



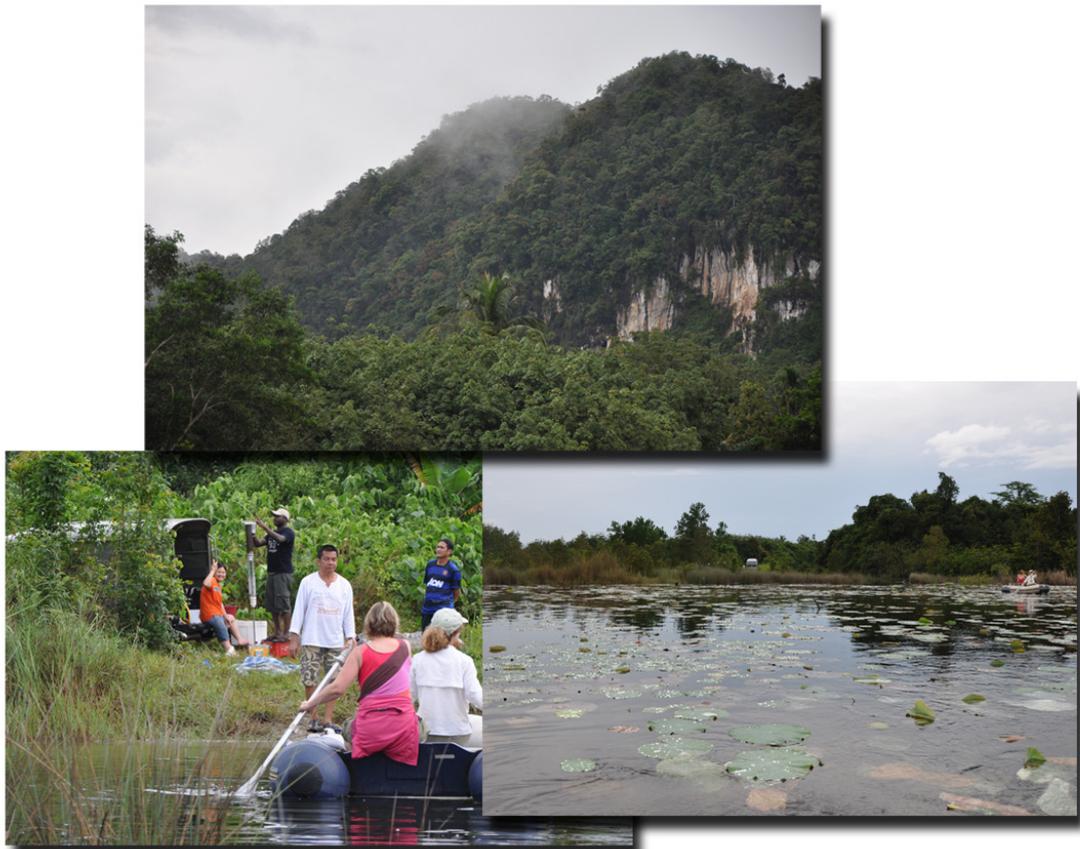
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A High Resolution Tropical Trophic Record of Lake Nong Thale Pron, Thailand, based on Lipid Biomarkers

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Bachelor Thesis, 15 credits

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Abstract

To be able to predict climate change caused by the Asian Monsoon system, an understanding of its long time variability is vital. The Asian Monsoon system affects a large area, and more specifically for this study, Thailand. This study entails in the interpretation of lipid biomarker records from the fresh water lake of Nong Thale Pron, Thailand. Here a 74 cm sediment core was retrieved and analyzed to investigate lake-level changes. The biomarkers analyzed, using a gas chromatograph-mass spectrometer (GC-MS), were: *n*-alkanes; highly branched isoprenoids (HBIs); and *Botryococcones*. The *n*-alkane results show that the primary input was from terrestrial plants, throughout the core, with a P_{aq} value showing mainly emergent macrophytes. The most significant change from the *n*-alkanes was that the C_{17} 's, produced by fast growing phytoplankton, concentration increased from 0.6 [$\mu\text{g g}^{-1}$ dw sample] around 30-50 cm depth to about 54 [$\mu\text{g g}^{-1}$ dw sample] at the top of the core. The results from the HBIs also showed a shifting trend at this depth. The *Botryococcene*, which is a specie of green algae indicating oligotrophic conditions, results had a higher concentration at the deeper depths which then decreased, around the same depth as the other biomarkers, towards the top. As these *Botryococcones* decreases in concentration the C_{17} increases and outcompetes the *algae*. This points to a change in the lake's trophic levels, evolving from an oligotrophic to a more mesotrophic lake. This change could either be a result from human influence, through eutrophication, or climate change, e.g. increased precipitation causing mixing of the lake. With further analysis, such as dating, these results could give more insight on the Asian Monsoon system.

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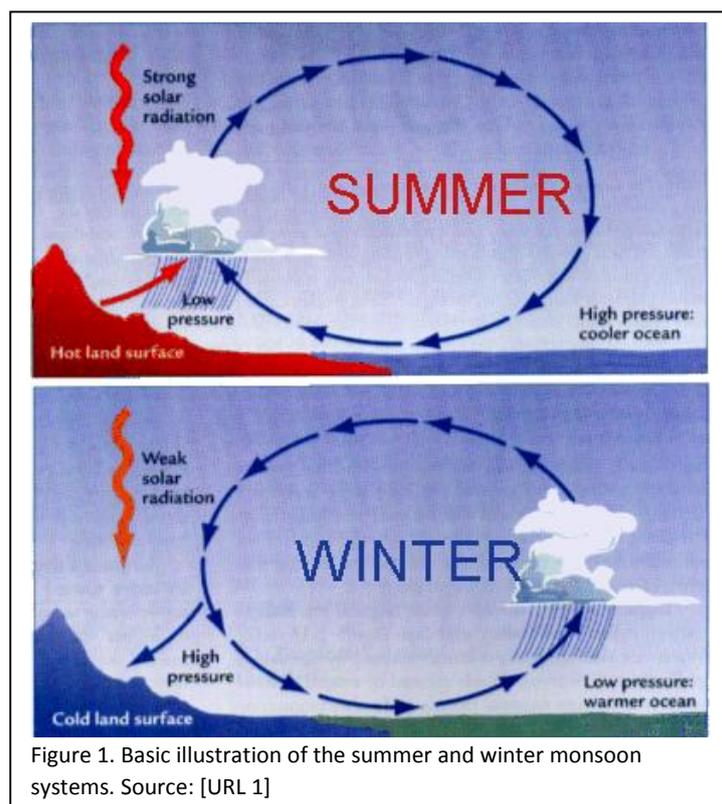
Introduction

Monsoon

The Asian monsoon system is one of the most dynamic climate systems and affects about half of the world's population (Wen et al., 2012). It is characterized by a distinct wet and dry season. The variability associated with the monsoon affects agriculture, economy and social life of people living in these regions. The Asian monsoon exerts a great impact on the global climate and has also had an effect on both flood and drought conditions in many countries (Wen et al., 2012).

Floods are caused by breaks in the active monsoon which produce unstable weathers with frequent storms and rainfalls. Drought conditions are caused by a dormant phase in the monsoon producing hot, clear and dry climates which could induce hardship or even famine in the monsoon regions (Webster, 1987). The monsoon is basically driven by incoming solar radiation, which is bound by a global divergent circulation. Solar radiation heats the landmass and oceans during the summer. But due to the higher heat capacity of water the landmasses are heated up faster. This creates a low pressure system on the landmasses and the denser more cooler air over the ocean creates a high pressure system. The pressure gradient causes the humid sea air to flow towards land, where convection takes place, causing precipitation (Haque, 2012). The conditions during the winter time are reversed (Figure 1).

Monsoon weather disturbances can last for years or even decades (Ummenhofer et al., 2013) affecting both humans and organisms living in these regions.



The Asian monsoon system is divided into four subunits: the Southwest Asian monsoon; the Northeast Asian monsoon; the East Asian monsoon; and the Indian monsoon. The Southwest Asian monsoon occurs only in the summer and receives winds from the high pressure systems built in the Indian Ocean and moves towards the landmasses. The Northeast Asian monsoon is a winter monsoon with northerly winds and dry weather. The Indian monsoon affects both the summer and

winter components. The summer monsoon receives low atmospheric southerly winds from the ocean which gives high humidity and high precipitation. The effects of the Indian monsoon in the winter is opposite from the summer monsoon. Similar to the Indian monsoon the East Asian monsoon is also composed of summer and winter monsoon. Here the summers are also warm and wet while the winters are cold and dry (Webster 1987).

Although the current monsoon climate is fairly well known, there is still much uncertainty governing the long-term variability. Yet this is critical for understanding and predicting potential shifts in rainfall patterns as a consequence of global climate change.

In the last decade a surge of studies on the science behind Asian monsoons have been made attempting to generate record of past variability. For instance, Strong et al. (2013) showed a clear divergence between biomarkers from 2000 years ago (2ka) to present day, probably due to local anthropogenic cultivation 2ka. This was done by analyzing biomarkers, bulk organic $\delta^{13}\text{C}$ and compound-specific $\delta^{18}\text{O}$ from sediment cores retrieved from the Pearl River Estuary in China.

An area in Thailand has been researched to predict the impact of climate change on groundwater recharge rates, waterlogging and saline groundwater analysis, by Saraphirom & Wirojanagud (2013). Their results pointed to higher groundwater recharge and higher precipitation in the future and that this climate change will have minimal effect on the saline distribution in the sub-watershed.

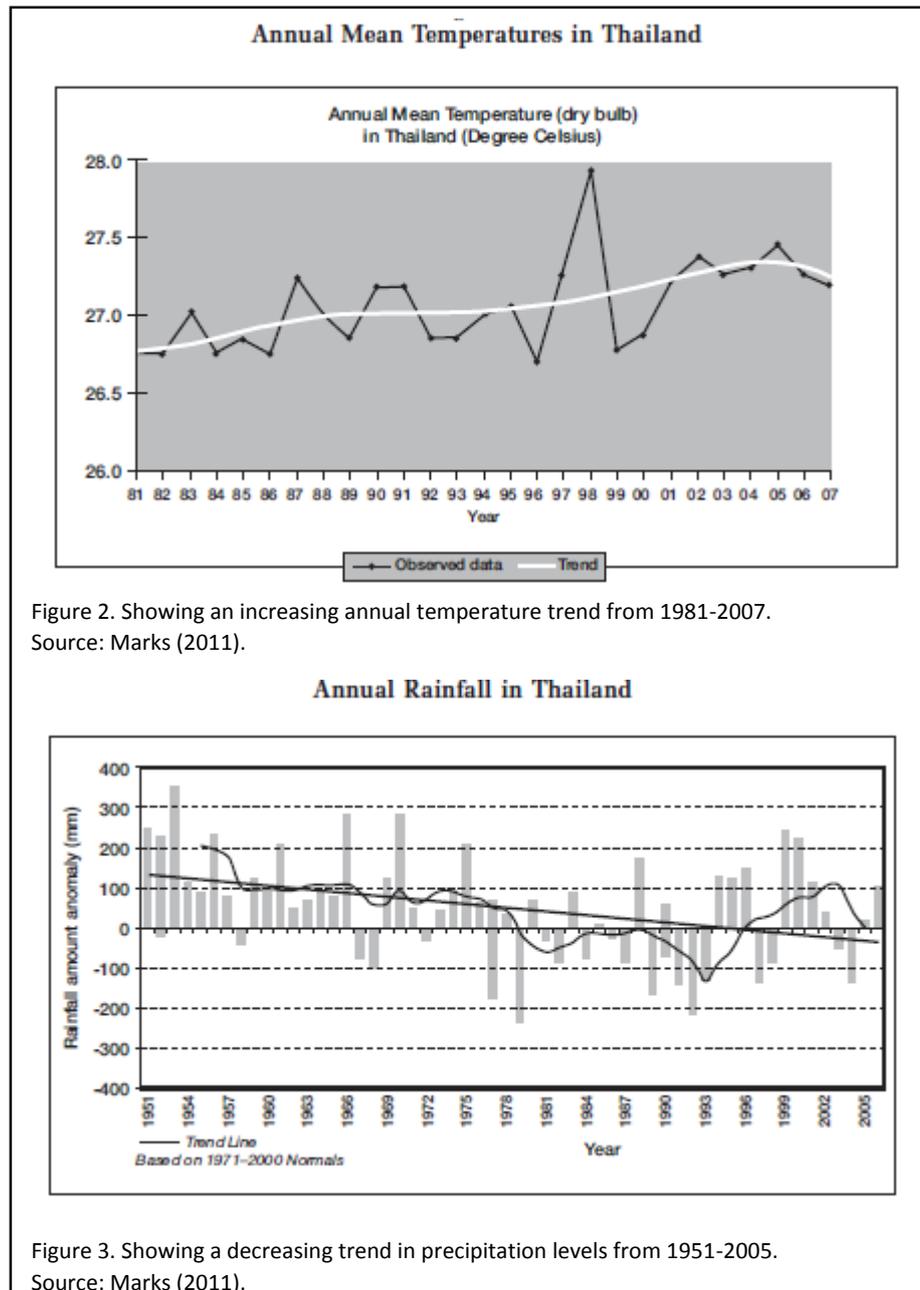
Other studies on the Asian monsoon systems effect include: the Asian summer monsoon inter-annual variability by Sato & Xue (2012), biomarker analysis on a crater lake in central India by Sarkar et al., (2014), long changes of Sea surface temperature (SST) over seas of China over the last 140 years by Bao & Ren (2014), and many more.

The need for accurate reconstructions and predictions of the monsoon variability is essential due to the fact that more than half of the human population is affected by the monsoon. Understanding how the monsoon's past changes affected the climate can contribute in the behavior of future climate changes (Strong et al., 2013).

The studied area for this thesis lies in the Southwestern part of Thailand in the Thung Yai district. Here sediment cores from Lake Nong Thale Pron were taken in December 2012, and brought to SU to be analyzed at the Department of Geological Sciences as part of a larger effort to learn more about the Asian Monsoon.

Thailand climate

Given its geographical location, Thailand will drastically be affected by climate change. In 2010 Thailand suffered a drought not seen in 20 years with one of its rivers water level falling to its lowest point in 50 years (Torsri et al., 2012). Thailand is located in the tropics south of China and affected by both a southwest and a northeast monsoon. The southwest monsoon affects the summer period, between May and October, and brings warm moist air from the Indian Ocean which then precipitates over land. While the northeast monsoon, affecting the winter period between November and April, brings dry cool air from the northeast (Torsri et al., 2012). According to Thailand's Department of Meteorology Thailand has already been influenced by climate change. Looking at chart trends from Figure 2 and 3, between the years of 1981 to 2007 the annual mean temperature had risen while the annual precipitation level shows a decrease in rainfall over the last 50 years (Marks, 2011).



Considering the monsoon variability in Thailand there are not many studies made to reconstruct climate and environmental changes due to monsoonal variations.

Aim

The main aim of this thesis entails in the analysis and interpretation of a lipid biomarker record from sediments of Lake Nong Thale Pron, Southwestern Thailand. This is the first step towards a stable hydrogen isotope-based reconstruction of past hydroclimate of the region. Better insight about the sources of lipid biomarkers are needed, which can be used to infer past limnological and environmental changes.

n-Alkanes

Biomarkers that are sensitive to environmental change are very useful to understand how past ecosystems and environments have changed through time. This is done by tracking the occurrence and amount derived from organisms with said biomarkers (Smittenberg et al., 2005). Hydrocarbons are molecules consisting of only carbon and hydrogen atoms, the smallest being CH₄,

methane. We focus on several types of hydrocarbons: 1. short- and long-chain *n*-alkanes; 2. Botryococcenes; and 3. highly branched isoprenoids (HBIs). These are further discussed below.

Identifying long chain hydrocarbons from lake and marine sediments, for the reconstruction of past climates, have been published for decades (e.g. Leider et al., 2013, Smittenberg et al., 2005, Strong et al., 2013). This through numerous methods of understanding the source of the sedimentary organic matter, such as the occurrence of molecular weight distribution and relative abundance of *n*-alkane, cycloalkane, isoprenoid and polycyclic aromatic hydrocarbons (Requejo et al., 1983). There are two different biosynthetic pathways that lead to two different types of hydrocarbons: acetogenic and isoprenoidal. Long-chain hydrocarbons are produced through an acetogenic pathway while highly branched isoprenoids and botryococcenes are produced by linking together isoprene units in an isoprenoidal pathway (Requejo et al., 1983).

Organisms produce normal alkanes (*n*-alkanes), which are part of the hydrocarbon family, but with an odd-over-even chain preference. Mutual to all forms of life, these organisms build unbranched carbon chains by assembling two carbon atoms at a time. Combining an acetic acid, $C_2H_4O_2$, with a malonic acid, $C_3H_4O_4$, forms a four-carbon acid with the loss a “carbon” (more exactly losing CO_2). Combining this four-carbon acid with another malonic acid, with the loss of yet another “carbon” (CO_2), will form a six-carbon acid, which can go on to create longer even chains (Figure 4). *n*-Alkanes are produced by snipping the carboxyl carbon, $COOH$, off the end which produces chains with odd numbers of carbon atoms (Gaines et al., 2009).

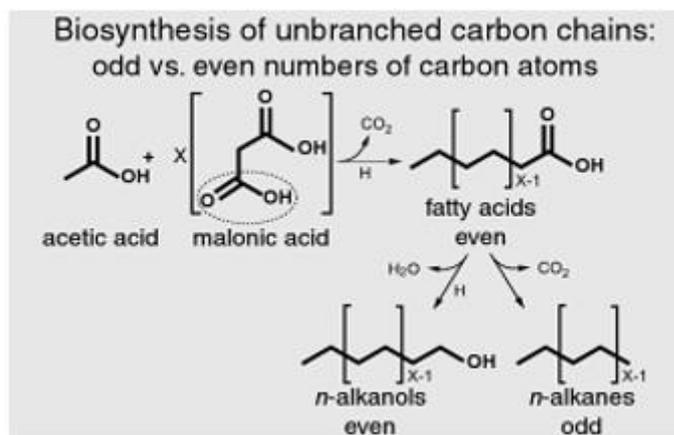


Figure 4. Showing how organism build unbranched carbon chains through an acetogenic pathway.
Source: Gaines et al., 2009

n-Alkanes are used as proxies and biomarkers. Depending on their origin, the mixture of *n*-alkanes can vary in dominant chain length, carbon number distributions and isotopic compositions (Castaneda & Schouten, 2011). Plants produce long-chain *n*-alkanes that contribute to the hydrophobic properties of their leaf wax, protecting the plants interior from the external environment (Bush & McInerney, 2013). This “barrier” is also what helps preserve the plant, making it robust and resistant to breakdown, once the plant has died and settled in sediment. Many studies have used these leaf wax *n*-alkanes as proxies to reconstruct past climates (Bush & McInerney, 2013). Long-chain *n*-alkanes (C_{23-35}) are usually produced by terrestrial higher plants (plants with seeds), for example the C_{31} is usually dominant in grasses and C_{27} & C_{29} are dominant in deciduous trees (Castaneda & Schouten, 2011). Large quantities of these compounds can be introduced into lake or marine environments by either fluvial or wind transport. Lake and marine environments also have aquatic organisms, such as algae, bacteria, and aquatic macrophytes (water plants), which produce short to mid chain *n*-alkanes. The molecular weight of the *n*-alkanes will be averaged out and thus not show a great dominance in the chain lengths of the *n*-alkanes (Duursma & Dawson, 1981). Lake

sediments can be used as a good indicator for lake-level change by looking at the relative input of terrestrially versus aquatic produced lipids (Yuxin He 2013). For example calculating the average chain length (ACL), gives information about the most dominant chain.

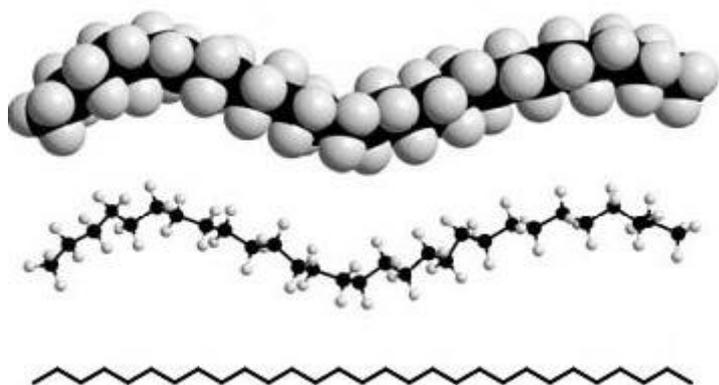


Figure 5. Nonacosane, $C_{29}H_{60}$, a typical long-chain n -alkane. Figure shows different means of drawing the structure. Source: Gaines et al., 2009.

Botryococcenes

Botryococcenes are derived from the green algae known as *Botryococcus braunii* (Smittenberg et al., 2005 and references therein). These microalgae's produce liquid hydrocarbons of C_{30} to C_{34} oils, which can potentially be converted into fuels such as gasoline, kerosene and diesel fuels (Weiss et al., 2010), although these algae do not grow very fast. *Botryococcenes* can also be used as indicators for oligotrophic lakes (Rosén, 1981), which are low in nutrient concentrations and have low plant growth. These algae are special because they have an ethyl group attached to their structure which is specific for the *botryococcenes*. In this paper the occurrence of botryococcenes will be used as a proxy and biomarker for environmental change.

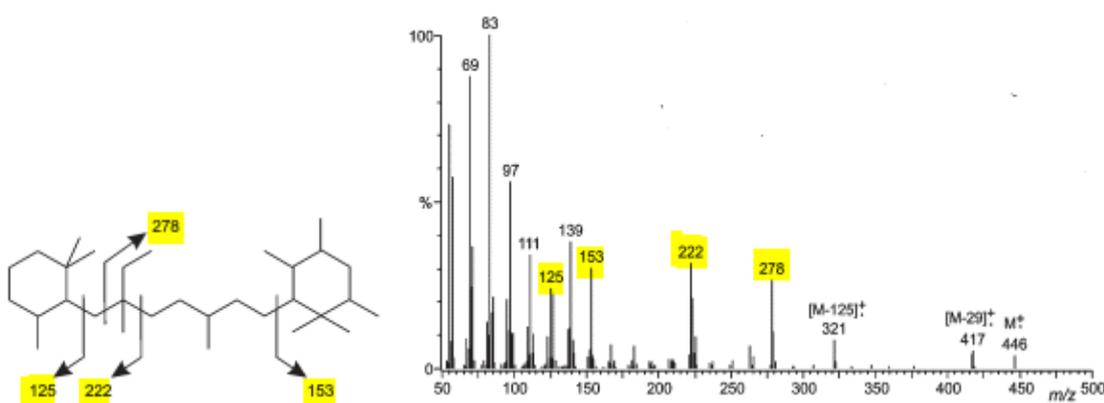


Figure 6. Showing an example of a C_{32} botryococcene structure and its mass spectra (x-axis is the molecular mass, y-axis is the abundance). The numbers and arrows on the structure indicates the break point in molecular mass which is shown in the mass spectra. Source: Smittenberg et al., (2005).

Highly branched isoprenoids (HBI)

A range of diatoms, ranging from benthic and planktonic to marine and fresh water species, have been shown to produce highly branched isoprenoids (HBIs) (Belt et al., 2001). HBIs have their structures built from a set of isoprenes (Figure 7), which are composed of a five carbon molecule structure that can either be saturated or unsaturated. For example five isoprenes would build up a C₂₅ HBI (Figure 8).

By analyzing the concentrations of HBIs in lake or marine sediments it is possible to correlate them with different growth stages of diatoms (Hird & Rowland 1995).

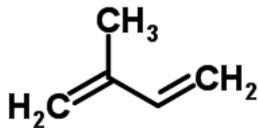


Figure 7. C₅ isoprene structure, this structure can be built in many different ways, with or without double bonds. Source: [URL 3].

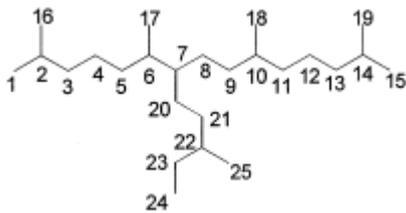


Figure 8. A C₂₅ HBI structure fully saturated. Source: Belt et al., 2000.

Macrophytes

Macrophytes are vascular plants that have been adapted to aquatic life (Sarkar et al., 2014) and grow in or near waters. The different macrophytes are: 1. Emergent; 2. Floating; or 3. Submerged (Figure 9). The emergent macrophytes have their vegetative parts over the water surface and the roots in shallow waters. Having their roots in sediment under water and their photosynthesis taking place in the air a perfect state is created for the plant (Mohammad R. Hasan et al., 2009). The floating macrophytes can either be rooted in the lake sediment at low depths with calm waters or be free floating with a few or no roots. Finally the submerged macrophytes are completely beneath the surface waters rooted firmly in the lake bottom (Mitchell & Prepas, 1990).

Macrophytes are important in shallow lake ecosystems because of their ability to produce oxygen, improve the water quality by influencing the nutrient influx and providing shelter and structure for other organisms (Tamire & Mengistou, 2012). Some macrophytes species have even been proven to inhibit the growth of algae bloom, but only for emergent and floating plant species (Zhao et al., 2013).

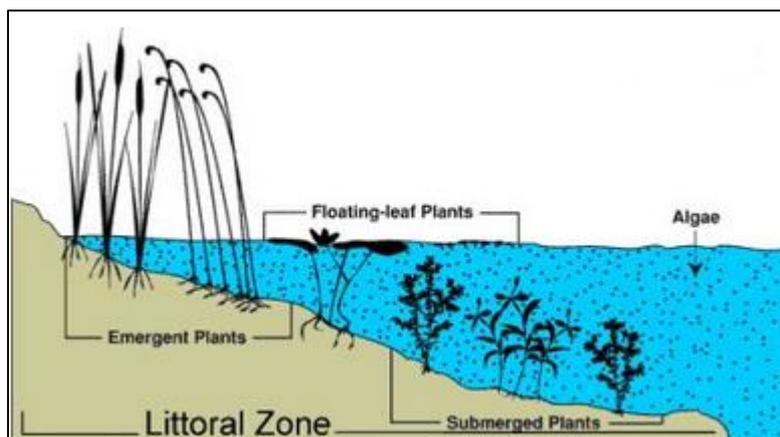


Figure 9. Showing how macrophytes are represented in lake systems. Source: [URL 2].

Gas chromatography Mass spectrometry (GC-MS)

There are different types of chromatography, such as: Gas-, Liquid, Thin layer-, Ion, Size exclusion- and Supercritical- chromatography. The basic principle is to separate and analyze compounds in either a liquid (mobile) or gaseous phase. Gas chromatography- mass spectrometry (GC-MS) is one of the most important and is widely used in the field of analytical chemistry (Gross, 2011).

Three processes are used when running samples through a Gas chromatograph (GC). The first process utilizes the *injector* to transports the sample, at a fixed speed, using an inert carrier gas (e.g. He, N₂ or H₂). Second process separates the molecules of the sample in a *column* from those with greater affinity to be in a stationary phase and those with an affinity to be in a mobile phase. Molecules are then retained by the *column* and come of at different retention times, which is recorded by the third process: *the detector* (Gross, 2011).

After the GC run, the molecules enter the Mass spectrometer (MS) where the characteristics of each individual molecule is measured. The MS has three functional components for this process. The first, *Ion source*, uses a high-energy electron beam to break the molecules into charged fragments, or ions, usually by loss of an electron. *Analyzer* is the second component which sorts and separated the ions according to their mass and charge. The third is the *detector*, here the ions are measured and results are demonstrated on a computer chart in the form of a mass spectra, which is a ratio of intensity of abundance in the molecule to the mass to charge ratio (Gross, 2011). See examples of compounds that are analyzed in this thesis in Figures 10, 11 and 12.

This paper presents the concentration and distribution of *n*-alkanes and biomarkers through a sediment core retrieved from Nong Thale Pron.

The methods entailed in the extraction of lipids through normal column- and silver nitrate column- chromatography. These lipids were then analyzed using a GC-MS and the data was imported from LabSolutions GCMS Version 4.11 SU1 into Microsoft Excel where it was quantified.

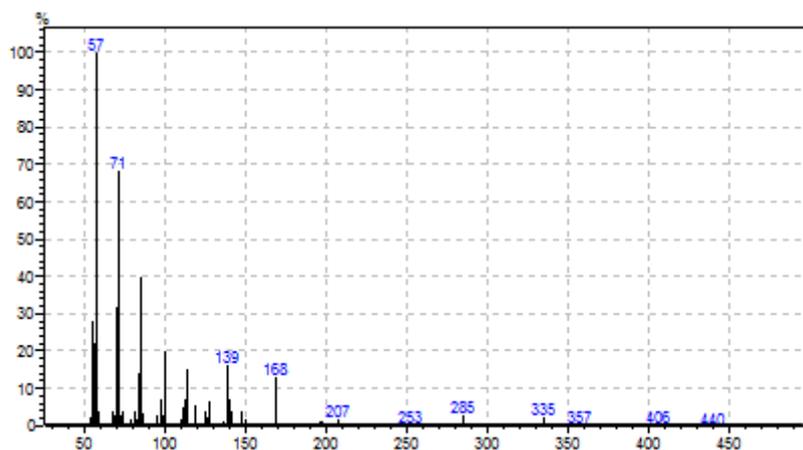


Figure 10. Spectrum showing the C_{17} n-alkane, the x-axis= molecular mass while the y-axis= the ratio of intensity of abundance in the molecule.

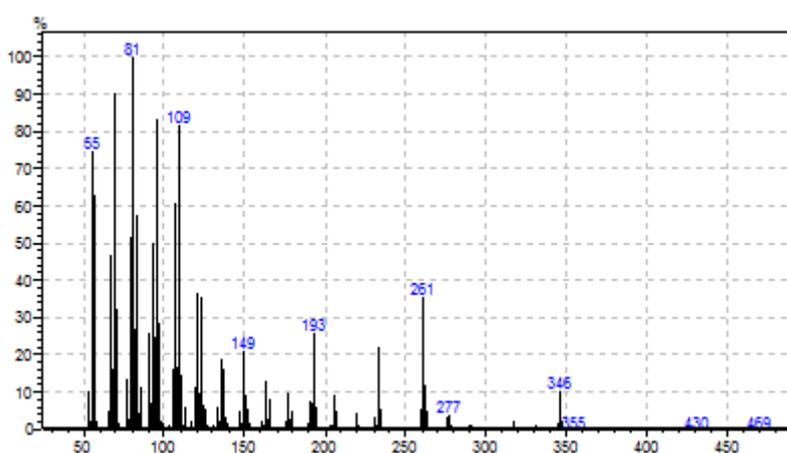


Figure 11. Spectrum showing the C_{25} HBI shown on Figure 25. The x-axis= molecular mass while the y-axis= the ratio of intensity of abundance in the molecule.

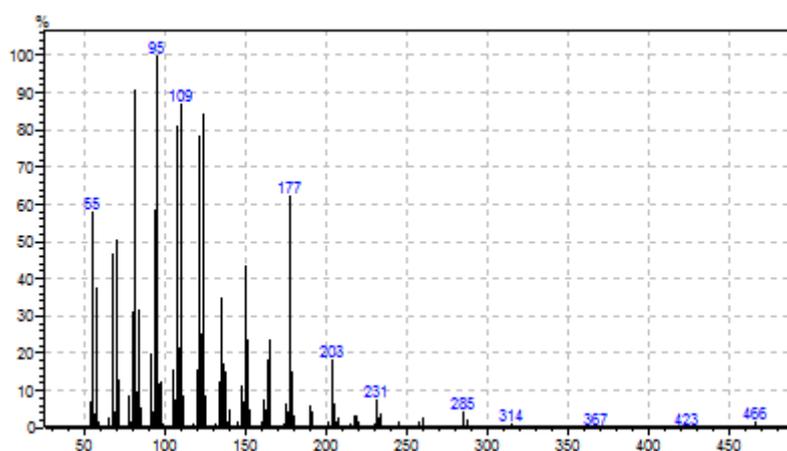


Figure 12. Spectrum showing the $C_{34:5}$ Monocyclic Botryococcene, with x-axis= molecular mass while the y-axis= the ratio of intensity of abundance in the molecule.

Setting

GPS (UTM) coordinates: 0541369 0902628 (set after map Figure 13).

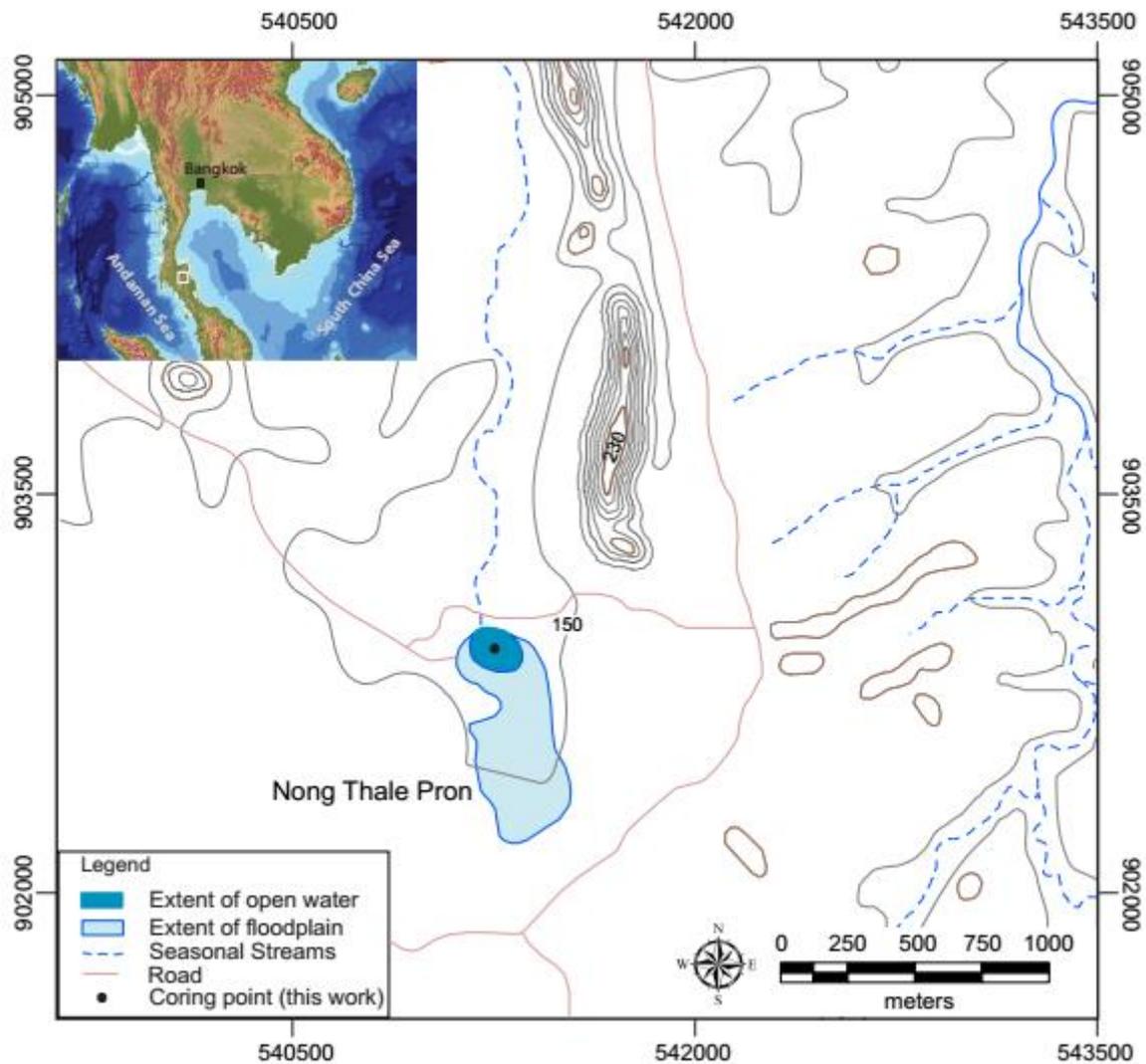


Figure 13. Showing the point (square in the little map) of the sampling area. The larger map is a black and white elevation map (elevation map in color in presented further below). Source: Map created by Akkaneewut at Stockholm University.

Nong Thale Pron, located in the Thung Yai district in the Southwestern part of Thailand. Here the summer monsoon is warm and wet coming from the southwestern direction, and the winter monsoon, coming from the northeastern direction, is cool and dry. This lake is slightly protected from the winter monsoon due to high mountain ranges blocking part of the winter monsoon in this region. Sampling was done on the 15th of December 2012 using a gravity corer.

The lake is positioned in a sinkhole with nearby limestone mountain ranges, surrounded by sedimentary sandstone, laterite and carbonate rocks. Visible caves are seen near the lake located in the mountains. The average water depth of the lake is 3.5 meters. 150 meters south of Nong Thale Pron another lake is situated and believed to be linked to this one.

Material & Methods

Tools for sampling

Sediments were taken from Lake Nong Thale Pron utilizing a gravity corer. The gravity corer was composed of a plastic tube with a weight attached. The corer had a wire, attached to the top, which was used to lower it to the lake bottom. Here the corer penetrated the sediment and a vacuum was made at the top, due to pressure differences, to bring the sediment up intact. The bottom of the tube was sealed using a rubber cork. The core was sliced into 1 cm pieces, starting from the top, which were sealed in separate plastic bags.

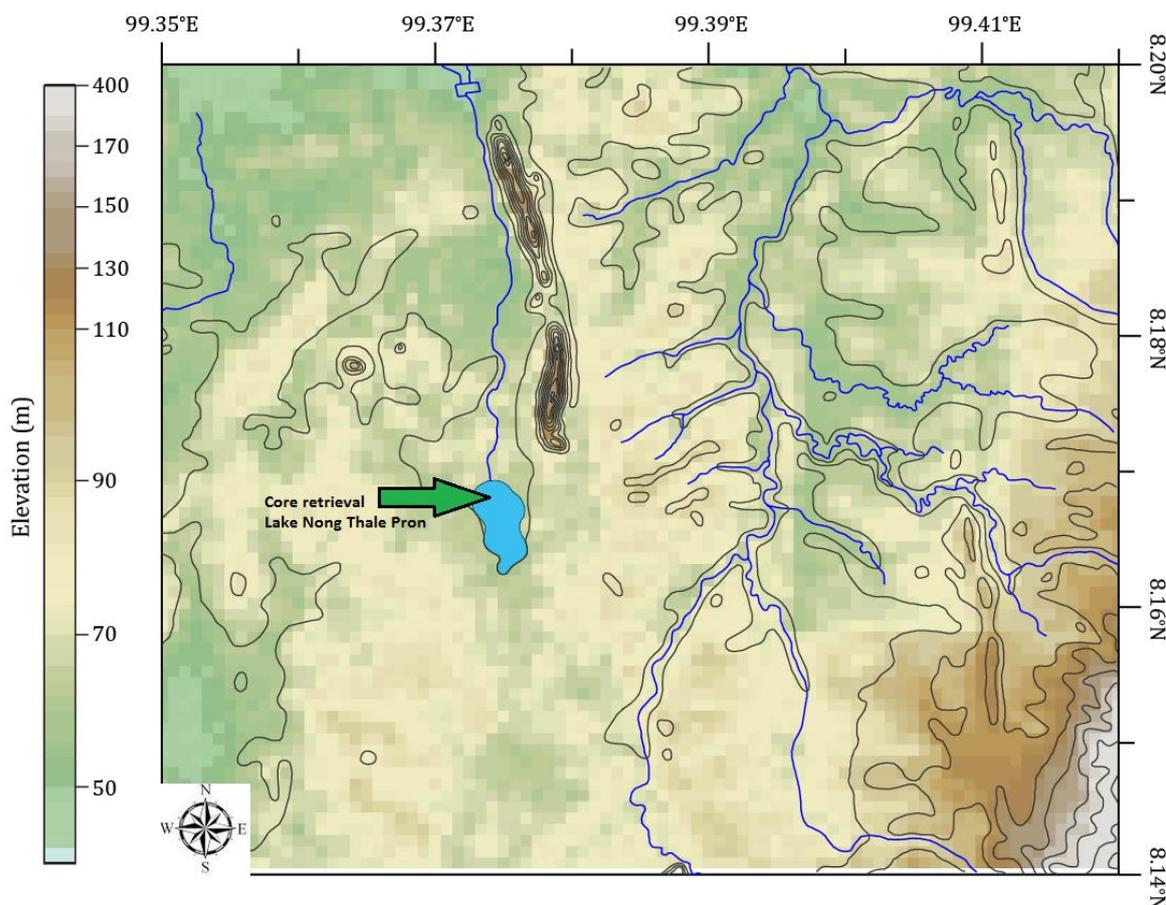


Figure 14. Map of Lake Nong Thale Pron with elevations in meters shown, green arrow indicates the area in which the core was retrieved from. Source: Map created by Akkaneeuwat at Stockholm University.

Sample preparation

Many steps were made to avoid contaminants (e.g. plasticizers or organic matter etc.). The sediment samples were analyzed every cm (0-74 cm). The sediment samples were freeze-dried and homogenized with a mortar before extraction. The weight ranged from 0.6-1.0 grams.

The extraction of lipids started with a freeze-drying process, to remove the water from the samples. To the sediment samples a mixture of dichloromethane (DCM) and methanol (MeOH), with an exact ratio of 9 to 1 v/v, is added to extract the lipids. Extraction was performed in a microwave (Milestone Ultrawave) designed for digestion/extraction and the extracts were separated from the solid material after centrifugation. The extraction was repeated two more times to ensure that all lipids/compounds were extracted. The sediment residue was kept for future analysis, among other things bulk $\delta^{13}\text{C}$ analysis. Each individual sample was then mixed with a 5% deactivated silica gel which was used in the column chromatography.

Normal column chromatography

The normal chromatography rinses the compounds with different solvents to get fractions with the least- to more- and more-polar compounds. The least polar fraction will yield the hydrocarbons and other low polar compounds, this is done by rinsing the column with hexane. The second fraction yields more polar compounds, this by rinsing the column with hexane: DCM solution with a 1 to 1 v/v ratio. The third fraction yields all the remaining compounds with a DCM: MeOH solution with a 1 to 1 v/v ratio. Only the first fraction is analyzed.

Silver nitrate column chromatography

To acquire separation of hydrocarbons with different amounts of double bonds, for better identification by GC-MS, silver nitrate chromatography was performed on two hydrocarbon fractions. They were added to a column individually filled with silver nitrate gel. Here the column was first rinsed with Hexane, the second fraction with Hexane: DCM (1 to 1 v/v ratio), the third with DCM, the fourth with DCM: Acetone (9 to 1 v/v ratio), the fifth with DCM: Acetone (1 to 1 v/v ratio) and the final was rinsed with Acetone: Acetonitrile (1 to 1 v/v). Both of the samples were analyzed with the GC-MS.

GC-MS properties

The lipids were quantified using a gas chromatography-mass spectrometry (GC-MS) on a Shimadzu GCMS-QP2010 Ultra, equipped with an AOC-20i auto sampler and a split-splitless injector operated in splitless mode. A Zebron ZB-5HT Inferno GC column (30m x 0.25mm x 0.25 μ m) was used for separation. The GC oven temperature was programmed to start at 60°C holding for 2 minutes and then increased to 180°C (at a rate of 15°C minute⁻¹) and then ramped to 320°C (at a rate of 4°C minute⁻¹) with a hold time of 20 minutes (Figure 15). The sampling time was set to 1 minute using pure helium as the carrier gas with a pressure of 57.4kPa.

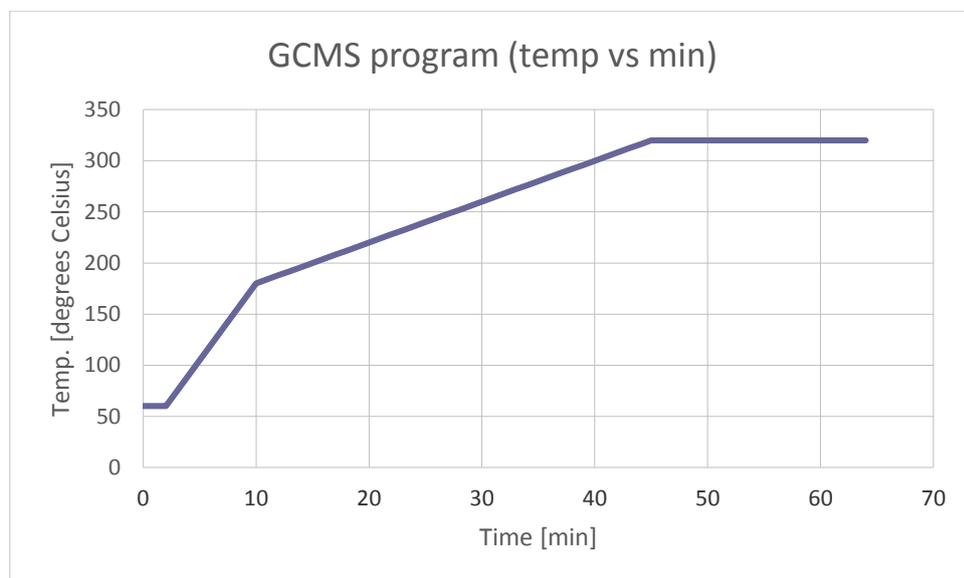


Figure 15. Showing the program that the GCMS was set to follow.

Note: *this temperature program was set for the lipids extracted from the normal chromatography.
*the silver-nitrate GC-MS program differed somewhat so that especially *botryococenes* and HBIs would separate well.

Results

- Graph data with calculated total concentration *n*-alkanes, HBI and botryococcenes of dry weight Nong Thale Pron sample ($\mu\text{g g}^{-1}$ dry weight (dw) sample) versus depth (cm).
Concentration calculated: $C_x [\mu\text{g g}^{-1} \text{ dw sample}] = \frac{(\text{Area of peak received from GCMS})}{(\text{average peak area from Quantification standard}) * (\text{volume of GCMS vial } [\mu\text{l}])} / \text{dry sediment weight [g]}$; where x is the length for a carbon.

Note: *Volume of GCMS vial differs on each vial, going from 1300-1500 μl .

*The Quantification standard was run first and after every eight Nong Thale Pron sample. The difference between each Quantification standard was calculated to have a standard deviation of 0.17%. Thus the average area for each *n*-alkane was the same in each calculation.

*Specific depth samples that were missing [in cm]: 0.5, 3.5, 6.5, 10.5, 13.5, 17.5, 21.5, 25.5, 29.5, 35.5, 42.5, 46.5, 51.5, 56.5, 60.5, 65.5, 69.5 and 73.5. These were kept for aside for future Pb^{210} dating.

* C_{19} and C_{21} were not represented due coelution by the C_{25} HBIs.

- The silver-nitrate column chromatograph was only used to ensure that no background data was present in the *Botryococcenes*. Only two sample depths were analyzed: 45.5 cm and 67.5 cm. These had no effect on the results, the spectrums can be found in the appendix (Figures 35-40). These results will not be shown in the results or be discussed.
- $P_{\text{aq}} (C_{23}+C_{25})/(C_{23}+C_{25}+C_{29}+C_{31})$, (Ficken et al., 2000) quantifies the ratio of mid-chain length to long-chain length homologous and can be used as a tool to estimate the input of submerged/floating aquatic macrophytes versus emergent and terrestrial plant input to sediment. Results were interpreted as follows:
 $P_{\text{aq}} < 0.1$ =terrestrial plants
 P_{aq} between 0.1-0.4=emergent macrophytes
 P_{aq} between 0.4-1.0=submerged/floating species
- ACL is the average chain length plotted here versus depth [cm].
$$\text{ACL} = \frac{(23 * C_{23} + 25 * C_{25} + \dots + 35 * C_{35})}{(C_{23} + C_{25} + \dots + C_{35})}$$

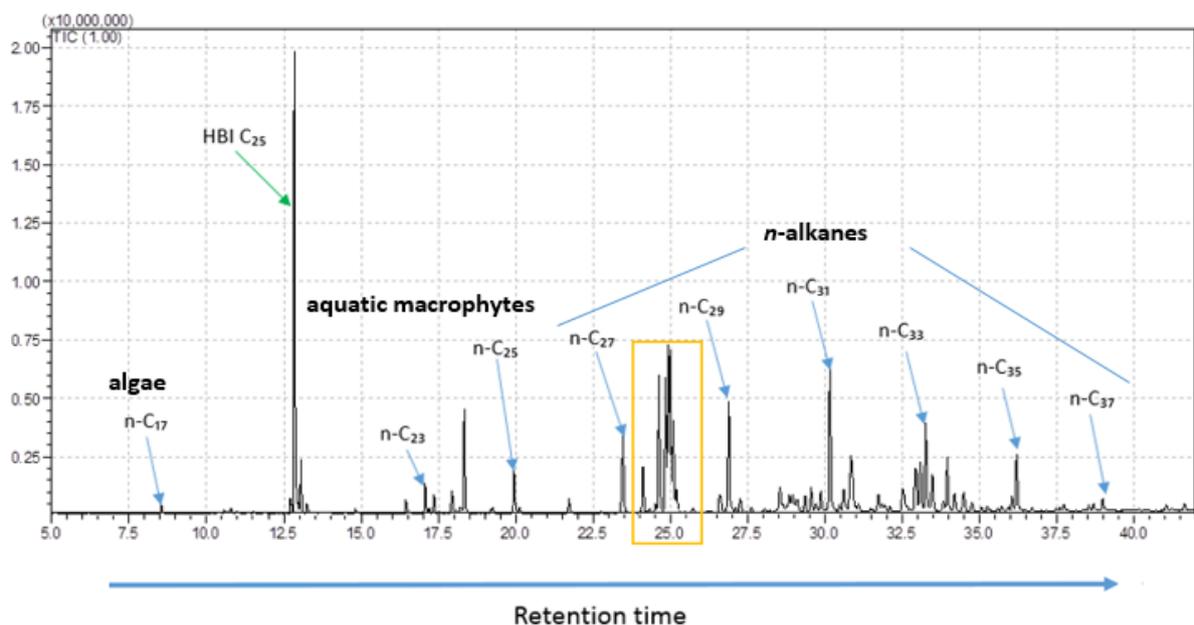


Figure 16. A GC-MS chromatogram of Nong Thale Pron sample, depth 70.5 cm. X-axis= retention time [min] and y-axis= abundance of compound

Note: *the total run time is not shown due to no more visible peaks

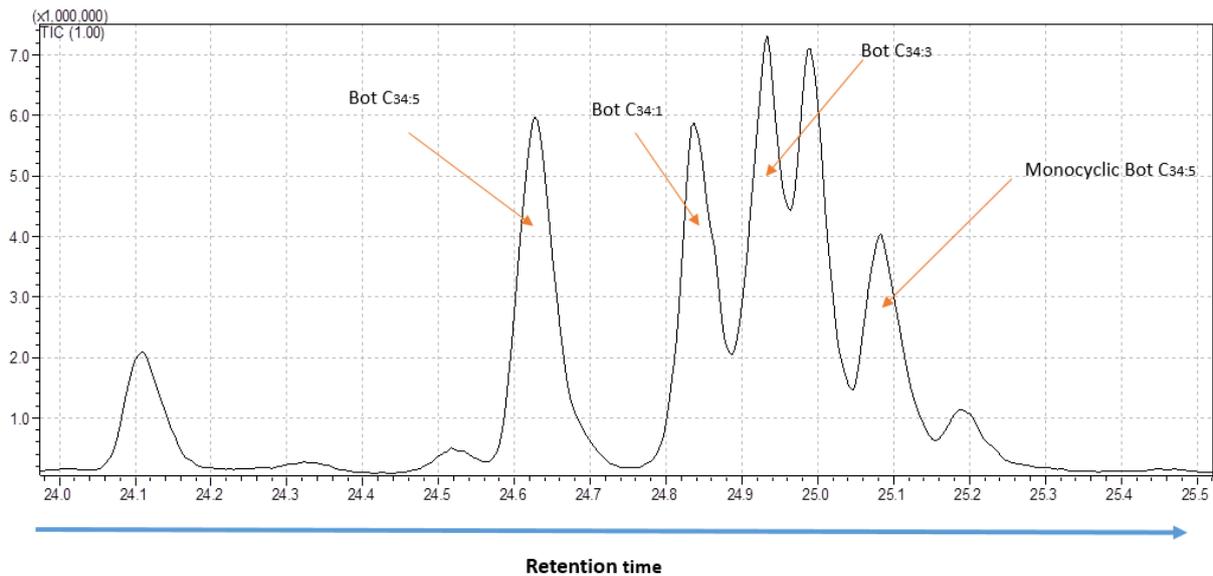


Figure 17. Figure shows an enhancement of the marked area in figure 16. Here the botryococcenes with their retention time and corresponding peak heights/areas. X-axis= retention time [min] and y-axis= abundance of compound

n-Alkanes

The odd numbered *n*-alkanes ranging from C₁₇ and C₂₃-C₃₅ (Figures 18, 19, 20 and 30-34) were quantified from the sediments retrieved from the Lake Nong Thale Pron.

The overall trend of the C₁₇ *n*-alkane is that it decreases with depth, rapidly (Figure 18), going from 54 [µg g⁻¹ dw sample] at the 2.5 cm mark then decreasing to 0.6 [µg g⁻¹ dw sample] at 44.5 cm depth. From 44.5 cm to the 72.5 cm mark C₁₇ is holding more steadily with an average concentration of 0.5 [µg g⁻¹ dw sample]. Overall, this *n*-alkane is extremely high in concentrations the first 20 cm but is continuously decreasing to values as low as 0.3 [µg g⁻¹ dw sample].

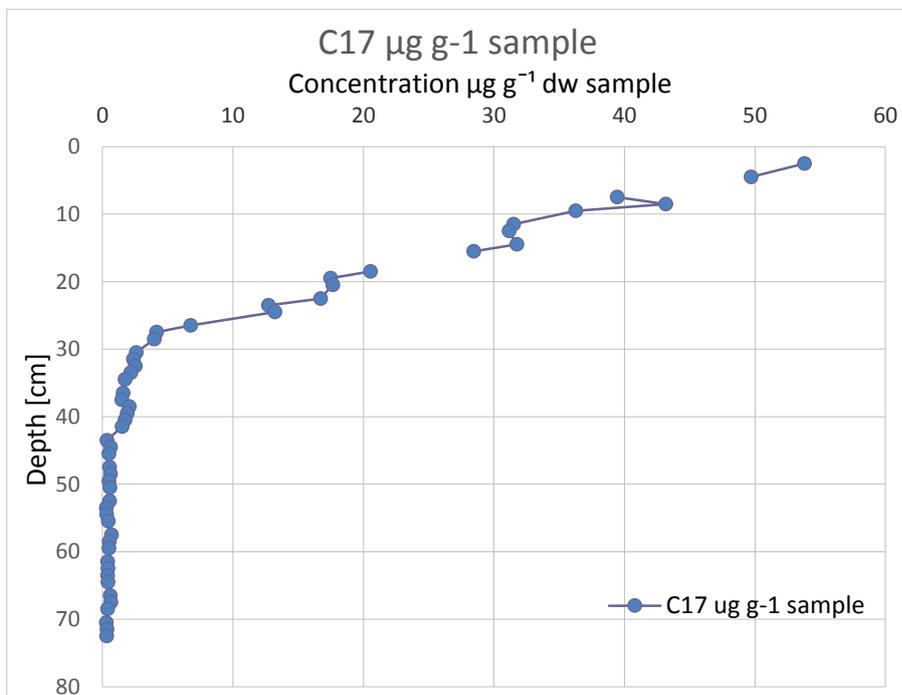


Figure 18. The allotment of the *n*-alkane C₁₇ [µg g⁻¹ dw sample] versus depth [cm].

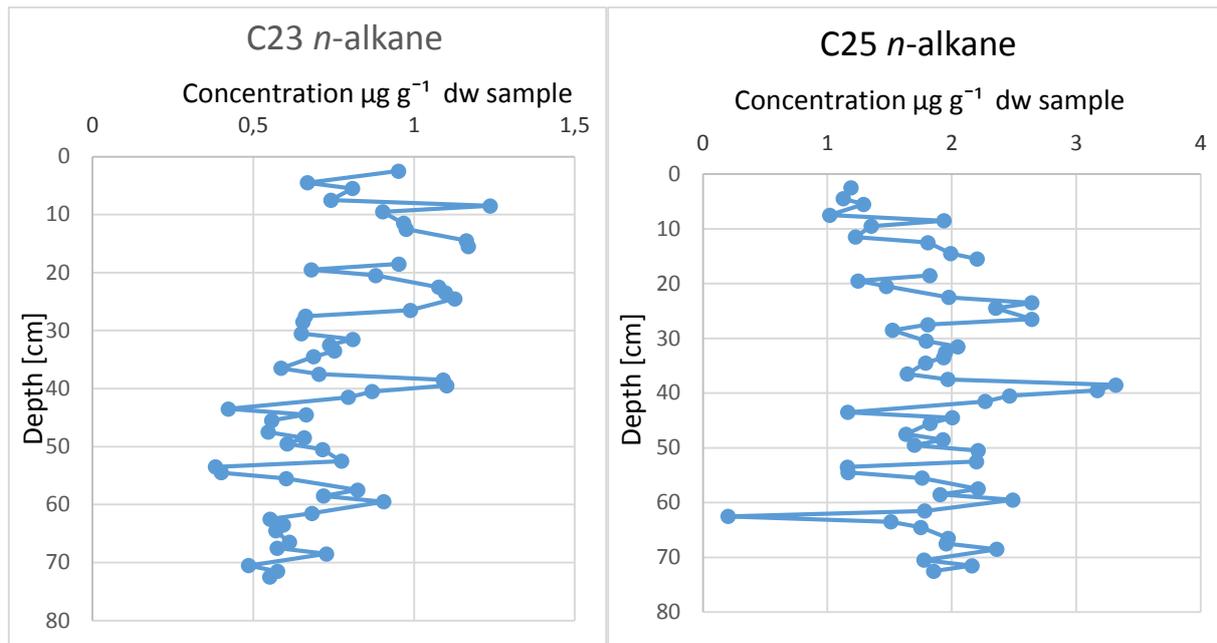


Figure 19 (left) and 20 (right). The left one shows the allotment of the C_{23} *n*-alkane [$\mu\text{g g}^{-1}$ dw sample] versus depth [cm]. While the right one shows the allotment of the *n*-alkane C_{25} [$\mu\text{g g}^{-1}$ dw sample] versus depth [cm].

The C_{23} *n*-alkane shows a decreasing concentration trend (Figure 19), decreasing from 0.95 [$\mu\text{g g}^{-1}$ dw sample] at 2.5 cm to 0.55 [$\mu\text{g g}^{-1}$ dw sample] at 72.5 cm.

The C_{25} *n*-alkane shows a slight increase in concentration with depth (Figure 20), increasing from 1.2 [$\mu\text{g g}^{-1}$ dw sample] at 2.5 cm to 1.9 [$\mu\text{g g}^{-1}$ dw sample] at 72.5 cm.

The long-chain C_{27-35} *n*-alkanes are averaged out both because their trends through depth are similar, all increase slightly (appendix Figures 30-34), and the fact that they are mainly produced from terrestrial leaf waxes. The average of C_{27-35} is done by adding all the concentrations from a depth and dividing it by 5. Looking at Figure 21, the trend shows a clear increase in concentration the deeper the sediment samples go (from 2.8 to 3.5 [$\mu\text{g g}^{-1}$ dw sample] from top to the bottom of the core).

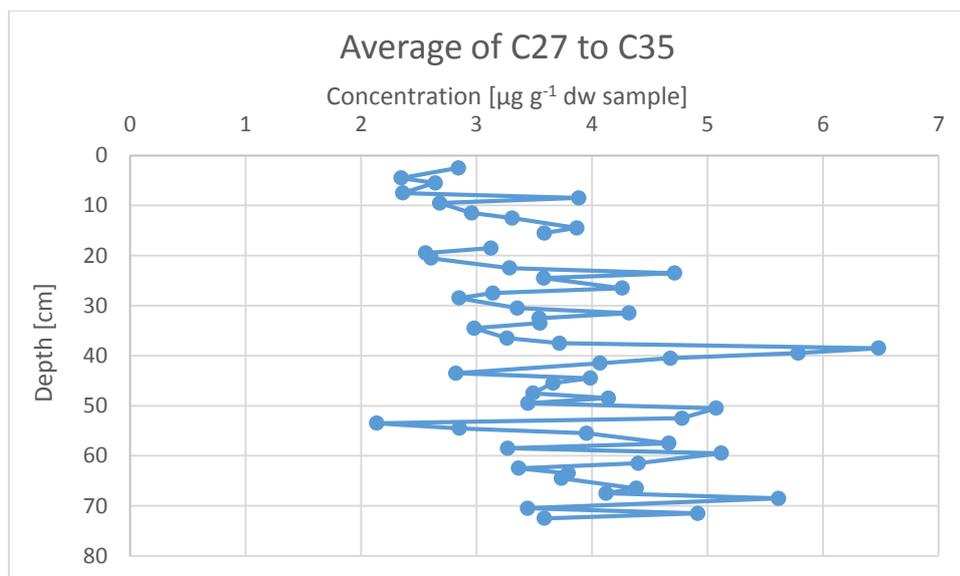


Figure 21. Shows the average allotment of C_{27-35} *n*-alkanes [$\mu\text{g g}^{-1}$ dw sample] versus depth [cm].

The average chain length (C_{23-35}) shows the *n*-alkanes to be very near a length consisting of 30 carbon atoms. From a depth of 2.5 cm to around 26.5 cm the overall trend shows a decrease with depth

going from 30 to 29.5, while the other half of the core (26.5-72.5 cm) shows a clear increase with depth to going from 29.5 to 30.5.

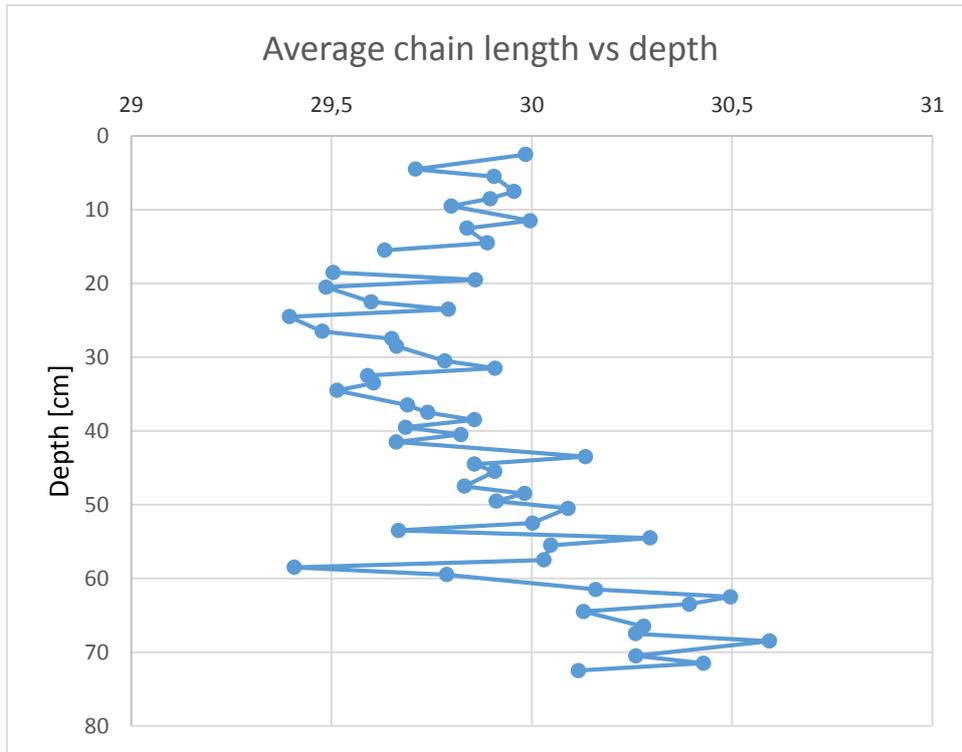


Figure 22. Showing the ACL through depth.

Note: *the C₃₇ is not represented, because it could not be distinguished from background data.

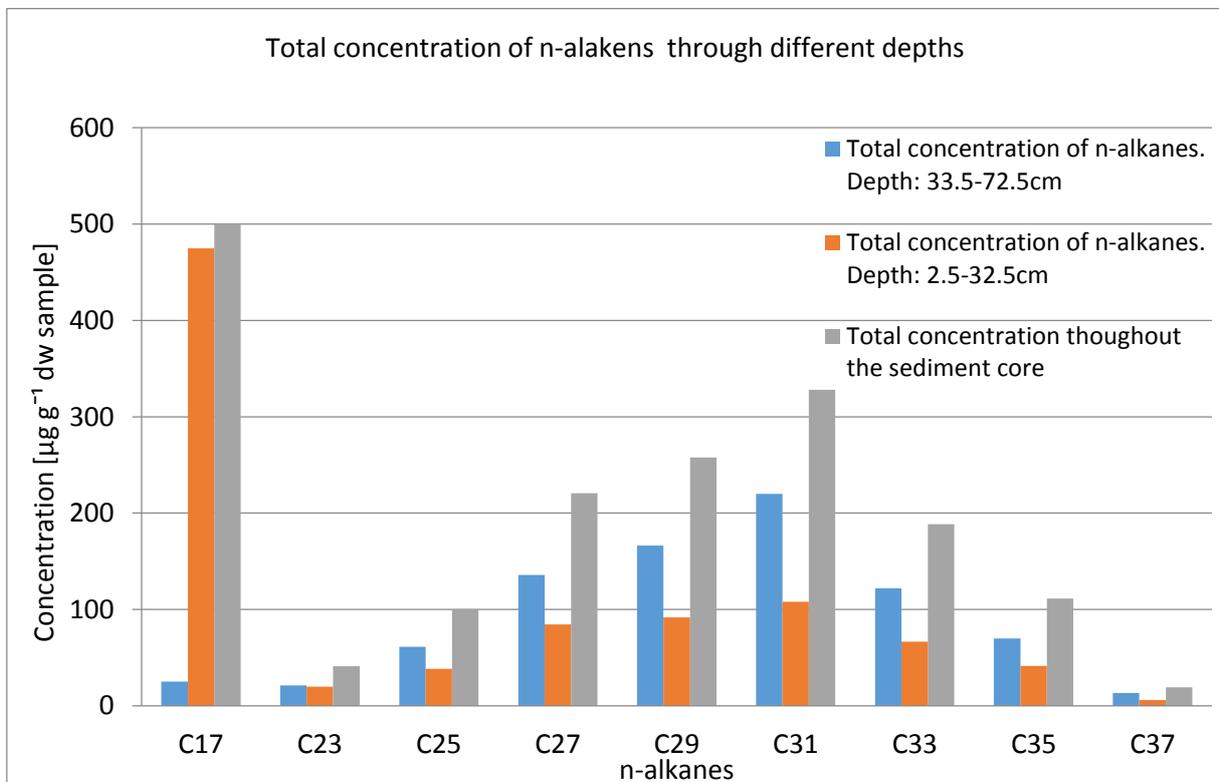


Figure 23. Showing total concentration of *n*-alkanes throughout and at different depths of core.

Even though C_{17} almost completely disappears at the end, it still has the highest total concentration of all n -alkanes (Figure 23). C_{27} , C_{29} , C_{31} and C_{33} are the most abundant of the long chain n -alkanes in the total concentration throughout the core with C_{31} being the most abundant off them all.

Botryococcenes

Each of the measured *botryococcenes* had a carbon chain length of 34, but with a different structure. $C_{34:5}$ has a five unsaturation and/or ring structure with 34 C atoms.

The *botryococcenes* trends in the results (Figure 24) have a notably small increase from 0.5 and 2 [$\mu\text{g g}^{-1}$ dw sample] (in the Bot $C_{34:1}$ and Bot $C_{34:3}$, respectively) at 2 cm depth to about 1.5 and 5 [$\mu\text{g g}^{-1}$ dw sample] at 30cm depth.

In the 32-60 cm depth an increasing shift is seen and new *botryococcenes* appear, which also increase and become more abundant in concentration, these increase up to about 14, 10, 12 and 6 [$\mu\text{g g}^{-1}$ dw sample] in the Bot $C_{34:1}$, Bot $C_{34:3}$, Monocyclic Bot $C_{34:5}$ and Bot $C_{34:5}$, respectively. However, at a depth of 60cm the trend changes and the concentrations decrease slightly going to an average, for all *botryococcenes*, of around 5 [$\mu\text{g g}^{-1}$ dw sample] at 72.5 cm depth.

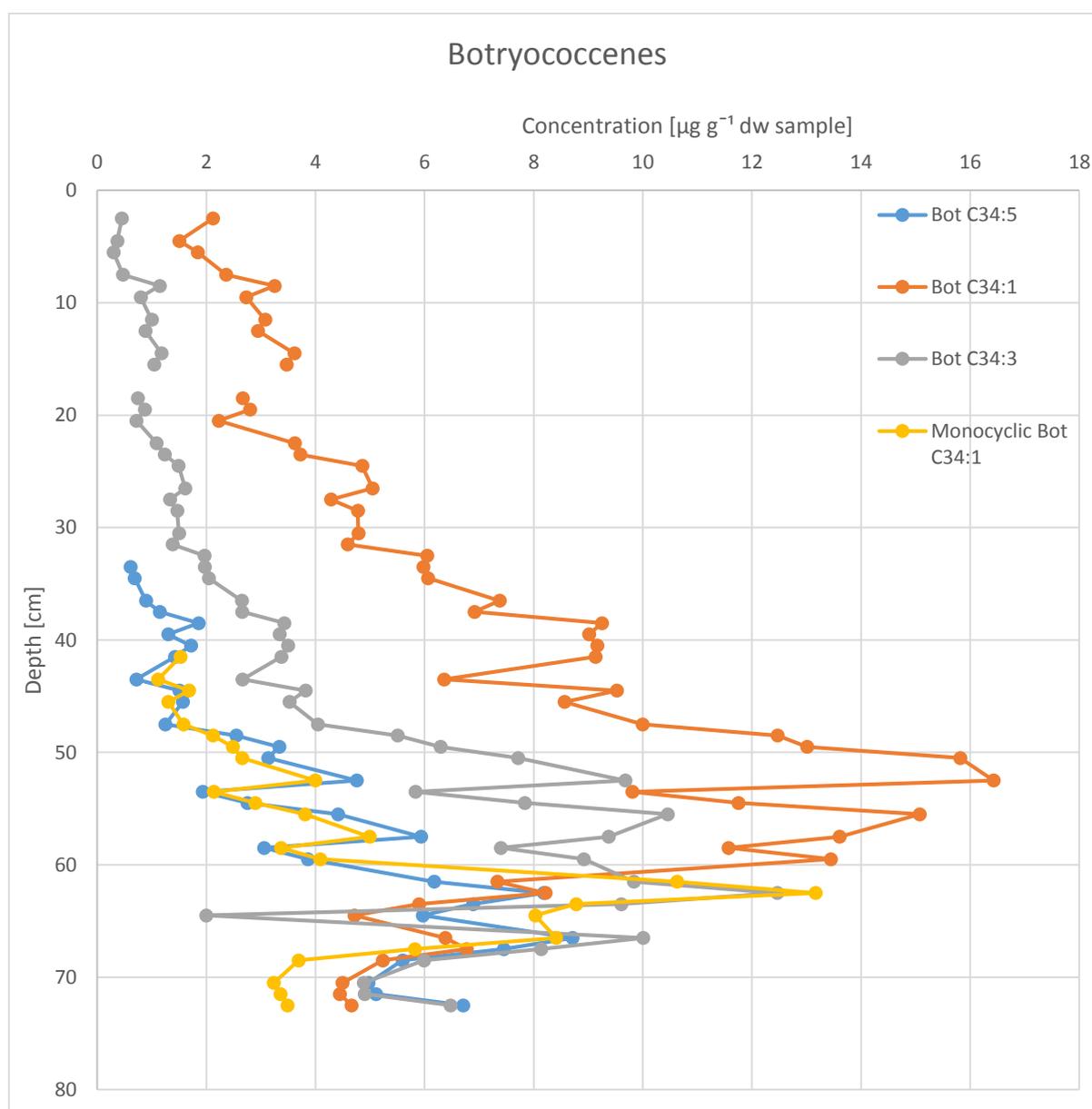


Figure 24. Showing the concentration of botryococcenes (all C_{34}) [$\mu\text{g g}^{-1}$ dw sample] through depth [cm].

HBI

The concentration of the HBI varies in the upper 0-30 cm (Figure 25) and a clear trend can be made that the concentration is increasing through this depth, from 75 to as high as 220 [$\mu\text{g g}^{-1}$ dw sample]. At around 30 cm the trend shifts to a decreasing trend from 220 to 150 [$\mu\text{g g}^{-1}$ dw sample] from 30-42 cm. However, at the 42 cm mark the results shows further but now more rapid decrease, going from 150 to 25 [$\mu\text{g g}^{-1}$ dw sample] in just a one cm. After this the concentrations decrease more slowly with depth, going to 11 [$\mu\text{g g}^{-1}$ dw sample] at 72.5 cm.

Note: *Only one measured HBI is presented because co-elution with other compounds made quantification unreliable.

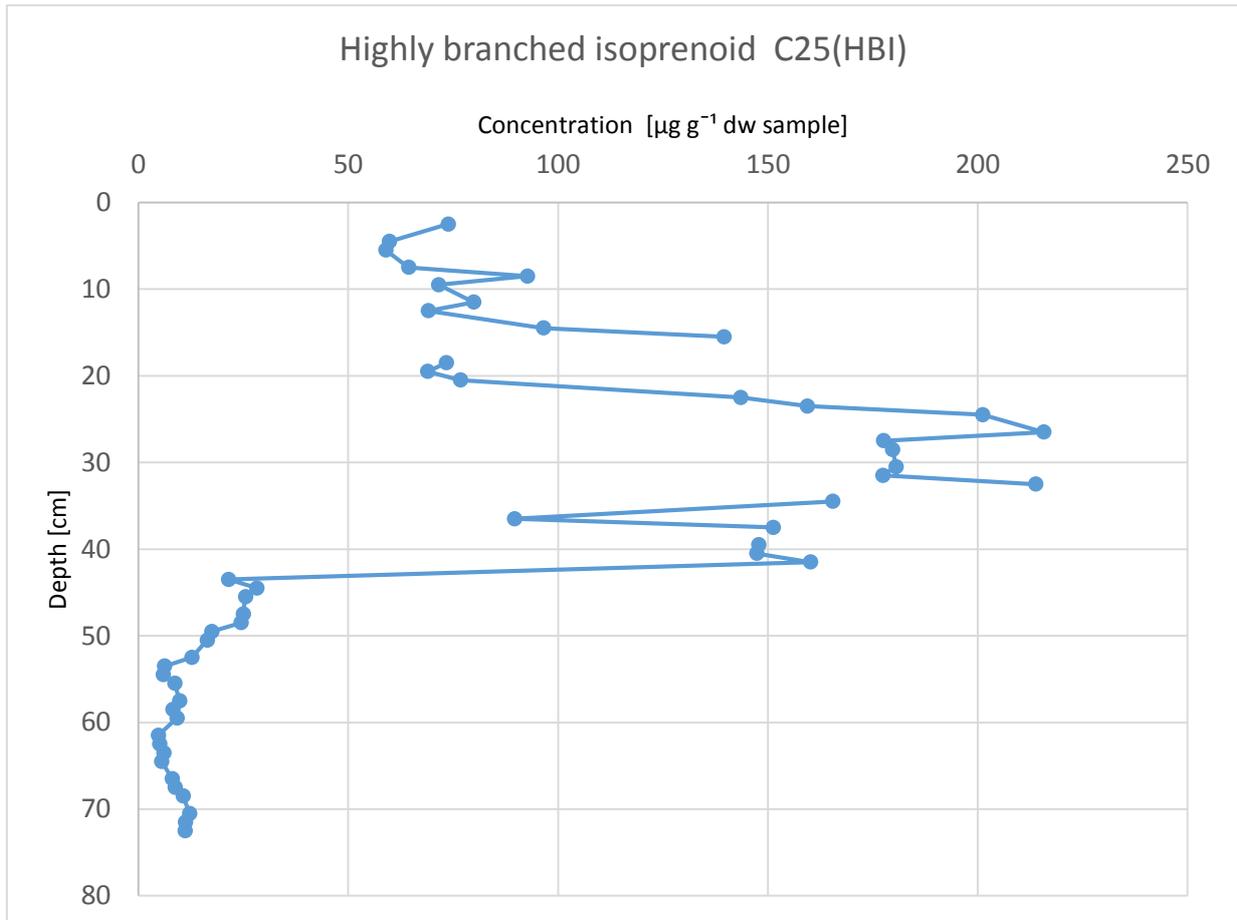


Figure 25. Showing the C₂₅ HBI concentrations [$\mu\text{g g}^{-1}$ dw sample] through depth [cm].

P_{aq} and ACL

The values for the P_{aq} , according to the results (Figure 26), seem to all be in the 0.1-0.4 values indicating that the input into the lake is mostly that of emergent plant types.

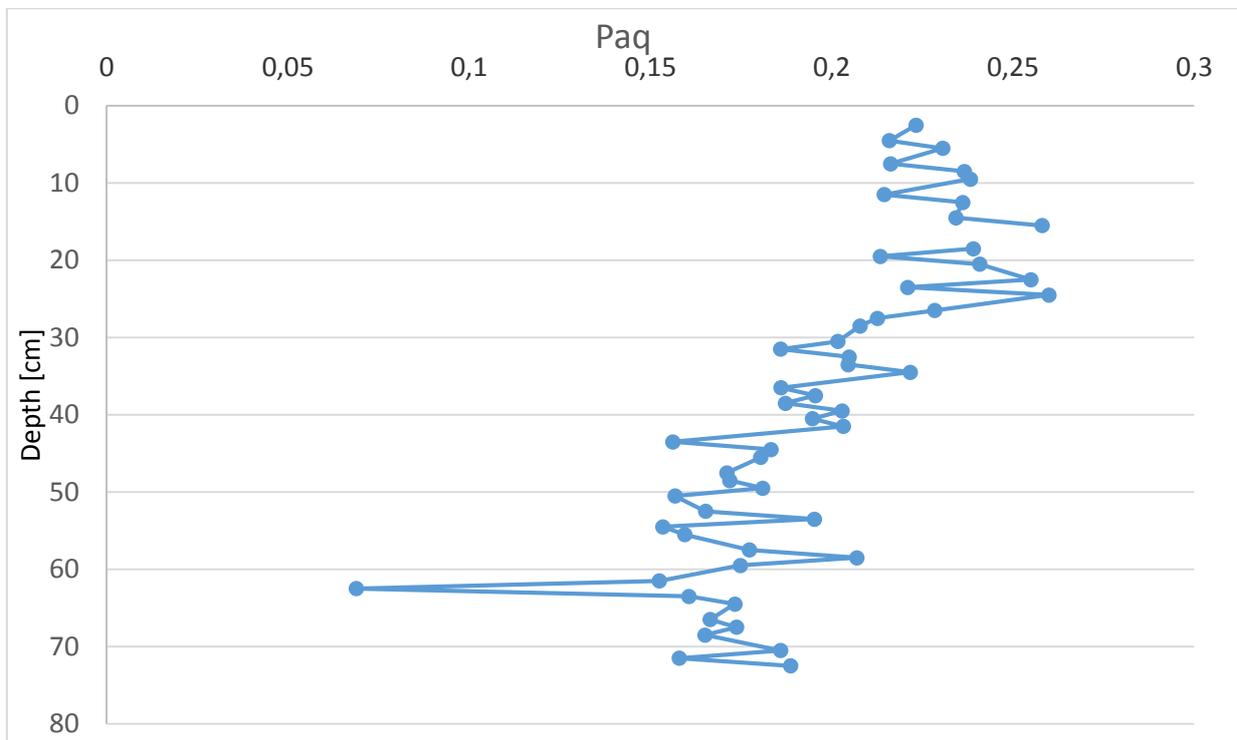


Figure 26. Showing the P_{aq} values through depth [cm].

Discussion

n-Alkanes

The most dominate odd numbered long-chain *n*-alkanes in this paper are C₂₇ – C₃₃ (Figure 23). These are usually derived from terrestrial plants (Punyu et al., 2013). High amounts of terrestrial plant *n*-alkanes lead to higher ACL (Figure 27) and lower P_{aq} (Figure 26), (Punyu et al., 2013) which is consistent with the presented results especially in the deeper samples. C₁₇ is a short chain *n*-alkanes which indicates the contribution of phytoplankton, such as algae and cyanobacteria (Punyu et al., 2013).

Botryococenes and C₁₇ algae

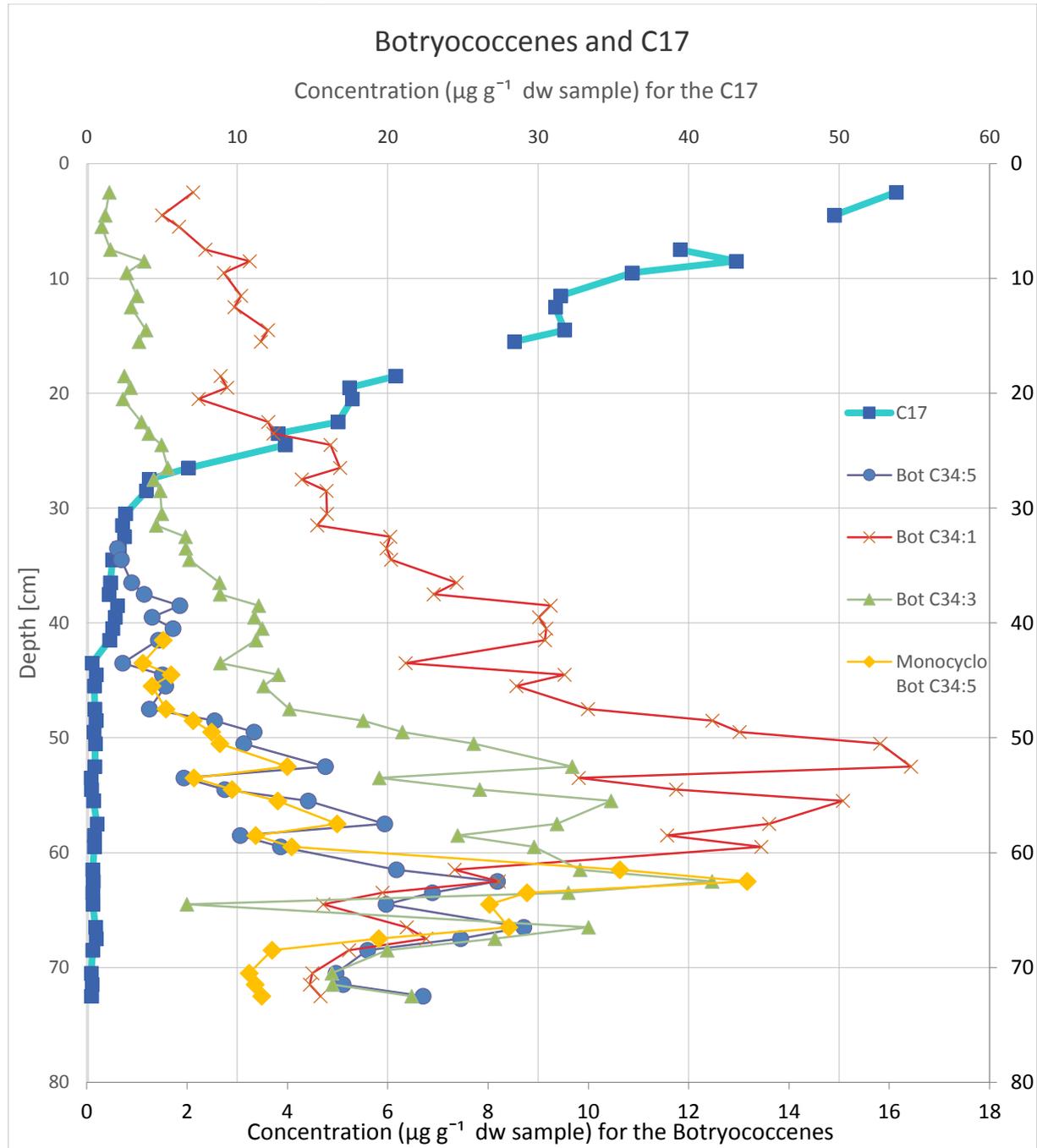


Figure 28. Logarithmic scale concentration of C₁₇ and the *botryococenes* versus depth. The decrease of the C₁₇ *n*-alkane correlates with the increase in the *botryococenes*.

As mentioned above the *botryococenes* can be used as indicators for oligotrophic lakes, the decrease in concentration suggests that something must have happened to the lake. The clear increase in C_{17} , derived from algae or cyanobacteria, which flourish when nutrients are present, also suggest a change in the lake. If C_{17} and the *Botryococenes* are plotted against each other a clear correlation can be seen (Figure 28). This suggests that the lake has evolved from an oligotrophic conditions to more mesotrophic or even eutrophic conditions.

Circumstances that might have induced this shift in trophic state of the lake: 1. a drought or reduction of precipitation would evaporate more water and the nutrient levels would become more concentrated, but with this “drought” the algae bloom would not flourish in the top section of the core as shown by the C_{17} algae (Figure 18); 2. human activities that enrich the lake system through fertilizing soils that increase nitrogen and phosphorus levels, because precipitation which would bring nutrients to the lake through soil penetration and run off into the sinkhole lake it would further enrich the lake; 3. More activity and mixing in the sediment and lake would induce higher nutrient concentrations. These are just some of the possible conditions that could change the trophic level of the lake.

C_{17} vs P_{aq}

The P_{aq} suggests that the dominant macrophytes are emergent ones (Figure 26). However these emergent macrophytes seem to have no effect on the C_{17} algae bloom when plotted against each other (Figure 29). Although the macrophyte species are unknown in this lake, an assumption can be made that the macrophytes are not algae inhibiting, as the ones found by Zhao et al. (2013).

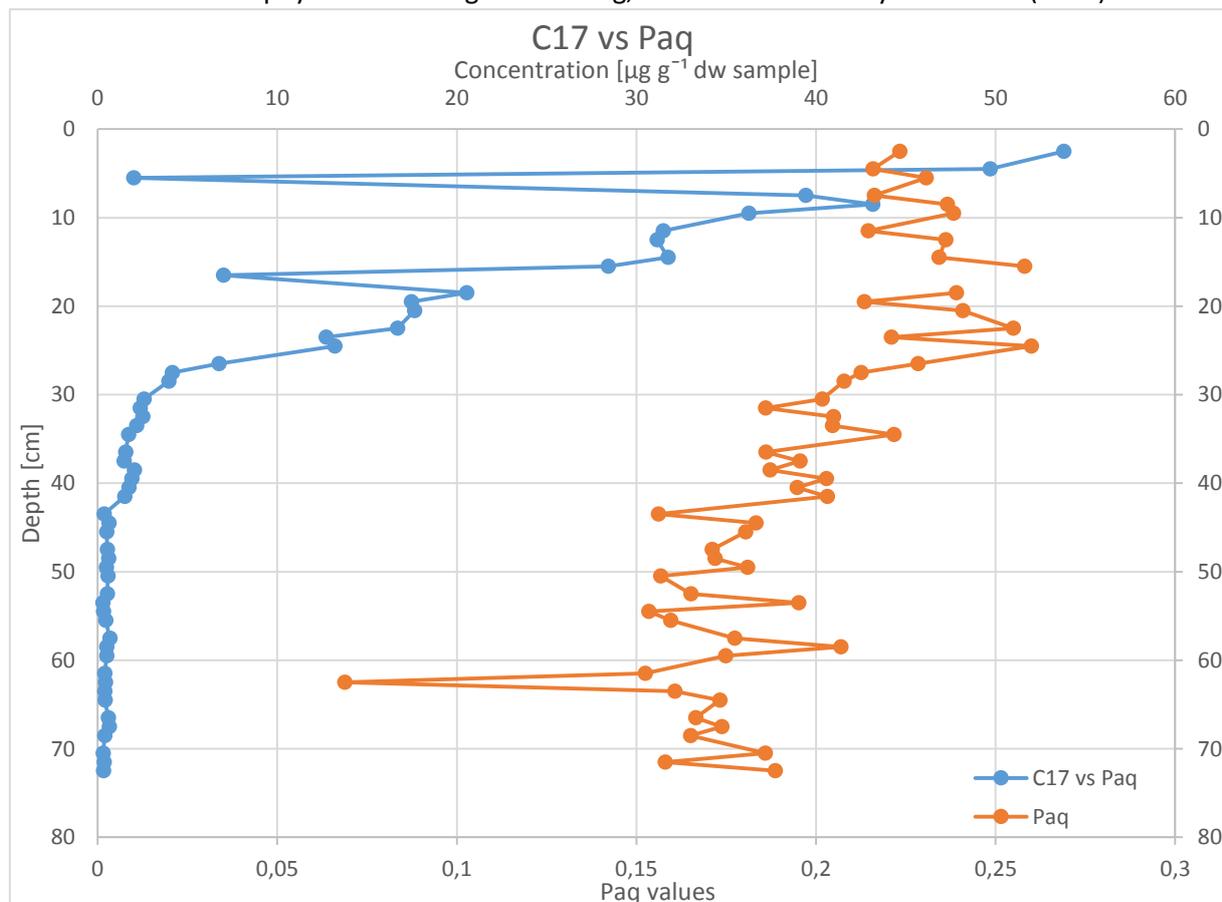


Figure 29. Plotting the C_{17} alkane concentrations with P_{aq} values through depth.

HBI

The HBI results show a rapid increase at the same depth (around 30-40 cm) as the other biomarkers. However the increasing shift is not constant and decreases around 24 cm. A distinct correlation cannot be made other than that the lake's condition change affected the HBI as well as the other biomarkers.

Possible errors and data

GC-MS instrumental and data errors.

All lipids from the depth of 1.5 cm and 16.5 cm are not represented due to instrumental errors creating concentration values that are not realistic.

Outlook:

Further studies and analyses that can be made on the sediment core retrieved from Nong Thale Pron included: 1. Stable isotope analysis of both stable carbon C and hydrogen (H/D ratio), which can tell us more about the conditions of the botryococenes and the HBIs; 2. Dating of the sediment core using ^{210}Pb , which can be used to correlate changes in lipids with climate events from the past; 3. TOC (total organic carbon) and/or LOI (loss on ignition) data.

Conclusions

This study used hydrocarbon biomarker records from a 74 cm sediment core retrieved from Lake Nong Thale Pron, Thailand, to estimate trophic changes in the lake. The result shows a change in the hydrocarbons concentrations. The *Botryococenes*, green algae specie, starting out low and then increasing, around 30-40 cm, in concentration down core, while the C_{17} , induced by phytoplankton, starts very high and decreases dramatically (around the same depth) in concentration down core. The HBI concentration also starts out high and increases rapidly at the 20 cm mark, after this shift the biomarker shifts again, now at the same depth as the other biomarkers, decreasing rapidly down core.

These biomarkers all have their shifts occurring roughly at the same record point. Considering the fact that *botryococenes* are indicators for oligotrophic lake environments, are very sensitive to environmental changes and grow quite slowly. It can be argued that the disappearance of the *algae* could be due to eutrophication, towards a more mesotrophic lake. Furthermore the C_{17} algae needs more nutrients, compared to the *botryococenes*, and is also fast growing. This suggests that the C_{17} outcompetes the *botryococenes*. This change in the lake could be due to human influence or mixing of the lake through climate change which could be because of the monsoon system.

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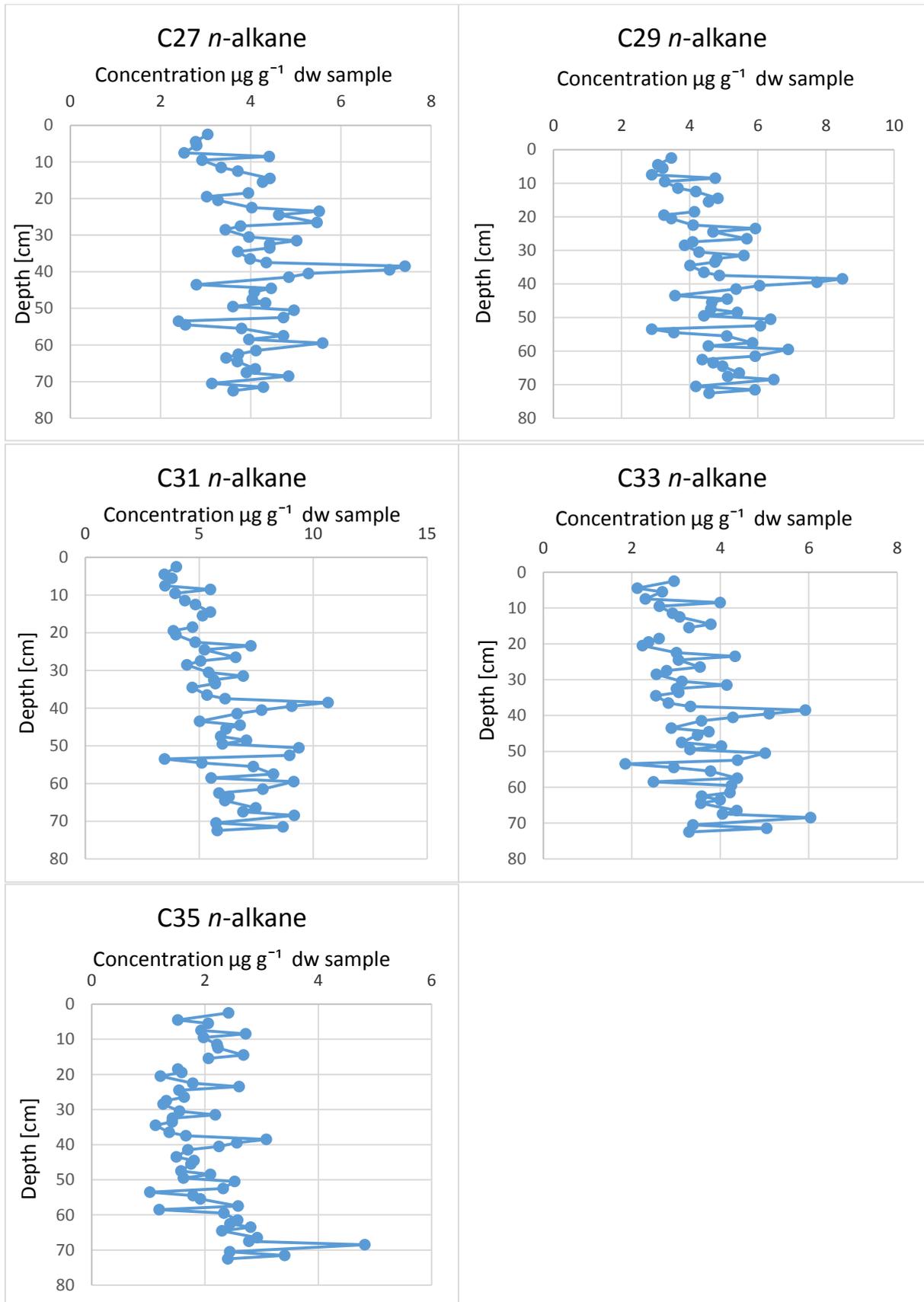
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Appendix



Figures 30, 31, 32, 33 and 34. Showing the allotment of C₂₇, C₂₉, C₃₁, C₃₃ and C₃₅ n-alkanes [$\mu\text{g g}^{-1}$] versus depth [cm].

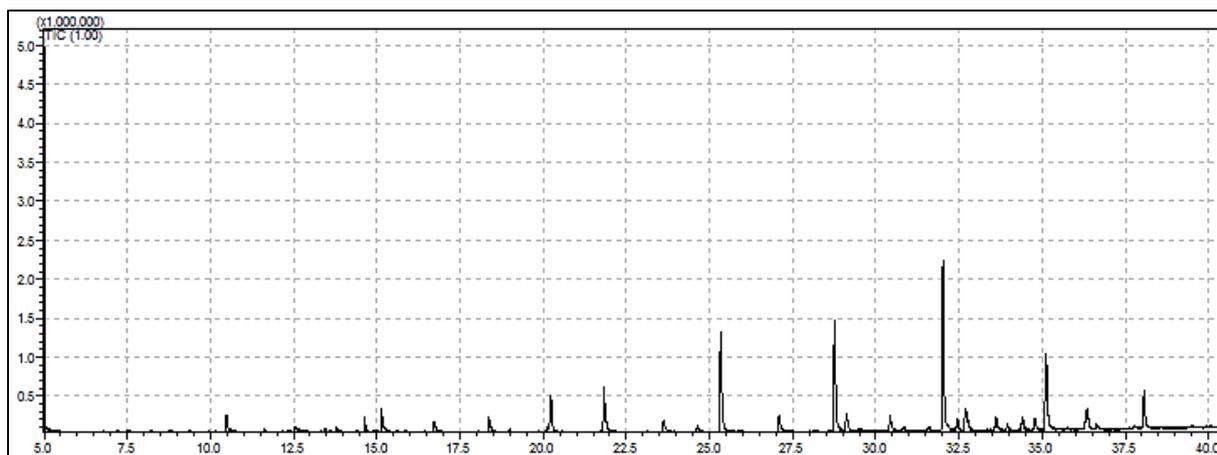


Figure 35. Showing chromatogram of depth 45.5 cm from the first fraction. Here *n*-alkanes are peaking, x-axis=retention time [min] and y-axis= abundance of compound.

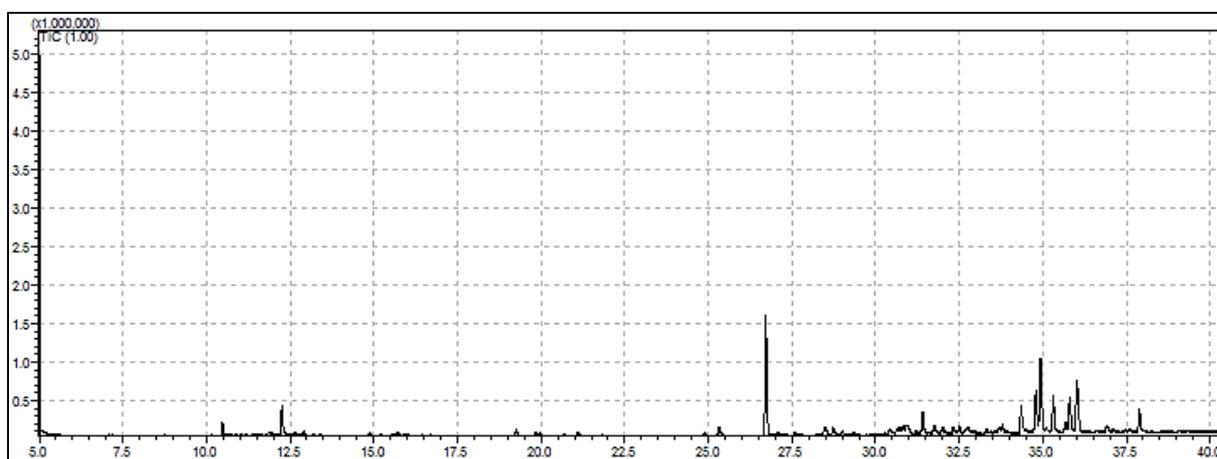


Figure 36 Showing chromatogram of depth 45.5 cm from the second fraction. Here the *botryococcenes* are peaking, x-axis=retention time [min] and y-axis= abundance of compound.



Figure 37. Showing chromatogram of depth 45.5 cm from the fourth fraction. Here the HBIs are peaking at the retention time of 15, x-axis=retention time [min] and y-axis= abundance of compound.

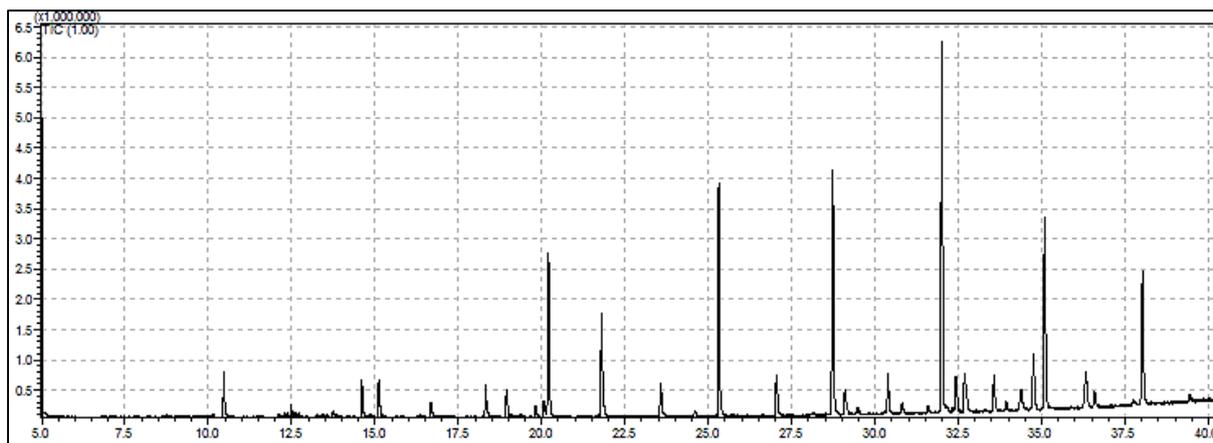


Figure 38. Showing chromatogram of depth 67.5 cm from the first fraction. Here the *n*-alkanes are peaking, x-axis=retention time [min] and y-axis= abundance of compound.

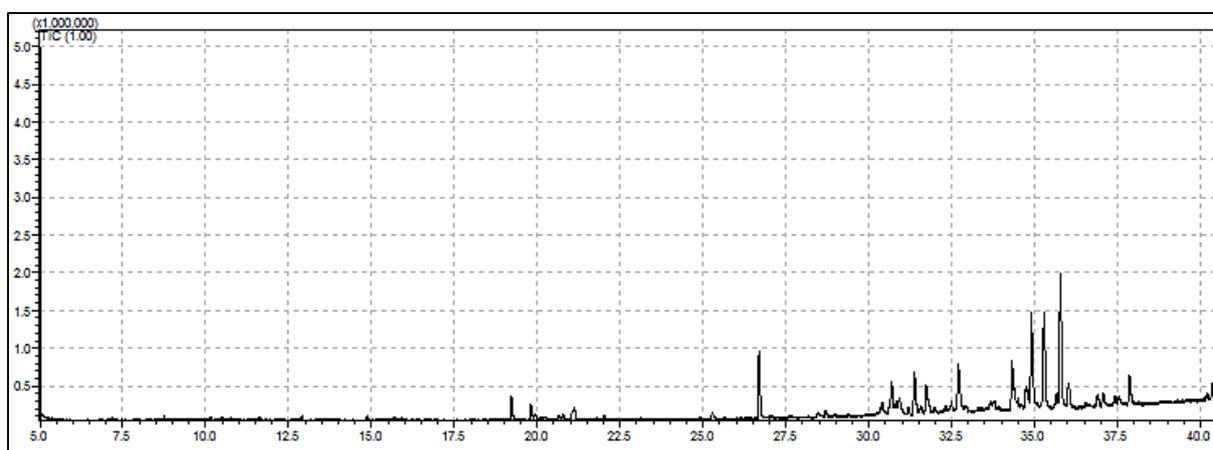


Figure 39. Showing chromatogram of depth 67.5 cm from the second fraction. X-axis=retention time [min] and y-axis= abundance of compound.

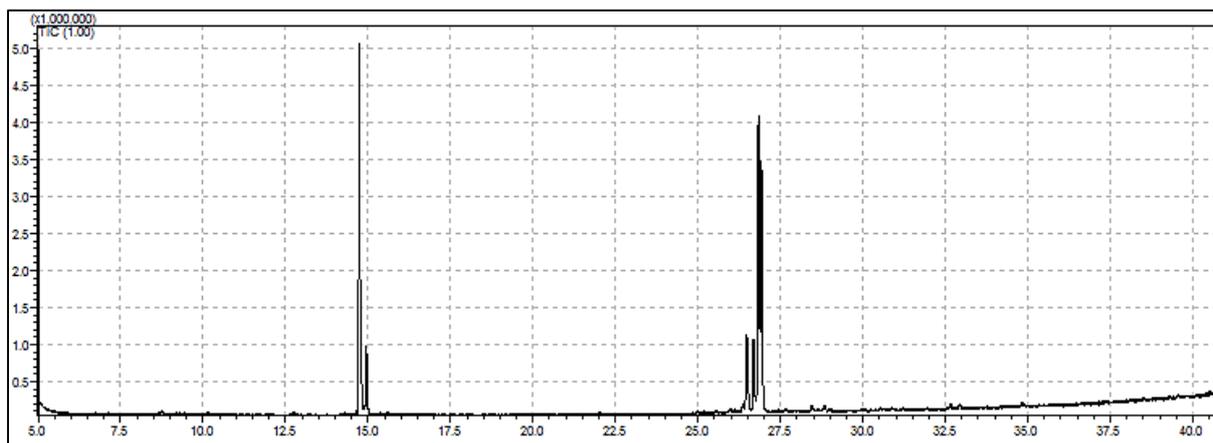


Figure 40. Showing chromatogram of depth 67.5 cm from the fourth fraction. Here the HBIs are peaking at the retention time of 15, x-axis=retention time [min] and y-axis= abundance of compound.