and palynology records suggests relative human-induced (cultural) eutrophication in the 1960s, which is increasingly evident since the 1980s AD. Dinocysts with aberrant cyst morphologies become dominant. These dinocysts, as '*Spiniferites miratorii*', probably reflect lower salinity environments. As a result of human deforestation, population growth and urbanization activities, runoff and erosion increased in the CH area. This led to higher sedimentation rates, higher input of terrestrial OM and increased primary productivity due to higher nutrient inputs. Deforestation is visible in the pollen record from 1971 AD onwards. Nitrate levels of the Peace River rose exponentially around 1975 AD. Estuarine primary production of both diatoms (based on the highly branched isoprenoids (HBI) biomarker record) and of dinoflagellates (increased dinocyst flux) increased. It is thought that eutrophication and human disturbances make the estuary unstable and more sensitive for natural and anthropogenic changes. The more dynamic records from 1970s AD onwards indicate an increased sensitivity to salinity changes. It is possible that the increased variability reflects the natural climate oscillations as the Atlantic Multidecadal Oscillation (AMO) or the El Niño Southern Oscillation (ENSO).

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CHAPTER 1: INTRODUCTION

Many coastal areas are directly affected by human influence, e.g. due to agriculture intensification, urbanization, land use changes, drainage and deforestation, but also climate change and sea level rise. There is an ongoing debate to what extend anthropogenic global change impacts the natural environment. Human disruption can cause problems as eutrophication and red tide development which can result in fish kills, human health risk and harmful algal blooms (HABs). Florida is a well-studied area concerning eutrophication. Reconstructions of the changes in the Holocene on different localities in southwest Florida show more marine conditions and in the last century enhanced eutrophication (Soelen et al., 2012). Human disturbances and eutrophication are detected in the southwestern Florida area in both biogeochemistry as in palynology, e.g. in Tampa, Rookery and Florida Bay (Xu et al., 2007; Soelen et al., 2010; Lammers et al., under review). Charlotte Harbor (Chapter 1.2) has been progressively shaped by the Holocene sea level rise through transgression of marine waters into the estuary. Natural variability in river runoff is amongst others influenced by the Atlantic Multidecadal Oscillation (AMO) and the El Niño Southern Oscillation (ENSO) (Chapter 1.3) In more recent years agriculture intensification, deforestation, eutrophication and urbanization shaped the estuary and its watershed. Eutrophication and changes in primary productivity are evident (Chapter 1.4). It is important to look on a high resolution to these problematic developments to be able to unravel the human and natural influence on this estuary. In this study recent and sedimentary archives of the last 140 years are used for this purpose.

1.1 Aims of this study

Eutrophication trends in North America has become noticeable in the last century. As the CH area has been seen as a relatively undisturbed estuary, it is thought that human impact will be visible in different proxies in the last decades. In most cases eutrophication is caused by anthropogenic nutrient input. Many organisms leave (fossilized) chemical components behind in sedimentary records. When these components are specific to a certain organism or a group of organisms, they can be referred to as biomarkers. As the specific components are of biological origin, they can reflect different parameters of the environment where the organisms lived in. Biomarkers are useful tools in reconstructing past environments and their biologic activity. For an overview of the biomarkers used in this study, see Appendix 7. This study will combine high resolution palynology and biogeochemical proxies. The biological changes are reconstructed with dinocyst analysis and certain biomarkers. The data obtained can be used to reconstruct the human impact on the until recently relatively untouched estuary. Possibly, natural rainfall oscillation trends can be found in this high resolution sampled sediment core. When the combined palynological and biomarker data can be linked to these natural rainfall patterns, the signals could possibly be used to reconstruct the natural variability in rainfall in the CH area further back in time.

We compared phytoplankton and biomarker analysis in the surface water with the sedimentary record. First, a growth experiment is conducted to understand the response of phytoplankton in CH to elevated organic and inorganic nutrient levels (Chapter 3.1). Second, a transect from the Peace and Myakka rivers is sampled for biochemistry to connect the algal growth experiment to the sediment core data and add a spatial dimension to the biomarker data (Chapter 3.2). Changes in (quantity of) biomarkers can indicate changes in runoff,

salinity and biological activity in and around the estuary. As *K. brevis* blooms are a nuisance to the Southwest Florida area, there will be extra emphasis on finding a biomarker for this HAB species, which, if found, could be used to reconstruct past HABs in the CH estuary on a high resolution. This would greatly help the understanding of what makes HABs tick.

1.2 Study area: Charlotte Harbor estuary

CH is a relatively shallow (on average 3 meters deep), coastal plain estuary of the Peace, Myakka and Caloosahatchee rivers, situated in the southwestern coast of Florida, enclosed by barrier islands (Fig. 1). Florida has a humid, subtropical climate, with a long dry season (usually October-May) and a wet season (June-October). CH is subjected to water exchange with the Gulf of Mexico, primarily at the southern end of the harbor (Boca Grande Pass). The waters of the estuary are well mixed due to the tidal flow, wind and relatively shallow water depth. In the northernmost parts of the estuary strong vertical-density stratification occurs, during periods of moderate to high river runoff (Montgomery et al., 1991). It has a significant hypoxic zone (less than 2 mg (dissolved) oxygen per liter water) (Camp et al., 1998 in: Turner et al., 2006). CH is, after Tampa Bay, the largest estuarine area in Florida. The watershed is 12950 km^2 into 800 km^2 surface area. CH has ~2500 km^2 of wetlands which includes mangrove forests and submerged macrophytes. The Peace and Myakka Rivers contribute more than 60% of the total tributary inflow into the CH estuarine system (Hammett, 1990). The river input of freshwater shows a marked seasonal variation due to the occurrence of a wet and a dry season.

Humans have been inhabiting Florida for 10 000 years and started agriculture around 800 years ago (Brown, 1998; Deagan, 1985). From ca. 1565 AD Florida has been colonized by Europeans, with a huge impact on the Florida ecosystem. This caused a gradual deforestation of a large area of northwest Florida, where forests were cleared for agriculture, lumber and fuel. Citrus plantations had additional impacts on the ecosystem, both in alteration of water by drainage and nutrient levels of the soil with fertilizers and pesticides. In the 19th and 20th century drainage, upstream river diversions and canal dredging affected the area. The changed hydrology can affect the flora and fauna on land, as well as in the estuary (salinity and nutrients). Human activities in Florida were at first mainly concentrated in the northwestern part, but largely in the last century the human activities spread. CH has been quite undisturbed until recently, where changes related to human impact are largely seen in the last 50 years (Turner et al., 2006).

Figure 1 depicts the CH watershed. It shows that the amount of urban areas increased rapidly between 1980 and 2000 AD, especially in the areas where the Peace River enters the estuary and around the Caloosahatchee River. Population grew exponentially; the Charlotte County experienced a 37-fold increase since 1950 AD (http://www.census.gov). The area experienced deforestation, reclamation and agricultural intensification including heavy citrus planate (with high fertilizer usage). In the 1950s and 1960s AD a ‘construction frenzy’ was going on in the CH area (Roat et al., 2002). In 1970 the development of a large scale waterfront urban area started (the Rotunda in Punta Gorda). The estuary is still relatively undisturbed when compared to Tampa Bay, the largest estuary in Florida.

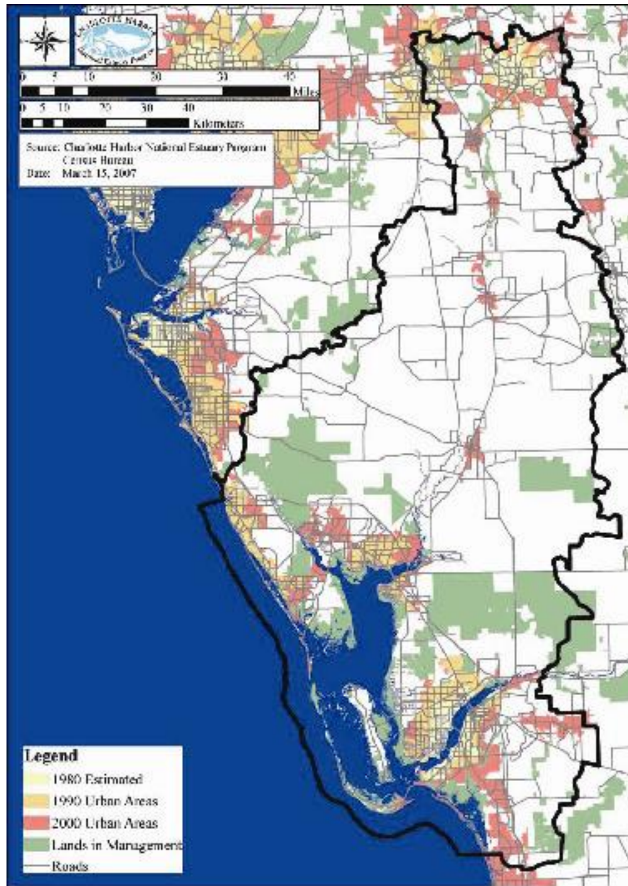

Figure 1: Changes in urban areas in the Charlotte Harbor area. Adapted from Beever et al. (2009).

The phytoplankton growth in CH is controlled by the availability of nitrogen (N) (McPherson et al., 1996; Montgomery et al., 1991;(Turner et al., 2006) as the Peace River is naturally enriched by P from phosphorites formations in the Bone Valley. Occasionally the CH estuary is subjected to hydrophilic phosphatic clay slime spills as a result of P-mining activities, which induce fish mortality and P enrichment of CH estuary (Froelich et al., 1985; Martin & Kim, 1977). At Arcadia, some 40 kilometers inland, long-term measurements are made by the U.S. Geological Survey (USGS) and give a long time series for the CH estuary in terms of runoff and nutrients. In the late 1970s AD stricter regulations concerning the P load of the rivers lowered the inorganic and total river phosphorus (P) load (Dixon et al., 2009). The P load of the Peace River increased again in the last 10 years. As the estuary is primarily susceptible to the changes in N load, human development along the coast and in the watershed area will have a profound influence on the water quality and the production of phytoplankton. Although since the mid-1980s the domestic waste input in the Peace River gradually decreased there is an increased trend of total N and productivity since the late 1980s (Dixon et al., 2009; SWFWMD, 2004). The amount of N measured in the Peace River increased with two orders of magnitude between the end of the 1950s and the 1990s AD (Turner et al., 2006).

1.3 Natural and anthropogenic factors affecting river discharge dynamics

Rainfall patterns, albeit natural variations or human induced hydrological changes, affect the river discharge into CH. The runoff influences CH nutrient content and salinity, important parameters for algal primary production.

The Atlantic Multidecadal Oscillation (AMO) is a natural shift in North-Atlantic Ocean surface temperature, with a cycle of about 60-80 years (Enfield et al., 2001). AMO warm phases have higher than average Atlantic sea surface temperature. When the AMO is in its warm phase, Florida has more rainfall, unlike most of the other states. Kelly & Gore (2008) showed that the Peace River had a higher summer and autumn runoff during the last AMO warm phase (calculated for the period 1940-1969), see Figure 2. From 1970 to 1999 there was on average a 39% decrease of Peace River flow during the wet season (gauged at Arcadia), for Myakka River (gauged near Sarasota) this was 22.5%, which is probably party offset due to agriculturally related increases in flow (Kelly & Gore, 2008). Other proxy-based evidence states that the stream flow of the Peace River decreased in the period 1931-1984 due to rainfall pattern changes in the wet season (Turner et al., 2006). There are on average more tropical cyclones during the warmer AMO phases (1930-1969) compared to the cooler AMO phases (1970-1994) (Basso & Schultz, 2003). Category 3 to 5 storms occurred 14 times within a radius of a few miles of the estuary from 1873 to 2004. The two most recent strong storms were Donna (1960 AD, cat. 5) and Charley (2004 AD, cat. 4). Warm AMO phases are reconstructed at ca. 1869-1893, 1926-1969 and after 1995 AD, and cool phases at 1894-1925 and 1970-1995.

Superimposed on the long-term AMO related change in rainfall, other oscillations could affect the rainfall pattern. On a shorter timescale, the El Niño-Southern Oscillation (ENSO) influences the rainfall patterns in Florida (Cronin et al., 2002). Mean river flow in the CH area showed a strong response to the ENSO phase during winter months (Schmidt et al., 2001). This means increased discharge in El Niño years in winter and spring months.

River and groundwater withdrawals are frequent in the rivers that flow into the CH estuary. There are major water withdrawals in the aquifers of the Peace River, and relatively minor withdrawals of surface water. These water withdrawals are done for agriculture (including citrus), phosphate mining and urban requirements. These withdrawals are quite large, e.g. in 2000 there was a 2.1 billion liters withdrawal per day, mostly from groundwater sources (SWFWMD, 2004). It is expected that these withdrawals are more pronounced in the dry seasons than in wet seasons. Therefore, as Kelly & Gore (2008) summarized, anthropogenic effects on the river flow should be most pronounced in the drier seasons. Changes in river flow are hence the result of both anthropogenic activity (stronger in drier seasons) and natural climate variability (mostly during the wetter seasons).

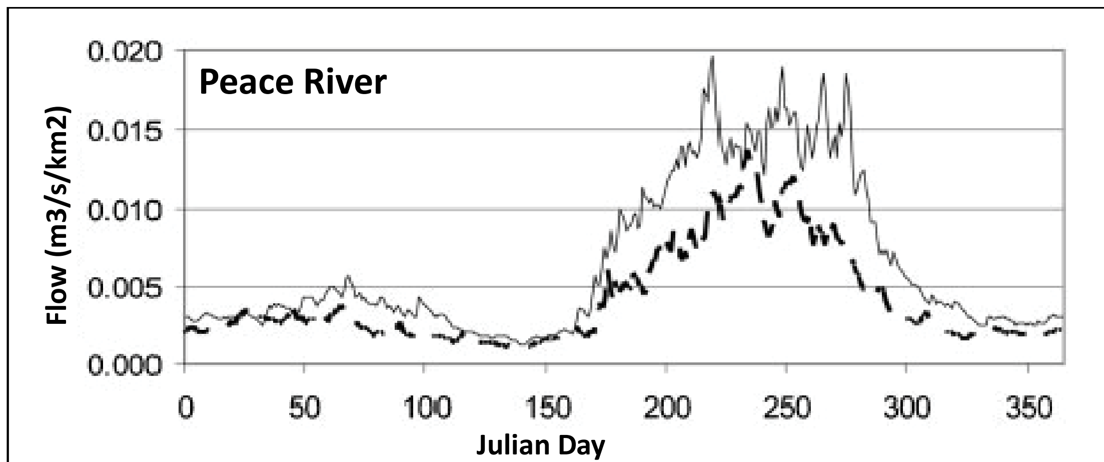

Figure 2: Peace River flow ($m^3/s/km^2$ catchment area) measured at Arcadia for two time periods. The solid line is the period of 1940 to 1969, within an AMO warm phase. The dashed line is the period of 1970 to 1999, within an AMO cool phase. In AMO warm phase the Peace River shows higher summer and autumn runoff. Modified from Kelly & Gore, 2008.

The salinity profile of the estuary shows a classical estuarine circulation, with more fresh water overlying a salt wedge (Sheng, 1998). The CH estuary has an increasing problem with seasonal hypoxia. This is deemed increasingly important, as the economical stakes in the area increase due to urban development, commercial fisheries and recreation. The hypoxic zone seems to increase, it is proposed due to increased nutrient and phytoplankton growth (Turner et al., 2006). The growth of the hypoxic zone could be caused by enhanced stratification (related to runoff) or increased primary productivity due to eutrophication. The sediments of CH are enriched in P. Low bottom water conditions can induce remineralization of sedimentary bound P and hence the water column (Mortimer, 1941; Ingall et al., 1993; Slomp et al., 1996a, 1996b).

1.4 Phytoplankton and harmful algal blooms

Dinoflagellates constitute, together with coccolithophorids and diatoms, the majority of marine eukaryotic phytoplankton. They are major primary producers, especially in coastal areas. Some dinoflagellates produce toxins that can harm wildlife as well as humans, and can have autotroph, heterotroph and mixotroph lifestyles (Bronk et al., 2007; Burkholder et al., 2008; Kudela et al., 2008; Stoecker, 1999; Vargo, 2009). Mixotrophy is an important feeding strategy making the dinoflagellates versatile. Many species known to generate Harmful Algal Blooms (HABs) are known to be mixotrophic (Burkholder et al., 2008). Some (toxic) species kill other phytoplankton or fish, and can use the nutrients released during decomposition to form blooms. Dinoflagellates belong to two major groups: Peridinioids and Gonyaulacoid. Most of the Peridiniod (and Protoperidiniod) dinoflagellates are heterotrophs, feeding on diatoms, other dinoflagellates and organic matter (OM). The Gonyaulacoid are usually autotrophs. About 20% of the dinoflagellates produce a (temporary) non-motile resting cyst made of organic biopolymers that has a high preservation potential (the organic-walled dinocyst). The generally different feeding strategies of P and G dinoflagellates can give insight into the nutrient content of the environment, as Peridinioids are thought to occur usually at higher nutrient levels, where higher overall primary productivity implies more food for the heterotrophs. Higher P/G ratios have thus been connected to cultural eutrophication,

but also used as indicators of industrial pollution; discussions are ongoing (Matsuoka, 1999; Dale, 2000; Sangiorgi et al., 2004; Matsuoka et al., 2003; Dale, 2009).

There are various factors affecting phytoplankton biodiversity and primary productivity, such as light, nutrients, temperature, salinity and grazing by zooplankton. The major nutrients required by phytoplankton are N and phosphorus. P is often present as phosphate (PO_4^{3-}). The N is available in its inorganic form as nitrate (NO_3^{2-}), nitrite (NO_2^-) and ammonium (NH_4^+). Organic nitrogen is often seen as largely refractory and thus not important for plankton nutrition, but recent studies have shown that some phytoplankton can use DON for their N nutrition (Bronk et al., 2007). Micronutrients as iron are also important.

The composition of the phytoplankton community in CH varies with location and season. McPherson et al. (1990), McPherson et al. (1996), Montgomery et al. (1991), among others, have collected data on phytoplankton species assemblages from CH estuary in the 1980s and 1990s AD. Below is a combined inventory of species found in these studies for lower, intermediate and high salinity in small (<5µm), average and large (>20µm) size fractions. It seems that diatoms dominate most samples with *Skeletonema costatum* being the most widespread species. In late spring and summer dinoflagellates and cyanophytes could become dominant. In lower salinities they found abundant small non-flagellated green cells, probably including Cyanophyceae *(Synechoccus* spp*., Chroococcus* spp*.,* and *Anacystis* spp*.),* Chlorophyceae (*Nannochloris* spp. and *Chlorella* spp.) and phytoflagellates (*Chlamydomans* spp., *Carteria* spp., *Chroomonas* spp., and *Cryptomonas* spp.). Larger size fractions consisted of Chlorophyceae (*Ankistroidesmus* spp., *Coelastrum* spp., *Crucigienia* spp., *Pediastrum* spp., *Scenedesmus* spp. and *Tetraedron* spp.), diatoms *(Cyclotella spp., Nitschia spp., Navicula spp. and Fragillaria* spp*.)*, and N-fixing cyanobacteria (*Anabaena* spp. and *Anacystis* spp.). In intermediate and higher salinities often the same assemblage of phytoplankton is found. The small size fraction was dominated by Cryptophyceae (*Chroomonas* spp. and *Cryptomonas* spp.) and diatoms (*Thalassiosira* spp., *Nitzschia* spp. and *Navicula* spp.). The large fraction consists of mostly diatoms (*Skeletonema costatum*, *Asterionella glacialis, Odontella sinensis, Corethron criophilum, Coscinodiscus centralis, C. eccentricus, Chaetoceras* spp*., Rhizosolenia* spp., and others). Seasonally, Dinophyceae (*Ceratium* spp., *Peridinum* spp., *Prorocentrum micans, P. minimum, Gymnodinium* spp*.,* and *Gyrodinium* spp.) were important.

The Southwestern Florida shelf area is prone to frequently occurring Harmful Algal Blooms (HABs) with varying duration and strength (Sellner et al., 2003; Hu et al., 2006). The naked non-cyst making brevetoxing producing dinoflagellate species of *K. brevis* can cause so called red tides. Another known HAB species in the area is *Pyrodinium bahamense*, which does form a preservable cyst (*Polysphaeridium zoharyi*) and thus can be documented back in time with palynological (dinocyst) analysis. Already when Europeans 'discovered' Florida there were sightings of massive fish kills and discolored water. Some documented *K. brevis* blooms in CH are in 1878, 1946-47, 1953-55 and 1996 AD (Barnes et al., 2006; Vargo, 2009). Possible consequences of red tides are fish, seabirds and marine mammal mortalities, oxygen depletion of the water column, resulting in a hypoxic or even anoxic water column and even human illness or death via consumption of seafood contaminated by the toxin producing algae (Kirkpatrick et al., 2004).

There seems to be a trend towards more abundant, severe and further offshore extending red tides along the Florida coast from 1954 AD onwards (Brand & Compton, 2007). It is thought that cultural eutrophication - that is, eutrophication due to human activity - is important in red tide proliferations and expansion (Heisler et al., 2008; Anderson et al.,

2008). Naturally, nutrients are provided to CH by river runoff, upwelling along the coast, nutrient recycling and windblown dust. The increased availability and mobility of N and P are due to amongst others wastewater discharge, fertilizer application, N-fixation by leguminous crops and atmospheric deposition of oxidized N from combustion of fossil fuels. Furthermore submarine groundwater discharge has been proposed as a source for nutrients, which could trigger and support the recurrent red tides (Brand & Compton, 2007; Hu et al., 2006). Other hypotheses of increased and more severe HAB occurrences are amongst others. long-term changes and ecosystem variability linked to natural climate oscillations. Storms and hurricanes are recurrent in the Gulf of Mexico and cause enhanced precipitation. Higher precipitation and runoff enhances the nutrient flux into the estuary. This implies that both anthropogenic nutrient input and climate change could stimulate phytoplankton proliferation and the occurrence of HABs.

In the discussions about HABs there are a lot of stakeholders, as safety (public safety, human health), money (tourism, recreation, seafood industries) and other institutions (e.g. monitoring programs, water quality, politics, lobbies) are involved. As the Gulf of Mexico is economically important, a process based understanding of cultural eutrophication and HAB occurrences is needed as a basis for policy makers to provide a foundation for remediation measures. In these discussions the distinction between anthropological and natural causes of HABs are important, as it is difficult to interfere in naturally occurring blooms, but proper management could perhaps reverse bloom initiations where human activities are the biggest influence (Sellner et al., 2003). Sediment core analyses are useful tools in evaluating the anthropogenic impact and eutrophication trends of coastal environments (e.g. used by Matsuoka, 1999; Sangiorgi & Donders, 2004, Dale, 2009). High resolution reconstructions of changes in productivity and runoff can help unraveling the human vs. natural causes of HABs and eutrophication in the CH area.

As this study consists of both sediment and filtered water samples of different origin and age, this is discussed in the order of age, i.e. first the algal growth experiment (Chapter 2.1), then the CH transect (Chapter 2.2) and at last the sediment core with organic geochemical work and palynology (Chapter 2.3). The total study area is depicted in Figure 3. This method section describes the extraction, qualification and quantification of different sediment and filtered water samples.

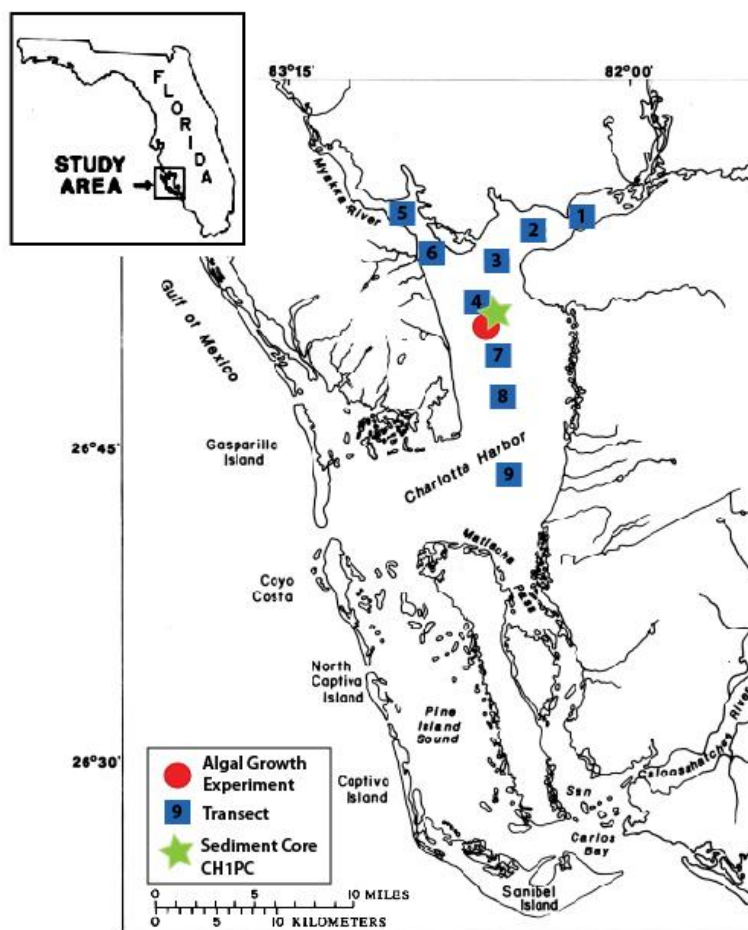

Figure 3: Multiple types of samples are taken from Charlotte Harbor, Florida. For the algal growth experiment water was taken (red circle); water samples for organic geochemistry sampling were taken on a salinity transect (blue squares); the sediment core was taken at the green star.

2.1 Water sampling for algal growth experiment

It is known that nutrients affect phytoplankton growth. However, knowing how different types of nutrients influence a natural plankton community is important but difficult at the same time. Most studies about the response of algae to different nutrient inputs are conducted with (sterile) phytoplankton cultures on monospecific algae. This is useful for physiological studies of the species, but pure culturing experiments cannot be used directly for studies on how processes work in nature on specific sites, as in reality zooplankton, bacteria and viruses interfere with phytoplankton growth. Thus for this research natural CH seawater is chosen, to attempt to study the relationship between phytoplankton growth and nutrient concentrations

with different types of nutrients in a laboratory environment as close as possible to natural conditions. The use of natural seawater has some drawbacks, as grazing together with bacterial and viral influence on the populations could affect the phytoplanktonic response.

According to (Dixon et al., 2009), dinoflagellates in the CH area show no significant seasonal patterns, although in late summer and early fall there are seasonal maxima. This means that sampling water in the spring should give a sufficient overview of the dinoflagellate species present, although concentrations are not expected to be high.

2.1.1 Material

In March 2011, 19 liter of natural surface seawater was collected in a 5 gallon cooler in CH (26°52,2' N, 082°07,5' W). Also 19 liters of 'concentrated' surface seawater was collected. The natural seawater was taken as a control, as the experiment is conducted with the 'concentrated' seawater. Scouting expeditions showed a low concentration of phytoplankton was present in CH waters. Therefore it was decided that the phytoplankton had to be concentrated in order to conduct a phytoplankton growth experiment. Ideally this concentrated water would enable accurate semi daily microscopic counts of the phytoplankton species present. The water was concentrated using a 15 µm mesh plankton net that was constantly submerged in seawater. 132 liters of natural seawater were poured through the submerged plankton net onboard. This resulted in 19 liters of 7 times concentrated seawater. Of the surface water specific conductance, pH, water temperature and dissolved oxygen (DO) were measured in the field.

2.1.2 Methods

The samples were stored in the dark and transported to the algal culturing laboratory of Florida International University, Miami. The process of sampling and transportation was conducted in 4.5 hours. The day after sampling, 15 Corning polycarbonate 1 liter bottles were filled with the concentrated seawater. A portion of the collected water, from both the 'natural seawater' and the 'concentrated seawater' was filtered through 142 mm Whatman GF/F glass fiber filter for biomarker analysis. Nutrients were analyzed for the natural and concentrated water.

The experiment included four treatments in triplicate, with dissolved organic N (DON), dissolved organic phosphorus (DOP), dissolved inorganic nitrogen (DIN) and dissolved inorganic phosphorus (DIP) nutrient additions. A blank experiment with natural waters was also performed in triplicate. Table 1 is an overview of the different treatments, including the concentration of nutrients added, based on high levels of nutrients measured in the CH estuary (Duffey et al., 2007), to represent naturally occurring high nutrient episodes.

Table 1: the five different treatments of the algal growth experiment

Treatment	Nutrient	Concentration	Bottles
DOP	B-glycero-phosphate disodium salt (penta) hydrate	15,12 mg / l	ABC
DON	Urea	10,96 mg / l	DEF
DIN	Sodium nitrate	9,38 mg / l	GHI
DIP	Potassium Phosphate monobasic	27,80 mg / l	JKL
Blank	-	-	MNO

Due to technical difficulties it was not possible to use an incubator with fixed temperature and light cycle for the experiment. Therefore a homemade incubator was used. This comprised of a black cloth to cover a shelf with the bottles, a ventilator to prevent the 'incubator' to overheat, a thermometer and fluorescent light bars which were automatically switched on and off to produce a day and night alteration. Due to technical difficulties it was not possible to enhance the light range with incandescent bulbs, which would make the light range more within the range of natural irradiance. The photoperiod was a 16/8 hours light/dark cycle. The irradiance of the fluorescent light source was 600 lux (lumen/m^2). This is in the range of typical light saturated photosynthesis rates of many phytoplankton species. The fluorescent light source emitted between 400 and 700 nm. During the experiment, the temperature ranged from 24 to 28°C which is in the natural temperature range of the CH estuary in spring. As the temperature only rose during the light cycle, this would mimic the natural heating of seawater due to the influence of sunlight.

Samples for microscopic analysis were collected every other day and preserved immediately with Lugol solution (4% volume). Lugol solution is better for phytoplankton than formaldehyde. Due to supply difficulties the samples of the first 4 days were only treated with Lugol at day 5. The identification and enumeration of the different species of phytoplankton were conducted with a light microscope with mounted camera. The phytoplankton was identified in different groups: diatoms, 'smalls', zooflagellates, flagellates and indet. Due to low phytoplankton concentrations only experiment H was counted for more than one sample-day in a row.

Chlorophyll-α was measured every other day. Samples for chlorophyll-α analysis were filtered through Whatman GF/F 25 mm filters and stored in 1.5 ml Eppendorf vials. The filters were extracted in 1.5 ml 90% acetone in a dark freezer for 24 hours. And centrifuged for 3 minutes. The chlorophyll-α working standard of FIU 2010 was used. The absorbance was measured with a spectrophotometer. The residual growth on the inside of the experiment bottle was not actively included in any chlorophyll-α measurements.

At the end of the experiment an array of samples was taken from the 15 'cultures', including samples for chlorophyll-α, nutrients (both filtered and unfiltered). The pH of the samples was measured on day 2 and day 20 with an UltraBasic Denver UB-5 Meter, standardized at a pH of 7. Total P (TP), Soluble Reactive Phosphorus (SRP), NO_2, N+N, and NH_4 were determined colorimetrically (USEPA-365.1 following dry ashing (Solorzano & Sharp, 1980); MDL of 0.08 uM; Apple leaves based on certified values by the National Bureau of standards, with P (0.159+-0.011 weight percent) and N (2.25+-0.19 weight percent)) by the FIU Biogeochemistry Laboratory in Miami.

The remaining sample was filtered over precombusted Whatman GF/F 142 mm filters. The residue in the bottle was not included in the chlorophyll-α measurements, but it is included in samples A to L for the biomarker filters. After filtering, the filters were dried at 60°C, weighed and stored in a refrigerator. A 10° wedge was cut out of the filters for TOC measurements. The TOC content of all filters was determined after decalcification. For this the wedges of filter were treated with HCl to remove inorganic carbon by placing the sample together with a few ml HCl (38%) in a dessicator for two days, then drying by replacing the HCl with silica gel, and weighed again. The filters were rolled into tin cups for TOC measurement on a Fison Instrument NA 1500 NCS analyzer. From every different batch one filter was selected based on microscopic analysis and weight for biomarker analysis. The samples used are CH A, CH E, CH H, CH J, CH O, 'CH natural' and 'CH concentrated'. The remainders of the filters were cut in small pieces and extracted ultrasonically five times five

minutes with a methanol/dichloromethane (DCM) mixture (1:1 v/v). The extracts were rotary evaporated and transferred into preweighted vials. The TLE was methylated with a few drops of diazomethane (CH_2N_2), dried under N_2, cleaned over a small silicagel 60 column with EtOAc and dried under N_2. This methylation was necessary to convert fatty acids into methyl esters. The methylated samples were subsequently silylated with 25µl pyridine and 25µl N.O-Bis(trimethylsilyl)trifluoroacetamide (BSTFA), which was heated for 20 minutes at 60°, in order to convert alcohols into TMS ethers. The samples were analyzed with gas chromatography (GC) and gas chromatography mass spectrometry (GC-MS). A HP Gas Chromatograph fitted with a CP-Sil 5CB fused silica capillary column (30m x 0.32mm i.d.) and a flame ionization detector (FID) was used. 1 µl sample in EtOAc (polar and TLE) or hexane (apolar fractions) at a 2 mg/ml concentration was co-injected on column with 1 µl 50µg/ml squalane for quantification. A flame photometric detector (FPD) was used to check for remaining sulfur. The oven program was pre-programmed to start at 70°C to 130°C with 20°C per minute, up to 320°C with 4°C per minute with 20 minutes end time at the 320°C. The peak area integration of the chromatogram of the GC was used for quantification purposes. For the qualification of the sample a ThermoFinnigan Trace GC-MS was used, with the same oven program and column as for the GC. The compounds of the samples were identified comparing published mass spectra and (relative) retention times data from libraries (as NIOZlib, Geolib, ect) and additional publications.

2.2 Water sampling along a salinity gradient

2.2.1 Material

On February 22nd, 2011, a transect in the CH estuary was sampled. In total 9 samples with a volume ranging between 8 and 20 liters were collected for filtering and subsequent biomarker analysis. In addition, salinity, temperature, dissolved oxygen (DO) and conductivity was measured on board with a field STD measurement device. The GPS position is noted, as well as the water depth (measured with onboard device). The sampling of the transect started around slack tide with sample 1, and tide was incoming during collection of the other samples. The precleaned Nalgene jugs were transported back and filtered within 24 hours.

2.2.2 Methods

The filters were extracted at the Organic Geochemistry Lab in Utrecht in the previously mentioned manner of biomarker analysis of the algal growth experiment. The TLE was methylated, silylated and measured on the GC and GC-MS with similar column and oven programs as described before. The peaks of the identified compounds were integrated and calculated to µg per TOC (g) per liter water to allow for comparisons of different compounds along the transect.

2.3 Sediment core CH1PC

2.3.1 Material

During a fieldwork campaign in spring 2008, multiple sediment cores were collected in CH using a vibracorer deployed from the R/V *G.K. Gilbert*. In this study we use a push core from 26°52,799'N/082°07,612'W that was taken next to one of the vibracores. The push core was photographed (Figure 5), described visually and dated with ^{210}Pb at Eckerd College, St. Petersburg, U.S.A. This push core CH1PC was sampled every centimeter, samples were

freeze-dried and grinded, the water content was determined, and samples were dated with ^{210}Pb.

2.3.2 Chronology

The age of the push core sediments were determined in 2008 with ^{210}Pb dating at Eckerd College, U.S.A. Based on these datings, the core comprises about 130 years in the top 24 centimeters (Figure 5). The dry bulk density (DBD; gram/cm^3) was measured at Eckerd College. With the Linear Accumulation Rate (LAR; cm/year) the Mass Accumulation Rate (MAR; gram/cm^2/year) was calculated (MAR=LAR*DBD). The MAR of carbonate was calculated for the top 22 samples. With the MAR, fluxes of palynomorphs and biomarkers can be calculated, e.g. dinocysts/cm^2/year.

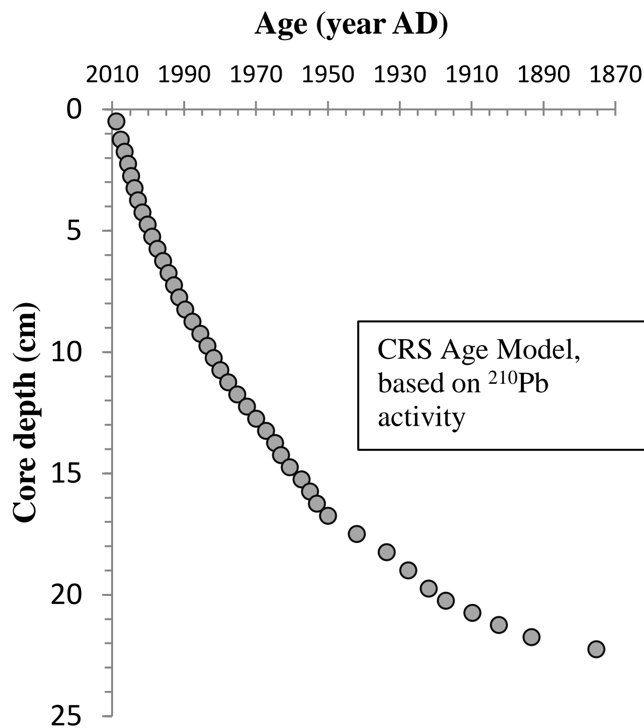

Figure 4: CRS age model of sediment core CH1PC from Charlotte Harbor, Florida, based on ^{210}Pb activity.

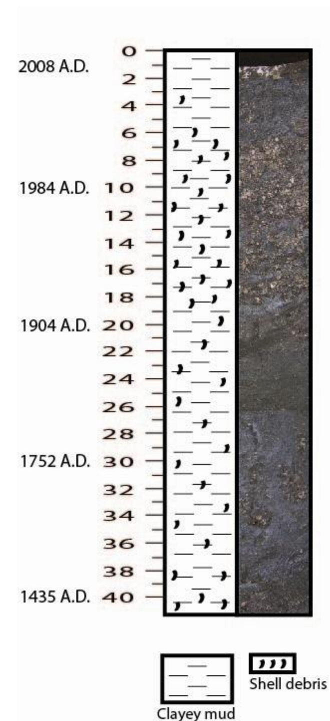

Figure 5: Core CH1PC with lithology and ages (year AD) calculated with ^{210}Pb dating

2.3.3 Palynology

The sediment samples were processed with the standard palynological method used at the LPP at Utrecht University, with cold HCl (10%) and HF (40%) and sieved at 10 micrometers. A *Lycopodium clavatum* tablet (containing 18583 spores) is used for quantification of the dinocyst counts (cysts per gram dry weight sediment). The sieved residues were mixed with glycerine and mounted on glass slides. Dinocyst relative abundances and fluxes (dinocysts/cm^2/year) were calculated.

Slides were counted for dinocysts, pollen, spore and other palynomorphs, up to 200 to 250 dincysts, using a light microscope at a magnification of x400. Dinoflagellate cysts taxa identification was based on (Cremer et al., 2007; Marret & Zonneveld, 2003) and the knowledge of the BioMarine Sciences staff at Utrecht University. When species were identified in the sample but not counted they are indicated as 'present' but is not considered for further analysis and interpretations. The dinoflagellate cysts assemblages studied are derived from high resolution sampling, taken every centimeter in the top 25 cm of the core, and every 4 cm down to 41 cm depth. In total 28 samples were studied for both dinoflagellate cyst assemblages and a rough pollen and spore assemblage. Light micrographs of frequently occurring taxa were taken with a Leica microscope with mounted camera at x400 magnification; scale bars (20 µm) were added with Adobe Photoshop.

2.3.4 Biomarkers

The 23 top sediment samples of the CH1PC core were extracted with Accelerated Solvent Extraction (ASE) by a Dionex 200 ASE extractor with DCM/MeOH (9:1 v/v) solvent, resulting in a total lipid extract (TLE). Approximately 3 gram of freeze-dried and grounded sediment was mixed with diatom-sand. The method used is the standard method of the Organic Geochemistry lab at the University of Utrecht. The samples were rotary evaporated and transferred into pre-weighed vials. Elemental sulphur was removed using activated cupper churnings and transferred over a small sodium sulfide column to remove CuS and water traces. When necessary, the samples were desulphurized twice or trice. The TLE was separated into two fractions, using a small column (Pasteur pipette) with activated Al_2O_3. An apolar fraction was obtained by eluting the column with hexane/DCM (9:1 v/v) and a polar fraction with DCM/MeOH (1:1 v/v). Subsequently a small silicagel 60 column was used to make the samples cleaner. The polar fraction of each sample was methylated with diazomethane, silylated with BSTFA and measured on the GC and GC-MS with oven program as described above.

Thin Layer Chromatography (TLC) is used to divide the TLE of three sample into multiple fractions. This way constituents can be identified, even if they have a (very) low concentration and compounds that co-elute on GC chromatograms can be separated. The aim is to find brevesterol, a possible biomarker for the HAB dinoflagellate *K. brevis*. The top of the CH1PC core was chopped into three samples, namely 0-11 cm, 11-22 cm and 22-33 cm. In addition, the top 7 cm of the CH11 core was used as a more marine signal. A water filter from the Caloosahatchee River was used as experiment as well. The sediments were dried in the stove at 40°C and crushed. The sediments (around 20 gram per sample) and the Caloosahatchee River filter were extracted with the Soxhlet method with DCM/MeOH (7.5/1 v/v) in precleaned Whatman extraction thimbles with a 85°C water bath. The samples were extracted for 24 hours and evaporated near vacuum with Büchi Rotavapor R-114 with Laboxact in a 30°C Büchi waterbath B-480 and desulphurized overnight. A small aliquot of TLE was methylated and silylated and measured on GC and GC-MS. The remainder was used for the TLC.

Precoated Merck silicagel 60 0.25x20x20 cm glass TLC plates were marked with pencil and precleaned by filling an air tight glass container with a few centimeters of DCM/MeOH (1:1 v/v) and placing the TLC plates vertically in it. When the liquid reached the top the plate was removed and blow-dried on the glass side of the plate. The top centimeter was scraped off as this centimeter contains the contamination from the cleaned plate. The TLE is dissolved in some DCM and applied on the baseline of the plate. Two solvent-

mixtures were prepared, Skipski 1 and Skipski 2, after the Skipski method for TLC for lipids (Skipski & Barclay, 1969). Skipski 1 consisted of diisopropylether and acetic acid (96:4 v/v) and Skipski 2 of petroleumether (40:60), diethylether and acetic acid (100%) (90:10:1 v/v). Skipski 1 was allowed to run up to 10 centimeters from the bottom of the plate and marked 'Skipski 1', the plate was removed and blow-dried. Then the plate was placed in Skipski 2 for approximately 1 hour, marked 'Skipski 2' and blow-dried. Visually the plate with banded compounds was divided into fractions, subsequently the plate was visually divided under a UV light source. According to Skipski's method the plate had to be sprayed with Rhodamine B solution (40 mg / 100 ml EtOH 96%) to allow different compound bands to show. This step was skipped as it did not show any differences compared to visual fractions in normal and UV light. Initially 5 standards (2 hexadecanol; 5α-androstan-3β-ol; nonadecanoic acid; 5α-androstane and heptatriacontane) were co-applied to the plate to allow identification of different bands of compounds. This did not work properly and the fractions were identified visually, no standards were used.

The fractions were marked with a pencil from A to I, A being the top fraction and I being the fraction with the non-soluble compounds at the bottom. For an overview of the plate see Figure 6. With a clean spatula the fractions were scraped off the glass plate and the silica powder containing the sample was extracted in a centrifuge tube. The apolar compounds (fractions A to E) were extracted from the silica-powder using hexane and the polar compounds (fractions F to I) by using EtOAc. Extractions were done by placing the centrifuge tubes in an ultrasonic-bath for 5 minutes, extractions were repeated 4 to 6 times per tube. The fractions were transferred into pre-weighed vials, using DCM. A small column with defatted cotton wool was used to remove remaining silica residue. The apolar fractions were ready for GC and GC-MS (oven program as described above), where the polar fractions were methylated and silylated prior to these analyses.

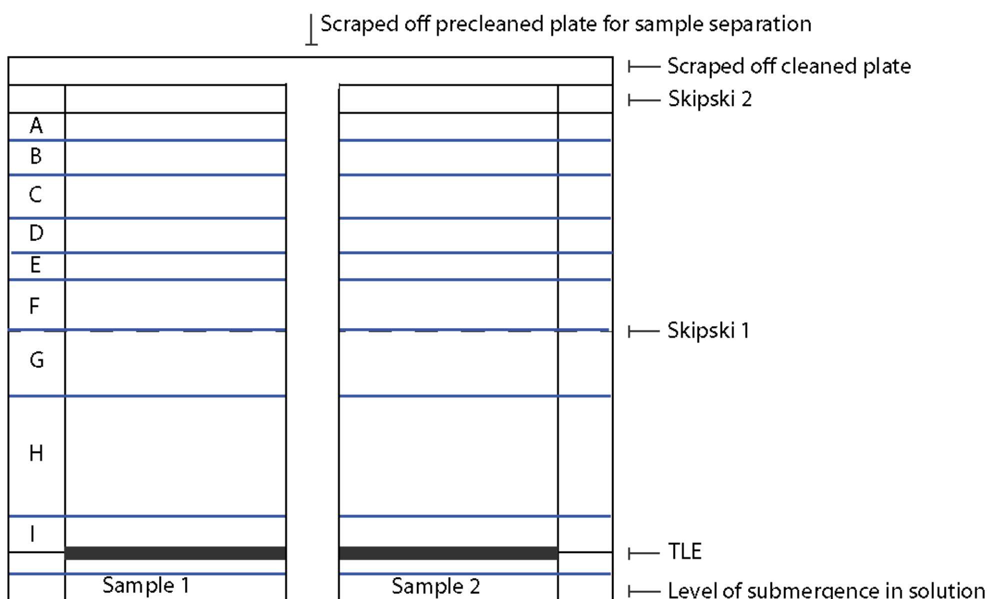

Figure 6: TLC plate for two different samples with visually divided fractions.

Some samples were saponified prior to TLC. The TLE of CH1PC 0-11 / 11-22 / 22-33 cm were saponified, next to the sediment residue (after Soxhlet extraction) of CH1PC/0-11 cm. For saponification of extracts, approximately 2 mg TLE is transferred into a centrifuge tube. 2 ml of 1N KOH (in 96% MeOH) was added and left to reflux for 2 hours at ~100°C on a heater/stirrer device, cooled down and transferred into a funnel. The pH is elevated to a pH of 5 with 2N HCl/MeOH (1:1 v/v). 2 ml of bidistilled water and 3 ml of DCM is added, shaked and washed with 2 ml DCM, 3 to 5 times. After each washing step the DCM layer is collected, rotary evaporated and transferred over a small Na_2SO_4 column into a preweighted vial.

For saponification of the sediment sample 4.4 gram sediment of previous Soxhlet extracted sediment was weighed into a centrifuge tube. 5 ml of KOH (in 96% MeOH) was added and left to reflux at ~100°C on a heater/stirrer device, cooled down and transferred to a funnel. The residue of sediment is washed twice with 2.5 ml MeOH/H_2O (1:1 v/v), twice with 2.5 ml MeOH and trice with 2.5 ml DCM, centrifuged for 2 minutes and pipetted off into the funnel. 7.5 ml bidistilled water was added to the collected solution in the funnel, pH is checked at 3 pH, and washed twice with 3 ml DCM and rotary evaporated near vacuum. This is transferred over a small Na_2SO_4 column into a preweighted vial.

The biomarker data is normalized to the TOC content of the samples and the MAR and presented as µg/TOC(g)/year. The TOC content of the sediment samples was determined after decalcification with HCl on a Fison Instrument NA 1500 NCS analyzer. Standards were used as described above.

3.1 Algal growth experiment

3.1.1 Biomass

Chlorophyll-α is used as a measure for biomass of photosynthetic organisms. As seen in Figure 7, the four different experiments with different nutrient conditions are averaged and compared to the blank experiment. The concentration of chlorophyll-α at the first half of the experiments (up to day 10) was below 1 µg/l; only in the DIN experiment biomass increase was seen after day 6. The highest concentration of chlorophyll-α occurred at day 14 in both DIP and DIN up to 6.3 µg/l, while the lowest concentrations were maintained by the blank experiment, where at day 14 the chlorophyll-α was still below 1 µg/l. The experiments which were enhanced with inorganic N and P show the highest average biomass and chlorophyll-α levels. The experiments contained some identifiable organisms (Figure 8) but not enough to count.

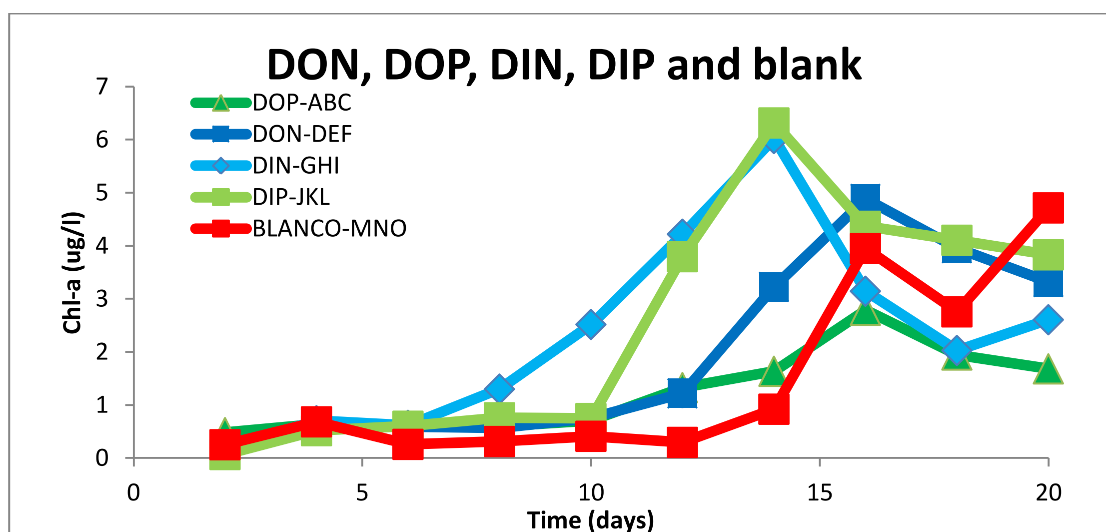

Figure 7: average response to additions of DOP, DON, DIN and DIP. Time is in days and chlorophyll-α is measured in µg/l.

3.1.2. Biomarkers

The lipid content of the different bottles varied markedly. Not only is the variability between the different nutrient treatments huge, but also the differences between the beginning and the end of the experiments are interesting. In Table 2 the lipids of the five different nutrient environments is shown. A-DOP, H-Din, J-DIP and O-blank have a low total lipid content (between 0.54 and 7.9 µg/l), E-DON contains an intermediate total lipid content (42.2 µg/l) and the start values of natural and concentrated water has the highest total lipid content (106 to 269 µg/l). All samples contain $C_{16:0}$ and $C_{20:5(n-3)}$ fatty acids, phytol and sterols $C_{27:1w5}$, $C_{27:2w5,22}$ and $C_{28:2w5,22}$. The rest group of fatty acids contain some unidentified MUFAs, PUFAs and branched fatty acids. One sample contains some loliolide, a biomarker for diatoms. The natural and concentrated water contain the same lipids, albeit in different amounts. An example of the GC chromatogram can be found in Figure 9.

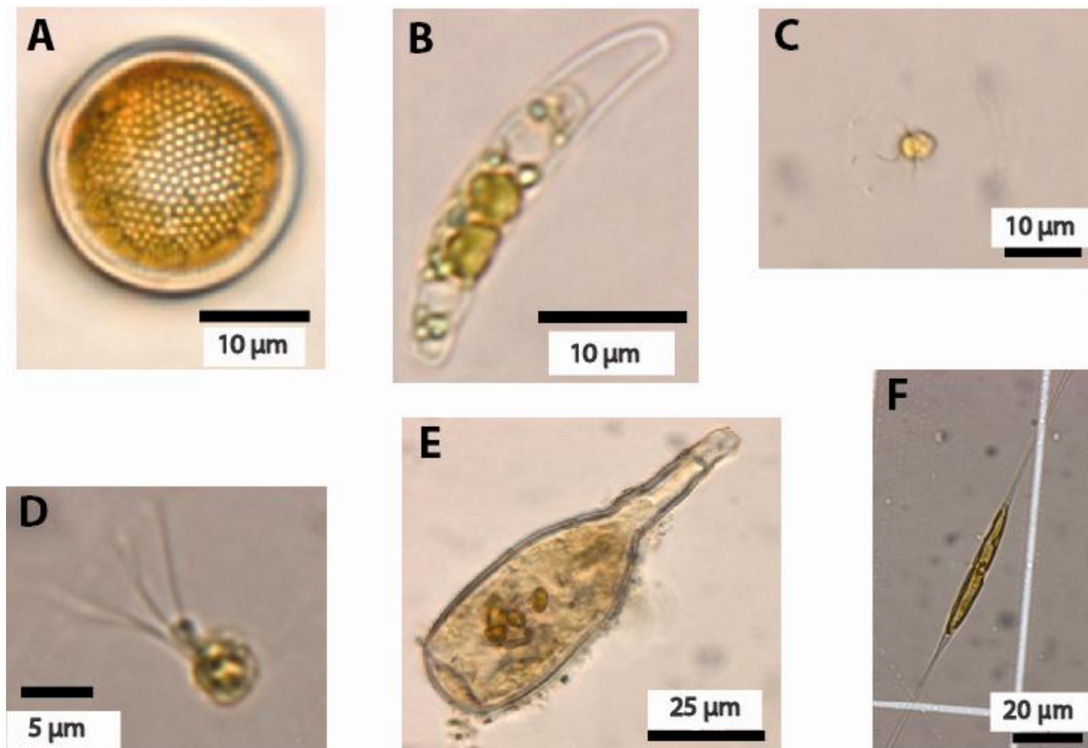

Figure 8: some phyto- and zooplankton from the various algal growth experiments. Picture A shows a centric diatom (*Thalassiosira* sp.; pers. comm. H. Cremer); B is the possible girdle ring of a diatom; C is a Choanoflagellate zooplankton, probably *Diaphanoeca*; D is a flagellate; E is a sort of zooplankton representative and F is a large pennate diatom (*Nitzschia closterium*; pers. comm. H. Cremer). Dinoflagellates were not found in the experiments, but it is evident from the microscopic analyses and the chlorophyll-α measurements that there was primary productivity.

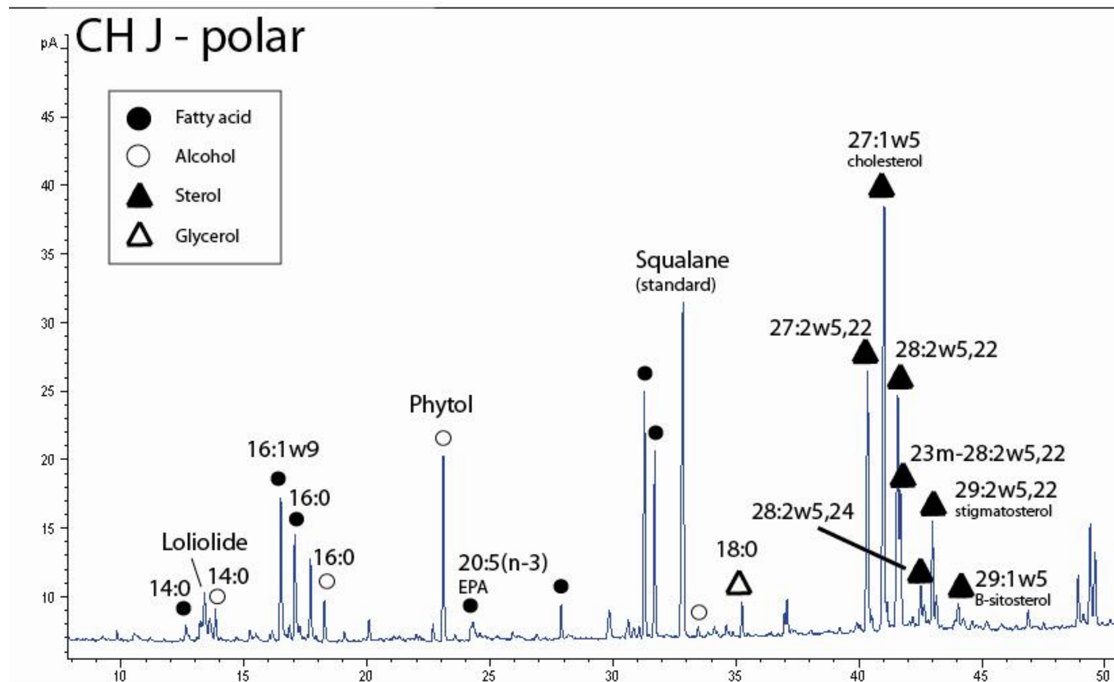

Figure 9: Chromatogram of sample CH J (polar fraction), of the DIP experiment. Fatty acids, alcohol, sterols and one glycerol are indicated.

Table 2: Lipid content of the algal growth experiment, in µg per liter, divided in fatty acid, alcohol, sterol and other biomarkers.

Nutrient addition	A-DOP	E-DON	H-DIN	J-DIP	O-Blank	Nat.	Conc.
Fatty acids							
C14:0	0,23	1,52	0,00	0,03	0,00	4,29	2,71
C15:0	0,03	1,15	0,00	0,00	0,00	2,26	2,26
C16:0	1,90	4,13	0,05	0,35	0,11	27,35	18,06
C16:1w9	2,21	14,93	0,03	0,46	0,08	9,02	14,30
C17:0	0,00	0,00	0,00	0,00	0,00	0,00	0,31
C18:0	0,10	0,00	0,00	0,00	0,01	18,15	7,53
C18:1w9	0,04	1,91	0,00	0,00	0,00	4,48	3,32
C18:1w10?	0,06	0,22	0,00	0,00	0,01	3,64	5,37
C20:5(n-3)	0,77	2,72	0,00	0,04	0,01	1,73	1,13
C24:0	0,00	0,00	0,02	0,00	0,00	0,00	0,00
C28:1	0,00	0,00	0,02	0,31	0,00	2,29	0,56
rest FA	0,30	3,76	0,06	0,50	0,08	9,21	6,45
C16:1/C16:0	1,2	3,6	0,5	1,3	0,7	0,3	0,8
C16/C18	19,9	9,0	-	-	8,1	1,5	2,2
C18 %	3,7	7,0	0,0	0,0	7,8	31,9	26,2
Total Fatty Acids	*5,6*	*30,3*	*0,2*	*1,7*	*0,3*	*82,4*	*62,0*
Alcohol							
C14:0	0,00	0,50	0,00	0,07	0,00	9,58	1,64
C16:0	0,04	0,62	0,00	0,12	0,00	10,15	2,72
C18:0	0,00	0,00	0,01	0,00	0,00	15,77	4,81
C20:0	0,00	0,00	0,00	0,00	0,00	7,96	2,33
C20:1	0,00	0,00	0,00	0,00	0,00	6,43	1,28
C21:0	0,17	0,00	0,00	0,00	0,00	0,00	0,00
C24:0	0,00	0,00	0,00	0,00	0,00	2,84	0,86
batylalcohol	0,02	0,14	0,00	0,02	0,00	1,40	1,02
Total Alcohols	0,23	1,26	0,01	0,22	0,00	54,13	14,67
Sterols							
Nor-C27:2w5,22	0,02	0,00	0,01	0,00	0,03	5,20	0,41
C27:1w5	0,86	4,98	0,10	1,70	0,09	97,21	23,62
C27:2w5,22	0,07	0,66	0,04	0,95	0,02	9,29	1,88
C28:2w5,22	0,61	2,37	0,09	0,89	0,12	7,18	1,52
C28:2w5,24	0,00	1,01	0,04	0,12	0,00	1,89	0,27
C29:1w5	0,00	0,56	0,02	0,06	0,00	4,38	0,63
C29:2w5,22	0,00	0,27	0,03	0,08	0,00	0,85	0,65
C29:2w5,24	0,00	0,00	0,00	0,00	0,00	4,16	0,53
C29:1w5 / C28:2w5,24		0,36	0,35	0,33	-	0,70	0,70
Total sterols	1,6	9,8	0,3	3,8	0,3	130,2	29,5
Other biomarkers							
Phytol	0,51	0,76	0,02	0,53	0,01	2,25	0,16
loliolide	0,00	0,00	0,00	0,06	0,00	0,00	0,00
bsito/bsito+phytol	0,00	0,42	0,49	0,10	0,00	0,66	0,79

Total lipids (µg/l)	**7,93**	**42,21**	**0,54**	**6,31**	**0,58**	**268,97**	**106,36**

As seen in Table 3 the concentration of lipids is different in the different batches. C_{16} fatty acids are dominant in all samples, while C_{18} fatty acids are mainly found in the start condition, and in low concentrations in the DOP and DON experiment. The DIN and DIP experiment show no C_{18} fatty acids but contain larger concentrations of $C_{28:1}$ fatty acid.

Table 3: concentration of lipids in the algal experiment. Fatty acid, alcohol and sterol concentrations are of total fatty acids, alcohol and sterols, respectively. Phytol and loliolide are a percentage of total lipids.

Nutrient addition	**A-DOP**	**E-DON**	**H-DIN**	**J-DIP**	**O-Blank**	**Nat.**	**Conc.**
Fatty acid **(% of total fatty acids)**							
C14:0	4	5	0	2	0	5	4
C15:0	1	4	0	0	0	3	4
C16:0	34	14	28	20	37	33	29
C16:1w9	39	49	14	27	27	11	23
C17:0	0	0	0	0	0	0	1
C18:0	2	0	0	0	5	22	12
C18:1w9	1	6	0	0	0	5	5
C18:1w10?	1	1	0	0	3	4	9
C20:5(n-3)	14	9	3	3	4	2	2
C24:0	0	0	11	0	0	0	0
C28:1	0	0	12	18	0	3	1
rest FA	5	12	32	30	24	11	10
Alcohol **(% of total alcohol)**							
C14:0	0	40	0	32	0	18	11
C16:0	16	49	0	57	0	19	19
C18:0	0	0	100	0	100	29	33
C20:0	0	0	0	0	0	15	16
C20:1	0	0	0	0	0	12	9
C21:0	76	0	0	0	0	0	0
C24:0	0	0	0	0	0	5	6
batylalcohol	9	11	0	11	0	3	7
Sterol **(% of total sterol)**							
Nor-C27:2w5,22	1	0	4	0	11	4	1
C27:1w5	55	51	29	45	35	75	80
C27:2w5,22	5	7	12	25	8	7	6
C28:2w5,22	39	24	27	23	46	6	5
C28:2w5,24	0	10	12	3	0	1	1
C29:1w5	0	6	6	2	0	3	2
C29:2w5,22	0	3	10	2	0	1	2
C29:2w5,24	0	0	0	0	0	3	2
Other biomarkers **(% of total lipids)**							
Phytol	6	2	4	8	2	1	0
loliolide	0	0	0	1	0	0	0

3.2 Salinity transect

Two transects can be distinguished from river to sea, namely the Myakka River Transect (samples 5-6-4-7-8-9) with a total length of 24.0 km, and the Peace River Transect (samples 1-2-3-4-7-8-9) with a length of 25.6 km. The samples are arranged in increasing salinity. The transect reflects both spatial differences and salinity differences, as the salinity correlates with the distance from the start of the transect.

Table 4: Data collection in Charlotte Harbor, 22nd of February, 2011, kilometers normalized to Peace River. Carbon and nitrogen measured by elemental analyzer, TOC calculated. The measurements with an asterisk are influenced by low sample weight and is not deemed accurate.

Salinity	kilometer transect	sample nr	Latitude °N	Latitude °W	Depth (m)	Salinity (PSU)	T (°C)	DO (mg/l)	Conductivity (mS/cm)	N %	C%	TOC %
19,2	0,0	1	26 56.994	82 02.598	0,9	19,2	21,9	6,9	30,9	0,3	3,1	3,1
21,3	1,6	5	26 57.525	82 11.834	3,4	21,3	22,8	7,5	32,5	1,2	9,7	4,3
24,0	4,7	2	26 56.475	82 05.392	2,7	24,0	21,4	7,5	35,1	0,2	1,6	1,7
24,8	7,2	6	26 54.974	82 10.011	3,4	24,8	21,8	7,4	36,6	0,3	2,3	2,5
25,2	8,0	3	26 55.297	82 06.873	4,4	25,2	21,2	7,7	36,6	2,6*	17,8*	25,6*
26,6	12,7	4	26 52.841	82 07.660	5,9	26,6	21,3	7,9	38,5	0,6	3,9	4,4
27,0	16,0	7	26 51.153	82 07.189	5,0	27,0	20,9	7,7	38,7	0,4	2,5	2,8
27,7	19,1	8	26 49.518	82 06.781	4,6	27,7	21,1	7,6	39,8	0,2	1,0	1,0
29,6	25,6	9	26 46.014	82 06.666	3,7	29,6	20,6	7,2	41,7	0,3	1,7	1,9

The fatty acid content is divided into three groups: saturated fatty acid (SAFA), mono unsaturated fatty acid (MUFA) and poly unsaturated fatty acid (PUFA). They are identified as fatty acid methyl esters, as the samples are methylated prior to measurement. The total amount of fatty acid in the samples ranged from 1.49 to 7.84 µg/l (or 71 to 1590 µg/TOC(g)/l). C_{14} to C_{18} fatty acids occur commonly, with one occurrence of a C_{24} FA. There are saturated (C_{14}, C_{15}, C_{16}, C_{17}, $C_1$8 and C_{24}) and unsaturated fatty acid ($C_{16:1}$, $C_{18:1}$, $C_{18:2}$) and three unidentified poly unsaturated fatty acids (PUFAs) present. Only two samples contain an unidentified hydroxy fatty acid (1 and 4).

Phytol is found in samples 1 to 8. In the more saline samples C_{18} and C_{20} saturated long-chained alcohols were identified (as TMS derivatives). Sample 8 contained an $C_{19:2}$ alkene. The average chain length of alcohols or alkanes is not calculated in the transect data, as the alcohol and alkanes data is too limited. Sample 9 contains Parsol MCX.

Nine sterols are identified (1.8 to 4.5 µg/l; 1 to 276 µg/TOC/l). The sterols 24-ethylcholest-5-en-3β-ol ($C_{29:1w5}$) and 24-methylcholesta-5,24(28)-dien-3B-ol ($C_{28:2w5,24(28)}$) are found in all samples. Cholest-5-en-3B-ol ($C_{27:1w5}$; except sample 8) and 23,24-dimethyl-cholest-22E-en-3β-ol ($C_{29:1w22}$, dinosterol; except sample 8 and 9) are found in most samples. 24-nor-cholesta-5,22E-dien-3B-ol ($C_{27:2w5,22}$), cholest-5,22E-dien-3B-ol ($C_{27:2w5,22}$), 24-methylcholest-5-en-3B-ol ($C_{28:1w5}$), 24-methylcholesta-5,22E-dien-3B-ol ($C_{28:2w5,22}$) and probably 24-ethylcholesta-5,22E-dien-3B-ol 9 ($C_{29:2w5,22}$) are found in some samples. The same can be noted for the fatty acids. There are substantial changes in amount of fatty acids (1.5 to 7.8 µg/l; 1 to 494 µg/TOC/l) and the internal composition of fatty acids change along the transect.

Salinity		**19.2**	**21.3**	**24.0**	**24.8**	**25.2**	**26.6**	**27.0**	**27.7**	**29.6**
Distance from start, km		0	1,6	4,7	7,2	8,0	12,7	16,0	19,1	25,6
Sample number		1	5	2	6	3	4	7	8	9
Fatty acids										
14:0		0,0	75,1	0,0	6,2	2,3	5,7	24,2	0,0	0,0
15:0		0,0	0,0	0,0	2,0	1,0	1,1	9,9	33,7	29,6
16:0		150,8	77,2	96,1	110,6	15,7	22,3	143,1	493,1	424,3
16:1a		155,9	185,7	189,9	80,2	22,7	28,5	107,2	295,6	175,9
17:0		32,7	0,0	0,0	0,0	0,0	0,0	6,0	52,2	42,2
18:0		40,8	1,6	0,0	0,0	0,0	0,0	28,9	152,2	170,9
18:1w9		26,9	8,0	0,0	14,0	0,0	7,9	22,1	0,0	64,0
18:2w9,12		17,3	0,0	0,0	6,6	0,0	2,8	9,0	63,1	0,0
24:0		0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	38,3
16:1/16:0		1,0	1,4	2,0	0,7	1,4	1,3	0,7	0,6	0,4
C18 %		15,1	2,0	0,0	9,0	6,4	15,3	11,8	13,6	18,1
SAFA%		55,0	57,6	38,2	61,6	51,4	39,6	63,5	58,8	64,4
MUFA%		9,8	1,9	15,4	7,3	13,6	17,6	7,8	22,6	17,2
PUFA%		35,1	40,5	46,5	31,1	35,1	42,8	28,7	18,6	18,5
C16/C18		3,6	33,0	-	7,1	8,3	3,0	4,9	3,7	2,6
Total Fatty Acids		563,1	478,8	408,6	397,4	71,4	111,0	510,2	1586,6	1299,6
Sterols										
Nor-C27:2w5,22	24-norsterol	0,0	1,9	0,0	0,0	0,0	0,0	0,0	46,8	0,0
C27:1w5	Cholesterol	0,0	2,9	28,3	17,9	3,6	11,2	19,3	0,0	99,4
C27:2w5,22		0,0	1,9	0,0	0,0	1,0	3,2	0,0	169,7	0,0
C28:1w5	Campesterol	0,0	0,0	0,0	0,0	0,0	4,9	35,0	0,0	0,0
C28:2w5,24(28)		19,1	18,8	40,4	13,9	3,8	10,4	46,2	169,2	132,7
C29:1w5	Sitosterol	10,5	26,9	38,3	27,4	3,3	7,0	5,7	204,6	276,2
C29:2a		0,0	0,0	9,7	0,0	0,8	0,0	0,0	0,0	0,0
C29:1w22	Dinosterol	39,9	29,9	91,4	46,7	4,2	8,8	37,2	0,0	0,0
Total sterols		69,5	95,3	221,3	112,4	18,7	55,7	151,5	713,2	508,2
Other biomarkers										
Phytol		42,6	25,9	80,5	21,4	3,5	3,8	17,4	14,8	0,0
Parsol MCX		0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	23,0

Table 4: Charlotte Harbor transect lipid content, in µg/TOC/liter. A: position of double bond is uncertain. SAFA= saturated fatty acid, MUFA= mono unsaturated fatty acid, PUFA=poly unsaturated fatty acid.

3.3 Sediment Core CH1PC

3.3.1 Description

The site of push core CH1 was chosen for coring as it is situated in a small hypoxic basin. In total the core is 92 cm long, but only the first 41 centimeters are used in this project. It consists of black, clayey, organic rich mud with shell remains and shell fragment layers (pers. comm. E. van Soelen). The lowest part used, from 41 to 39 cm, consists of black clayey organic rich mud with an intermediate amount of shell fragments. From 39 to 18 cm there is a small amount of shell fragments. From 6 to 18 cm there are more shell fragments. The top 6 cm consist of black mud/clay with a low amount of shell fragments.

3.3.2 Age model

It is known that sediment deposition rates increased in CH during this period (Soelen et al., 2012). Therefore, the older sediments which could not be dated with ^{210}Pb cannot be directly extrapolated. To overcome this problem the age model (E. van Soelen, pers. comm.) of core of another CH core (CH1) was used for samples 22.5 – 40.5, where the ^{210}Pb dating is used for samples 1 – 21.5. As these cores were taken next to each other, and the push core ^{210}Pb dating was used for the top part and radio carbon dating of the lower sediments of the age model for the CH1 core, this usage of the CH1 age model for the older samples of CH1PC seems to be no problem. This means that the part of core CH1PC sampled for palynology goes back to 1435 AD.

3.3.3 Palynology

3.3.3.1 Dinocysts

In total 23 different dinocysts species found in CH1PC. Some species occur sporadically where other species are present in all the samples. In Figure 10 (relative abundance) and Figure 11 (flux) the most abundant species are plotted. In Appendix 1 a detailed distribution and environmental interpretation of the cysts can be found. Environmental interpretation and dinocyst identification is based on Marret & Zonneveld (2003), Tomas (1997), Cremer et al. (2007) and F. Sangiorgi (pers. comm.).

Different genus and species of dinocysts can have different preservation potentials. In general the P-cysts are more sensitive to aerobic decay than most of the G-cysts (e.g., Zonneveld et al., 1997). The lithology is fairly consistent throughout the core, the sediments are very recent and deposited in a hypoxic depression. We hence exclude that differential preservation represents a bias for the results.

Species that had a relative abundance below 2% and / or species that occurred in low amounts in less than 5 samples are not plotted in Figure 10, but all species relative abundances and fluxes can be found in Appendix 2 and 3, respectively. Therefore *Nematosphaeropsis labyrinthus*, cyst of *Pentapharsodinium dalei*, cyst of *Protoperidinium stellatum*, *Selenopemphix nephroides*, *Spiniferites bulloides*, *S. delicatus*, *S. hyperacanthus*, *Tuberculodinium vancampoae*, *Echinidinium delicatum* and *E. granulatum* were omitted from the results, although they are accounted for in the (sub)totals of P cysts, G cysts, *Spiniferites* total, *Echinidinium* total and dinocyst total. Some species are grouped, although they were counted seperately: Round Brown Spiny consists of *E. delicatum*, *E. granulatum*, *E.* spp. and other spiny round brown P-cysts that could not be identified. *Spiniferites mirabilis* complex consists of *S. mirabilis*, *S. mirabilis* with extensive membranous development and *S. hyperacanthus*. *Operculodinium* spp. is the sum of *O. israelianum* and *O. centrocarpum*. *Lingulodinium machaerophorum* complex consists of *L. machaerophorum* with normal and reduced processes.

L. machaerophorum and *Spiniferites* are the two dominant dynocysts in this core, together accounting for 75 to 95% of total dinocysts. Most species have a higher and more dynamic flux from 1960s AD, even species with constant (e.g. *P. zoharyi*) or decreasing (e.g. *L. machaerophorum)* relative abundances. *L. machaerophorum* decreases in relative abundance. A typical morphotype with well-developed processes is found together with other *L. machaerophorum* morphotypes with relative short, or sometimes bulbous processes. *L. machaerophorum* is present at relative high levels throughout the core (13 to 59%; 400-2100 cyst/cm^2/yr) and shows a decrease from 1930s AD onwards, in relative abundance but not in flux. The flux of *L. machaerophorum* shows a rather different signal; with a high

variable flux. *Spiniferites* is, unlike *L. machaerophorum* the main species reacting on changes in CH. *L. machaerophorum* has a highly variable flux, but compared to total dinocyt flux this is almost a constant input. The flux of Spiniferites is increasing and shows a higher variability. In this core three species of *Spiniferites* often occur (*S. bentorii, S. mirabilis* and *S. hyperacanthus*), together with a morphotype, here named *S. miratorii* (see Figure 17), which shows features of the *S. mirabilis* (antapical flange) and the *S. bentorii* (apical protuberance). Some cysts of other *Spiniferites* species are found in a very low abundance (*S. bulloides*, *S. delicatus* and *S. ramosus);* they are grouped into the *Spiniferites* total. *S. bentorii* is often seen as a transgression indicator and is restricted to higher salinity ranges and full marine settings, although it is found below 34 SSS. The species increases but never dominates the *Spiniferites*. The *S. miratorii* morphotype is present at a steady level of 2% until 1695 AD while it increases afterwards and constitutes up to 50% of total dinocyst count in 1986 AD.

The P/G cyst ratio shows some variability in the sediment core, ranging from 0.03 to 0.15, average 0.08 (Figure 10). In the record it is seen that the relative abundance of total P cysts -which due to their heterotrophic lifestyle are often taken as indicative of productivity- is relatively constant throughout the core. There are two peaks of increased P cyst flux, namely around 1966 AD and 1992 AD. The most abundant heterotrophic cyst genus, *Brigantedinium* spp., shows higher fluctuations from 1960 AD onwards.

Polysphaeridium zoharyi is characteristic for euryhaline (sub)tropical mesotrophic coastal sites. It has been an important HAB species in the CH area (pers. comm. E. Lammertsma). In CH1PC *P. zoharyi* is found in relative stable relative abundances, although there seems a rise in flux together with most other species from the 1950s AD onwards. The heterotrophic *Brigantedinium* spp. is the most occurring P-cyst in this core. *Selenopemphix quanta* can be used as an indicator species of (nutrient and heavy metal) pollution (pers. comm. F. Sangiorgi; Sætre et al., 1997). This species is not found in high abundances, but can be indicative of possible eutrophication.

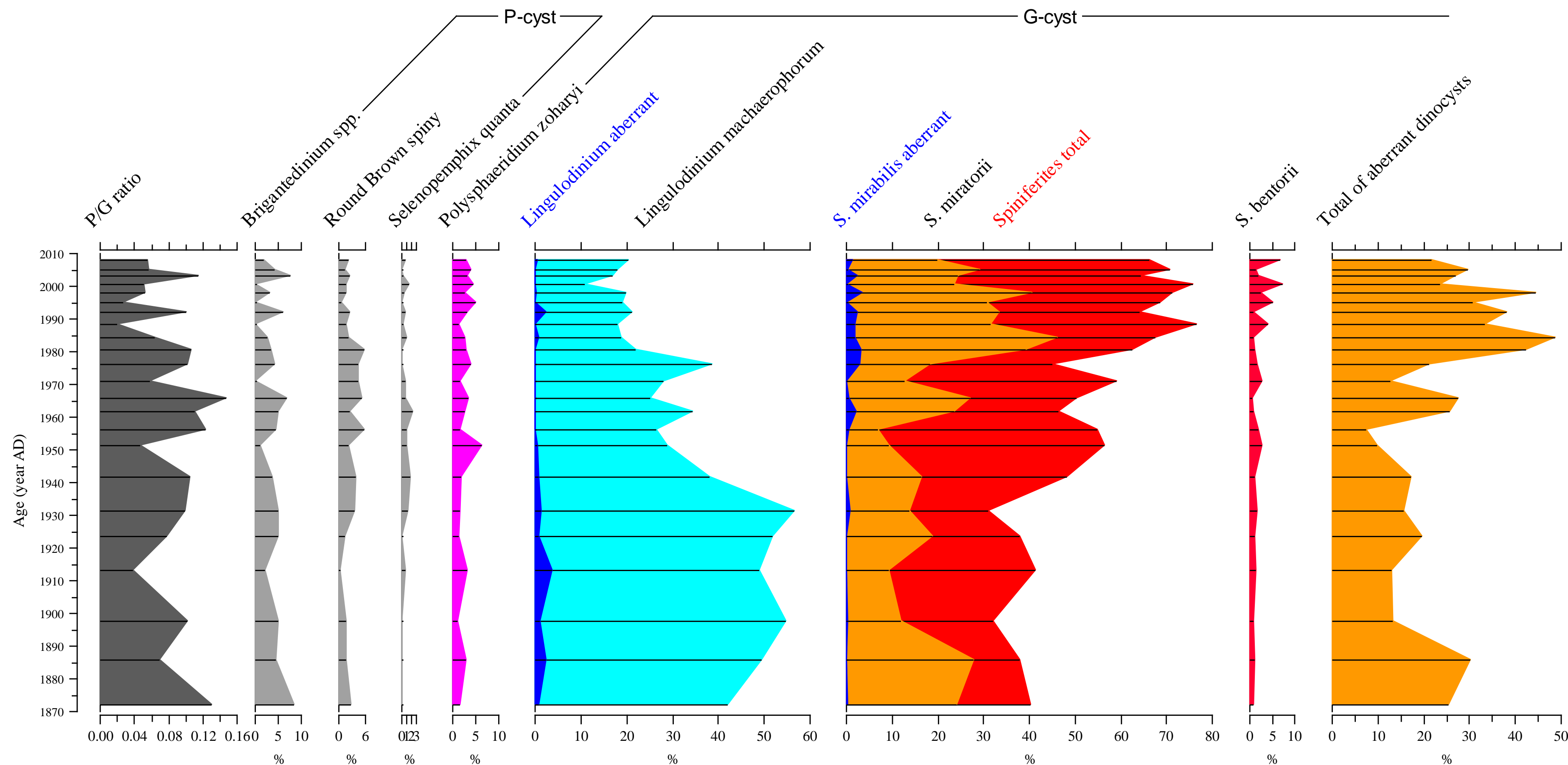

Figure 10: the relative abundance of dinocysts in sediment core CH1PC from Charlotte Harbor, Florida. In the *Lingulodinium machaerophorum* record the dinocysts with aberrant processes are marked by the darker blue. It is only a small amount of the total *L. machaerophorum* signal. The *Spiniferites* signal is at times dominated by the aberrant morphology (*S. miratorii*) and a small percentage of *Spiniferites* consist of S. mirabilis with a surplus of membranes.

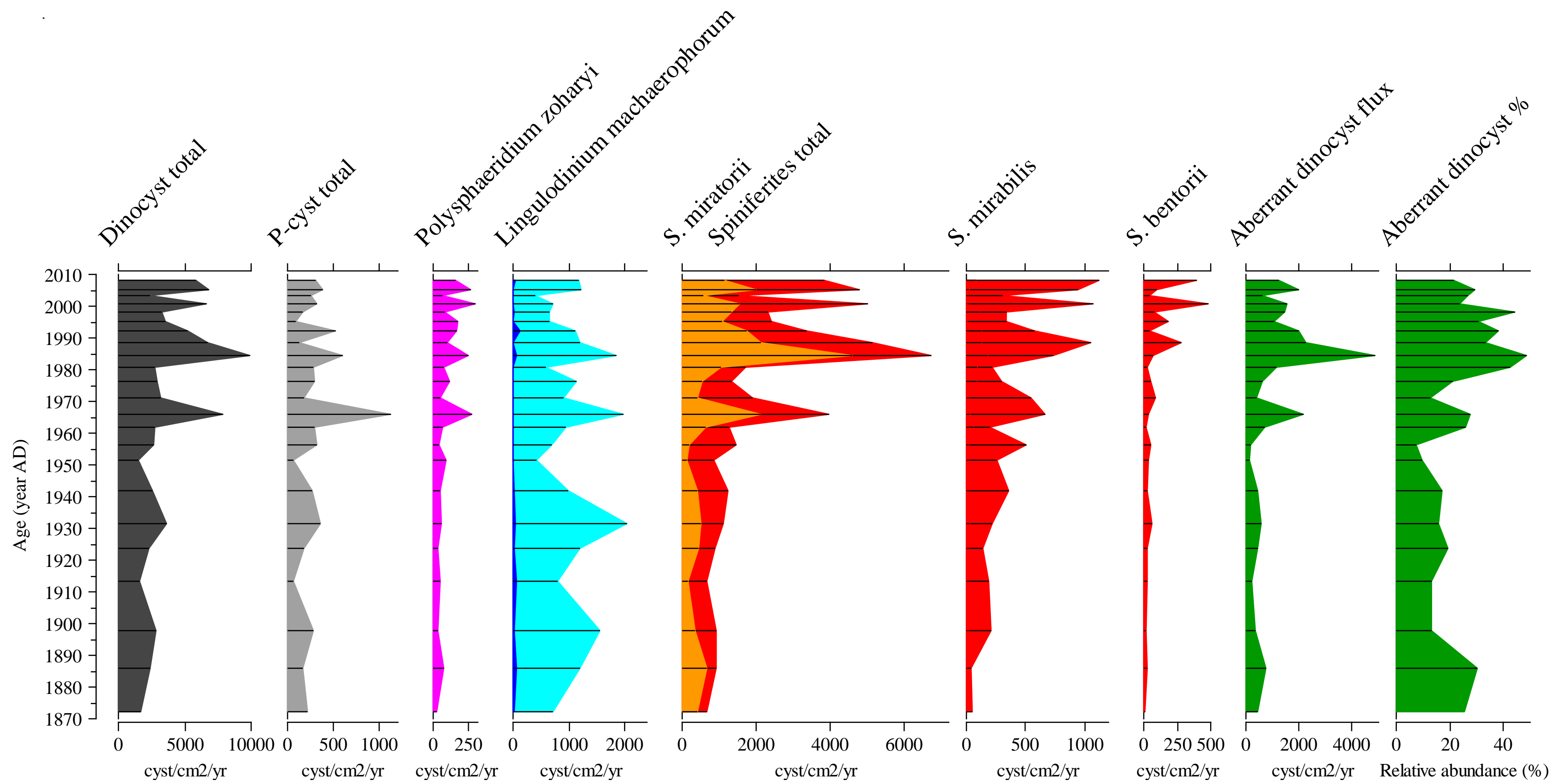

Figure 11: the flux of dinocyst of sediment core CH1PC of Charlotte Harbor, Florida. It is calculated in cyst/cm^2/yr.

3.3.3.2 Pollen

In 28 samples pollen were identified and counted, see Appendix 4 for an overview of species and their common name. Overall, 19 species were identified; for the flux of the most abundant species see Figure 12. The most widespread are the bisaccate pollen, which are present between 42.3 and 78.3% of the total pollen and spore counts. When the bisaccate pollen are excluded from the count, *Quercus laurifolia* (13.7-46.5%), Amarantaceae (*Amaranthus australis*) (1.3-42.3%), *Myrica cerifera* (2.0-19.0%), Asteraceae (Asteraceae Tubuliflorae (1.0-19.2%); *Ambrosia artemisiifolia* (0.9-13.2%)) and *Cyperaceae* spp. (0-11.1%) are most abundant. In smaller abundances *Cephalanthus occidentalis* (0-9.1%), *Casuarina equisetifolia* (0-8.2%), *Typha* spp. (0-7.0%), *Iva* sp. (0-5.3%), *Taxodium distichum* (0-4.8%), *Phlebodium aureum* (0-3.9%), *Poacea* spp. (0-3.9%), *Carya* sp. (0-3.2%) and *Salix* sp. (0-2.2%) were found. Species with a single occurrence or a single identification per slide are cf. *Toxicodendron*, cf. *Corylus* and *Ulnus* sp. The flux of total pollen (pollen per cm^2 per year) is between 14 and 89 thousand. This is 36 to 60 percent of the total palynomorphs found.

Before large-scale land use alterations in the area around CH, the landscape was covered by evergreen conifer and mixed hardwood forests with patches of prairie (Myers and Ewel, 1990; E. Lammertsma, pers. comm.). The vegetation surrounding the CH estuary is presently dominated by mangrove and marsh vegetation. Pines (*Pinus* spp.) is part of the pine flatwoods. Oak (*Quercus* spp.) and wax myrtle (*Morella cerifera*) are part of both the hardwood forests and hammocks. The mesic hardwood forest also commonly includes hickory (*Carya* sp.) and the more moist hammocks elm (*Ulmus* sp.). Pioneer species (as *Asteraceae Tubuliflorae*, *Amaranthus australis, Ambrosia artemisiifolia, Casuarina equisetifolia* and *Phlebodium aureum*) are grouped as well, as these species are known to show (anthropogenic) disturbance. As species of Asteraceae Tubuliflorae can be hard to distinguish, this is grouped in Asteraceae Tubuliflorae (Aster. Tub.).

The flux of pollen increased fourfold over the last 100 years, from on average ca. 5500 pollen at 1900 AD to ca. 18 900 pollen at 2000 AD. The amount of bisaccates seems to be decreasing from 1875 AD onwards. Species that show a relative and an absolute increase are Asteraceae Tubuliflorae, *Ambrosia artemisiifolia* and *Myrica cerifera*, while *Amaranthus australis* shows an absolute and relative decrease towards the top. *Quercus* sp. shows an absolute increase, but stays relatively constant.

The marine-terrestrial ratio was calculated based on marine palynomorphs (dinocysts) over total (marine + terrestrial) palynomorphs. This ratio decreases, with a maximum of 0.64 in 1898 to 0.34 in 2008.

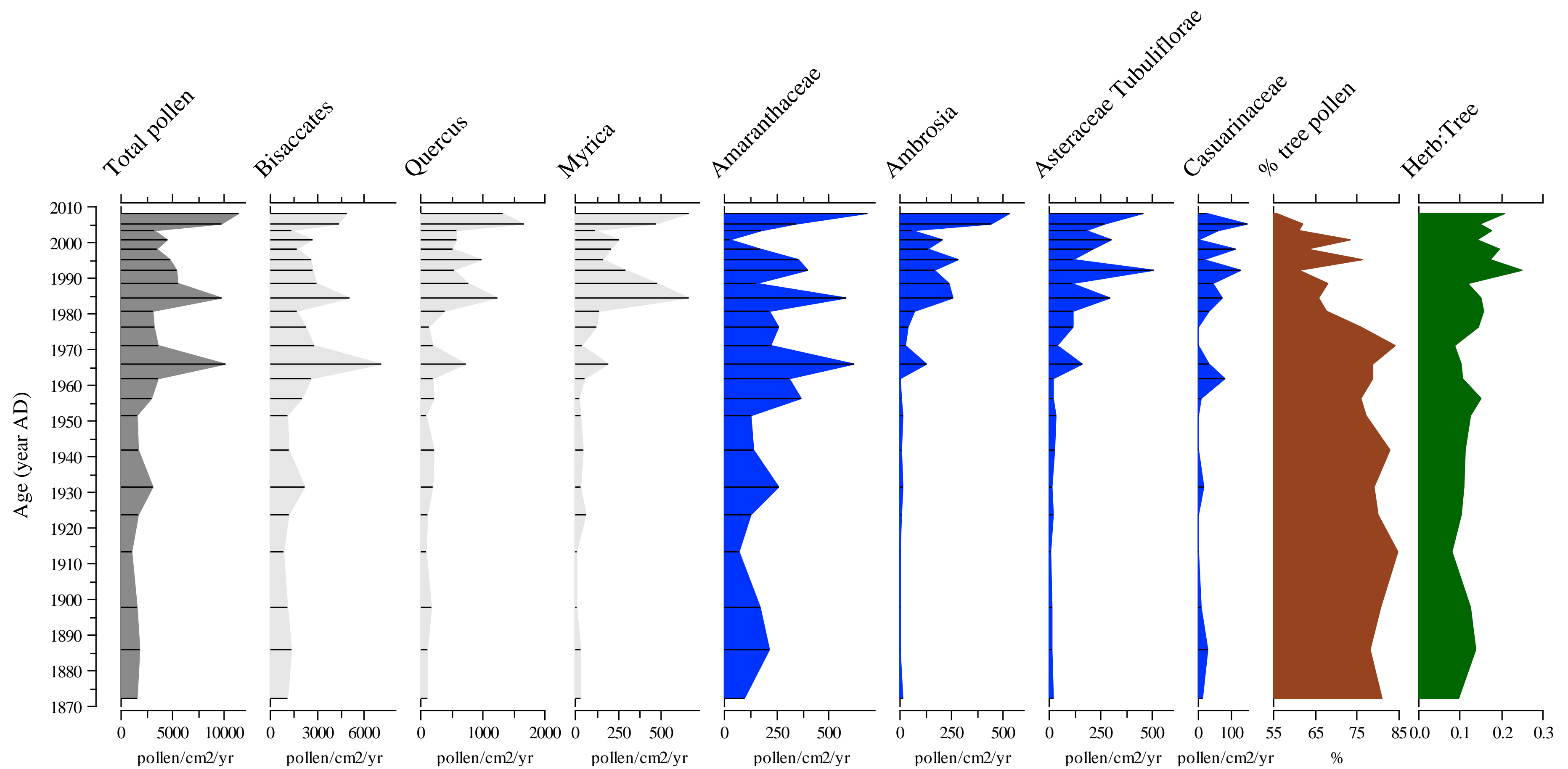

Figure 12: The pollen flux of sediment core CH1PC, Charlotte Harbor, Florida. The flux of pollen with a high abundance is shown. Tree species, as bisaccates and *Quercus*, and the shrub *Myrica cerifera* are light grey. Pioneer species (blue) as well as *Casuarinaceae*, a species often used as the start of human influence as it is not native to the American continent, are plotted. The percentage of tree pollen and herbs over tree pollen are indicative of deforestation, disturbances and/or more open vegetation.

3.3.3.3 Other palynomorphs

Next to pollen and dinocysts other palynomorphs are identified. The most abundant are *Cymatiosphaera*, fresh water algae. *Cymatiosphaera* increase slowly up to 1971 AD, decrease towards the 1990s and show a large increase in the 2000s AD. Some occurrences of *Botryococcos* (in 2 samples), tintinnids (5x), *Pediastrum* (1x), copepod eggs (4x) and in more samples acritarchs, foram linings (chains and spiral) and fungal spores are found.

3.3.4 Biomarkers

Peaks in the GC chromatogram are identified (with GC-MS), quantified with GC spectrum integration, calculated relatively (in µg/gTOC) and as flux (in µg/cm^2/yr), see Appendix 8 and 9. The polar lipids are more abundant than the apolar lipids. The total lipids identified range between 2 and 22 µg lipids per gram sediment with a three to four fold increased lipid content from 1976 AD onwards. The relative abundances of the different interesting lipids is shown in Figure 13. The two biggest contributors to the total lipid are n-alkanes and long-chain alcohols. The CPI fluctuates between 5.1 and 7.2. The C_{30}/C_{32} diol index ranges from 56 to 75 and shows smaller fluctuations (between 65 and 75) from 1930s AD onwards. $ACL_{alkanes}$ ranges from 28.0 to 29.5. The sample at 13.5 cm (1966 AD) is omitted from the CPI and ALC data. The samples contain almost no detectable fatty acids.

Most biomarkers show large fluctuations and remain at low levels until 1960, with a slight increase to 1976 after which they increase sharply. Between 1980 and 1990 AD most biomarkers (phytol, taraxerol, β-sitosterol and HBIs) show a large peak. When the top part (2008 to 1960 AD) is compared to the lower part of the core (1960 to 1880s AD) a large difference is seen in average lipid biomarker content. The lower part contains on average 2.4 µg/gTOC phytol, where the upper part has 11.0 µg/gTOC. The same is with taraxerol (16.1 to 36.6 µg/gTOC), β-sitosterol (3.8 to 19.7 µg/gTOC), dinosterol (0.2 to 5.4 µg/gTOC), C_{30}-C_{32} diols (50.8 to 81.3 µg/gTOC), $HBI_{25:4}$ (3.7 to 63.6 µg/gTOC), $HBI_{30:5}$ (0.8 to 4.5 µg/gTOC). The CPI shows an increase in these two periods, from 5.6 to 6.7. The $ACL_{alkanes}$ decreases slightly from 29.2 to 29.1, while the diol index increases (69.2 to 70.7). The biomarkers show a larger oscillation. For example, phytol in the top period 1960 to 2008 AD ranges from 1.6 to 21.8 µg/gTOC, when in the lower period it ranged from 0.7 to 5.2. When detected, dinosterol ranged from 0.3 to 2.3 µg/gTOC in the bottom part, up to 0.4 to 14.0 µg/gTOC in the upper part. The same holds for β-sitosterol (bottom 0.1-19.9 µg/gTOC; top 9.4 to 42.4 µg/gTOC), $HBI_{25:4}$ (bottom 0.5-11.1 µg/gTOC; top 5.9-88.0 µg/gTOC) and $HBI_{30:5}$ (bottom 0.2-1.8 µg/gTOC ; top 1.1-5.8 µg/gTOC).

The C/N ratio decreases gradually from ca. 12 in 1870 AD to ca. 10 in 1900 AD. From 1971 AD onwards C/N decreases more markedly to ca. 9 in the 21th century.

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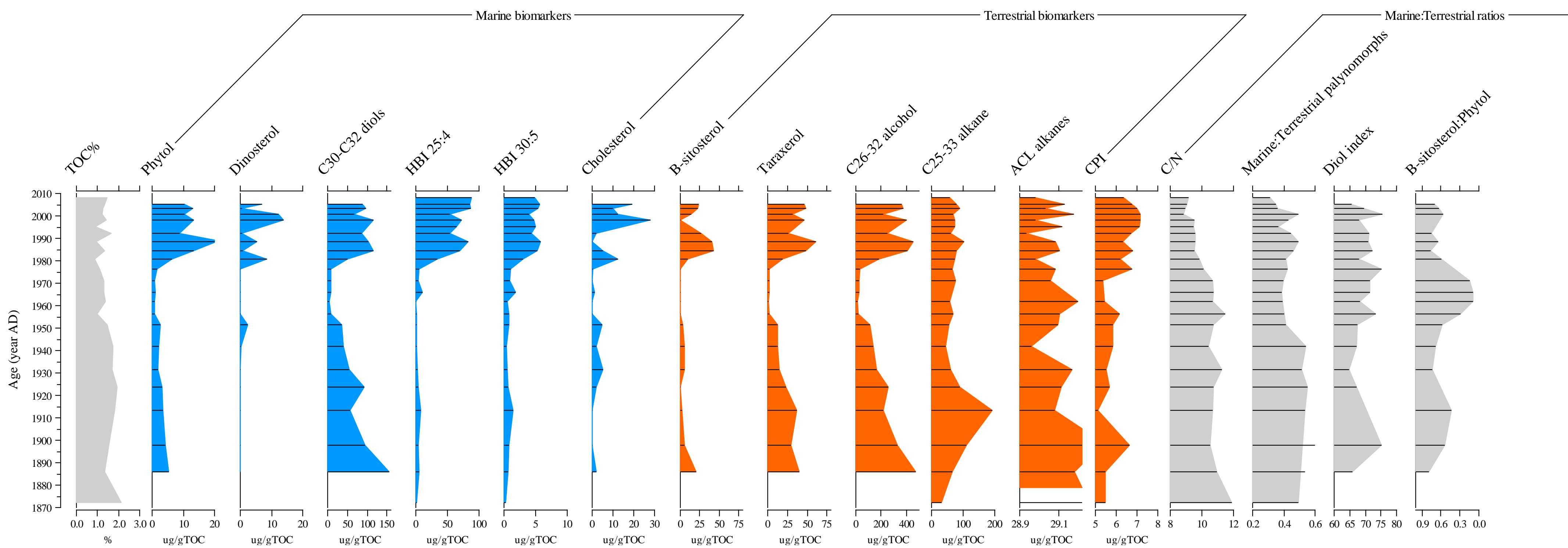


Figure 13:Biomarkers of sediment core CH1PC of Charlotte Harbor, Florida. The detected biomarkers are in µg per gram TOC. The lipids are divided in three clusters. First, the marine biomarkers are comprised of phytol, dinosterol, cholesterol, $HBI_{25:4}$, $HBI_{30:5}$ and C_{30}-C_{32} diols. The terrestrial biomarkers are comprised of B-sitosterol, taraxerol, C_{26} to C_{32} alcohols, C_{25} to C_{33} alkanes, the average chain length and carbon preference index of alkanes. The last cluster are the marine over terrestrial ratios, as the C/N ratio, marine over terrestrial palynomorphs, the relative salinity index based on diols and a marine over terrestrial biomarker record of B-sitosterol over phytol (reversed x-axis).

CHAPTER 4: DISCUSSION

4.1 Algal growth experiment

The ratio between TN:TP in the CH estuary is usually <2 due to natural P enhancement (McPherson et al., 1996). In the algal growth experiment this ratio TN:TP ratio is 1:2.13 and falls in the order of TN:TP ratios found in previous studies. The experiment where DIN was added resulted in the lowest amounts of lipids and biomarkers, and shows the quickest chlorophyll-α increase and collapse. Autotrophic organisms assimilate dissolved inorganic P (orthophosphate), and alter it to organic phosphorus. Heterotrophs obtain P by their feeding strategy of autotrophic organisms (organic phosphorus). When organisms die (or defecate) phosphate is again available by bacterial decomposition of the OM. It seems that inorganic N and P additions give the quickest phytoplankton response in CH, while the experiments with organic nutrients give a slower and lower response. This can be explained by the amount of autotrophic organisms, as they can use inorganic nutrients.

It is interesting to see if and how different nutrient additions (DIN, DIP, DON, DIN) affect the chlorophyll-α levels of the experiment. This can be compared to the batch without nutrient additions. The difference between organic and inorganic nutrients is important, as this can say something about the type of organisms in the experiment. For example, with inorganic nutrient additions it is thought that the autotrophic phytoplankton will experience growth, as they can take up the inorganic nutrients. Heterotrophic organisms will feed on the autotrophic phytoplankton, decreasing the growth of the phytoplankton by grazing. With organic nutrient additions it is thought that there is direct competition between the phytoplankton and the grazers.

As the heterotrophs are not included in the biomass estimate (which is based on chlorophyll-α only) it is thought that organic nutrient addition would show slower chlorophyll-α spikes and a lower value of the chlorophyll-α due to the direct competition. This is reflected in the results of this experiment. Both DIP and DIN show a faster and higher increase than DOP and DON, possibly indicating more competition of auto- and heterotrophs. Figure 8 shows a selection of organisms seen in the various experiments.

All samples contain cholesterol, campesterol and $C_{27:2w5,22}$ sterol. Most samples contain 24-norsterol, $C_{28:2w5,24}$ and β-sitosterol. That samples contain β-sitosterol is interesting, as this is usually seen as a biomarker of terrestrial origin. It is possible to retain β-sitosterol in the water without degrading it. Another possibility is that it has an in situ origin of marine phytoplankton, cyanobacteria or haptophytes (Volkman, 1986). Phytol, the degradation product of chlorophyll-α, was found in all samples, mostly in the DOP, DON and DIP experiments. This has most likely a diatom origin, as the common $C_{16:0}$ and $C_{16:1}$ fatty acids are widespread in diatoms and bacteria, while the $C_{20:5(n-3)}$ fatty acid is common in diatoms and dinoflagellates. In the DOP, DON and start samples general bacterial activity biomarkers are found, such as $C_{15:0}$ and $C_{17:0}$ fatty acid. Sample A to L contained organisms that grew on the inside of the bottle. As they were excluded in samples M to O and not present in visible high concentrations at the start, it could be possible to determine what species these organisms belong to. But in the results only the phytol concentration differs greatly between A-DOP, E-DON, H-DIN- J-DIP (including residue) and O-blank and the start conditions. In µg/liter there seems to be no discrepancy. The biomarkers are not indicative for the presence of specific organisms or species.

Microscopically, small green phytoplankton were identified as Crypophyceae in sample H-DIN (V. Palubok, pers. comm.). 24-ethylcholesta-5,22E-dien-3B-ol is present in some Cryptophyceae. This sterol is indeed found in most experiments. Most was found in the E-DON sample (0.27 µg/l), but it consisted of 10% of all sterols in H-DIN.

Due to the low amount of cells in the surface waters of CH at the time of sampling, several aspects of the algal growth experiment were not executed. The aim, dinoflagellate growth with different nutrient additions was not possible due to the lack of dinoflagellates in the sampled water. This could be due to the concentration step, as some species cannot handle that amount of stress, although measures were taken to reduce stress as much as possible (keeping the phytoplankton net submerged, by pouring the water slowly, by transporting the water with as low amount of shaking or stirring as possible). Although chlorophyll-α measurements give an indication of biomass, it was not possible to count cells, convert this to biovolume or to estimate the different phytoplankton group changes during the experiments.

Most importantly for future research is the use of not only surface waters, but also deeper waters. As the upper CH area has a classical salt wedge structure, the more saline water is near bottom. Changes in the salinity profile can change the phytoplankton community. As only surface phytoplankton is used in the algal growth experiment, and only surface water is filtered as part of the transect sampling, the representativeness declines, especially when one wants to compare estuarine waters to the sedimentary record of CH. In the more seaward area of the estuary the mixing is better, so in these areas mixing is less of a problem.

For determining the biological origin of the phytoplankton in future experiments, an alternative could be HPLC pigment analysis. This analysis can qualify and to some extent quantify the different phytoplankton groups and species. DNA analysis could provide this as well, but this method is less advanced at this period of time, but it has great potential. With more accurate species determination, sampling at greater water depth or as a mesocosm experiment, this could be a valuable approach.

4.2 Transect

The transect is sampled from river to sea from slack tide onwards, so the samples in the transect would be influenced increasingly more (with time and place) by the incoming tide. The C/N ratio of the transect shows a marked decrease from 11 to 6 seawards, which reflect the seawards increase of marine OM, thereby lowering the C/N ratio.

The $C_{16:0}/C_{16:1}$ ratio ranges from ~2 to 0,4; C_{16}/C_{18} ratio ranges between 2,6 and 33. Both the $C_{16:0}/C_{16:1}$ and C_{16}/C_{18} ratios both show a decreasing trend seawards while total fatty acids increase towards the sea, see Figure 14. The comparable trend in both ratios indicate that from river to sea there is a change in dominant phytoplankton assemblage composition from a diatom dominated river to more (dino)flagellate dominated assemblages closer to sea. Phytol is derived from chlorophyll-α, and shows a photosynthesis algal input. The amount of phytol is decreasing towards the sea. This could indicate on the one hand a general lower productivity of photosynthesizing algae, or on the other hand that a shift occurred in phytoplankton composition. As nutrients come mainly from the rivers, it seems reasonable that towards the sea less productivity occurs. This is reflected in the biomarkers, as not only total lipids yield but also phytol and diatom markers decrease seawards. It is curious that B-sitosterol increases seawards, as it is mostly used as higher plant terrestrial biomarker. The C/N ratio shows a decrease of terrestrial input seawards, while this sterol increases. As B-sitosterol can also be found in some organisms, it is thought that the C/N ratio shows a

more reliable trend. The sample with the highest salinity (CH Transect 9), contains Parsol MCX compound, which is an UV-B blocking agent used in sunscreen. This could be due to sunscreen pollution by sampling error or other recreants, or due to sewage disposal in the area, as these compounds could end up in household grey wastewater (Eriksson et al., 2003). Most transect samples contain residues of burned plastic.

As a test, transect sample 7 was saponified, both the extract and the residual filter. As shown in Figure 15 there are some differences in the GC chromatogram. The same lipids are seen in all three chromatograms. The idea was that bound lipids or large molecules would be visible after saponification, but for these samples this was not the case. In the sterol compartment it did clean up and less peaks co-eluted. But without saponification the chromatogram was nice as well so for the rest of the samples saponification is deemed not essential.

In the transect dinosterol is not present in the higher salinity ranges (sample 8 and 9), which is unexpected. The dinocysts found in the sediment core are thought of as relatively high salinity species (e.g. *S. bentorii*) so it seems odd that in the higher salinity ranges no dinosterol was found. Some possible explanations are that fresh or brackish water dinoflagellates produce more dinosterol than marine species. Maybe the season of sampling was not right for finding dinoflagellates altogether, although (Brand & Compton, 2007) found that the dinoflagellates were year round present in CH. Or the dinoflagellates were not present in the samples surface water layer, but were present in deeper water layers. At least many Gonyaulax species (which produces *Spiniferites*) and *Lingulodinium* produce dinosterol (e.g. Sangiorgi et al., 2005).

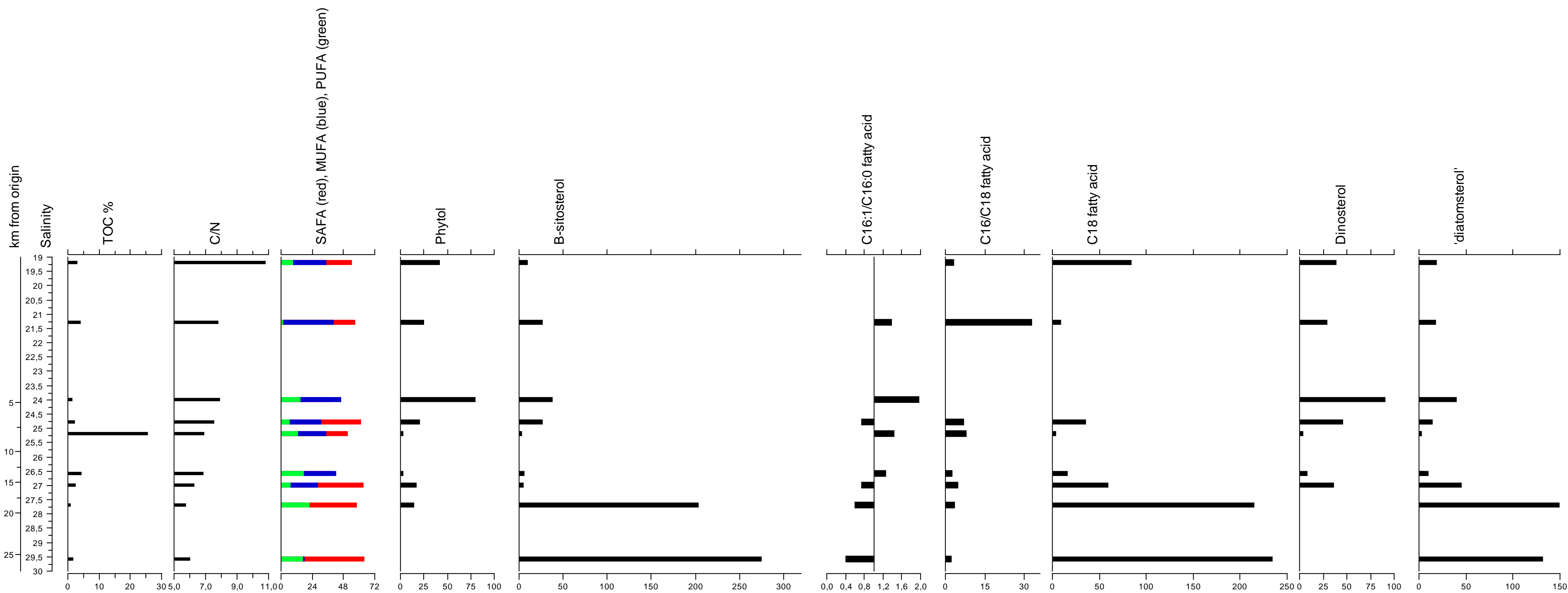


Figure 14: Charlotte Harbor transect biomarkers on salinity scale. Secondary y-axis is the distance from the first sample, with Myakka River normalized to the Peace River samples. A shift is evident from diatom dominated phytoplankton assemblages towards assemblages dominated by other phytoplankton, e.g. flagellate (seen in the increase in C_{18} fatty acid). This general shift is seen with the $C_{16:1}/C_{16:0}$ and C_{16}/C_{18} fatty acid ratios as well. β-sitosterol increases seawards, a biomarker usually interpreted as higher plants OM input, while the C/N ratio decreases seawards. This means that the β-sitosterol cannot be used as a terrestrial OM biomarker in this specific experiment, as the C/N ratio is more robust and logical. TOC, fatty acid distribution (in SAFA, MUFA and PUFA) in percentages; phytol, β-sitosterol, C_{18} fatty acid, dinosterol and diatomsterol in µg/TOC/liter.

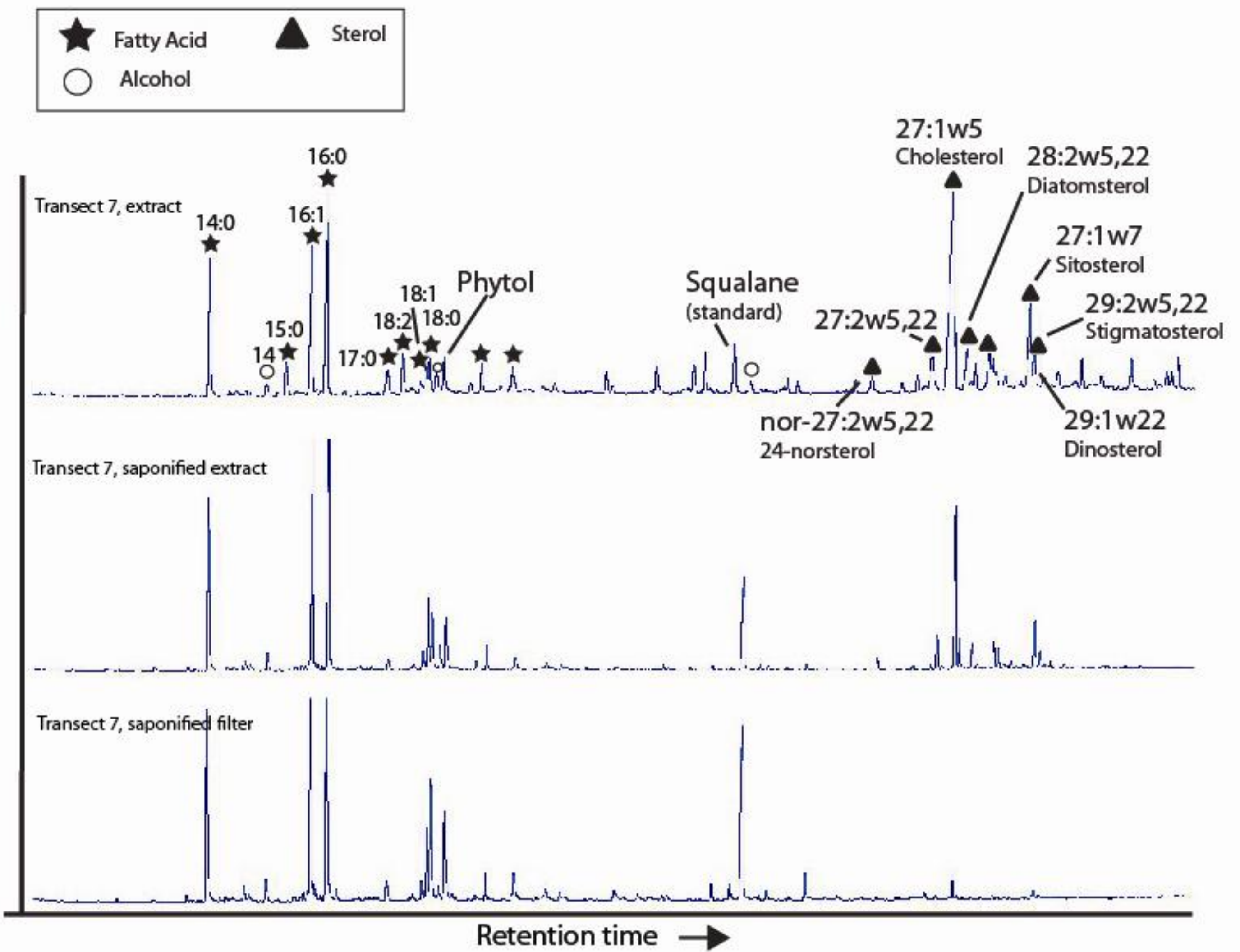


Figure 15: the GC chromatogram of transect sample 7. The upper chromatogram is the TLE, middle chromatogram is this TLE but then saponified, and lower chromatogram is the saponified residual filter. As can be seen, all peaks in the extract are found in the saponified extract and filter. The sterols in the saponified extract are somewhat better separated. The saponified filter yielded no extra peaks although the fatty acids have higher intensities.

4.3 Sediment core CH1PC

4.3.1 Chronology

The ^{210}Pb activity nicely fits the modeled activity and the age model used. The age model of the sediment core is deemed accurate. The core has an increasing sedimentation rate towards the top, from on average 0.10 cm^2/yr in the bottom half (1872-1956 AD) to 0.31 cm^2/yr in the upper half (1962-2008 AD), with a maximum of 0.54 cm^2/yr in 2005 AD. This is partly due to a lack of compaction, but is likely to reflect changes in erosion as well as deforestation and changes in land use have a big impact on erosion and thus on sedimentation (Soelen et al., 2010; and references herein). Next to a higher sedimentation rate, the biomarker and palynomorph accumulation rates increase from the 1960-70s AD onwards. This could be an artifact of increased preservation due to higher productivity, so the pronounced increase in biomarkers that is found in the sediment core could be a tradeoff between increased OM delivery and preservation signal. The TOC% content of the core is relatively stable. This indicates that the increase in lipids is not a result of depositional alteration of OM but an actual increase in primary production. This is most likely due to enhanced human activity, as this can increase nutrients, productivity and thus higher biomarker and palynomorph fluxes. Between 6 and 18 cm more shell fragments are found.

In other cores from the CH area, storm deposits are found in the sedimentology (Soelen et al., 2012). In CH1PC distinct coarser grained layers that could be linked to tropical cyclone activity in the Gulf of Mexico are not identified.

4.3.2 Human impact on land

The vegetation reconstruction based on pollen analysis indicates changing land use during the 20th century AD. The tree pollen, including *Pinus*, shows a sharp decrease at 1972 AD; from a relatively constant 75-85% from 1870 to 1972 AD, down to 55% tree pollen in 2008 AD (Figure 16). This can indicate human induced change in land usage, e.g by deforestation. Since *Pinus* pollen are easily transportable with wind or water, the changes could be partly related to water discharge decrease. This does not explain the ongoing decrease of *Pinus* even though discharge increased in the last decade. Asteraceae Tubuliflorae is a family with a wide environmental arrangement and increases from 1960s AD. Ragweed (*Ambrosia)* is an early succession plant that can quickly occupy cleared sites and is increasingly present from 1962 AD (with a few earlier occurrences). A sudden increase in abundance of *Ambrosia* pollen has been used in North America to mark deforestation and urban development (Willard et al., 2003). The herbs over tree pollen ratio indicates a gradual increase in herbs from the 1970s AD onwards. The increase in herbs, pioneer species and the decrease in tree pollen indicates that the area becomes more open, relatively dry and disturbed (Figure 16). There are greater abundances of weedy pioneer species from the 1960s AD onwards. This corresponds to trends of modern agricultural practices and urbanization seen in other anthropogenically impacted sites around the world (Willard et al., 2003; Donders et al., 2008). A development 'frenzy' occurred in the CH area in 1950-1960, culminating amongst others in large development projects as the 'Rotunda' in Punta Gorda. The *Ambrosia*, Asteraceae Tubuliflorae and *Pinus* disturbance signal is likely related to these developments on the land surrounding CH.

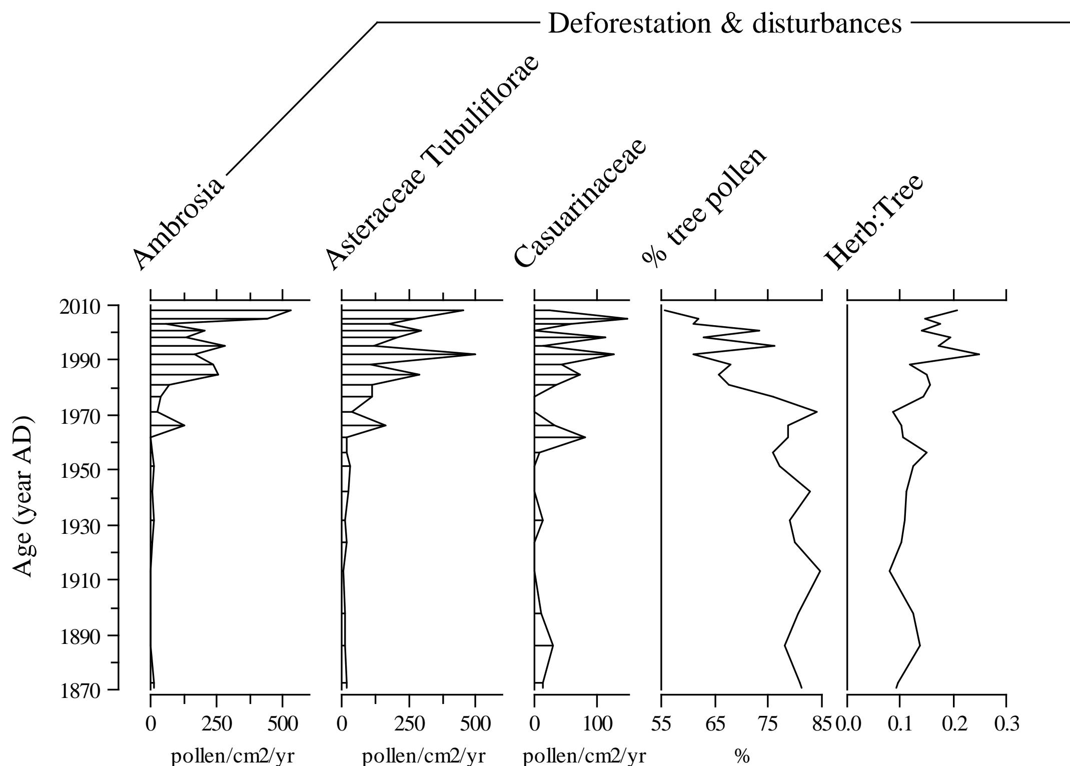


Figure 16: Deforestation and human disturbances in the pollen data of core CH1PC, Charlotte Harbor, Florida. The percentage of tree pollen (including *Pinus* spp.) and the herbs over tree pollen (including *Pinus* spp.) indicate a marked decrease in tree pollen from the 1970s AD and an increase in herbs. Early succession plants as *Ambrosia* and Asteraceae Tubuliflorae indicate more open and disturbed environments. The pollen record points towards deforestation and disturbances, which all fit with the increased urban development and population growth in the area.

4.3.2 Increased terrestrial and marine input

The biomarker and palynology record of CH1PC shows differences in influx of terrestrial and marine material. It can be difficult to separate terrestrial and marine OM influxes as they show parallel increases. Increased runoff enhances the flux of terrestrial biomarkers and pollen and increases the nutrient input in the estuary. Different proxies show an increased terrestrial input. The palynology-based marine:terrestrial ratio decreased in the 20th century AD, with a few peaks of relatively higher marine input in 1942, 1989 and 2001 AD, in this general trend of increased terrestrial input towards the top of the core. The CPI, ACL and C/N ratio are expected to reflect changes in terrestrial input into the CH area. The core shows a CPI ranging between 5.1 (1913 AD) and 7.2 (1995 to 2001 AD) with a minimum in the 1960s. This is a measure of terrestrial plant input and is in the range of high terrestrial input (Rieley et al., 1991). Just as the CPI increases over time, the ACL shows a general decrease. The decrease of the C/N ratio indicates a relatively higher marine OM input as C/N ratios in marine OM is close to 5-7, while terrestrial OM has a ratio above 15 (Meyers, 1997). This decrease in C/N is caused by the increased primary productivity that is indicated by biomarkers and palynology. The marked increase of both $HBI_{25:4}$ and $HBI_{30:5}$ from the 1980s AD onwards indicates an increase in diatom dominance and/or a changed diatom assemblage. *R. setigera* probably played a more dominant role in the CH estuary since the 1980s, as both HBIs, biomarker of this diatom species, increase steeply. According to Versteegh et al. (1997) the diol index is between 25 and 67 in most freshwater or restricted marine environments, where indices between 68 and 79 are the marine environments (highly productive areas;

tropical upwelling area's). From the 1930s AD onwards, the diol index fluctuates between 64.7 and 75.4, near and in the range of highly productive areas (Versteegh et al., 1997). This biomarker seems not of use in this study.

The period between 1956 and 1976 AD is more or less a transition period, with the first large peak of total lipids and palynomorphs at 1966 AD. In this sample, the P-cyst flux is thrice the average P-cyst flux. It is the only peak of *Operculodinium* spp. and the first peak in *S. miratorii*. *P. zoharyi* and *Lingulodinium machaerophorum* increase as well. Moreover, there is a peak flux of foraminifera linings, *Pinus*, Amaranthaceae, bacterial hop-22(29)ene (Volkman et al., 1992) and terrestrial 24-nor-lupane. The terrestrial biomarker B-sitosterol and taraxerol are minimal in these two decades, while dinosterol is also absent. This could indicate that between 1956 and 1976 there was less runoff, resulting in a higher salinity and lower terrestrial input. An additional biomarker possibly indicating a lower terrestrial input is the depth profile of C_{24} alcohol, as it is mainly derived from vascular plants, and some phytoplanktons (Cranwell, 1982; Volkman et al., 1980).

Up until the 1970s AD the flux of most biomarkers is relatively low (except 1966 AD). After this low period a large increase in most biomarkers and palynomorphs up to the most recent sediments with large variability, indicating strong fluctuations in runoff and primary productivity. These fluctuations could be partly explained by the higher resolution in the top part of the core due to an increased sedimentation rate. But it is very likely that the shown variability is real, as anthropogenic influence increases from the 1960s AD onwards.

The marine-terrestrial ratio decreased from 0.64 in 1898 to 0.34 in 2008. This indicates a lower proportion of marine palynomorphs and a higher input of terrestrial palynomorphs. The increase of fresh water *Cymatiosphaera* indicate increased runoff in the 1950s and 1960s and in the 2000s.

The gauged Peace River flow decreased between ca. 1960 and 1990 AD while the flux of land derived biomarkers (taraxerol, B-sitosterol, long chain alcohols, CPI) and freshwater and lower salinity biomarkers (*S. miratorii*, *Cymatiosphaera*, B-sitosterol:phytol ratio) increased. Not only river discharge is an important medium for land derived biomarkers to enter the estuary. Urbanization, industrialization, deforestation, agriculture and land use change influences the amount of sheet flow. With increased human disturbances on land, the sheet flow is expected to increase.

4.3.4 Dinocyst flux and aberrant cyst morphologies

The increasing dinocyst record is interpreted as a productivity signal. This general eutrophication trend is seen in many areas around the globe. Around 1930 AD (Figure 10) the dinocyst assemblage shifts from being mostly represented by *L. machaerophorum,* a coastal nutrient loving species generally indicative of warm eutrophic waters (Marret & Zonneveld, 2003; Sangiorgi & Donders, 2004), to a *Spiniferites* spp dominated assemblage. The *Spiniferites* spp. flux mainly increases in the 1980s, after a short peak at 1966 AD. In general, more *Spiniferites* indicate more saline conditions. This may imply a shift towards a more open marine setting, which could be due to relative lower runoff hence relatively more influence of marine waters in the area. *L. machaerophorum,* as a cosmopolitan species, seems to have a large range of occurrences, but is usually found in higher abundances in upwelling and high river input regions. Seen in the light of eutrophication the relative decline of *Lingulodinium* is puzzling, as some consider *L. machaerophorum* to be a harmful marine species, or use it a cultural eutrophication indicating species (Dale, 2009).

A known HAB species, *Polysphaeridium zoharyi*, is present in variable but low relative abundance throughout the core. Generally *P. zoharyi* is a more saline species and has been a major HAB species in the CH area during the Holocene (pers. comm. E. Lammertsma). It is also a stratification loving species, which can thrive on both low and high stratification and salinity although in relative low amounts, the flux of *P. zoharyi* shows four peaks: at 1966, 1984, 2001 and 2005 AD.

The P/G ratio is variable and shows a slight decline, which could be interpreted as a decline in productivity, but as total dinocyst and biomarker flux indicate increased primary productivity, in this study the P/G ratio cannot be used as a eutrophication proxy as used in other studies (e.g. Sangiorgi & Donders, 2004; Dale, 2009; and references herein). The influence of high amounts of autotrophic primary productivity overwhelms any (high) heterotrophic productivity signal when present.

The large increase in productivity is stimulated by the increased nutrient input in the estuary. This does not explain why the aberrant cysts become dominant from 1976 AD onwards. The first peak in *S. miratorii* is in 1966 AD, while the large increase in N load of the Peace River increases later, around 1975 AD. Moreover, *Lingulodinium* is known to thrive in nutrient rich stratified coastal environments but is not dominant in CH after 1930. It still shows a relative constant but fluctuating flux. The shift from *Lingulodinium* to *Spiniferites* dominated dinocyts assemblages cannot be explained by the large peak of N input in the estuary as the shift predates large N load increases. Other environmental parameters must be more important. Salinity is an important ecological variable controlling various aspects of food-web organization in coastal systems. It is known that large rainfall fluctuations between (the wet and dry) seasons affect the salinity in CH strongly (Barnes et al., 2006).

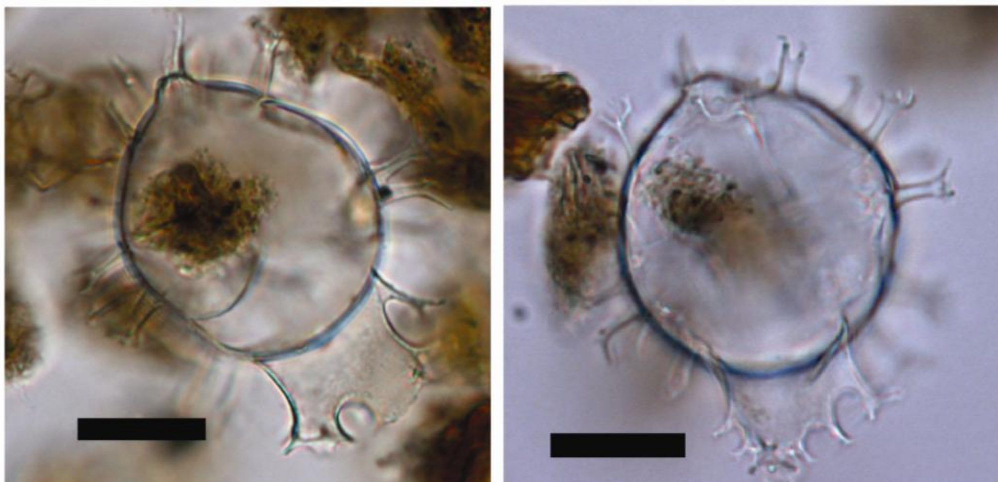


Figure 17: Two photos of *S. miratorii*, scale bar is 20 micrometer. It shows both the apical protuberance typical of *S bentorii*, and the antapical flange typical of *S. mirabilis*.

Some dinoflagellate species are known to build processes with uncommon morphologies under salinity stress. It is known for *L. machaerophorum* to make cysts in lower salinity environments, both in nature and in cultures (Dale, 1996; Ellegaard, 2000; Lewis & Hallett, 1997; Mertens et al., 2009; Wall & Dale, 1973; Wall et al., 1973) and *Operculodinium centrocarpum* (e.g., Mertens et al., 2010). *Spiniferites* are known for reduced processes in lower salinities, but also for membranous development and changes in the morphology of the

central body (Ellegaard, 2000; Matthiessen & Brenner, 1996; Wall & Dale, 1973; Kouli et al., 2001). Overall, it is thought that dinoflagellates grown in lower salinities produce reduced, bulbous processes with or without extra membranous development. If salinity is too low, processes are mostly absent. This indicates that the cyst morphology of various dinoflagellate species can reflect past changes in salinity. We propose that the *S. miratorii* cyst is produced at low(er) salinities.

These changes in salinity, linked to runoff, could be influenced by natural climate variability such as ENSO and AMO, but also influenced by anthropogenic activities such as water withdrawals.

4.3.5 Natural salinity variability

In the CH area both human and natural factors play a role in changing runoff and salinity. In this study we look at the changes in the last 140 years. The big Holocene transgression has already occurred. Hence, changes in salinity can be thought of as runoff signals.

Hypoxia and stratification are known phenomena in CH. Hurricanes and storm activities, linked to processes of ENSO, AMO and/or global warming, can cause both mixing (wave action) and stratification (high fresh water input) of the water column. This stratification induces a bigger difference between the salt wedge deep water and the fresher surface waters. Due to the salt wedge structure of the estuarine water column, shallow depth and relative deep penetration of sunlight it could be that at one point in time remains of two different phytoplankton assemblages are deposited. Lower runoff can cause the salt wedge to protrude more land inwards, thereby allowing higher salinity species. This could result in different assemblages and relative abundances of dinocysts found in the core. Sedimentary archives shows the average signal of a longer time period, while the phytoplankton water column sampling represents only one moment in time. This could explain the differences between the phytoplankton sampling and the sediment core fossil remains.

Humans influence i.e. the water withdrawal mostly in winter as demand for water withdrawal is higher during the dry season. Natural variability can influence the timing and amount of rainfall and river discharge. Hurricanes affect stratification and mixing rates of the water column for a short period. These effects may be too short-lived to be reflected in the palynology and biomarker record. It is possible that longer periods with higher runoff (as influenced by ENSO and AMO) create the major differences. The AMO is thought to enhance summer runoff (Enfield et al., 2001; Kelly & Gore, 2008), while El Niño enhances winter runoff (Schmidt et al., 2001). More winter runoff means more nutrients and lower salinity in the surface waters of CH. Increased winter runoff can prolong the usual summer water column stratification. This could in theory favor the coastal eutrophication and stratification species *L. machaerophorum* and *P. zoharyi.*

To see if this natural variability is reflected in the data, the ENSO and AMO signals (as departures from mean temperatures) are compared to the palynology record. The fluxes of *L. machaerophorum* and *P. zoharyi* are compared to the ENSO record to see whether El Niño events are reflected in the signal of these species that thrive on stratification (Figure 18). As *S. miratorii* is interpreted as a lower salinity species, the ENSO recorded is also compared to this species with disturbed processes.

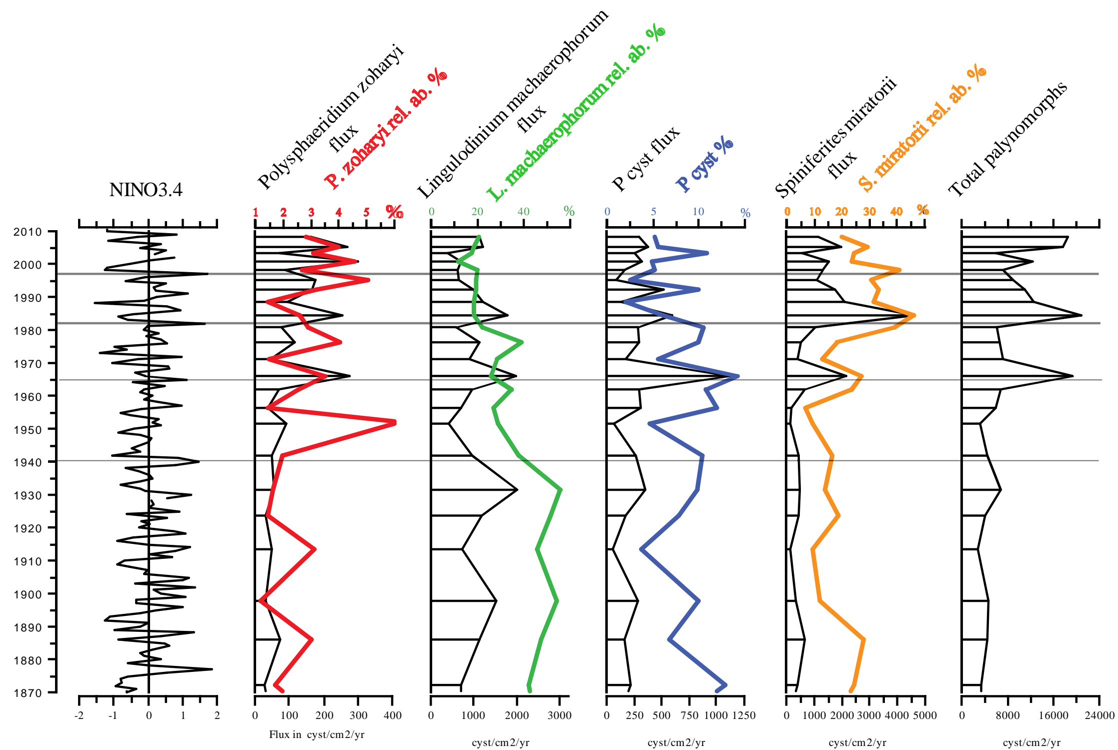


Figure 18: The Kaplan et al. (1998) NINO3.4 from http://climexp.knmi.nl was used as index reflecting the ENSO events. *P. zoharyi*, *L. machaerophorum*, *S. miratorii* and total P cyst are shown in flux (cyst/cm^2/yr) and in relative abundances (percentage of total dinocysts). The grey lines indicate El Niño events. The 1982-83 and 1997-1998 AD events are known as the strongest El Niño events of the 20th century.

The ENSO, a quasi-periodic natural variability of warming of the eastern Pacific ocean, oscillates on a short time-scale (ca. 5 years). It is therefore difficult to detect possible effects on the dinocyst record on this time-scale, especially down-core where the sampling resolution is lower (decadal) and the standard deviation of the age model increases. In 1982-83 and 1997-1998 AD there were strong, prolonged or heavy El Niño events, known as the strongest El Niño events of the 20th century. As the El Niño events influences the winter runoff, the expected changes are an increase in species that like lower salinities and/or stratification as *L. machaerophorum* and *P. zoharyi*. *L. machaerophorum* shows no tight correlation with the ENSO events. The ENSO events are not one to one reflected in the record of *P. zoharyi* although a general pattern of relative abundance of *P. zoharyi* and large changes in the NINO3.4 is suggested.

The natural shift in North Atlantic ocean surface water temperature has a cycle of ca. 60-80 years (Enfield et al., 2001). This AMO influences the Florida rainfall patterns in summer, where warm phases indicate higher Florida summer precipitation (Kelly & Gore, 2008), including higher runoff, lower salinity and higher primary productivity in warm AMO phases. The CH1PC record is in theory large enough to contain two whole cycles.

Phytol is one of the degradation products of chlorophyll-α from in situ algal primary production, while β-sitosterol is thought to be mainly derived from vascular plants (as used in van Soelen et al., 2010), but can occur in marine phytoplankton, haptophytes and cyanobacteria (Volkman, 1986). The ratio between β-sitosterol (terrestrial origin) and phytol (marine origin) shows a marked similarity to the AMO curve (Figure 19). The β-sitosterol:phytol ratio is closer to 1 with low influence of organic matter of marine origin and

is closer to zero if the marine OM is very abundant. As the AMO phase influences the summer runoff in CH, this could be reflected in terrestrial to marine OM distribution, as more runoff means more transport of terrestrial biomarkers. On the other hand, enhanced runoff increases the nutrient flux into the estuary, and thus can enhance primary productivity. That the AMO curve shows the same trend as this β-sitosterol:phytol ratio can be explained by higher summer runoff in warm AMO years that increase the β-sitosterol flux, while it is not clearly influencing the phytol record. The flux of phytol is not responding to the enhanced summer runoff.

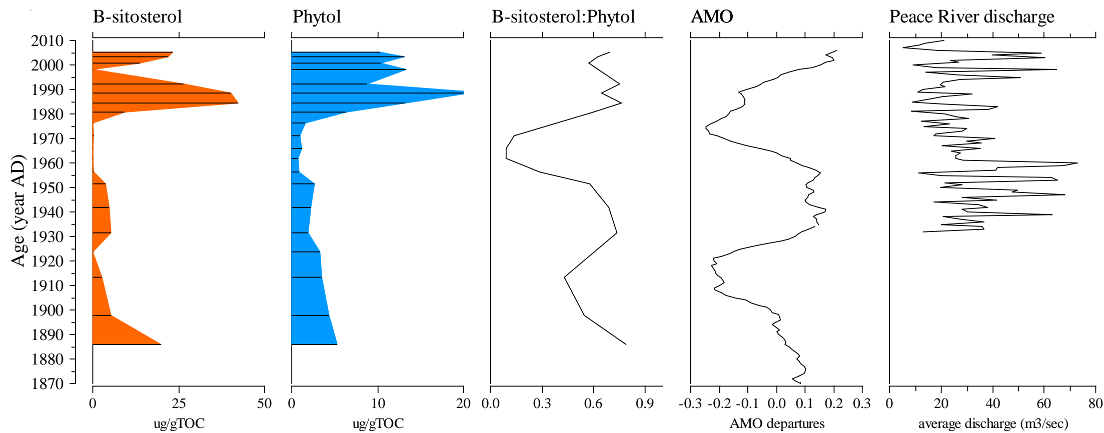


Figure 19: a comparison between a land derived biomarker (B-sitosterol) and a marine primary productivity biomarker (phytol). The ratio calculated as B-sitosterol/(B-sitosterol+phytol) shows a comparable trend to the temperature departure of the mean North Atlantic surface ocean water. The AMO data is retrieved from http://www.ncdc.noaa.gov. The Peace River discharge (in cubic meters per second) shows a decrease since the early 1960s AD, in line with the AMO phase and again an increase from ca. 2000 AD onwards (in line with the AMO departure). The discharge data is retrieved from http://waterdata.usgs.gov for USGS 02296750 Peace River at Arcadia, Florida.

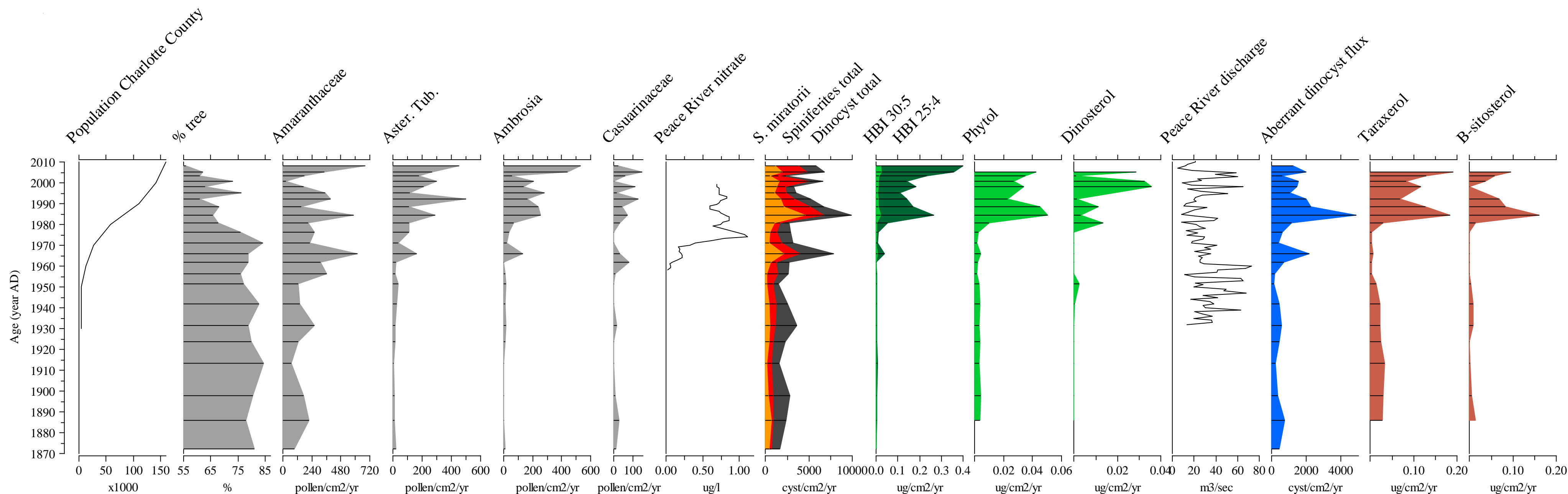


Figure 20: three large developments are plotted. First, the human influence on land. The population exponentially increased, while the pollen data shows a decrease in tree and an increase in herbs. The pioneer species (Ambrosia, Aster. Tub.) increase much after the 1960s. Second, there is a clear increase in nutrients and primary productivity. The first peak flux of dinocysts predates the large increase in nitrate loading of the Peace River. Input of nitrate increased exponentially in the early 1970s AD. The increase in HBIs, phytol and dinosterol lags behind the increase in N. Third, there is a salinity or runoff signal. Terrestrial biomarkers as taraxerol and B-sitosterol increase. The Peace River discharge is influenced by natural variability (AMO, ENSO), but also by anthropogenic water withdrawals. The aberrant dinocyst flux peaks first at 1966 and has a bigger peak around 1985 AD. This top flux of aberrant dinocysts either lags behind the maximum N load of the Peace River, or it predates the increase in mean annual Peace River discharge.

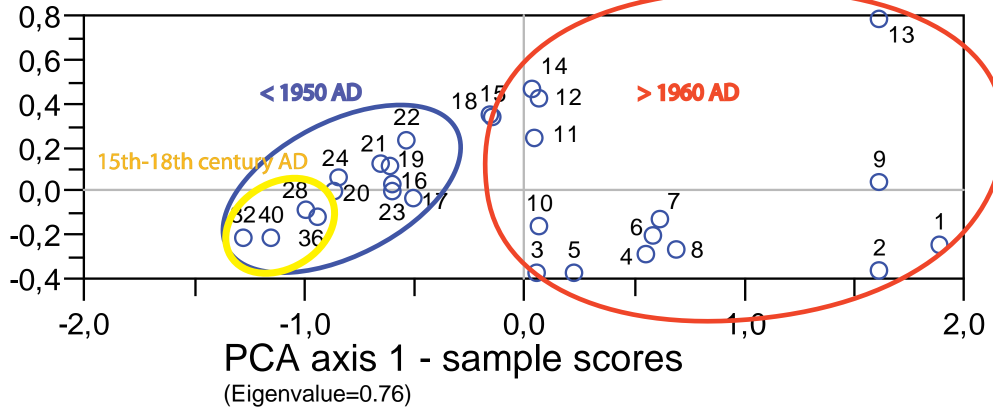
4.3.6 Human versus natural changes

It is often difficult to separate anthropogenic changes from natural variability and climate change. The multi-proxy approach of this study helps with differentiation the changes seen in the pollen, dinocyst and biomarker records. The high resolution sampled core CH1PC also covers a period before large-scale anthropogenic influence started, such as deforestation and population increase. Large anthropologic disturbances are not expected before 1950 AD, although aboriginal inhabitants have been in Florida since 10 thousand years, European colonization of the state started in 1565 AD and phosphorite mining in the upstream Peace River basin started at the end of the 19th century AD. Still, CH has been relatively undisturbed, especially when compared to other Florida estuaries.

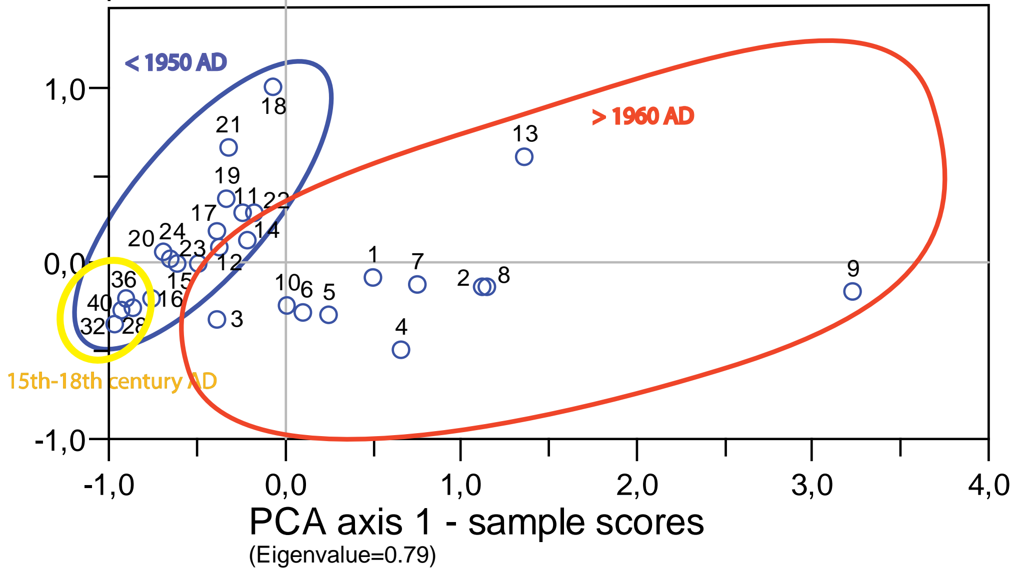
The concentrations of phytoplankton biomarkers ($HBI_{25:4}$, $HBI_{30:5}$, phytol, dinosterol and dinocysts) substantially increased around 1980 AD, and reflect an increase in algal in situ primary productivity. The main source of primary production seems to be diatoms, but also dinoflagellates and eustigmatophytes contribute to the OM. The increase in (Figure 20) occurs after the large increase in nitrate concentration of the Peace River in 1975 AD (Turner et al., 2006). The HBIs increase between the sample at 1976 and 1981, while most dinocysts increase after 1981 AD (with the exception of the first dinocyst peak at 1966 AD). The nitrate concentration remains relatively high after this first large increase. The dinocysts seem to predate the large recorded N load increase (first dinocyst peak at 1966 AD) while the diatom-HBIs lag behind this nutrient increase.

Principal component analysis can indicate the similarity of different samples based on their dinocyst, pollen and lipid content. In Figure 21 it can be seen that there is a large difference between older and more recent sediments, in palynology and in lipid content. The pollen and dinocyst assemblage both can be divided in 3 clusters, the 15th-18th century (samples at 28.5, 32.5, 36.5 and 40.5 cm depth), samples before 1950 AD and samples after 1960 AD. The biomarker record is similar to the palynomorphs, except there seems to be a transition zone between 1966 and 1980 AD, where biomarkers are in between the biomarker composition of low and upper part. As described before, the difference between the two (pollen and dinocyst flux) or three (biomarker flux) clusters clearly show that conditions changed in the estuary. The same pattern of change around 1960 AD is seen in the diatom assemblages (pers. comm. E. Gaiser).

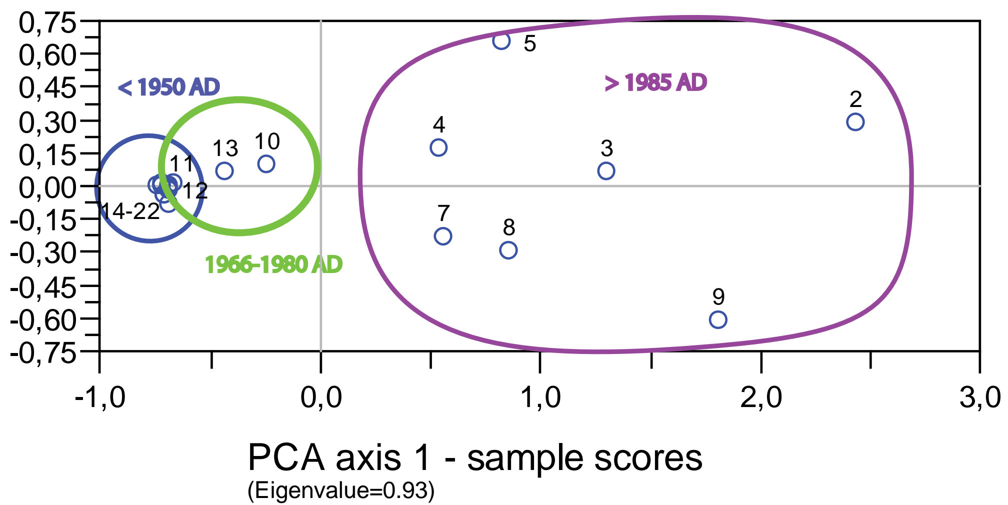
Figure 21 (next page): principal component analyses of the palynology and organic geochemistry biomarkers of core CH1PC from Charlotte Harbor. <u>Top</u>: PCA of pollen and fresh water algae (excluding dinocysts), the red cluster includes sample 1-14 and represents samples younger than 1960 AD; the blue cluster (sample 16-40 excl. 18) are samples older than 1950 AD; the yellow cluster is included into the blue cluster and represents samples older than the 19th century AD. <u>Mid</u>: PCA of dinocysts, the red cluster includes sample 1-14 and represents samples younger than 1960 AD; the blue cluster (sample 12-40 excl. 13) are samples older than 1950 AD; the yellow cluster is included into the blue cluster and represents samples older than the 19th century AD. <u>Bottom</u>: PCA of selected biomarkers (Phytol ,Cholesterol, 23,24 dimethylcholesta 5,22 dien 3b ol, B-sitosterol, dinosterol, HBI 25:4, HBI 30:5, 24-nor-lupane and hop-22(29)ene), the red cluster includes sample 1-14 and represents samples younger than 1960 AD; the blue cluster (sample 12-36 excl. 13) are samples older than 1950 AD. As the oldest samples (below 24 cm depth) are not analyzed for biomarkers, the oldest cluster found in the palynology is missing. The biomarkers show an overall similar picture, although the changes seem reflected in the biomarkers somewhat later than in the palynological record (only from 1985 AD compared to the change in palynology around ca. 1960 AD)



PCA: **palynology without dinocysts**. Sample scores
PCA axis 2 - sample scores
(Eigenvalue=0.08)
PCA axis 1 - sample scores
(Eigenvalue=0.76)
< 1950 AD
> 1960 AD
15th-18th century AD
0,8
0,6
0,4
0,2
0,0
-0,2
-0,4
-2,0
-1,0
0,0
1,0
2,0



PCA: **Dinocysts**. Sample scores
PCA axis 2 - sample scores
(Eigenvalue=0.08)
PCA axis 1 - sample scores
(Eigenvalue=0.79)
< 1950 AD
> 1960 AD
15th-18th century AD
1,0
0,0
-1,0
-1,0
0,0
1,0
2,0
3,0
4,0



PCA: **Biomarkers**. Sample scores
PCA axis 2 - sample scores
(Eigenvalue=0.05)
PCA axis 1 - sample scores
(Eigenvalue=0.93)
< 1950 AD
1966-1980 AD
> 1985 AD
14-22
0,75
0,60
0,45
0,30
0,15
0,00
-0,15
-0,30
-0,45
-0,60
-0,75
-1,0
0,0
1,0
2,0
3,0

Known large disruptive events as storms, slime spills and flooding events in the 1940s AD are not reflected in the biomarker, palynology or sedimentary record. In the 1940s five storms of category 3-5 landed within a few miles of the estuary. There was also a big P slime spill in Polk Country and two Peace River flooding events. This is seen in this multi-proxy record. There are no large changes in the lithology (Figure 5), although between 1932 and 1989 AD relatively more shell fragments are found. Either the events in the 1940s had no impact, the sample resolution is too low, or the human impact could have made the whole system more labile and receptive to changes (since the 1970s). In this latter period, the impact of human disturbances, changes in river runoff and natural variability are reflected in the palynology and biomarker records.

Biomarker records from Florida Bay (FB) (Xu et al., 2007) and Rookery Bay (RB) (Lammers et al., under review) also indicate a more dynamic environment during the past century as a result of human activity in the area. The timing though is different in the three areas of south-west Florida. RB shows an increase in certain biomarkers (C_{30}-$C_{32\ 1,15}$ diols, $HBI_{25:4}$, B-sitosterol, taraxerol) from the beginning of the 20th century AD, where Florida Bay shows an increased biomarker signal from 1970 AD. Additional pollen data from RB also indicates disturbances from ca. 1900 with an intensification of disturbances from 1950 AD (Donders et al., 2008). Compared to the RB area the biomarker signal of CH shows a similar response but with a different timing. For example phytol peaks around 1970 AD in RB, while in CH this is around 1990 AD. The $HBI_{25:4}$ in RB increases from the 1920s, where in CH this is at ca. 1976 AD. Same for β-sitosterol, which increases in RB before the 20th century and shows large fluctuations from 1930s onwards. In CH it increases only after 1980s AD. Thus, South-Florida estuaries show a comparable development with human disturbance. The onset of changes is related to the disturbance caused by human development of the area and thus are dependent on the timing of regional human disturbances.

4.3.7 Biomarker for *K. brevis*

The aim of the TLC analysis of lipids was to find a biomarker for *K. brevis*. This is not found. TLC is a great method although time consuming method to separate different compound classes, as shown in

Figure 22. As no *K. brevis* biomarker is found, it could be that the biomarker is too labile for sedimentary biomarker reconstructions, or that the CH area is not suitable for this analysis. This core is possibly not located at the right spot as *K. brevis* blooms do occur here but not recurrent enough to allow a detectable amount of brevesterol to accumulate in the sediment.

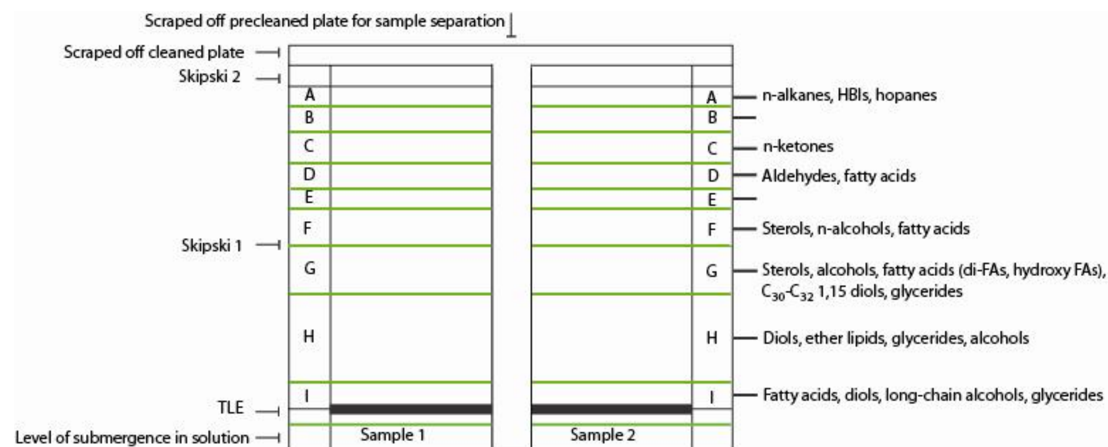


Figure 22: Thin Layer Chromatography plate with method indicators and banded result lipids

CHAPTER 5: CONCLUSION

Human impact in CH is evident in biomarkers and palynology of a sediment core of the last 140 years. The data strongly presents a disturbance in the second half of the 20th century. Towards the top of the core, both palynology and biomarker records become more dynamic. Increases in fluxes of both biomarkers and palynology coincides with significant human activities in the CH area. The population in Charlotte County has been rising exponentially since the 1950s. The N load of the Peace River peaks around 1970 AD. The pollen record shows a decrease in tree pollen since 1971. This is often seen in areas with increased human activity and deforestation. From the 1980s, species associated with deforestation and open vegetation (*Ambrosia* and Asteraceae Tubuliflorae) increased. Human impact in the area is also seen in the increase of primary production. There is enhanced primary productivity of not only dinoflagellates (as evident in the dinocyst flux) but also of some diatom species (as evident in the large flux of $HBI_{25:4}$ and $HBI_{30:5}$ from 1981 AD). General primary productivity increase is detected in the phytol record. The primary productivity fluxes ($HBI_{25:4}$, $HBI_{30:5}$, phytol and dinosterol) substantially increase from 1976 AD onwards, lagging behind the maximum N load increase. A more dynamic and variable ecosystem was present in the last 50 years, reflected in the dinocyst data from 1966 AD and the biomarker record from 1976 AD. The biomarker record shows trends similar to other Florida estuaries, e.g. Tampa Bay, Rookery Bay and in Florida Bay (Xu et al., 2007; van Soelen et al., 2010; Lammers et al., in press;). Thus, there is increased human disturbance of the CH catchment and coastal area by deforestation (lower tree pollen, increased early succession species as *Ambrosia*), urbanization (population rise, large-scale waterfront development), pollution (waste water, P mining and slime spills) and agriculture (fertilizer usage, irrigation, water withdrawals).

As the terrestrial input intensifies (sedimentation rate, B-sitosterol, taraxerol, marine-terrestrial palynomorphs ratio) it is thought that runoff increased. With enhanced runoff not only biomarker fluxes, nutrient fluxes and coupled primary productivity would increase, but the salinity would lower too. This is supported by the dinocyst data, where the aberrant *Spiniferites* morphology *S. miratorii* dominates the assemblage from the mid-1970s AD. Some dinoflagellate species are known to make aberrant cysts in lower salinities. Nutrient enhancement cannot explain the disturbed cyst morphology as *S. miratorii* is present in all samples and peaks before the large N load of the Peace River. Moreover, *Lingulodinium machaerophorum* is generally known as a species that would thrive in more eutrophic conditions, but shows a relative decrease since the 1930s. Changes in the timing of river discharge could explain the lower mean discharge until the 1990s and is compatible with the occurrences of dinocysts with aberrant morphologies. Human disturbances and natural variability can change the timing of rainfall.

It is due to this presumed connection that an attempt is made to link El Niño events (which cause enhanced winter precipitation) to aberrant cyst fluxes. Enhanced winter precipitation can prolong stratification. As stated, the aberrant dinocysts are thought to reflect lower salinities, possibly linked to stratification of the water column. As the ENSO has a short periodicity, it is difficult to link the changes in dinocysts to specific El Niño years. Probably some of the El Niño events in the last 40 years are reflected in the *S. miratorii* record. Although the quality of the age model of the sediment core is very good, it is still hard to pinpoint exactly the age of peak fluxes. Therefore, the link between El Niño events and the dinocyst record is not conclusive.

Warmer than average mean North Atlantic water temperature brings about more summer rainfall in Florida. The B-sitosterol:phytol biomarker ratio seems to co-vary with the AMO phases, as more runoff would indicate more B-sitosterol. It would be interesting to investigate if the link between B-sitosterol:phytol and the AMO curve holds in other sediment cores and/or on longer timescales.

The salinity driven cyst morphology distortion hypothesis needs more research. In future algal growth experiments it would be valuable to look at phytoplankton assemblage responses to salinity changes, in order to test the salinity-hypothesis as the origin of the aberrant cyst morphologies found in the sediment core.

The human activity is superimposed on the record of natural variability. It seems that the system reacts stronger to events after it has been disturbed. Furthermore, as the CH area is increasingly disturbed and developed, rainfall can erode and transport more terrestrial matter and sheet flow becomes more important.

Next to the analyses on the sediment core, two different experiments were conducted in Florida. An algal growth experiment was set up to investigate the response of dinoflagellates on nutrient enrichment. Although no dinoflagellates were found in this study, the phytoplankton response to inorganic nutrients surpassed the biomass response to organic nutrients as expected. The second experiment was conducted to find the spatial relationship of the phytoplankton community and biomarker distribution. Salinity seems to be one of the driving forces in dinocyst morphology, so comparison between filtered surface water samples along a salinity transect and the sediment core are valuable.

APPENDIX 1: DINOCYST SPECIES OF CH1PC

Order Gymonodiniales (Apstein)

Polykrikos schwartzii (Butschli 1873) is a temperate-subtropical species living in a wide range of environments, from coastal to open ocean. It is a heterotrophic species which can live from oligotrophic to eutrophic waters and uses an active phagocytic nutrition.

Order Gonyaulacales (Taylor)

Lingulodinium machaerophorum (Deflandre & Cookson; Wall 1967), cyst of *Lingulodinium polyedrum* ((Stein; Dodge 1989). It is a temperate to tropical coastal euryhaline autotrophic or mixotrophic species. Its occurrence is often related to eutrophic conditions, and increased cyst relative abundances in sediments can be related to (anthropogenic) nutrient input (Marret & Zonneveld, 2003). Usually *L. machaerophorum* is interpreted as a transition species between brackish and marine environments. The living dinoflagellate is typically found in stratified warm water conditions. (Dale, 2009) suggests that an increase in *L. machaerophorum* could indicate cultural eutrophication. It is also found abundant in environments characterized by seasonal stratification (seasonal upwelling and river runoff).

Nematosphaeropsis labyrinthus/*Rigida* complex (Wrenn 1988), cysts of *Gonyaulax spinifera* complex (Head 1996). The species has a wide range of environmental parameters (temperature, salinity, nutrient availability) as an autotroph cosmopolitan. Some research suggests that *N. labyrinthus* and *S. ramosus* have the same motile affinity but *N. labyrinthus* only forms when the salinity is higher (25-30 spu; (Rochon et al., 2009).

Operculodinium centrocarpum sensu (Wall et Dale 1966), cyst of *Protoceratium reticulatum*, lives in a wide range of environmental parameters (temperature, salinity, nutrient availability) with mostly low abundances in tropic regions. This species is generally regarded as a signal for environmental changes.

Polysphaeridium zoharyi (Rossignol, Bujak 1980), cyst of *Pyrodinium bahamense* var. *bahamense / compressum*, is a (sub)tropical, coastal, euryhaline red tide and HAB species which can live in oligotrophic to mesotrophic environments and occurs mostly in the Gulf of Mexico. It is mostly regarded as an autotrophic species but has a possible mixotrophic feeding mechanism. The two variations form the same dinocyst; *P. zoharyi* var. *bahamense* is nontoxic and bioluminiescent but var. *compressum* forms toxic blooms.

Spiniferites bentorii (Wall & Dale 1970), the cyst of *Gonyaulax digitalis* (Kofoid 1911) is a temperate to tropical coastal autotrophic species, which can live up to a salinity of 40 ppt. The cyst has a distinct apical 'hump'. According to (Marret & Zonneveld, 2003) *S. bentorii* is restricted to fully marine settings, but could be living in lower SSS than 34. ***Spiniferites mirabilis*** (Rosignol 1967, Sarjeant 1970) and ***Spiniferites hyperacanthus*** (Deflandre et Cookson 1955; Cookson et Eisenack 1974), are the cysts of *Gonyaulax spinifera* and are usually grouped as *S. mirabilis*. It is a temperate to tropical, oligotrophic to eutrophic species, predominately fully marine but also common in coastal sediments. The species are only found in salinities exceeding 28.5 (Marret & Zonneveld, 2003). Tomas (1997) describes this species

as an oceanic, estuarine and neritic cosmopolitan species. Some use the species as an indicator of seasonal hypoxia and broad salinity fluctuations (Willard et al., 2003). Difference between *S. hyperacanthus* and *S. mirabilis* is the presence (*S. mirabilis*) or absence (*S. hyperacanthus*) of the antapical flange. "***Spiniferites miratorii***" is the working name for the morphological amalgamate between *S. mirabilis* and *S. bentorii*. This morphological amalgamate shows a distinct apical protuberance like *S. bentorii*, but also shows the distinct flange and intergonal processes of *S. mirabilis*. Origin unknown. ***Spiniferites ramosus*** (Ehrenberg 1838; Mantel 1854) includes the species ***Spiniferites bulloides*** (Deflandre et Cookson 1955; Sarjeant 1970), can live in a broad range of environments (temperature, salinity and nutrient wise) and is considered to be a cosmopolitan species.

Tuberculodinium vancampoe (Rossignol 1962; Wall 1967), cyst of *Pyrophacus stenii* (Wall & Dale 1971), is a (sub)tropical, coastal autotroph that lives mainly in oligotrophic conditions. It can live in brackish to fully marine conditions and is often seen as a species living in reduced or enhanced SSS.

Order: Peridiniales (Haeckel)

***Brigantedinium* spp.** (Reid 1977) cyst of *Protoperidinium conicoides* (Balech 1974 (*B. simplex*) 1973 Tomas). These 'round browns' are a heterotrophic genus that are often difficult to classify to species level. In general the genus is occurring worldwide and can be found in environments that have a wide range in temperature, salinity and nutrient availability. They are considered cosmopolitan (Tomas, 1997).

Echinidinium spp is a relatively newly recognized genus of dinoflagellate cysts with an unknown motile affinity. All *Echinidinium* species are grouped into 'Round Brown Spiny'. ***Echinidinium delicatum*** (Zonneveld 1997) is a (sub)tropical species that is present in eutrophic environment sediments. The species is found in non-upwelling nutrient-rich conditions. ***Echinidinium granulatum*** (Zonneveld 1997) is a (sub)tropical species that is present in mesotrophic to eutrophic environments with a broad salinity range.

***Lejeunecysta* spp.** (Reid 1977) has a probable living affinity of *Protoperidinium leonis* (Balech 1974) and lives worldwide in preferably warm temperate to tropical and coastal to oceanic environments. It is a heterotrophic species.

Pentaphasodinium dalei (Indelicato et Loebich III 1986) is considered to be a cosmopolitan species that can live in a broad spectrum environment (temperature, salinity, nutrients).

Protoperidinium stellatum (Rochon 1999) is the living affinity of what is called the "cyst of" *Protoperidinium stellatum*. The heterotrophic cysts are found in temperate to tropical, brackish to fully marine and oligotrophic to eutrophic environments.

Round Brown Spiny is the name for brown colored P-cysts with (many) processes, with an heterotrophic lifestyle. As these cysts are difficult to identify on species level, the cysts are grouped. Could include *Echinidinium* spp., *Selenopemphix quanta* and other brown cysts with lots of processes.

Selenopemphix nephroides (Benedek 1972; Benedek et Sarjeant 1981), cyst of *Protoperidinium subinerme*, can be found from fully marine to coastal waters with a broad temperature, salinity and nutrient range. It can live from oligotrophic to eutrophic environments. ***Selenopemphix quanta*** (Matsuoka 1985), with a living affinity *Protoperidinium conicum* (Balech 1974) is a cold to tropical, coastal to oceanic species which is more abundant in mesotrophic to eutrophic environments. It is a cosmopolitan species in temperate to tropical waters (Tomas, 1997). It has a broad range of salinity, temperature and nutrient availability. This species can be used as a (relative) indication of (nutrient) pollution.

APPENDIX 2: RELATIVE ABUNDANCE OF DINOCYSTS IN SEDIMENT CORE CH1PC

Relative abundances of dinocysts of core CH1PC, in respect to total dinocysts

Sample depth (cm)	Age AD	Brigantedinium spp	Echinidium spp.	Echinidium delicatum	Echinidium granulatum	Selenopemphix nephroides	Selenopemphix quanta	Lejeunecysta	Pentaphasodinium dalei	cyst of Protoperidinium stellatum	Indet. spiny round browns	P-cyst total	Lingulodinium machaerophorum	Lingulodinium gek	Lingulodinium total	Nematosphaeropsis labyrinthea	Operculodinium centrocarpum	O. israelianum	Operculodinium spp.	Polykrikos schwartzii	Polysphaeridium zoharyi	Spiniferites spp.	S. bentorii	S. bulloideus	S. delicatus	S. ramosus	S. mirabilis complex	S. hyperacantus	S. mirabilis	S. miratorii	S. mirabilis extra membraneous	Spiniferites total	Tuberculodinium vancampoae	Deviant G-cysts total	G-cyst total	Indet. Dinocyst	P/G ratio [P/P+G]
1,0	2008	1,7	0,7	1,3	-	-	0,9	-	-	0,4	0,2	5,2	19,7	0,7	20,4	-	0,7	-	0,7	-	2,8	19,8	6,7	-	0,2	0,4	2,2	15,8	19,3	19,7	1,3	66,3	-	21,7	90,1	4,7	0,05
2,5	2005	4,2	-	0,7	-	-	-	-	-	-	0,7	5,6	17,9	-	17,9	-	-	-	-	-	4,0	12,3	1,4	-	-	-	1,1	12,5	13,9	29,2	0,4	70,8	-	29,6	92,6	1,8	0,06
3,5	2003	7,7	-	0,4	0,4	-	0,4	-	-	0,4	1,8	10,9	16,8	-	16,8	-	-	-	-	-	3,0	11,8	2,0	-	-	-	2,2	8,4	13,1	24,4	2,5	64,3	-	26,9	84,2	4,8	0,11
4,5	2001	-	0,3	1,4	-	-	1,4	-	-	1,0	-	4,9	10,8	-	10,8	-	-	-	-	0,7	4,5	11,0	7,3	-	0,3	1,0	1,7	14,5	16,2	23,6	-	75,7	-	23,6	91,1	4,0	0,05
5,5	1998	3,0	-	0,4	-	-	0,4	-	-	-	1,3	5,1	19,3	0,4	19,7	-	-	-	-	-	2,7	7,8	2,5	-	-	-	2,7	4,2	10,2	40,5	3,4	71,2	-	44,3	93,6	1,3	0,05
6,5	1995	-	-	-	-	-	-	-	-	-	0,4	2,5	18,9	-	18,9	-	-	-	-	2,1	5,1	12,8	5,3	-	0,4	-	2,1	7,6	9,7	30,7	-	68,6	0,4	30,7	93,1	4,4	0,03
7,5	1992	6,2	-	-	-	-	0,8	-	-	0,4	2,6	9,9	18,9	2,4	21,3	0,4	-	-	-	-	3,2	8,2	0,8	-	-	-	1,6	7,0	10,9	33,4	2,4	64,2	-	38,2	89,1	1,0	0,10
8,5	1989	-	0,6	0,6	-	-	0,3	-	-	-	0,3	1,9	18,0	-	18,0	-	-	0,3	0,3	-	1,4	9,5	4,2	0,3	-	-	4,8	8,8	15,6	31,5	1,9	76,6	-	33,4	96,3	1,8	0,02
9,5	1984	2,7	-	-	-	-	1,1	-	-	-	2,2	6,0	17,9	0,7	18,7	-	0,7	-	0,7	-	2,6	5,3	0,7	0,7	-	-	0,4	5,1	7,3	46,2	1,8	67,6	-	48,7	89,6	4,4	0,06
10,5	1981	3,5	-	0,8	-	-	-	0,8	-	0,4	4,9	10,5	21,8	-	21,8	0,2	1,2	-	1,2	-	2,9	6,0	1,0	0,4	-	-	0,4	4,3	8,0	39,1	3,3	62,6	-	42,4	88,7	0,8	0,11
11,5	1976	4,2	-	-	-	-	-	1,3	-	0,2	4,2	10,0	38,6	-	38,6	0,4	0,4	-	0,4	-	4,0	4,7	1,7	-	-	-	-	7,2	10,2	18,2	3,0	44,9	-	21,2	88,3	1,7	0,10
12,5	1971	-	1,5	0,4	0,4	-	0,7	-	-	-	2,2	5,5	28,1	-	28,1	-	-	1,3	1,3	0,4	1,5	8,3	2,8	0,9	-	-	7,4	9,8	17,2	12,8	-	59,1	1,1	12,8	91,1	3,3	0,06
13,5	1966	6,8	-	0,8	-	-	0,8	0,2	-	0,8	4,3	14,3	25,2	-	25,2	-	3,3	-	3,3	0,4	3,5	6,0	0,4	-	-	-	2,5	5,6	8,5	27,1	0,4	50,4	-	27,5	82,4	3,3	0,15

Sample depth (cm)	Age AD	Brigantedinium spp	Echinidium spp.	Echinidium delicatum	Echinidium granulatum	Selenopemphix nephroides	Selenopemphix quanta	Lejeunecysta	Pentaphasodinium dalei	cyst of Protoperidinium stellatum	Indet. spiny round browns	P-cyst total	Lingulodinium machaerophorum	Lingulodinium gek	Lingulodinium total	Nematosphaeropsis labyrinthea	Operculodinium centrocarpum	O. israelianum	Operculodinium spp.	Polykrikos schwartzii	Polysphaeridium zoharyi	Spiniferites spp.	S. bentorii	S. bulloideus	S. delicatus	S. ramosus	S. mirabilis complex	S. hyperacantus	S. mirabilis	S. miratorii	S. mirabilis extra membraneous	Spiniferites total	Tuberculodinium vancampoae	Deviant G-cysts total	G-cyst total	Indet. Dinocyst	P/G ratio [P/P+G]
14,5	1962	4,9	-	0,4	-	-	2,2	0,4	-	0,4	2,2	10,7	34,4	-	34,4	-	2,0	-	2,0	0,4	2,5	7,6	0,7	-	-	-	3,6	1,4	7,2	23,6	2,2	46,4	0,2	25,7	85,5	3,8	0,11
15,5	1956	4,3	-	0,6	0,3	-	1,0	1,0	-	-	4,7	11,9	26,4	-	26,4	0,3	1,3	-	1,3	-	1,4	8,1	1,9	-	-	-	3,9	14,5	19,0	6,8	0,6	54,8	0,3	7,4	84,5	3,5	0,12
16,5	1951	1,0	0,3	0,3	0,7	-	1,0	-	-	-	1,0	4,6	28,2	0,7	28,9	0,3	-	1,0	1,0	0,3	6,2	9,2	2,6	1,3	-	-	6,1	11,0	17,1	9,2	-	56,5	0,3	9,9	93,3	2,1	0,05
17,5	1942	3,6	-	0,4	-	0,4	1,8	0,7	-	-	3,6	10,4	37,5	0,7	38,2	0,4	-	-	-	-	2,0	3,0	1,1	-	-	-	4,3	9,5	13,8	16,5	-	48,2	-	17,2	88,7	0,9	0,10
18,5	1932	5,0	0,8	0,8	-	-	1,2	-	-	-	2,0	9,8	55,4	1,2	56,6	-	0,2	-	0,2	-	1,6	3,6	1,6	-	-	-	2,0	3,2	6,0	13,7	0,8	30,9	-	15,7	89,4	0,8	0,10
19,5	1924	5,0	-	-	-	-	-	1,4	-	-	1,4	7,8	51,1	0,7	51,8	0,4	-	-	-	-	1,4	6,4	1,1	-	-	-	2,5	3,4	5,9	18,8	-	37,9	-	19,5	91,5	0,7	0,08
20,5	1913	2,2	-	0,4	-	-	0,7	-	-	0,4	-	3,7	45,2	3,7	48,9	-	-	-	-	-	3,1	6,6	1,5	0,4	-	-	7,2	4,6	11,8	9,4	-	41,4	0,4	13,1	93,8	2,6	0,04
21,5	1898	5,1	-	-	-	-	-	2,4	0,3	0,5	1,7	1-	53,7	1,0	54,7	-	-	-	-	-	1,2	4,9	0,7	-	-	-	3,7	3,2	7,3	11,8	0,3	31,9	-	13,2	87,8	2,2	0,10
22,5	1886	4,4	0,4	-	-	-	-	0,4	-	0,4	1,2	6,8	47,0	2,4	49,4	0,4	-	-	-	-	3,0	5,8	1,2	-	-	-	0,8	0,8	1,6	27,7	-	38,0	-	30,1	90,8	2,4	0,07
23,5	1872	8,3	-	0,4	-	-	-	1,5	-	-	2,3	12,8	41,3	0,8	42,1	0,8	0,6	-	0,6	0,4	1,7	9,6	0,8	-	-	-	0,8	1,7	2,8	24,2	0,4	40,2	-	25,3	85,3	1,9	0,13
24,5	1858	5,3	-	-	-	-	1,6	-	-	-	0,3	7,1	43,8	2,5	46,3	-	0,6	-	0,6	-	3,4	5,4	0,6	0,3	-	-	5,9	2,5	9,0	14,9	0,6	39,3	-	18,0	89,6	3,3	0,07
28,5	1787	8,9	-	1,4	-	-	1,0	0,3	-	0,3	0,3	12,3	31,4	1,0	32,4	0,7	-	-	-	-	7,2	6,5	2,0	-	0,3	-	6,1	6,7	13,5	8,2	0,7	44,0	-	9,9	84,3	3,4	0,13
32,5	1695	7,1	-	0,3	-	-	0,7	-	-	-	1,6	9,7	37,6	2,6	40,2	0,3	-	-	-	-	2,5	4,9	1,6	-	-	-	6,2	11,0	18,9	1,6	1,6	46,0	-	5,9	89,0	1,3	0,10
36,5	1579	5,8	-	-	-	-	0,6	-	-	-	-	6,7	39,3	0,9	40,2	0,3	0,3	-	-	0,3	4,7	3,8	1,7	-	-	-	6,2	12,8	19,3	1,8	0,3	46,0	-	3,0	91,2	2,1	0,07
40,5	1435	7,3	-	0,9	-	-	0,9	-	-	0,3	0,3	9,6	39,3	0,9	40,1	-	0,3	-	-	-	3,5	2,5	2,9	-	-	-	7,3	12,0	19,3	1,8	-	45,7	-	2,6	89,3	1,0	0,10

APPENDIX 3: FLUX OF DINOCYSTS IN SEDIMENT CORE CH1PC

Dinocyst flux of core CH1PC. Flux is expressed in dinocysts/cm^2/ year.

Age AD	Brigantedinium spp	Echinidium spp.	Echinidium delicatum	Echinidium granulatum	Selenopemphix nephroides	Selenopemphix quanta	Lejeunecysta	pentaphasodinium dalei	cyst of Protoperidinium stellatum	Indet. spiny round browns	P-cyst total	Lingulodinium machaerophorum	Lingulodinium gek	Lingulodinium total	Nematosphaeropsis labyrinthea	Operculodinium centrocarpum	O. israelianum
2008	100,8	37,8	75,6	ND	-	50,4	-	-	25,2	12,6	302,3	1146,2	37,8	1184,0	-	37,8	-
2005	281,8	-	49,0	-	-	-	-	-	-	49,0	379,8	1212,9	-	1212,9	-	-	-
2003	181,0	-	8,4	8,4	-	8,4	-	-	8,4	42,1	256,8	395,7	-	395,7	-	-	-
2001	-	23,0	92,1	-	-	92,1	-	-	69,1	-	322,4	713,9	-	713,9	-	-	-
1998	99,1	-	12,4	-	-	12,4	-	-	-	43,4	167,3	631,9	12,4	644,3	-	-	-
1995	-	-	-	-	-	-	-	-	-	14,7	88,4	662,7	-	662,7	-	-	-
1992	324,0	-	-	-	-	41,8	-	-	20,9	135,9	522,6	992,9	125,4	1118,3	20,9	-	-
1989	-	43,2	43,2	-	-	21,6	-	-	-	21,6	129,5	1208,7	-	1208,7	-	-	21,6
1984	273,4	-	-	-	-	109,3	-	-	-	218,7	601,4	1785,9	72,9	1858,8	-	72,9	-
1981	95,0	-	22,3	-	-	-	22,3	-	11,2	134,1	284,9	592,2	-	592,2	5,6	33,5	-
1976	124,8	-	-	-	-	-	37,4	-	6,2	124,8	293,2	1135,5	-	1135,5	12,5	12,5	-

Age AD	Operculodinium spp.	Polykrikos schwartzii	Polysphaeridium zoharyi	Spiniferites spp.	S. bentorii	S. bulloideus	S. delicatus	S. ramosus	S. mirabilis complex	S. hyperacantus	S. mirabilis	S. miratorii	S. mirabilis extra membraneous	Spiniferites total	Tuberculodinium vancampoae	Deviant G-cysts total	G-cyst total	Indet. Dinocyst	Dinocyst total
2008	37,8	-	163,7	1152,5	390,5	-	12,6	25,2	1121,0	126,0	919,5	1146,2	75,6	3847,9	-	1259,5	5233,4	270,8	580
2005	-	-	269,5	833,1	98,0	-	-	-	943,4	73,5	845,3	1984,7	24,5	4802,5	-	2009,2	6284,9	122,5	678
2003	-	-	71,6	277,9	46,3	-	-	-	307,3	50,5	197,9	572,6	58,9	1511,4	-	631,5	1978,7	113,7	234
2001	-	46,1	299,4	725,5	483,6	-	23,0	69,1	1070,9	115,2	955,8	1554,6	-	4997,6	-	1554,6	6010,9	264,8	659
1998	-	-	86,7	254,0	80,5	-	-	-	334,5	86,7	136,3	1325,8	111,5	2329,4	-	1449,7	3060,4	43,4	327
1995	-	73,6	176,7	449,2	184,1	-	14,7	-	338,7	73,6	265,1	1075,1	-	2400,5	14,7	1075,1	3254,7	154,6	349
1992	-	-	167,2	428,5	41,8	-	-	-	574,8	83,6	365,8	1755,9	125,4	3375,9	-	2006,7	4682,3	52,3	522
1989	21,6	-	97,1	636,7	280,6	21,6	-	-	1046,8	323,8	593,6	2115,2	129,5	5147,8	-	2244,7	6475,2	118,7	672
1984	72,9	-	255,1	528,5	72,9	72,9	-	-	728,9	36,4	510,3	4592,3	182,2	6724,5	-	4847,4	8911,3	437,4	99
1981	33,5	-	78,2	162,0	27,9	11,2	-	-	217,9	11,2	117,3	1061,5	89,4	1698,5	-	1150,9	2408,0	22,3	27
1976	12,5	-	118,5	137,3	49,9	-	-	-	299,5	-	212,1	536,6	87,3	1322,7	-	623,9	2601,7	49,9	294

1971	-	47,2	11,8	11,8	-	23,6	-	-	-	70,8	177,0	896,8	-	896,8	-	-	41,3	41,3	11,8	47,2	265,5	88,5	29,5	-	-	548,7	236,0	312,7	407,1	-	1888,1	35,4	407,1	2908,8	106,2	31
1966	536,7	-	65,0	-	-	65,0	16,3	-	65,0	341,5	1122,1	1984,0	-	1984,0	-	260,2	-	260,2	32,5	276,5	471,6	32,5	-	-	-	666,8	195,1	439,1	2130,4	32,5	3968,0	-	2162,9	6488,6	260,2	78
1962	135,0	-	10,0	-	-	60,0	10,0	-	10,0	60,0	294,9	949,8	-	949,8	-	55,0	-	55,0	10,0	70,0	210	20,0	-	-	-	200	100	40,0	649,8	60,0	1279,7	5,0	709,8	2359,5	105,0	27
1956	114,8	-	17,0	8,5	-	25,5	25,5	-	-	123,3	314,7	697,5	-	697,5	8,5	34,0	-	34,0	-	38,3	212,6	51,0	-	-	-	501,8	102,1	382,8	178,6	17,0	1446,0	8,5	195,6	2232,7	93,6	26
1951	14,7	4,9	4,9	9,8	-	14,7	-	-	-	14,7	68,5	420,5	9,8	430,3	4,9	-	14,7	14,7	4,9	92,9	136,9	39,1	19,6	-	-	254,3	90,5	163,8	136,9	-	841,0	4,9	146,7	1388,6	31,8	14
1942	93,0	-	9,3	-	9,3	46,5	18,6	-	-	93,0	269,8	972,1	18,6	990,7	9,3	-	-	-	-	51,2	79,1	27,9	-	-	-	358,1	111,6	246,5	427,9	-	1251,1	-	446,5	2302,2	23,3	25
1932	181,4	29,0	29,0	-	-	43,5	-	-	-	72,6	355,5	2002,5	43,5	2046,1	-	7,3	-	7,3	-	58,0	130,6	58,0	-	-	-	217,7	72,6	116,1	493,4	29,0	1117,4	-	565,9	3228,7	29,0	36
1924	116,0	-	-	-	-	-	33,1	-	-	33,1	182,3	1193,4	16,6	121-	8,3	-	-	-	-	33,1	149,2	24,9	-	-	-	136,7	58,0	78,7	439,2	-	886,8	-	455,8	2138,2	16,6	23
1913	35,9	-	6,0	-	-	12,0	-	-	6,0	-	59,8	735,7	59,8	795,5	-	-	-	-	-	50,8	107,7	23,9	6,0	-	-	191,4	116,6	74,8	152,5	-	672,9	6,0	212,3	1525,3	41,9	16
1898	143,9	-	-	-	-	-	67,2	9,6	14,4	48,0	283,0	1525,6	28,8	1554,4	-	-	-	-	-	33,6	139,1	19,2	-	-	-	206,3	105,5	91,2	335,8	9,6	906,7	-	374,2	2494,7	62,4	28
1886	107,2	9,7	-	-	-	-	9,7	-	9,7	29,2	165,7	1140,2	58,5	1198,7	9,7	-	-	-	-	73,1	141,3	29,2	-	-	-	39,0	19,5	19,5	672,5	-	921,0	-	730,9	2202,5	58,5	24
1872	140,5	-	6,4	-	-	-	25,6	-	-	38,3	217,2	699,5	12,8	712,2	12,8	9,6	-	9,6	6,4	28,7	162,9	12,8	-	-	-	47,9	12,8	28,7	408,8	6,4	680,3	-	428,0	1443,7	31,9	16
1858	81,8	-	-	-	-	24,0	-	-	-	4,8	110,6	678,2	38,5	716,7	-	9,6	-	9,6	-	52,9	84,2	9,6	4,8	-	-	139,5	91,4	38,5	230,9	9,6	608,5	-	279,0	1387,7	50,5	15
1787	81,2	-	12,5	-	-	9,4	3,1	-	3,1	3,1	112,4	287,2	9,4	296,6	6,2	-	-	-	-	65,6	59,3	18,7	-	3,1	-	123,3	56,2	60,9	74,9	6,2	402,8	-	90,5	771,2	31,2	91
1695	29,8	-	1,4	-	-	2,8	-	-	-	6,9	40,9	158,7	11,1	169,8	1,4	-	-	-	-	10,4	20,8	6,9	-	-	-	79,7	26,3	46,4	6,9	6,9	194,1	-	25,0	375,7	5,5	42
1579	52,0	-	-	-	-	5,5	-	-	-	-	60,2	352,8	8,2	361,0	2,7	2,7	-	-	2,7	42,4	34,2	15,0	-	-	-	173,7	56,1	114,9	16,4	2,7	412,9	-	27,3	819,0	19,1	89
1435	48,1	-	5,8	-	-	5,8	-	-	1,9	1,9	63,5	258,7	5,8	264,5	-	1,9	-	-	-	23,1	16,4	19,2	-	-	-	127,0	48,1	78,9	11,5	-	301,0	-	17,3	588,6	6,7	65

APPENDIX 4: POLLEN SPECIES OF CH1PC

Family	Genus and species	Common name
Amaranthaceae	*Amaranthus australis*	Southern amaranth
Asteraceae	*Ambrosia artemisiifolia*	Ragweed
Asteraceae	*Iva* sp.	Marshelders
Asteraceae Tubuliflorae	*“*Aster. Tub.*”*	
Juglandaceae	*Carya* sp.	Hickory
Casuarinaceae	*Casuarina equisetifolia*	Australian pine
Cyperaceae	*Cyperaceae* spp.	Sedges
Fagaceae	*Quercus laurifolia*	Laurel oak
Myricaceae	*Myrica cerifera*	Wax myrtle
Poaceae	*Poacea* spp.	Grasses
Polypodiaceae	*Phlebodium aureum*	Golden polypody
Rubiaceae	*Cephalanthus occidentalis*	Buttonbush
Salicaceae	*Salix* sp. *(*probably *S. caroliniana)*	Willow
Taxodiaceae	*Taxodium distichum*	Swamp cypress
Typhaceae	*Typha* sp.	Cat-tail
Ulmaceae	*Ulmus* sp.	Elm
Bisaccates	Probably*: Pinus* sp.	
cf. *Toxicodendron*		
cf. *Corylus*		

APPENDIX 5: RELATIVE ABUNDANCE OF POLLEN AND OTHER PALYNOMORPHS IN SEDIMENT CORE CHIPC

Relative abundances of pollen and spores of core CH1PC, in respect to total pollen and spores

Sample depth (cm)	Age AD	Bisaccates	Asteraceae Tubuliflorae	Ambrosia	Myrica	Quercus	Poacea	Phlebodium	Taxodium distichum	Amaranthaceae	Casuarinaceae	Typha spp.	Cephalanthus occidentalis	Cyperaceae	Carya	Iva	Salix	Cf. Toxicodendron	Corylus cf.	Monoleet	Ulnus	Indet. Pollen
1,0	2008	43,5	4,0	4,7	5,8	11,6	0,7	0,4	0,9	6,0	0,2	0,7	5,1	2,0	0,7	0,4	-	-	-	1,6	0,2	11,6
2,5	2005	45,0	2,8	4,5	4,8	17,1	0,5	0,5	1,3	3,5	1,5	1,3	1,3	3,5	-	0,5	-	-	0,3	0,5	0,5	10,8
3,5	2003	42,3	5,6	1,9	3,7	18,4	1,6	-	0,5	5,6	1,9	1,6	1,3	1,1	-	0,5	0,3	-	-	-	0,3	13,6
4,5	2001	60,3	6,7	4,6	5,7	12,9	1,5	1,5	0,5	0,5	-	-	-	-	-	-	-	-	-	-	-	5,7
5,5	1998	48,1	6,1	4,0	6,1	14,8	0,4	-	1,1	5,0	3,2	0,4	1,4	2,5	-	-	-	-	-	0,7	-	6,1
6,5	1995	55,6	2,5	5,9	3,4	20,7	0,3	0,6	0,3	7,4	0,3	-	-	-	-	-	-	-	-	-	-	3,1
7,5	1992	50,7	9,5	3,2	5,5	9,9	-	0,4	0,8	7,5	2,4	-	-	1,6	-	0,8	0,4	-	-	-	-	7,5
8,5	1989	54,1	2,0	4,4	8,7	13,9	1,6	-	0,4	2,8	0,8	0,4	0,8	3,2	-	-	-	-	-	-	-	7,1
9,5	1984	51,6	3,0	2,6	6,8	12,8	0,8	0,4	-	6,0	0,8	0,8	1,1	0,8	1,1	0,4	0,4	-	-	0,4	-	10,5
10,5	1981	54,7	3,6	2,2	4,3	12,6	-	0,4	1,8	6,8	1,1	0,7	0,7	1,8	0,4	0,4	-	-	-	-	-	8,6
11,5	1976	71,5	3,5	1,2	3,9	3,9	0,4	0,4	0,8	8,2	-	-	0,4	0,8	0,4	0,4	-	-	-	0,4	0,4	3,5
12,5	1971	78,1	1,0	0,7	1,0	5,3	0,3	0,3	-	6,3	-	-	-	2,0	0,7	-	-	-	-	-	-	4,3
13,5	1966	7-	1,6	1,3	1,9	7,1	0,6	-	0,3	6,1	0,3	-	0,6	0,6	1,0	0,3	0,6	-	-	0,6	-	6,8
14,5	1962	73,2	0,6	-	1,4	5,2	-	0,3	0,3	8,6	2,2	0,6	0,3	1,1	0,3	0,6	-	-	-	0,3	0,3	5,0
15,5	1956	69,0	0,6	0,3	0,9	7,0	-	-	0,3	12,6	0,3	0,6	0,3	1,8	-	0,9	-	-	-	1,2	-	4,4
16,5	1951	71,3	2,2	0,9	1,8	5,5	0,6	-	0,9	8,0	-	0,6	-	1,2	-	0,6	0,3	-	-	0,3	0,3	5,2
17,5	1942	69,5	1,7	0,6	2,8	13,3	0,6	-	0,6	8,3	-	0,6	-	0,6	-	-	-	-	-	-	-	1,7
18,5	1932	72,7	0,5	0,5	1,0	6,2	-	-	1,0	8,6	0,5	1,9	-	1,9	-	1,4	-	-	-	1,9	-	1,9
19,5	1924	72,9	1,0	0,5	3,5	6,5	-	-	-	7,5	-	0,5	-	0,5	-	-	0,5	-	-	2,0	-	4,5
20,5	1913	75,7	0,6	-	0,6	8,8	-	-	0,6	6,6	-	-	0,6	1,1	-	-	-	-	-	-	-	5,5
21,5	1898	70,2	0,6	-	0,6	10,3	-	-	0,6	10,9	0,6	0,6	-	0,6	-	0,6	-	-	-	-	-	4,3
22,5	1886	72,2	0,5	-	1,6	5,9	-	-	-	11,8	1,6	0,5	-	0,5	-	0,5	-	-	-	0,5	-	4,3
23,5	1872	73,4	1,3	0,8	2,1	7,6	0,4	-	0,4	6,3	0,8	0,8	0,4	3,0	-	-	-	-	-	0,8	-	1,7
24,5	1858	78,3	0,4	0,4	0,8	4,2	0,8	0,4	-	5,0	0,4	-	-	1,7	-	0,8	-	-	-	1,3	-	5,4
28,5	1787	75,4	1,9	0,8	1,1	7,2	-	-	-	4,5	-	-	-	1,5	-	0,4	-	0,4	-	0,8	-	6,1
32,5	1695	66,6	0,3	0,6	1,9	7,7	1,3	-	0,6	8,7	-	0,3	-	2,6	-	-	-	-	-	1,0	0,3	8,0
36,5	1579	68,2	0,6	1,2	1,9	6,5	0,3	-	0,3	7,1	-	0,9	1,5	0,9	-	0,3	-	-	-	0,9	-	9,3
40,5	1435	62,2	3,3	0,4	1,1	6,9	0,4	-	1,8	8,0	-	0,4	0,7	3,6	0,4	0,4	0,4	-	-	2,2	0,4	7,6

Relative abundances of other palynomorphs of core CH1PC, in respect to total palynomorphs. Marine-terrestrial ratio is calculated with (dinocysts)/(dinocysts+pollen).

Sample depth (cm)	Age AD	Acritarch	Botryococcus spp.	Copepod egg	Cymatiosphaere	Foram lining (chain)	Foram lining (spiral)	Fungal spore	Pediastrum	Tintinnid	Marine:Terrestrial ratio [M/M+T]
1,0	2008	0,8	0,1	0,3	0,8	0,1	4,6	0,3	0,1	0,2	0,34
2,5	2005	0,3	-	-	1,3	0,1	4,2	0,3	-	-	0,41
3,5	2003	0,3	-	-	0,3	0,1	6,6	0,1	-	-	0,43
4,5	2001	0,7	-	0,2	0,9	0,2	6,6	1,3	-	-	0,60
5,5	1998	0,3	-	-	0,3	-	5,7	0,3	-	-	0,49
6,5	1995	1,8	-	0,2	0,7	0,3	4,2	0,8	-	0,3	0,42
7,5	1992	0,4	-	-	-	0,4	4,0	-	-	-	0,50
8,5	1989	0,2	-	-	0,5	-	2,6	-	-	-	0,55
9,5	1984	0,5	-	-	0,2	-	4,0	0,7	-	-	0,51
10,5	1981	0,4	-	-	0,7	-	5,0	0,5	-	-	0,47
11,5	1976	-	-	-	0,8	0,4	4,8	-	-	-	0,48
12,5	1971	0,2	-	0,3	1,6	0,3	4,1	0,2	-	-	0,47
13,5	1966	0,2	-	-	0,5	0,2	5,8	-	-	-	0,44
14,5	1962	-	-	-	0,6	0,1	3,9	0,3	-	0,3	0,43
15,5	1956	-	-	-	1,3	-	3,8	-	-	-	0,48
16,5	1951	0,2	0,2	-	0,9	0,2	3,8	0,3	-	-	0,48
17,5	1942	0,2	-	-	0,8	0,4	3,1	-	-	-	0,61
18,5	1932	-	-	-	-	-	2,6	-	-	-	0,54
19,5	1924	-	-	-	-	0,2	3,0	-	-	-	0,59
20,5	1913	0,6	-	-	1,0	0,2	3,3	-	-	-	0,60
21,5	1898	-	-	-	0,4	0,4	2,5	-	-	-	0,64
22,5	1886	-	-	-	0,4	0,4	4,8	-	-	0,2	0,57
23,5	1872	-	-	-	0,2	0,8	3,8	-	-	0,4	0,53
24,5	1858	0,2	-	-	1,0	0,7	2,9	-	-	-	0,57
28,5	1787	0,2	-	-	1,7	0,2	3,9	0,3	-	-	0,53
32,5	1695	-	-	-	0,9	-	3,7	-	0,3	-	0,49
36,5	1579	0,4	-	-	0,9	0,4	4,4	0,4	-	-	0,50
40,5	1435	0,4	-	-	0,7	0,6	5,8	0,7	-	-	0,55

APPENDIX 6: FLUX OF POLLEN AND OTHER PALYNOMORPHS IN SEDIMENT CORE CHIPC

<u>Pollen and spores flux</u> of core CH1PC. Flux is expressed in palynomorphs/cm^2/ year.

Sample depth (cm)	Age AD	Bisaccates	Asteraceae Tubuliflorae	Ambrosia	Myrica	Quercus	Poacea	Phlebodium	Taxodium distichum	Amaranthaceae	Casuarinaceae	Typha spp.	Cephalanthus occidentalis	Cyperaceae	Carya	Iva	Salix	Cf. Toxicodendron	Corylus cf.	Monoleet	Ulnus	Indet. Pollen	Total pollen and spores
1,0	2008	4924,8	453,4	529,0	655,0	1309,9	75,6	50,4	100,8	680,1	25,2	75,6	579,4	226,7	75,6	50,4	-	-	-	176,3	25,2	1309,9	11323
2,5	2005	4386,0	269,5	441,0	465,6	1666,2	49,0	49,0	122,5	343,0	147,0	122,5	122,5	343,0	-	49,0	-	-	24,5	49,0	49,0	1053,6	9752
3,5	2003	1338,8	176,8	58,9	117,9	581,0	50,5	-	16,8	176,8	58,9	50,5	42,1	33,7	-	16,8	8,4	-	-	-	8,4	429,4	3165
4,5	2001	2694,6	299,4	207,3	253,3	575,8	69,1	69,1	23,0	23,0	-	-	-	-	-	-	-	-	-	-	-	253,3	4467
5,5	1998	1654,1	210,6	136,3	210,6	508,0	12,4	-	37,2	173,5	111,5	12,4	49,6	86,7	-	-	-	-	-	24,8	-	210,6	3438
6,5	1995	2650,9	117,8	279,8	162,0	986,7	14,7	29,5	14,7	353,5	14,7	-	-	-	-	-	-	-	-	-	-	147,3	4776
7,5	1992	2686,1	501,7	167,2	292,6	522,6	-	20,9	41,8	397,2	125,4	-	-	83,6	-	41,8	20,9	-	-	-	-	397,2	5299
8,5	1989	2946,2	107,9	237,4	474,8	755,4	86,3	-	21,6	151,1	43,2	21,6	43,2	172,7	-	-	-	-	-	-	-	388,5	5449
9,5	1984	5011,5	291,6	255,1	656,0	1239,2	72,9	36,4	-	583,2	72,9	72,9	109,3	72,9	109,3	36,4	36,4	-	-	36,4	-	1020,5	9713
10,5	1981	1698,5	111,7	67,0	134,1	391,1	-	11,2	55,9	212,3	33,5	22,3	22,3	55,9	11,2	11,2	-	-	-	-	-	268,2	3106
11,5	1976	2289,8	112,3	37,4	124,8	124,8	12,5	12,5	25,0	262,0	-	-	12,5	25,0	12,5	12,5	-	-	-	12,5	12,5	112,3	3200
12,5	1971	2784,9	35,4	23,6	35,4	188,8	11,8	11,8	-	224,2	-	-	-	70,8	23,6	-	-	-	-	-	-	153,4	3563
13,5	1966	7057,8	162,6	130,1	195,1	715,5	65,0	-	32,5	618,0	32,5	-	65,0	65,0	97,6	32,5	65,0	-	-	65,0	-	683,0	10082
14,5	1962	2649,4	2-	-	5-	19-	-	1-	1-	309,9	8-	2-	1-	4-	1-	2-	-	-	-	1-	1-	18-	3619
15,5	1956	2003,1	17,0	8,5	25,5	204,1	-	-	8,5	365,7	8,5	17,0	8,5	51,0	-	25,5	-	-	-	34,0	-	127,6	2904
16,5	1951	1131,9	34,2	14,7	29,3	88,0	9,8	-	14,7	127,1	-	9,8	-	19,6	-	9,8	4,9	-	-	4,9	4,9	83,1	1586
17,5	1942	1167,4	27,9	9,3	46,5	223,2	9,3	-	9,3	139,5	-	9,3	-	9,3	-	-	-	-	-	-	-	27,9	1679
18,5	1932	2198,4	14,5	14,5	29,0	188,6	-	-	29,0	261,2	14,5	58,0	-	58,0	-	43,5	-	-	-	58,0	-	58,0	3025
19,5	1924	1205,8	16,6	8,3	58,0	107,7	-	-	-	124,3	-	8,3	-	8,3	-	-	8,3	-	-	33,1	-	74,6	1653
20,5	1913	819,5	6,0	-	6,0	95,7	-	-	6,0	71,8	-	-	6,0	12,0	-	-	-	-	-	-	-	59,8	1082
21,5	1898	1108,2	9,6	-	9,6	163,1	-	-	9,6	172,7	9,6	9,6	-	9,6	-	9,6	-	-	-	-	-	67,2	1578
22,5	1886	1315,7	9,7	-	29,2	107,2	-	-	-	214,4	29,2	9,7	-	9,7	-	9,7	-	-	-	9,7	-	78,0	1822
23,5	1872	1108,3	19,2	12,8	31,9	115,0	6,4	-	6,4	95,8	12,8	12,8	6,4	44,7	-	-	-	-	-	12,8	-	25,6	1510
24,5	1858	904,3	4,8	4,8	9,6	48,1	9,6	4,8	-	57,7	4,8	-	-	19,2	-	9,6	-	-	-	14,4	-	62,5	1154
28,5	1787	621,3	15,6	6,2	9,4	59,3	-	-	-	37,5	-	-	-	12,5	-	3,1	-	3,1	-	6,2	-	5-	824
32,5	1695	287,7	1,4	2,8	8,3	33,3	5,5	-	2,8	37,4	-	1,4	-	11,1	-	-	-	-	-	4,2	1,4	34,7	431
36,5	1579	603,0	5,5	10,9	16,4	57,4	2,7	-	2,7	62,9	-	8,2	13,7	8,2	-	2,7	-	-	-	8,2	-	82,0	884
40,5	1435	328,9	17,3	1,9	5,8	36,5	1,9	-	9,6	42,3	-	1,9	3,8	19,2	1,9	1,9	1,9	-	-	11,5	1,9	40,4	529

Flux of other palynomorphs of core CH1PC. Flux is expressed in palynomorphs/cm^2/ year.

Sample depth (cm)	Age AD	Acritarch	Botryococcus spp.	Copepod egg	Cymatiosphaere	Foram lining (chain)	Foram lining (spiral)	Fungal spore	Pediastrum	Tintinnid	Total palynomorphs	Marine:Terrestrial ratio [M/M+T]
1,0	2008	138,5	12,6	63,0	138,5	25,2	843,9	50,4	25,2	37,8	18465	0,34
2,5	2005	49,0	-	-	220,5	24,5	735,1	49,0	-	-	17617	0,41
3,5	2003	16,8	-	-	16,8	8,4	395,7	8,4	-	-	5961	0,43
4,5	2001	92,1	-	23,0	115,2	23,0	806,1	161,2	-	-	12287	0,60
5,5	1998	24,8	-	-	24,8	-	408,9	24,8	-	-	7193	0,49
6,5	1995	162,0	-	14,7	58,9	29,5	382,9	73,6	-	29,5	9020	0,42
7,5	1992	41,8	-	-	-	41,8	439,0	-	-	-	11079	0,50
8,5	1989	21,6	-	-	64,8	-	323,8	-	-	-	12583	0,55
9,5	1984	109,3	-	-	36,4	-	838,3	145,8	-	-	20793	0,51
10,5	1981	22,3	-	-	44,7	-	312,9	33,5	-	-	6235	0,47
11,5	1976	-	-	-	49,9	25,0	312,0	-	-	-	6532	0,48
12,5	1971	11,8	-	23,6	118,0	23,6	295,0	11,8	-	-	7240	0,47
13,5	1966	32,5	-	-	97,6	32,5	1105,8	-	-	-	19222	0,44
14,5	1962	-	-	-	4-	1-	259,9	2-	-	2-	6728	0,43
15,5	1956	-	-	-	76,6	-	221,1	-	-	-	5843	0,48
16,5	1951	4,9	4,9	-	29,3	4,9	122,2	9,8	-	-	3251	0,48
17,5	1942	9,3	-	-	37,2	18,6	139,5	-	-	-	4479	0,61
18,5	1932	-	-	-	-	-	174,1	-	-	-	6813	0,54
19,5	1924	-	-	-	-	8,3	124,3	-	-	-	4123	0,59
20,5	1913	17,9	-	-	29,9	6,0	95,7	-	-	-	2859	0,60
21,5	1898	-	-	-	19,2	19,2	115,1	-	-	-	4572	0,64
22,5	1886	-	-	-	19,5	19,5	214,4	-	-	9,7	4512	0,57
23,5	1872	-	-	-	6,4	25,6	127,8	-	-	12,8	3376	0,53
24,5	1858	4,8	-	-	28,9	19,2	81,8	-	-	-	2838	0,57
28,5	1787	3,1	-	-	31,2	3,1	71,8	6,2	-	-	1855	0,53
32,5	1695	-	-	-	8,3	-	33,3	-	2,8	-	898	0,49
36,5	1579	8,2	-	-	16,4	8,2	84,8	8,2	-	-	1909	0,50
40,5	1435	5,8	-	-	9,6	7,7	75,0	9,6	-	-	1296	0,55

APPENDIX 7: BIOMARKERS OF CH1PC

N-alkanes

Long-chained saturated hydrocarbons occur in different groups of organisms. Where marine organisms typically contain short-chain n-alkanes (C_{16}-C_{24}), there terrestrial leaf-waxes contain long-chain n-alkanes (C_{25}-C_{35}) (Eglinton et al., 1967). The vascular land plant long-chain n-alkanes show a odd-over-even predominance.

The Carbon Preference Index (CPI) can describe the molecular distribution characteristics of long chain n-alkanes, the odd-to-even carbon number predominance. The CPI can be used as a proxy for the origin of the n-alkanes, and is also thought to be dependent on climate (Rao et al., 2009). The larger the CPI, the bigger the difference between odd versus even n-alkanes is. The CPI can be calculated based on the n-alkane data. Where terrestrial plant input is high, the CPI will reach values around 5 to 10, but where marine input is dominant the CPI will approach 1 (Rieley et al., 1991).

The average chain length (ACL) of n-alkanes is often used as a proxy for the source and as a check of consistency of the OM input. Generally rivers have higher ACL than marine sediments, as rivers have more terrestrial input. It is calculated in the following manner:

$$ACL_{alkanes} = (23*[C_{23}\ alkane]+25*[C_{25}\ alkane]+ 27*[C_{27}\ alkane]+29*[C_{29}\ alkane]+ 31*[C_{31}\ alkane]+33*[C_{33}\ alkane]+ 35*[C_{35}\ alkane]) / ([C_{23}\ alkane]+ [C_{25}\ alkane]+ [C_{27}\ alkane]+ [C_{29}\ alkane]+ [C_{31}\ alkane]+ [C_{33}\ alkane]+ [C_{35}\ alkane])$$

Combined, the CPI, ACL and C/N ratio are expected to reflect changes in terrestrial input into the Charlotte Harbor area.

HBI

The monocyclic highly branched isoprenoid (HBI) 25:4 is produced amongst others by the bloom forming diatom *Rhizosolenia setigera* (Sinninghe Damsté et al., 1999; Massé et al., 2004; Volkman et al., 1994; Xu et al., 2006), *Haslea* spp. (*H. ostrearia*) species (Sinninghe Damsté et al., 2004; Volkman et al., 1994) and some freshwater diatoms (Belt et al., 2001a; 2001b). $HBI_{30:5}$ is also found in *R. setigera* (Belt et al., 2001; Volkman et al., 1994; 1998).

Fatty Acids

Fatty acids are the main constituents of the total lipid content of algae. The fatty acid content of samples is dependent on the presence of species and influenced by environmental parameters as temperature, nutrient and light availability. Differences in saturation and chain length can say something about the different groups of organisms present. (branched) $C_{15:0}$ and $C_{17:0}$ fatty acids are general bacterial biomarkers (Kaneda, 1991), while $C_{14:0}$ is a general algal biomarker. The fatty acid content is not sufficiently specific to describe the algae assemblage, but are shown to correlate with different plankton communities and thus still

useful (Reuss & Poulsen, 2002). The saturated C_{14} and C_{16} fatty acids are a major component of the fatty acids of most algae. The ratio of $C_{16:1w7}/C_{16:0}$ fatty acid is commonly used as a biomarker for the dominance of diatoms (Reuss & Poulsen, 2002). If $C_{16:1w7}/C_{16:0}$ is close to or bigger than 1 diatoms are thought of to dominate the planktonic assemblage. The $C_{16:1w7}/C_{16:0}$ FA ratio is generally used to see differences in diatom dominance along the transect, and is compared to the C_{16}/C_{18} ratio. Flagellates are more abundant in C_{18} FA, where diatoms are more abundant in C_{16} (Reuss & Poulsen, 2002).

Alcohols

Long chain (fatty) alcohols are mostly derived from wax esters. Sources of the wax esters can be higher plants, terrestrial plants and zooplankton. In some work the long-chain alcohols $>C_{22}$ are used as terrestrial markers as e.g. C_{24} originates mainly from vascular plants (Volkman, 1980; Cranwell, 1982).

The average chain length (ACL) of the alcohols is calculated:

$ACL_{alcohol}$ = (22*[C_{22} alcohol]+24*[C_{24} alcohol]+ 26*[C_{26} alcohol]+28*[C_{28} alcohol]+ 30*[C_{30} alcohol]+32*[C_{32} alcohol]) / ([C_{22} alcohol]+ [C_{24} alcohol]+ [C_{26} alcohol]+ [C_{28} alcohol]+ [C_{30} alcohol]+ [C_{32} alcohol])

The Diol Index can be calculated based on the relative abundances of C_{30} and $C_{32\ 1,15}$ diols. These diols are present in the polar fraction of the sedimentary core and can indicate differences in salinity and / or upwelling environments (Versteegh et al., 1997). According to (Versteegh et al., 1997) in most freshwater or restricted marine environments, the diol index falls between 25 and 67, where indices between 68 and 79 are more typical of marine environments (highly productive areas; tropical upwelling area's). Fresh and brackish water environments have relatively low diol indices compared to marine water environments. As the diol index can indicate relative salinity changes (e.g. by more freshwater input or incursions of more saline waters), it could be a good tool in relative salinity reconstructions of the Charlotte Harbor estuary.

Sterols

Sterols are compounds produced by marine and terrestrial eukaryotes. Some are generally occurring sterols, e.g. cholesterol. Sterols are made by organisms by the biosynthesis of squalene and can be species specific due to differences in carbon atoms and saturation level. Sterols can be used as tracer for terrestrial input or autochthonous marine algal production. Generally C_{27} and C_{28} sterols are produced by marine plankton, where C_{29} sterols are major constituents of terrestrial plants (Volkman, 1986).

β-sitosterol (24-ethylcholest-5-en-3β-ol or $C_{29:1w5}$) is a terrestrial biomarker, although it can be found in some diatoms, haptophytes and cyanobacteria as well (Volkman, 1986). A comparison between the C_{22} long chain alcohols and the β-sitosterol composition of the samples can indicate terrestrial versus diatom origin of β-sitosterol. is abundant in vascular plants (as used in Soelen et al., 2010), but also in marine phytoplankton, haptophytes and cyanobacteria (Volkman, 1986). Taraxerol is a sterol used as a biomarker for mangrove

forests. This is transported via mangrove leaves into the estuary. Dinosterol (23,24-dimethyl-cholest-22E-en-3β-ol or C29:1w22) is a generic dinoflagellate biomarker, and produced by some other algae (Rampen et al., 2010). It is thought by some that low salinity species produce more dinosterol than higher salinity species (E. van Soelen, pers. comm.) where others hypothesize that by measuring dinosterol and its diagenesis products one can estimate the amount of dinoflagellate input into the sediment (Mouradian et al., 2007). It must be stated that dinosterol is also produced by non-cyst making dinoflagellates, thus discrepancies between dinosterol content and dinocysts will be present.

Some dinoflagellates and diatoms produce 24-nor-cholesta-5,22E-dien-3B-ol (trivial name: NOR-$C_{27:2w5,22}$ or 24-norsterol) (Rampen et al., 2007; 2010). Cholest-5,22E-dien-3B-ol ($C_{27:2w5,22}$) is mainly present in diatoms and red algae, but also in some dinoflagellates (Volkman, 1986). Cholest-5-en-3B-ol ($C_{27:1w5}$ or cholesterol) is present in most zoo- and phytoplankton and haptophytes and can have terrestrial origin. 24-methylcholest-5-en-3B-ol ($C_{28:1w5}$ or campesterol) is present in diatoms and higher plants (Volkman, 1986). 24-methylcholesta-5,24(28)-dien-3B-ol ($C_{28:2w5,24(28)}$) is present in some diatoms (Volkman, 1986; Rampen et al., 2010). 24-methylcholesta-5,22E-dien-3B-ol ($C_{28:2w5,22}$, “Diatomsterol” or brassicasterol) is present in diatoms, haptophytes, prymnesiophytes and higher plants (Volkman, 1981; 1986; Rampen et al., 2010). 24-ethylcholesta-5,22E-dien-3B-ol ($C_{29:2w5,22}$ or stigmasterol) is present in diatoms, green algae and Cryptophyceae.

Other lipids

Hop-22(29)-ene is produced by Prochlorophyta (Volkman et al., 1998). Loliolide is produced by diatoms.

APPENDIX 8: RELATIVE ABUNDANCE OF BIOMARKERS IN SEDIMENT CORE CHIPC

The relative abundance of biomarkers in the sediment core CH1PC. Values are in µg/gTOC.
a = bad measurement (polar lipids).
ND = not detected.

Sample depth (cm) Age AD	1 2008	2,5 2005	3,5 2003	4,5 2001	5,5 1998	6,5 1995	7,5 1992	8,5 1989	9,5 1984	10,5 1981	11,5 1976	12,5 1971	13,5 1966	14,5 1962	15,5 1956	16,5 1951	17,5 1942	18,5 1932	19,5 1924	20,5 1913	21,5 1898	22,5 1886	23,5 1872
C15 alcohol	*a*	1,4	2,3	1,3	6,9	*a*	2,6	4,0	2,8	2,1		ND	0,4	0,1	ND	0,5	1,2	ND	1,8	1,7	1,8	1,8	*a*
C16 alcohol	*a*	10,4	10,2	6,5	10,9	*a*	8,0	16,6	10,2	8,2	1,6	1,0	1,2	0,7	1,4	5,9	4,7	6,8	9,2	7,5	8,4	11,1	*a*
C17 alcohol	*a*	3,3	3,0	1,7	4,4	*a*	2,7	5,7	4,0	2,6	0,5	0,3	0,5	0,2	0,1	0,8	1,5	2,1	1,9	1,4	2,4	2,5	*a*
C18 alcohol	*a*	10,4	11,3	8,4	12,7	*a*	9,7	20,9	13,1	13,7	2,2	1,4	1,8	1,0	1,7	7,4	5,1	7,0	9,2	6,2	10,5	11,6	*a*
C19 alcohol	*a*	3,1	2,8	ND	3,5	*a*	2,9	4,5	3,2	ND	0,3	0,2	0,3	0,2	0,3	0,5	1,7	2,1	3,1	1,5	3,0	4,4	*a*
C20 alcohol	*a*	17,4	15,5	8,7	13,4	*a*	11,6	30,4	15,2	12,4	2,6	1,7	2,0	1,1	2,0	8,7	6,4	7,6	8,6	7,8	11,6	12,0	*a*
C21 alcohol	*a*	6,4	4,1	2,0	5,8	*a*	2,4	7,7	3,7	2,3	0,9	0,6	0,7	0,4	0,6	1,8	1,6	2,3	5,1	5,8	6,2	4,8	*a*
C22 alcohol	*a*	199,2	96,0	26,6	37,6	*a*	49,1	213,4	55,5	25,6	12,3	3,3	5,5	2,7	6,4	39,9	34,4	19,5	23,6	12,6	17,1	21,5	*a*
C23 alcohol + squalane	*a*	84,4	89,4	86,8	82,1	*a*	51,6	77,0	60,8	131,7	ND	ND	ND	ND	ND	71,7	35,4	55,1	47,7	52,3	66,9	76,3	*a*
C24 alcohol	*a*	62,1	78,2	61,3	118,6	*a*	49,3	96,8	70,6	37,5	7,2	5,2	5,7	3,3	5,7	26,0	12,9	20,1	94,2	29,9	56,2	84,6	*a*
C25 alcohol	*a*	10,3	11,9	7,4	12,5	*a*	9,2	16,4	13,9	8,3	1,6	1,3	1,4	0,8	1,5	5,4	6,0	7,0	9,7	7,7	11,3	15,9	*a*
C26 alcohol	*a*	57,5	63,3	39,1	65,1	*a*	46,3	88,8	73,0	37,1	7,3	5,8	6,4	3,4	6,3	21,5	25,4	31,6	44,4	38,2	32,3	74,8	*a*
C27 alcohol	*a*	11,9	15,2	11,1	16,0	*a*	12,1	ND	22,8	11,0	2,2	1,8	1,9	1,0	1,9	7,1	8,2	8,5	16,1	ND	13,8	27,4	*a*
C28 alcohol	*a*	142,0	152,9	72,5	143,9	*a*	91,6	238,7	139,8	69,3	10,1	8,5	8,4	4,0	7,8	41,9	42,8	54,7	77,5	70,6	97,8	141,0	*a*
C30 alcohol	*a*	98,8	96,8	73,1	128,0	*a*	70,3	79,1	127,8	52,3	7,6	7,0	6,1	2,8	4,9	33,1	43,2	53,4	81,7	81,9	121,4	154,9	*a*
C32 alcohol	*a*	50,6	43,8	23,7	48,1	*a*	28,2	44,1	44,5	12,6	3,6	3,6	2,6	0,6	1,3	11,0	18,1	19,1	37,4	28,3	61,2	74,0	*a*
C33 alcohol	*a*	19,2	20,2	10,3	24,4	*a*	14,7	17,1	21,3	ND	1,1	0,5	0,4	0,2	0,6	3,4	6,4	9,5	20,2	ND	23,2	43,7	*a*
C34 alcohol	*a*	7,2	7,7	5,9	9,5	*a*	5,8	5,3	7,5	3,4	0,3	0,4	0,3	ND	ND	2,6	3,9	3,6	6,8	2,6	8,4	12,7	*a*

Sample depth (cm)	1	2,5	3,5	4,5	5,5	6,5	7,5	8,5	9,5	10,5	11,5	12,5	13,5	14,5	15,5	16,5	17,5	18,5	19,5	20,5	21,5	22,5	23,5
Age AD	**2008**	**2005**	**2003**	**2001**	**1998**	**1995**	**1992**	**1989**	**1984**	**1981**	**1976**	**1971**	**1966**	**1962**	**1956**	**1951**	**1942**	**1932**	**1924**	**1913**	**1898**	**1886**	**1872**
Unknown, m/z 131	*a*	37,5	30,6	13,1	32,4	*a*	24,1	33,0	29,5	9,3	ND	0,3	0,3	0,6	1,6	0,5	0,6	ND	0,3	ND	ND	2,8	*a*
1-monopalmitin	*a*	12,3	9,3	13,7	6,3	*a*	6,2	9,6	8,2	9,3	1,8	0,7	2,1	1,3	2,5	5,3	2,5	9,3	4,2	1,9	3,9	4,2	*a*
bis-monostearin	*a*	1,2	9,5	13,5	8,3	*a*	3,0	9,4	5,9	12,7	2,3	1,3	2,1	1,4	2,4	6,5	4,4	7,2	13,0	ND	6,5	7,2	*a*
Phytol	*a*	10,3	13,1	10,4	13,4	*a*	8,8	21,8	13,3	6,3	1,6	0,9	1,2	0,7	0,9	2,6	2,2	1,9	3,2	3,5	4,3	5,3	*a*
B-sitosterol	*a*	23,2	22,0	13,8	ND	*a*	26,5	40,1	42,4	9,4	ND	0,2	0,1	0,1	0,3	3,6	4,9	5,3	ND	2,6	5,2	19,9	*a*
Cholesterol	*a*	19,4	1ND	12,3	28,0	*a*	2,1	ND	5,1	12,6	0,5	0,1	1,2	0,1	0,2	4,7	1,8	5,3	1,9	ND	ND	2,1	*a*
Dinosterol	*a*	7,0	0,4	12,3	14,0	*a*	0,5	5,4	0,9	8,4	ND	ND	ND	ND	ND	2,3	0,3	ND	ND	ND	ND	ND	*a*
Taraxerol + C 29 alcohol	*a*	46,1	48,4	31,2	46,2	*a*	26,6	61,2	48,4	19,2	2,2	1,8	2,0	1,0	1,9	12,9	13,1	14,4	23,1	36,4	3ND	40,5	*a*
23,24 dimethylcholesta 5,22 dien 3b ol	*a*	1,7	0,6	3,9	6,2	*a*	0,9	1,8	1,4	3,2	ND	ND	ND	ND	ND	ND	0,6	1,4	ND	ND	ND	ND	*a*
keto-ol = C30 1,15 keto-ol	*a*	15,0	8,0	5,8	6,5	*a*	9,4	47,1	13,4	6,1	ND	ND	ND	ND	ND	3,1	11,4	6,4	7,7	ND	30,7	31,8	*a*
C30 diol	*a*	57,9	67,3	45,5	78,8	*a*	61,7	73,4	83,4	34,2	7,0	6,3	6,2	3,6	6,4	23,9	26,9	35,0	62,2	31,8	70,5	103,2	*a*
C32 diol	*a*	30,2	29,3	21,2	37,2	*a*	25,0	29,9	32,0	16,0	2,3	2,5	2,5	1,2	2,3	11,5	13,1	19,1	30,3	24,3	23,4	53,2	*a*
C27 alkenone	*a*	31,9	22,6	ND	18,0	*a*	16,5	40,3	23,5	13,7	3,2	2,0	2,4	1,0	1,2	9,6	11,5	1ND	14,1	8,9	10,5	20,6	*a*
HBI 25:4	88,0	86,1	87,5	53,9	72,7	65,1	54,5	82,4	69,5	34,1	5,9	4,3	11,1	0,5	1,9	1,8	1,3	2,6	3,5	7,9	3,4	5,1	0,7
HBI 30:5	4,9	5,7	5,4	4,0	4,7	5,0	4,4	5,8	5,3	3,0	1,1	0,9	1,8	0,5	0,8	0,8	0,4	0,6	0,7	1,4	0,8	0,6	0,2
24 nor lupane	4,2	2,1	1,7	1,3	1,8	1,9	1,7	5,8	1,9	2,2	1,9	6,4	12,4	5,5	2,3	1,8	2,0	2,3	2,9	18,2	2,4	1,3	0,6
hop-22(29)ene	3,4	4,1	4,0	2,7	3,8	1ND	2,9	6,6	4,4	3,9	2,9	1,8	5,3	0,9	2,7	2,5	2,0	3,6	4,2	6,2	ND	2,3	1,4
C16 alkane	4,9	ND	ND	1,0	7,3	1,7	5,2	9,8	6,2	2,0	ND	ND	41,3	ND	3,7	4,0	0,7	5,0	15,5	2,9	0,7	0,9	ND
C17 alkane	1,0	0,4	0,5	0,5	0,6	0,8	0,9	1,5	1,5	1,0	0,4	3,0	5,7	3,2	1,1	1,6	1,4	1,2	1,7	1,1	0,4	0,2	ND
C18 alkane	8,8	1,9	7,2	3,8	9,4	5,5	5,3	15,8	7,5	5,8	2,3	3,7	40,2	2,8	10,2	9,2	5,2	7,9	23,2	11,2	3,9	3,6	0,9
C19 alkane	1,7	1,8	2,2	2,2	2,9	2,7	2,6	5,4	3,6	4,6	2,4	2,4	10,6	2,2	4,1	3,5	1,6	3,0	2,8	4,2	2,9	2,0	ND
C20 alkane	ND	1,7	5,3	2,7	2,1	1,3	1,1	3,0	2,0	2,0	1,1	1,6	3,2	3,1	2,2	2,1	1,1	1,5	2,6	ND	ND	1,3	ND
C19 alkene	17,5	2,6	12,5	ND	15,6	11,8	9,2	20,9	13,9	11,2	6,1	6,5	32,3	5,2	11,4	10,3	ND	7,7	14,0	17,0	2,6	1,8	ND
C21 alkane	1,2	0,7	0,8	0,3	1,6	0,9	0,8	2,0	1,4	0,9	0,4	0,4	3,3	ND	1,1	0,5	0,3	0,4	0,4	2,6	0,5	0,4	0,2

Sample depth (cm)	1	2,5	3,5	4,5	5,5	6,5	7,5	8,5	9,5	10,5	11,5	12,5	13,5	14,5	15,5	16,5	17,5	18,5	19,5	20,5	21,5	22,5	23,5
Age AD	**2008**	**2005**	**2003**	**2001**	**1998**	**1995**	**1992**	**1989**	**1984**	**1981**	**1976**	**1971**	**1966**	**1962**	**1956**	**1951**	**1942**	**1932**	**1924**	**1913**	**1898**	**1886**	**1872**
C22 alkane	2,9	1,1	3,9	2,1	4,2	3,9	2,1	4,2	3,0	3,3	1,8	0,7	8,7	0,6	2,7	2,4	1,1	2,0	2,8	5,5	3,5	2,6	0,7
C23 alkane	2,9	3,6	4,5	2,8	3,5	2,9	3,3	4,9	3,7	3,4	2,3	3,8	8,2	2,6	2,5	2,1	1,7	2,4	3,5	6,5	3,1	2,1	0,8
C24 alkane	2,6	2,9	3,7	2,2	2,6	2,7	2,5	4,5	3,0	2,9	2,2	2,2	7,1	1,3	2,1	1,9	1,4	2,0	2,7	5,7	2,9	2,1	0,8
C25 alkane	7,0	9,0	10,5	7,7	9,3	7,9	8,0	11,5	9,9	8,9	6,1	7,3	24,3	4,9	6,9	5,7	5,1	6,0	9,7	21,5	9,4	6,9	2,9
C26 alkane	2,6	3,4	3,6	2,7	3,1	3,0	2,2	4,0	2,6	3,1	2,1	7,0	9,4	4,8	2,6	2,0	1,7	2,2	3,5	8,3	3,9	2,4	1,1
C27 alkane	9,4	12,4	15,3	10,5	12,9	12,7	10,4	16,4	12,9	11,7	9,5	11,5	34,2	8,9	10,1	8,1	6,7	8,4	14,3	31,3	16,6	9,4	4,4
C28 alkane	2,3	3,2	3,9	2,8	3,0	2,8	2,4	4,3	3,3	3,2	2,7	3,5	12,8	3,3	2,6	2,6	2,2	2,9	4,3	6,4	5,5	3,2	1,5
C29 alkane	16,5	21,6	24,3	18,9	2-	20,3	15,6	25,6	19,7	19,3	20,7	19,3	44,9	14,4	18,8	14,6	12,4	15,1	20,1	44,8	28,8	15,8	8,0
C30 alkane	2,5	2,8	3,4	2,5	2,8	2,2	2,8	5,4	4,0	4,2	3,7	2,0	17,5	1,4	4,3	3,6	3,3	4,1	6,2	17,3	6,9	4,6	2,1
C31 alkane	10,1	14,2	16,4	11,8	12,4	13,4	9,8	19,5	14,0	12,8	11,8	13,0	29,3	11,0	12,8	10,4	8,2	11,8	18,7	32,6	22,9	12,9	6,5
C32 alkane	2,4	3,4	3,5	2,5	2,7	3,6	2,4	4,0	2,9	2,7	2,2	3,0	10,2	2,3	2,6	2,1	1,5	2,4	3,2	8,8	2,3	2,8	1,6
C33 alkane	6,3	9,1	9,9	7,9	8,1	8,1	6,2	10,8	9,6	7,3	6,4	9,1	21,6	7,4	7,3	5,9	4,5	6,6	10,2	21,6	13,8	7,0	3,5
C34 alkane	1,5	1,3	1,4	1,0	1,0	1,1	0,9	1,3	1,2	0,8	1,1	1,0	2,2	1,0	1,1	0,8	0,6	1,1	1,9	2,6	1,7	0,9	0,5
C35 alkane	3,2	5,3	5,4	4,9	4,5	4,5	4,1	6,4	5,5	4,1	2,6	3,2	ND	2,5	2,9	2,5	1,4	2,9	3,9	9,4	5,4	3,2	1,3
C36 alkane	ND	2,1	2,5	1,6	2,0	1,0	1,7	2,7	1,9	1,7	1,6	ND	ND	ND	1,3	0,9	1,0	1,1	2,3	ND	1,8	1,1	0,6

APPENDIX 9: FLUX OF BIOMARKERS IN SEDIMENT CORE CHIPC

The flux of biomarkers in the sediment core CH1PC. Values are in µg/cm^2/yr.
a = bad measurement (polar lipids).
ND = not detected.

Sample depth (cm)	**1**	**2,5**	**3,5**	**4,5**	**5,5**	**6,5**	**7,5**	**8,5**	**9,5**	**10,5**	**11,5**	**12,5**	**13,5**	**14,5**	**15,5**	**16,5**	**17,5**	**18,5**	**19,5**	**20,5**	**21,5**	**22,5**	**23,5**
Age AD	**2008**	**2005**	**2003**	**2001**	**1998**	**1995**	**1992**	**1989**	**1984**	**1981**	**1976**	**1971**	**1966**	**1962**	**1956**	**1951**	**1942**	**1932**	**1924**	**1913**	**1898**	**1886**	**1872**
C15 alcohol	*a*	5,8	6,0	3,5	17,6	*a*	6,8	8,3	10,6	3,3	ND	ND	1,4	0,4	ND	0,6	2,1	ND	2,0	1,6	1,8	1,2	*a*
C16 alcohol	*a*	43,1	27,5	17,2	27,9	*a*	20,8	34,6	38,9	13,2	2,9	1,8	4,5	2,0	2,7	6,8	8,1	11,1	9,8	7,1	8,5	7,6	*a*
C17 alcohol	*a*	13,5	8,0	4,4	11,2	*a*	6,9	11,9	15,0	4,2	0,8	0,5	1,9	0,5	0,3	0,9	2,6	3,5	2,0	1,3	2,4	1,7	*a*
C18 alcohol	*a*	43,1	30,5	22,1	32,4	*a*	25,2	43,4	49,9	22,0	3,8	2,6	6,5	2,8	3,4	8,4	8,7	11,5	9,8	5,9	10,7	7,9	*a*
C19 alcohol	*a*	12,6	7,5	ND	8,9	*a*	7,4	9,4	12,2	ND	0,6	0,4	1,0	0,6	0,6	0,6	2,9	3,5	3,3	1,4	3,1	3,0	*a*
C20 alcohol	*a*	71,9	41,5	23,0	34,1	*a*	30,1	63,3	57,7	19,9	4,6	3,1	7,1	3,3	4,1	9,9	11,0	12,5	9,2	7,3	11,8	8,2	*a*
C21 alcohol	*a*	26,4	10,9	5,2	14,8	*a*	6,3	16,1	13,9	3,6	1,6	1,2	2,6	1,3	1,3	2,0	2,8	3,8	5,4	5,5	6,3	3,3	*a*
C22 alcohol	*a*	823,9	257,8	70,2	95,7	*a*	127,4	443,6	210,9	41,0	21,8	6,2	19,8	7,9	12,8	45,5	58,8	32,0	25,2	11,8	17,3	14,8	*a*
C23 alcohol + squalane	*a*	348,9	240,1	229,1	208,9	*a*	133,8	160,2	230,9	211,2	ND	ND	ND	ND	ND	81,8	60,4	90,4	50,9	49,1	68,0	52,4	*a*
C24 alcohol	*a*	257,0	21-	161,9	301,9	*a*	127,8	201,3	268,4	60,2	12,7	9,7	20,6	9,6	11,5	29,6	22,1	33,0	100,5	28,1	57,1	58,0	*a*
C25 alcohol	*a*	42,6	31,9	19,5	31,7	*a*	23,9	34,0	52,7	13,3	2,9	2,4	5,2	2,2	3,0	6,1	10,3	11,5	10,4	7,3	11,5	10,9	*a*
C26 alcohol	*a*	237,8	170,1	103,2	165,8	*a*	12-	184,6	277,5	59,5	12,9	10,6	23,1	10,1	12,7	24,5	43,4	51,8	47,4	35,9	32,8	51,4	*a*
C27 alcohol	*a*	49,4	40,7	29,4	40,6	*a*	31,4	ND	86,8	17,7	3,8	3,3	6,9	3,0	3,9	8,1	14,1	13,9	17,2	ND	14,1	18,8	*a*
C28 alcohol	*a*	587,2	410,6	191,3	366,4	*a*	237,4	496,3	531,4	111,1	17,9	15,6	30,4	11,8	15,7	47,8	73,0	89,7	82,7	66,3	99,4	96,8	*a*
C30 alcohol	*a*	408,8	259,9	193,0	325,9	*a*	182,3	164,4	485,7	83,8	13,5	13,0	22,2	8,1	9,8	37,7	73,8	87,6	87,2	77,0	123,4	106,4	*a*
C32 alcohol	*a*	209,2	117,6	62,5	122,3	*a*	73,0	91,8	169,1	20,2	6,4	6,6	9,3	1,8	2,7	12,5	30,9	31,3	39,9	26,6	62,2	50,8	*a*
C33 alcohol	*a*	79,3	54,1	27,1	62,1	*a*	38,2	35,6	80,9	ND	1,9	1,0	1,3	0,5	1,1	3,9	11,0	15,6	21,6	ND	23,6	3-	*a*
C34 alcohol	*a*	29,7	20,6	15,7	24,2	*a*	15,0	11,1	28,4	5,4	0,6	0,7	1,1	ND	ND	2,9	6,6	5,9	7,3	2,5	8,5	8,7	*a*

Sample depth (cm) Age AD	1 2008	2,5 2005	3,5 2003	4,5 2001	5,5 1998	6,5 1995	7,5 1992	8,5 1989	9,5 1984	10,5 1981	11,5 1976	12,5 1971	13,5 1966	14,5 1962	15,5 1956	16,5 1951	17,5 1942	18,5 1932	19,5 1924	20,5 1913	21,5 1898	22,5 1886	23,5 1872
Unknown, m/z 131	*a*	155,1	82,1	34,7	82,6	*a*	62,6	68,6	112,2	14,9	ND	0,5	1,1	1,7	3,3	0,5	1,0	ND	0,4	ND	ND	1,9	*a*
1-monopalmitin	*a*	50,8	25,1	36,1	15,9	*a*	16,0	20,1	31,3	14,9	3,1	1,4	7,5	3,8	5,0	6,1	4,3	15,3	4,5	1,8	4,0	2,9	*a*
bis-monostearin	*a*	4,9	25,5	35,6	21,1	*a*	7,8	19,6	22,3	20,3	4,1	2,3	7,7	4,0	4,7	7,4	7,6	11,8	13,9	ND	6,6	5,0	*a*
Phytol	*a*	42,6	35,2	27,3	34,1	*a*	22,9	45,4	50,4	10,1	2,9	1,7	4,3	2,1	1,7	3,0	3,8	3,2	3,5	3,3	4,4	3,6	*a*
B-sitosterol	*a*	95,8	59,1	36,3	ND	*a*	68,7	83,4	161,2	15,0	ND	0,3	0,4	0,2	0,7	4,1	8,3	8,7	ND	2,4	5,3	13,6	*a*
Cholesterol	*a*	80,2	26,9	32,5	71,2	*a*	5,4	ND	19,5	20,2	0,9	0,2	4,2	0,2	0,3	5,3	3,1	8,7	2,0	ND	ND	1,4	*a*
dinosterol	*a*	28,8	1,2	32,5	35,6	*a*	1,4	11,2	3,3	13,5	ND	ND	ND	ND	ND	2,7	0,5	ND	ND	ND	ND	ND	*a*
Taraxerol + C 29 alcohol	*a*	190,5	129,9	82,4	117,6	*a*	69,0	127,3	184,0	30,9	4,0	3,3	7,2	2,9	3,9	14,7	22,4	23,6	24,7	34,2	30,5	27,8	*a*
23,24 dimethylcholesta 5,22 dien 3b ol	*a*	7,1	1,6	10,3	15,8	*a*	2,3	3,8	5,2	5,1	ND	ND	ND	ND	ND	ND	1,0	2,3	ND	ND	ND	ND	*a*
C30 1,15 keto-ol	*a*	62,0	21,5	15,3	16,6	*a*	24,3	97,9	50,8	9,7	ND	ND	ND	ND	ND	3,6	19,5	10,4	8,3	ND	31,2	21,9	*a*
C30 diol	*a*	239,4	180,7	120,1	200,7	*a*	160,1	152,5	317,0	54,8	12,4	11,6	22,5	10,5	12,9	27,2	45,9	57,4	66,3	29,8	71,7	70,8	*a*
C32 diol	*a*	124,9	78,5	56,0	94,8	*a*	64,9	62,2	121,7	25,7	4,1	4,6	9,0	3,4	4,7	13,1	22,3	31,3	32,3	22,9	23,8	36,5	*a*
C27 alkenone	*a*	131,8	60,7	ND	45,8	*a*	42,7	83,8	89,2	22,0	5,7	3,7	8,8	2,9	2,4	10,9	19,6	16,3	15,0	8,4	10,7	14,2	*a*
HBI 25:4	399,9	356,3	234,9	142,4	185,0	108,2	141,3	171,3	264,0	54,6	10,5	8,0	40,2	1,4	3,8	2,0	2,3	4,2	3,7	7,4	3,5	3,5	0,7
HBI 30:5	22,2	23,5	14,4	10,6	12,1	8,3	11,4	12,0	2ND	4,8	1,9	1,6	6,5	1,4	1,5	0,9	0,7	0,9	0,8	1,3	0,8	0,4	0,2
24 nor lupane	18,9	8,7	4,4	3,4	4,6	3,2	4,4	12,0	7,3	3,5	3,3	11,9	44,9	16,0	4,6	2,1	3,5	3,8	3,1	17,1	2,4	0,9	0,7
hop-22(29)ene	15,3	16,8	10,7	7,0	9,7	16,6	7,5	13,7	16,7	6,2	5,2	3,3	19,2	2,8	5,5	2,8	3,4	5,9	4,5	5,8	ND	1,6	1,4
C16 alkane	22,2	ND	ND	2,8	18,7	2,8	13,6	20,4	23,4	3,3	ND	ND	149,1	ND	7,4	4,6	1,2	8,2	16,6	2,7	0,7	0,6	ND
C17 alkane	4,8	1,5	1,3	1,2	1,6	1,4	2,4	3,0	5,8	1,6	0,8	5,5	20,6	9,3	2,2	1,8	2,5	2,0	1,8	1,0	0,4	0,2	ND
C18 alkane	39,9	7,8	19,3	9,9	24,0	9,2	13,8	32,8	28,5	9,4	4,0	6,9	145,4	8,1	20,5	10,5	8,9	12,9	24,8	10,5	4,0	2,5	0,9
C19 alkane	7,7	7,6	6,0	5,9	7,3	4,5	6,7	11,1	13,7	7,3	4,2	4,5	38,2	6,5	8,2	4,0	2,8	4,9	3,0	4,0	3,0	1,3	ND
C20 alkane	ND	7,1	14,1	7,1	5,4	2,2	2,9	6,3	7,6	3,3	1,9	3,0	11,6	9,2	4,5	2,4	2,0	2,5	2,7	ND	ND	0,9	ND
C19 alkene	79,7	10,8	33,5	ND	39,6	19,6	23,8	43,4	52,8	18,0	10,8	11,9	116,6	15,3	22,8	11,8	ND	12,6	14,9	16,0	2,7	1,2	ND
C21 alkane	5,3	2,8	2,1	0,8	4,0	1,5	2,2	4,2	5,3	1,5	0,6	0,7	11,9	ND	2,1	0,6	0,5	0,7	0,4	2,5	0,5	0,3	0,2

Sample depth (cm) Age AD	1 2008	2,5 2005	3,5 2003	4,5 2001	5,5 1998	6,5 1995	7,5 1992	8,5 1989	9,5 1984	10,5 1981	11,5 1976	12,5 1971	13,5 1966	14,5 1962	15,5 1956	16,5 1951	17,5 1942	18,5 1932	19,5 1924	20,5 1913	21,5 1898	22,5 1886	23,5 1872
C22 alkane	13,0	4,7	10,4	5,4	10,6	6,5	5,4	8,7	11,5	5,3	3,2	1,3	31,5	1,8	5,5	2,7	1,9	3,3	3,0	5,1	3,6	1,8	0,7
C23 alkane	13,2	14,9	12,1	7,4	8,9	4,8	8,5	10,3	13,9	5,5	4,1	7,1	29,6	7,6	4,9	2,4	2,9	3,9	3,7	6,1	3,1	1,5	0,8
C24 alkane	11,7	12,2	9,9	5,8	6,7	4,5	6,6	9,4	11,3	4,7	3,9	4,1	25,7	3,9	4,2	2,1	2,4	3,3	2,9	5,3	2,9	1,4	0,8
C25 alkane	31,9	37,4	28,2	20,4	23,7	13,1	20,8	24,0	37,8	14,2	10,8	13,6	87,8	14,3	13,9	6,5	8,8	9,8	10,3	20,2	9,6	4,7	3,0
C26 alkane	11,8	14,2	9,6	7,1	7,9	5,0	5,8	8,4	1-	4,9	3,6	13,0	33,9	14,0	5,2	2,3	3,0	3,6	3,7	7,8	3,9	1,6	1,1
C27 alkane	42,7	51,5	41,0	27,6	32,7	21,1	26,9	34,0	49,1	18,7	16,9	21,2	123,4	26,0	20,3	9,2	11,5	13,8	15,2	29,4	16,9	6,4	4,5
C28 alkane	10,7	13,3	10,4	7,5	7,6	4,6	6,1	8,9	12,4	5,1	4,8	6,5	46,2	9,8	5,3	3,0	3,7	4,7	4,6	6,0	5,6	2,2	1,5
C29 alkane	74,9	89,3	65,3	49,8	51,0	33,7	40,5	53,3	74,7	30,9	36,7	35,7	162,2	42,2	37,8	16,7	21,2	24,8	21,5	42,1	29,3	10,9	8,0
C30 alkane	11,2	11,6	9,0	6,7	7,2	3,6	7,2	11,2	15,4	6,7	6,5	3,7	63,3	4,1	8,7	4,0	5,6	6,8	6,7	16,3	7,0	3,2	2,1
C31 alkane	45,8	58,9	44,1	31,1	31,4	22,3	25,5	40,6	53,2	20,5	20,8	24,0	106,0	32,1	25,8	11,8	14,1	19,4	19,9	30,6	23,3	8,9	6,5
C32 alkane	10,8	14,1	9,4	6,6	6,9	5,9	6,3	8,3	10,8	4,3	3,9	5,5	36,8	6,6	5,3	2,4	2,5	4,0	3,4	8,2	2,3	1,9	1,6
C33 alkane	28,8	37,6	26,6	20,8	20,6	13,5	16,2	22,6	36,3	11,8	11,3	16,9	78,2	21,8	14,7	6,7	7,7	10,9	10,9	20,3	14,1	4,8	3,5
C34 alkane	6,9	5,4	3,7	2,5	2,5	1,8	2,4	2,7	4,7	1,3	2,0	1,9	7,8	2,9	2,3	0,9	1,1	1,8	2,0	2,5	1,8	0,6	0,5
C35 alkane	14,4	22,1	14,6	13,0	11,4	7,5	10,6	13,3	20,9	6,5	4,6	6,0	ND	7,3	5,7	2,8	2,5	4,8	4,1	8,9	5,5	2,2	1,4
C36 alkane	ND	8,6	6,8	4,2	5,2	1,6	4,5	5,6	7,4	2,7	2,8	ND	ND	ND	2,6	1,0	1,7	1,8	2,4	ND	1,8	0,7	0,6

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