Analysis of Biomarker Candidates from Plant Lipid Inputs into Galapagos Lacustrine Sediments

by

Katharine Ricke

Submitted to the Department of Earth, Atmospheric and Planetary Science
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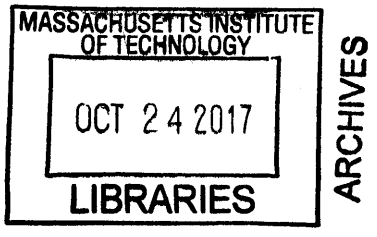

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Abstract

Paleoclimatological investigations into past precipitation and temperature patterns in regions of the tropical Pacific may be the key to resolving scientific disputes about the effects of global warming on the magnitude and frequency of the El Niño/Southern Oscillation (ENSO) phenomenon. Plant lipids identified in the sediment record of lakes in regions of high ENSO activity can act as biomarkers to reconstruct past precipitation patterns by measuring the D/H ratios preserved in these compounds to observe the local climate changes with global temperature variations. Twelve plant species and two sediment samples from in and around El Junco lake catchment on San Cristobal in the Galapagos Islands were solvent extracted, identified and quantified using gas chromatography and mass spectrometry. The analysis revealed evidence for significant aquatic and terrestrial vascular plant inputs to lake sediments. High concentrations of unsaturated C_{16} and C_{18} fatty acids were found in all plant samples, but these compounds appear to be degraded significantly in the sediment record. *n*-Alkane distributions suggest a strong hydrocarbon contribution from submerged and floating plants. Additionally, a terrestrial biomarker, fernene, was identified. The information in this study should be a helpful guide for further biomarker identification efforts at the El Junco lake and in other tropical crater lakes.

Thesis Supervisor: Julian Sachs
Title: Assistant Professor

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Chapter 1

Introduction

In the context of current debates about global warming, many scientists have hypothesized that with a rise in global average temperatures there will be a corresponding increase in number and severity of extreme climate events. Because of the severe flooding and droughts associated with the El Niño-Southern Oscillation (ENSO) disruption, the social and economic implications of increased duration or magnitude of the phenomenon are tremendous. Certain researchers have theorized that under the effects of global warming, El Niño—the disruption of the ocean-atmosphere system in the tropical Pacific that causes temperature and precipitation anomalies—will become more pronounced [25, 24]. Observational paleoclimatological research examining past sea surface temperatures in the eastern equatorial Pacific indicates the contrary. According to this research, the magnitude of sea surface temperature (SST) anomalies seems to diminish with warmer overall global temperatures [17]. In order to better understand the effects changing global temperatures have on El Niño, more past climate data is needed from those regions most affected by the ENSO phenomenon. This paper is a preliminary step in efforts to reconstruct precipitation patterns within one such region of high ENSO activity. Through identification of aquatic and terrestrial plant lipid inputs into lacustrine sediment, potential biomarkers may be identified for reconstruction of past water balance through deuterium/hydrogen (D/H) ratio analysis.

1.1 El Niño-Southern Oscillation (ENSO)

The ENSO phenomenon is the result of an aperiodic ocean-atmosphere fluctuation that causes temperature and precipitation anomalies in several regions of the equatorial Pacific. Under normal climate conditions, the easterly trade winds cause warm ocean water to "pile up" in the western tropical Pacific, leaving a relatively cold depression and shallow thermocline in the eastern equatorial along the coast of Central and South America. During an El Niño event, these winds relax causing warm waters to flow into the eastern Pacific, increasing atmospheric convection in the East and further exacerbating the collapse of the trade winds. The results of this collapse have major effects on climate patterns all over the globe [2]. Still, the effects of ENSO are most heavily concentrated within several distinct geographical nodes across the tropical Pacific [19]. It is within these core regions that evidence for past ENSO activities should be most pronounced.

Two of the most recent El Niño events-those occurring in 1982/83 and 1997/98-were the most intense in over a century; possibly as a result of rising greenhouse gas concentrations in the atmosphere [11]. Several models suggest ENSO behavior is closely linked to Milankovitch orbital forcing and climate cycles [4], but theoretical understanding of such variability is relatively poor. Thus one of the best ways to understand how El Niño may change under altered future climatic conditions is through paleoclimatological investigations of potential ENSO activity throughout glacial and interglacial climate periods.

1.2 The Galapagos Islands and the El Junco Lake

Darwin's famous desert islands, the Galapagos, lie directly along the equator at the edge of the cold tongue of the eastern equatorial Pacific. The typically arid climate of the island is inundated by a large positive precipitation and temperature anomaly during an ENSO event. [19] On the eastern-most island of San Cristobal, a closed basin crater lake, El Junco, lies at the top of an inactive volcano. Closed basin lakes

are particularly good targets for past precipitation studies because their often simple evaporation-precipitation hydrological systems and relatively small volume creates an environment with high sensitivity vis-à-vis water chemistry parameters. Another advantage of the El Junco crater lake is the lack of massive biological diversity that could be a hindrance in attempts to identify species-specific biomarkers in sediments in most of the world. The lack of plant species diversity in the highlands of the Galapagos Islands makes a thorough comparative investigation of sediment and species samples possible [8].

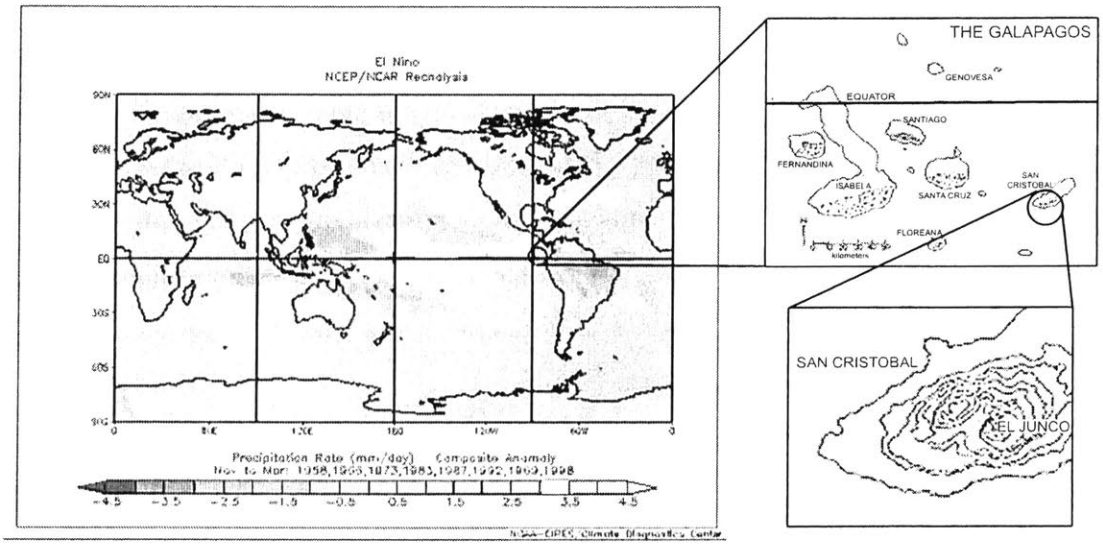

Figure 1-1: The largest El Niño precipitation anomalies are concentrated in nodes across the equatorial Pacific. The Galapagos Islands (inset) lie within one such node. The El Junco crater lake is at the top of a mountain on the eastern-most island of San Cristobal. (Figures adapted from http://www.cdc.noaa.gov/ENSO/ [19] and Colinvaux (1968) [6].)

1.3 Biomarkers and Reconstruction of Paleoclimate

Aquatic sediments are a long established source of paleoclimatological information. By identifying and quantifying a variety of mineralogical, biochemical and fossil indicators preserved in the sediment record, climate characteristics such as SSTs, precipitation magnitudes and biological productivity can be reconstructed. Only a small

fraction— 0.1–1%—of the biological material produced in an aquatic environment makes it into the sediment record [10]. Chemical and biological processes within the lake degrade the primary products of plant growth. Yet some forms of organic matter are less vulnerable to degradation than others. $CaCO_3$ traces from diatoms or SiO_4 produced by foraminifera can be used to gauge past environmental conditions such as salinity, temperature, species composition of nutrient level—all factors effected by climate variations. Pollen and macrofossil records have been a popular source for information about vegetation in lacustrine sediments [13]. A profile of alteration in microfossil remains of pollen grains from the submersed water fern, *Azolla microphylla*, and was taken from the El Junco lake sedimentation and used to reconstruct periods of relative moist and arid climate that corresponded with glacial and interglacial variations in global climate [7]. However, the bacterial and algal components, and a large amount of non-recognizable organic matter, is outside the analytical window of such studies. Lipid analysis provides the opportunity for a comprehensive view of all of the biological components of sedimentary input and the variations therein that are influenced by environmental change.

One of the most versatile indicators of past climate conditions is the biolipid content of sediment samples. Living organisms produce a wide variety of lipids—substances that can be extracted from organic matter by nonpolar solvents, such as hexane. Though lipids make up only a small fraction of the total biomass produced, in depositional environments they are better preserved than sugars and proteins [10] and easier to analyze than large macromolecular components like cellulose and lignin. Thus through the identification and quantification of specific lipid components, called biomarkers, organic matter in sediment can be used to reconstruct the environmental conditions under which it was originally produced. [9].

Examples of molecules that can be lipid biomarkers include fatty acids, n-alkanes, triterpenoids, and alcohols. Well-known biomarkers from vascular plants are long-chain n-alkanes (C_{25}-C_{31}). These compounds exhibit a strong bimodal distribution—with odd carbon numbered components occurring in far more abundance than even-numbered components—which are part of the waxy surfaces of terrestrial plant leaves

[10]. Ficken et al. (2000) compared the distributions of all n-alkyl lipids (n-alkanoic acid, n-alkanols and n-alkanes) between a number of plant species, both aquatic and terrestrial, and sediment samples from lakes on Mt. Kenya in East Africa and found a correlation between the carbon chain length of n-alkanes and the environmental growth conditions of the plant (submerged, emergent or terrestrial). Correlative analysis between lacustrine sediments and plant samples is feasible by way of other biomarkers as well. Baas et al. (2000) differentiated the lipid abundances across several categories for twelve species of peat moss through total lipid extraction and gas chromatography-mass spectrometry analysis and used compound distributions to identify a "chemotaxonomic fingerprint" for the species in peat bogs. A great number of studies have identified correlations between lipid content of vascular plant inputs and sediment deposits.

1.4 Precipitation Patterns and Hydrogen Isotope Analysis

The water balance of a small-basin aquatic body changes markedly during an El Niño event, within a node of high ENSO activity, with precipitation increasing relative to evaporation. Sternberg (1988)[23] showed that lipids of submerged aquatic plants record deuterium/hydrogen (D/H) ratios of environmental water. By looking at relative abundances of deuterium to normal hydrogen in lipids one can evaluate the magnitude of evaporative loss of water from the lake relative to gain via precipitation. Sauer et al. (2001) used algal sterols in lacustrine sediments to reconstruct past isotopic characteristics of the lake's water. Huang et al. (2002) examined the deuterium isotope abundance, or δD, of the ubiquitous lacustrine sediment compound, palmitic acid, in conjunction with fossilized pollen to show a correlation in the δD and pollen-inferred temperature [15]. Through identification of lipids characteristic to abundant submerged water plants, it may be possible to reconstruct the water balance of El Junco, and hence El Niño frequency and intensity during the past 30,000 years.

1.5 Thesis Outline

The goal of this paper is to draw a correlation between the lipid composition found in the preliminary analyses of available El Junco lacustrine sediment samples and the lipid content of samples of abundant aquatic and terrestrial plants in and around the El Junco lake. Through careful comparative spectrographic analysis, promising biomarkers are identified for D/H isotope ratio analyses of sediment samples to be collected in an upcoming expedition to El Junco. The information in this thesis and the recommendations should prove helpful to further comparative study of plant inputs and sediment for the El Junco lake in the future.

In Chapter 2, the methods for the extraction, identification and quantification of lipids from plant samples collected from the El Junco catchment are described and contrasted with the methods employed by Dr. Rienk Smittenberg, a post-doctoral researcher at M.I.T., in obtaining the same lipid information from El Junco sediment samples. Chapter 3 summarizes the results of the study by describing the general character of the lipid distributions in twelve plant samples and one sediment sample.

In Chapter 4, the features of the plant lipid extracts are compared to those of the sediment sample and some of the most remarkable features of the data are are further detailed. Examples of such features include the similarities and differences of distributions in the fatty acids and n-alkanes of the plant and sediment samples, and the identification of a terrestrial plant hydrocarbon biomarker, fernene. Finally, Chapter 5 highlights the most important observations about the lipid content of the plant samples and lacustrine sediment and makes suggestions for future investigations into biomarker identification for the El Junco lake, as relevant to the quest to reconstruct past water balance for the lake.

Chapter 2

Methods

In order to compare lipid components of the sediment with those of actual plant samples, both types of samples were extracted using organic solvents and measured by gas chromatography and mass spectrometry. With guidance from Dr. Rienk Smittenberg, the author was responsible for extraction and measurement of lipids in plants samples, while Smittenberg performed the corresponding analyses on the sediment samples. His methods are described in subsections 2.1.2 and 2.2.2, following the author's corresponding methods description, for convenient comparison.

2.1 Sampling

2.1.1 Plant Samples

While fresh plant samples—freeze-dried and sent directly from the Galapagos—would have been ideal for the analysis in the study, acquisition of such samples proved impossible on the timescale available for the project. Thus, conventional herbarium samples were used. All plant specimens were originally collected from the El Junco lake basin catchment on San Cristobal in the Galapagos; and were generously donated by Dr. Paul Colinvaux from his private herbarium.

All samples were collected by Colinvaux; primarily in July of 1966, with some secondary samples collected in February of 1972. Field collections were made in large

polyethylene bags. As soon after collection as possible, specimens were sorted, folded between sheets of old newspaper and stacked and pressed between aluminum dividers in hand-operated screw presses. The air-dried samples were shipped to Columbus, Ohio for final preparation by a trained herbarium curator and divided into two sets. One set was shipped to Dudley Herbarium at Stanford University for identification arranged by Ira Wiggins and the second was retained by Colinvaux. These specimens were mounted with glue to standard herbarium sheets or, in some cases placed within paper envelopes glued to herbarium sheets, and stored in manila folders in a steel herbarium cabinet. The cabinet remained in an air-conditioned laboratory in Columbus until 1990, after which it was shipped to the Smithsonisan Tropical Research Institute (STRI) in Panama, where it remained in an air-conditioned laboratory until 1998. It was then shipped to Woods Hole, Massachusetts, where it has since remained in the basement of a cottage. In 37 years of storage, the only known treatment to the cabinet was the introduction of the insect repellents, camphor or napthelene (moth balls). Subsamples were taken from the specimens stored in this cabinet by the author in Woods Hole in January 2004 [5].

Samples were taken of twelve aquatic and terrestrial native plant species found in abundance in and around the El Junco lake. Species of aquatic or semi-aquatic plants sampled include the water fern, *Azolla microphylla*; the submersed carnivorous water plant, *Utricularia foliosa*; the bed-forming reed, *Eleocharis mutata*; the semi-aquatic, reed bed-dwelling herb, *Hydrocotyle galapagensis*, also found in abundance in the pasture outside the crater; and the submersed water plant, *Najas guadalupensis*—found not in El Junco lake, but rather in divertica of streams running down from the crater. Terrestrial plants sampled include the abundant tree fern, *Cyathea weatherbyana*; the 2-3 meter tall flowering shrub, *Miconia robinsoniana*; the flowering herb, *Cuphea carthagenensis*, and the small flowering shrub, *Ludwigia leptocarpa*, that both grow on the marshy flat next to the lake; the weed, *Polygonum punctatum*, which grows on patches of lava mud within the catchment; and the invasive guava tree species, *Psidum galapageium*, which has become very abundant on the island over the past half century.

ID	*Species*	*Environment*	*Found in catchment?*	*Glue?*
A	*Utricularia foliosa*	aquatic, submerged	Y	N
B	*Azolla microphylla*	aquatic, submerged	Y	N
C	*Najas guadalopersis*	aquatic, submerged	N	N
D	*Eleocharis mutata*	aquatic, rooted	Y	Y
E	*Hydrocotyle galapagensis*	semi-aquatic, rooted	Y	Y
F	*Cyathea weatherbyana*	terrestrial	Y	Y
G	*Ludwiga leptocarpa*	terrestrial	Y	N
H	*Miconia robinsoniana*	terrestrial	Y	N
I	*Cuphea carthagenensis*	terrestrial	Y	Y
J	*Polygunum punctatum*	terrestrial	Y	Y
K	*Trichomanes Krausii*	terrestrial	N	Y
L	*Psidum galapageum*	terrestrial	N	N

Table 2.1: Plant samples analyzed listed with environmental information and indication of glue contamination.

At the time of subsampling in January 2004, specimens were intact, but aged to varying degrees from forty years of handling. It is likely that specimens accumulated some amount of extraneous biomass as a result of collection, storage and reference techniques that were not ideal for samples intended for organic geochemical analysis. In addition, though subsamples were taken from the envelopes containing glue-free surplus specimens whenever possible, half of the samples had been mounted with glue to standard herbarium sheets, and it proved impossible to completely remove all said glue from the surface of the samples.

2.1.2 Sediment Samples

Sediment cores were taken in 1972 by Colinvaux using a modified Livingstone piston corer, and portions were subsampled into 1 cm^3 parts. After transport they were stored in glass vials at 4°C in Columbus, Ohio; and after 1990, at Northern Kentucky University. During the years, the subsamples slowly desiccated. Two sediment samples, of 15 cm and of 208 cm core depth, respectively, were sent to MIT for biomarker analysis [22].

2.2 Lipid Extraction and Derivitization

2.2.1 Plant Lipid Extraction

Any visible remnants of glue on the surface of the leaves samples was removed with a set of tweezers and reserved for analysis. Stems and non-leaf material were discarded. All dry samples were weighed and rinsed with dichloromethane to remove surface contaminants and residues. These rinse fractions were then reserved for further analysis and the samples were crushed and packed with sand for lipid extraction. All samples were extracted using a soxhlet apparatus for approximately 15 hours in a dichloromethane/methanol (DCM:MeOH 9:1 v/v) mixture. A blank experiment and glue sample were run concurrently. After extraction, a 5α-cholestane standard was added to all samples (approximately 1 μg/10 mg dry weight of sample) for quantification purposes. The remaining plant material was reserved for further analysis through saponification. Solvents were removed by evaporation under nitrogen gas using a Zymark Turbovap LV (Caliper Life Sciences, Hopkinton, MA, USA) and the residues transferred to 4 mL vials using DCM.

Aliquots of total lipid extracts (TLEs) and sample rinses, equivalent to 25-50 mg of dry plant weight (or $\frac{1}{2}$ of the sample if total dry weight was less than 50 mg), were dried over pipette columns of anhydrous Na2SO4. To remove highly polar compounds they were subsequently eluted over pipette columns filled with 5% deactivated silica 60 with ethyl acetate. All solvents were evaporated under a stream of nitrogen and the residues were silylated with 5 μL of bis(trimethylsilyl)trifluoroacetamide (BSTFA) and 5 μL of pyridine for 20 minutes at 60°C to convert alcohols and acids to their trimethylsilyl ethers. Derivitized samples were then diluted in ethyl acetate for gas chromatography (GC) and gas chromatography–mass spectrometry (GC-MS).

The TLEs and remaining plant material of five samples identified for particular ecological importance (abundance and/or submersed aquatic growth environment) in the study, *A. microphylla, U. foliosa, E. mutata, C. weatherbyana*, and *M. robinsoniana*, were subjected to saponifaction. Aliquots equivalent to 25–50 mg dry weight (or $\frac{1}{2}$ of the remaining TLE, i.e. $\frac{1}{4}$ of the original sample) were heated (110°C) in 50

ml 1 N KOH solution (MeOH:H2O 95:5 v/v) for two hours. The resultant extracts were transferred to Teflon centrifuge tubes, shaken over distilled water and hexane and centrifuged three times. Neutral fractions were recovered by reservation of the hexane after each run. Acid fractions were recovered in an identical manner, after acidification of the alkaline solution with HCl to a pH level less than 3. Residues of the extracted plant leaves were saponified in the same way as the TLE fractions, resulting in neutral and acid fractions. All fractions were prepared for GC and GC-MS as described above. For quantification, 5a-cholestane standard was added to the hydrolyzed TLE acid fractions and to the saponified residue fractions.

2.2.2 Sediment Extraction

Sediment samples were freeze-dried and weighed. Except for small amounts kept as reference material, the sediments were extracted and prepared for GC and GC-MS analysis in a similar manner to that described for the plant samples. To transform fatty acids into their corresponding methyl-esters, half of the total lipid extract was dissolved in a mixture of BF_3 (14%) in methanol and heated to 60°C for 5 minutes. After addition of H_2O, lipids were extracted in a separatory funnel with DCM and dried over a pipette column filled with anhydrous $MgSO_4$. The fractions were subsequently eluted over 5% deactivated silica and derivatized with BSTFA as described above. The other half of the TLE of the sample from 15 cm-depth was saponified in a fashion similar to the methods described for the plant samples, and separated into an acid and a neutral fraction. The acid fraction was treated with BF_3/methanol to convert the fatty acids into their corresponding methyl esters. Both the acid and neutral fractions were separated into 4 fractions over a pipette column filled with 5% deactivated silica, using subsequently hexane (hydrocarbons), hexane:ethyl acetate (9:1 v/v) (neutral fraction: ketones; acid fraction: fatty acid methyl esters), hexane:ethyl acetate (8:2 v/v) (neutral fraction: alcohols; acid fraction: hydroxy-fatty acid methyl esters), and ethyl acetate (rinse) as eluents. The fractions were prepared for GC and GC-MS analysis using BSTFA as described above. Compounds were quantified by injecting known volume fractions (1/500) into the gas chromatograph,

and comparing the integrated peak areas in the chromatograms with peak areas of a standard mixture of four compounds with known concentration, which were analyzed immediately before and after the samples.

2.3 GC and GC-MS

Gas chromatography was performed using an Agilent 6890N Network GC System equipped with a PTV injector and monitored with a flame ionization detector. A CP-Sil 5 CB fused silica capillary column (60m $\times$ 0.32mm, 0.25μm film thickness) with helium as a carrier gas was used for separations. Samples were injected and held at 70°C for two minutes. The temperature was raised at 20°C/min up to a temperature of 130°C. The temperature was then raised to 320°C at the rate of 4°C/min and held for twenty minutes. Gas chromatography-mass spectrometry was performed on a Hewlett-Packard 6890 instrument with an Agilent 5973N Mass Selective Detector operated in electron ionization mode at 70 eV and with a mass range of m/z 50-800, using the same silica capillary column, carrier gas, and temperature program as the GC.

2.4 Identification and Quantitation

Lipids were identified from the GC-MS data with the aid of GC-MS Enhanced Data Analysis software and gas chromatographic reference literature, as well as AMDIS-Chromatogram with NIST MS Search software. Components were then quantified by integration of peak area generated by the gas chromatograph and comparison with the cholestane standard peak.

Chapter 3

Results

Twelve total lipid extracts (TLEs), five sets of neutral fractions (TLE and residue), five sets of acid fractions, one glue extract and a procedural blank were analyzed for lipid content using GC-MS data to identify compounds and GC to quantify each identified peak. A typical TLE gas chromatogram is shown in Figure 3-1. The numbers designating the peaks refer to the compounds identified and listed in Table 3.1. Lipid quantities for the 5 TLE neutral fractions are listed in Table ??. Biomarker content of the plant samples is organized by chemical classification in the first section of this chapter. Additionally, as the results of Dr. Smittenberg's sediment extractions are unpublished at this time, the sediment data are summarized in the second section of this chapter. Due to time constraints on the project, the results summarized below do not represent an exhaustive analysis of the data, but rather an organization of the most obvious results and those considered most interesting and/or important.

3.1 Lipid Content of Twelve Plant Species

3.1.1 Fatty Acids

The n-alkanoic acids, or fatty acids, were the most abundant free lipids extracted from the samples of all plants species. The summed concentrations vary between 413 to 2600 ug/g dry weight of the sample. Especially abundant in all samples

	Compound	Species ID											
		A	B	C	D	E	F	G	H	I	J	K	L
	Fatty Acids												
	n-Alkanoic Acids												
1	16:1	23	12	24	17	18	59	18	*	15	8	4	15
2	16	1068	1054	1151	1063	875	1148	1380	212	334	380	562	495
3	17	54	21	27	21	20	12	23	6	11	17	20	17
4	18:2	122	21	*	35	123	19	56	14	56	47	38	51
5	18:1	376	151	250	204	56	252	281	59	124	59	222	76
6	18	462	225	214	159	145	167	205	42	85	60	83	154
7	19:1	–	–	–	–	–	–	–	19	–	–	–	–
8	20	102	107	106	39	38	62	48	22	28	37	105	37
9	21	–	–	–	–	–	–	*	*	6	–	*	–
10	22	137	33	96	63	48	17	*	20	51	51	39	40
11	23	96	10	*	4	25	13	*	3	14	15	16	12
12	24	97	154	58	77	32	26	28	11	23	50	43	13
13	25	61	12	–	17	–	*	–	*	8	–	8	–
14	26	*	25	30	21	*	*	30	4	21	27	13	–
	Sum	2598	1824	1955	1721	1381	1776	2069	413	777	752	1152	909
	Alcohols												
	n-Alkanols												
15	phytol	41	–	–	28	–	9	65	3	–	–	7	42
16	19	–	–	–	–	–	–	–	–	–	–	–	–
17	20	–	–	–	20	–	–	–	–	–	–	–	–
18	22	111	*	–	–	–	–	–	–	–	–	*	–
19	24	–	9	–	–	5	–	–	2	–	–	–	–
20	26	–	–	–	–	–	–	–	6	–	20	–	–
21	28	–	18	–	–	57	–	–	11	–	–	10	–
22	30	–	–	–	–	*	–	–	–	–	–	–	–
	Sum	151	27		48	61	9	65	22		20	18	42
	Midchain Alcohols												
23	31	–	62	–	–	–	–	–	–	–	–	–	–
24	33	–	–	–	–	–	78	–	–	–	–	–	–
	Sterols												
25	cholesterol	–	14	–	–	–	–	–	–	–	–	–	–
26	28:1	–	51	–	91	–	–	39	–	–	–	–	–
27	29:2	120	–	–	–	–	–	–	13	–	–	–	–
28	29:2 stigma-	153	–	–	199	27	–	86	–	–	–	–	–
29	29:1	–	–	–	–	–	–	605	–	–	–	198	399
31	29:1 sito-	174	–	–	–	–	186	–	153	–	–	–	–
32	29 β-sito	–	288	–	441	–	141	–	22	–	–	–	–
33	30 lano-	73	–	–	–	–	–	–	–	–	–	–	–
	Sum	520	353		731	27	328	731	188			198	399
	Diols												
34	29-9,21	–	298	–	–	–	–	–	–	–	–	–	–
35	30-11,20	–	146	–	–	–	–	–	–	–	–	–	–
	Hydrocarbons												
	n-Alkanes												
36	n23	–	–	35	–	10	–	–	4	7	17	10	
37	n24	–	–	–	–	–	–	–	–	–	*	–	–
38	n25	–	–	*	*	*	–	–	5	*	*	*	–
39	n26	–	29	–	–	–	–	–	–	–	*	*	–
40	n27	–	31	30	–	18	–	–	5	*	*	25	15
41	n28	–	29	23	–	16	–	–	–	–	–	24	–
42	n29	–	19	20	–	21	–	–	6	10	–	33	128
43	n30	–	–	15	–	12	–	–	–	*	–	15	–
44	n31	–	–	–	–	17	–	–	12	10	–	19	263
45	n33	–	–	–	–	–	–	–	*	–	–	–	55
	Sum		109	123	*	94			32	26	17	125	461
	Triterpenoids												
46	fernene	–	–	–	–	–	240	–	–	–	–	–	–
47	dehydroabietic acid	581	324	–	12	32	10	49	9	–	–	–	–

(*) = coelutes, '–' = not identified

Table 3.1: The lipid identities and concentrations for the Total Lipid Extracts. The values represent μg of the given compound/g of dry weight of the plant sample. Asterisks denote compounds that coelute. Dashes indicate compounds that were not detected in the given sample.

Compound		Species ID				
		A	B	D	F	H
	Alcohols					
	n-Alkanols					
15	phytol	323	89	28	29	24
16	19	–	–	–	18	–
17	20	25	7	*	–	2
18	22	48	*	*	*	5
19	24	42	19	19	11	11
20	26	27	–	21	15	13
21	28	6	–	27	8	15
22	30	13	–	–	*	–
	Sum	484	116	95	81	70
	Midchain Alcohols					
23	31	–	53	–	–	–
	Sterols					
25	cholesterol	104	33	–	–	–
26	28:1	–	50	110	–	6
27	29:2	112	–	–	–	–
28	29:2 stigma-	–	–	235	–	–
29	29:1	126	–	–	–	–
31	29:1 sito-	–	–	543	160	204
32	29 b-sitσ	–	305	–	105	21
33	30 lano-	48	–	–	–	–
	Sum	390	379	889	266	231
	Diols					
34	29-9,21	–	296	–	–	–
35	30-11,20	–	158	–	–	–
	Hydrocarbons					
	n-Alkanes					
36	n23	–	13	9	–	–
37	n24	–	*	10	–	–
38	n25	*	*	*	*	–
39	n26	19	27	8	10	–
40	n27	18	29	6	13	–
41	n28	13	24	4	9	–
42	n29	10	21	9	12	–
43	n30	–	–	–	–	–
44	n31	–	–	7	–	–
45	n33	–	–	–	–	–
	Sum	60	113	53	44	
	Triterpenoids					
46	fernene	–	–	–	255	–

Table 3.2: The lipid identities and concentrations for the saponified neutral fraction of the TLEs of five selected samples. The values represent μg of the given compound/g of dry weight of the plant sample. Asterisks denote compounds that coelute. Dashes indicate compounds that were not detected in the given sample.

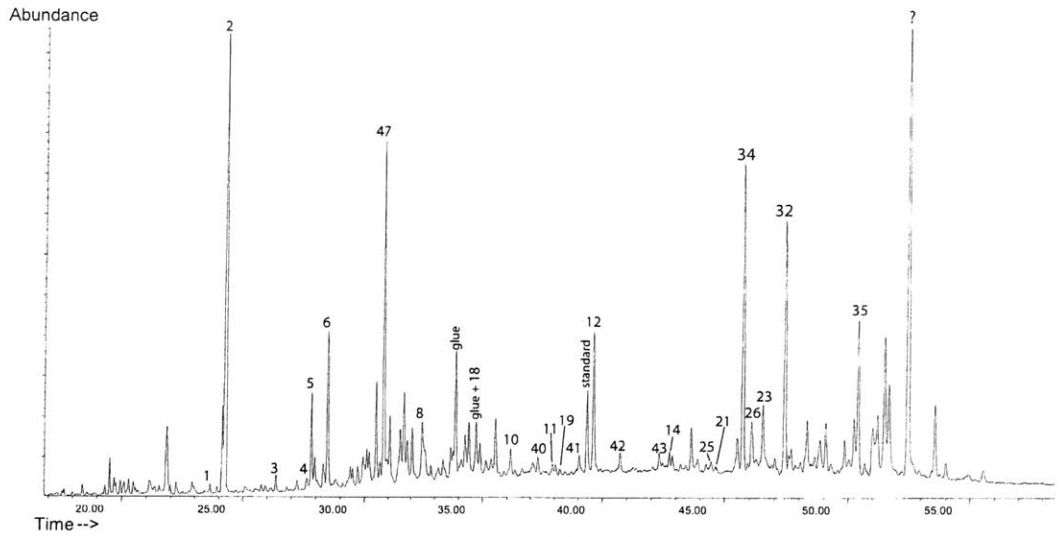

Figure 3-1: Typical Chromatogram shows the Total Lipid Extract results for *Azolla microphylla*. The numbers correspond to the compounds listed in Table 3.1.

was palmitic acid, the unsaturated C_{16} fatty acid. In all species, palmitic acid is at least twice as abundant as any other fatty acid and often the most abundant compound in the total lipid extract (see Figure 3-2). Also abundant were saturated and unsaturated C_{18} fatty acids; and even-numbered saturated compounds C_{22}–C_{26} were dominant over counterparts with odd carbon numbers. Most rinse fractions showed fatty acid peaks, as well, with distributions generally proportional to those found in the total lipid extracts, though much smaller. Submerged aquatic plant rinse fractions appear to have slightly higher fatty acid peaks proportionally than terrestrial plants do, though the large variations in contamination levels and sample quality make this observation impossible to quantify. In general, the peaks of major contaminants, such as glue molecules and dehydroabietic acid were much larger in the rinse fractions than the fatty acid peaks, the exception to this being in samples with very small or imperceptible levels of glue and paper contamination.

3.1.2 Hydrocarbons

n-Alkanes

With the exception of *P. galapagaeum*(L), all plant species contained relatively small amounts of n-alkanes. Total concentrations varied between 17 and 125 μg/ g dry weight. Most species showed either a unimodal or weakly bimodal distribution (see Figure ??). Aquatic plants generally showed a weaker bimodal distribution than terrestrial plants. This may be due to higher vulnerability to contamination from newspaper ink during drying. Ficken et al. (2000) found that lipid extractions from newspaper produce a smooth unimodal n-alkane distribution centered around n-C_{26}. Additionally, the C_{25} alkane coeluted with another compound in nearly every sample.

The only two specimens to show the strongly bimodal distribution associated with vascular plants were *P. galapageum* and *M. robinsoniana*(H). The terrestrial plant, *P. galapageum*, had a high total n-alkane concentration of 461μg/g dry weight, with C_{29} and C_{31} peaks that were among the largest compound peaks in the chromatogram. This may be explained by the specimen's waxy leaves. Strong bimodal long-chain n-alkane distributions are associated with waxy leaf surfaces.

Triterpenoids

The triterpenoid, fern-9(11)-ene (46), was found in considerable abundance in the TLE and neutral TLE fraction of the *C. weatherbyana*(F) lipid extracts. The concentrations were measured at 240 and 255 μg/ g dry weight, respectively. It was present in the saponified neutral residue fraction as well, in much smaller abundance. Fernene was not found in any amount in any of the other plant extracts. It is a compound that has only been isolated in ferns and closely related genera [1, 18].

3.1.3 *n*-Alkanols

The *n*-alkanols were the least abundant contributor to the n-alkyl lipid content in all samples. It was only possible to quantitate them accurately in the saponified neutral fractions, where observable concentrations ranged from 70 to 484 μg/ g dry

weight. In the total lipid fractions, n-alkanol components generally coeluted with other compounds or were too small to identify. The distribution of n-alkanols in the neutral fractions is shown in Figure ??. Aquatic plants appear to have a smaller peak carbon length (around C_{22}–C_{24}) than terrestrial plants (C_{26}–C_{28}). C_{30} was not quantifiable in many samples, as it coeluted with the saturated C_{29} sterol. Any presence of n-alkanols in the rinse fractions was too small to observe. Saponified residue neutral fractions showed clear n-alkanol peaks, in smaller concentrations than those observed in TLE fraction.

Phytol, a side-chain of chlorophyll, was present in most samples. It was particularly prevalent in the neutral fractions of *U. foliosa* and the saponified residue fractions of most species.

3.1.4 Sterols

Summed concentrations of sterols ranged from 188–731μg/ g in TLEs and 231–889μg/g in saponified neutral fractions, making sterols one of the most abundant lipid components in the samples. The only exceptions were *H. galapagensis*(E), for which the entire chromatograph showed evidence of high levels of contamination and *Cuphea carthagenensis*(I) and *Polygunum punctatum*(J), two samples with very high levels of glue contamination, for which sterol concentrations were not examined in detail. C_{29} sterols, especially stigmasterol and sitosterol, were far more abundant than any others, though cholesterol was found in concentrations of 33–104μg/ g in both El Junco submerged water plants. The only C_{30} sterol identified was 9,19cyclo,24methyllanosterol found in a concentration of 73μg/g in *U. foliosa*(A). This compound is a precursor and unlikely to be detected in the sediment record [14].

3.1.5 Diols and Midchain alcohols

The lipid extract for *A. microphylla* (B) revealed a large peaks for a C_{31} midchain alcohol and two diols, all which were not observed in any other species. The diols accounted for a sum contribution of 454 μg/ g dry weight of the sample. The lipid

extract for *A. microphylla* reveals large concentrations of three alcohol components not found in the TLE of any other plant species examined. These are a 9,21-C_{29} diol, a C_{31} midchain alcohol, and an 11,20-C_{30} diol with concentrations of 296, 53 and 158 μg/ g dry sample respectively. These compounds have not been detected in the sediment lipid extracts, and maybe precursors within the biogenesis, but because of the ideal growth conditions of *A. microphylla* for D/H sensitivity and its observed history in the lake sediment byway of pollen grain analysis [7], it is worth remarking on the components' abundance.

3.1.6 Contamination, Coelutions, and Errors

Levels of identifiable contamination varied greatly between the plant samples. The most obvious sources of contamination in all samples came from the glue used to attach specimens to herbarium boards and newspaper used to press the samples after collection. In some samples, such as *C. carthagenensis*(I) and *P. punctatum*(J), the largest peaks in the TLE chromatograms were those produced by glue remnants. Large peaks for dehydroabietic acid (DHA), a paper industry byproduct mainly derived from pine trees [27], in the plants, *U. foliosa* and *A. microphylla*(B), suggests aquatic plants were particularly vulnerable to contamination from the newspaper. Other samples with heartier leaves that had been stored in envelopes, such as *M. robinsoniana*(H) and *P. galapageum*(L), appeared to contain much smaller levels of contamination from glue, paper and other debris. A more detailed discussion of important effects of contamination on data analysis is included in the next chapter. Regardless, it is fair to say that contamination of samples at the time of subsampling and lipid extraction was probably significant for most species, and a dominant component in some.

Additional errors in quantitation were almost certainly produced by the uncertain measure of the standard compound, 5α-cholestane. This standard was chosen for these experiments because it is non-existent in vascular plant tissue and was readily available. While the standard and other compound peaks elute independently in the GC-MS chromatograms, on the GC (which produces a more reliable measure of

peak area) 5α-cholestane coelutes with the C_{24} fatty acid–TMS ether. As such, a more indirect approach to quantitation was taken. A ratio of areas of the coeluting compounds was taken from the GC-MS data and then applied to the quantification of the GC data. A number of compounds were unquantifiable because of coelution. Even the chromatograms of the fractionated extracts were sometimes crowded enough that coelution was still a problem.

3.2 Lipid Content of the El Junco Sediment

The chromatogram for the saponified total lipid extract from the El Junco lake sediment sample, taken from a depth of 15 cm, is shown in Figure 3-5. Like in the plant samples, the TLE is dominated by n-alkyl lipids, of which the most abundant compounds are fatty acids. Also present in relatively high abundance were the n-alkane-2-ones, or ketones, compounds found in barely detectable quantities in the plant extracts. Quantification was carried out using an external standard and error is at least 10% on an absolute basis. For relative abundance of compounds, however, quantification errors are limited to those generated by faulty GC-detection (< 0.5 %) As above, the results for the sediment data are summarized by chemical classes. The lipid distrubtion of the 208 cm depth core sample is not presented here, but is similar to that of the 15 cm sample.

3.2.1 Fatty acids

The n-Alkanoic Acids were the by far the most abundant n-alkyl lipid compounds in the El Junco sediment TLE with a total concentration of 132.4 μg/g of dry sediment. A bimodal distribution favored the even-numbered compounds, with especially high concentrations observed of the C_{22}, C_{26}, and C_{28} varieties (see upper right of Figure 3-6).

3.2.2 Hydrocarbons

n-Alkanes

The *n*-alkanes display a bimodal distribution generally centered around C_{27} (concentration 5.2 μg/g), though higher abundances are seen in mid-chain lengths (C_{23}-C_{26}) than in long chain lengths (C_{23}-C_{26})(see lower left of Figure 3-6). The sum concentration is 23.8 μg/g.

Fernene

Fern-9(11)-ene, the triterpenoid discussed in the previous section, occurs in relatively high abundance in the sediment of 2.1 μg/g. (see Figure 3-8). This lipid is a likely biomarker in the El Junco lake for *Cyathea weatherbyana*(F), the tree fern found throughout the catchment.

3.2.3 Ketones

Ketones, molecules 2-methylketones feature prominantly in the sediment extracts, especially the C_{27} and C_{29} homologues (Fig 3.5), which showed concentrations of 7.3 and 9.4 ug/g respectively (Fig. 3-6). However, these compounds are not abundant in the plant extracts.

3.2.4 Alcohols

Figure 3-9 shows the alcohol fraction, including the phytol peak, *n*-alkanols, and sterols. The *n*-alkanol concentrations are weakly bimodal (even carbon numbers are dominant) with a maximum peak at C_{28} (see upper right of Figure 3-6). The sum concentration is 22.1 μg/g. Unlike in the plant samples, the C_{30} (22) species is quantifiable in the sediment analysis and did not coelute with the saturated C_{29} sterol.

3.2.5 Sterols

While quantified data is currently unavailable, Figure 3-9 gives some indication of the general abundance of sterols in the saponified sediment sample. Sterols are generally more abundant than the *n*-alcohols and the most prevalent species are the C_{29} sterols that were identifed in the plant extracts, especially sitosterol (31). Large peaks are also produced by cholesterol (25), stigmasterol (28), and dinosterol— a biomarker for phytoplanktonic dinoflagellates.

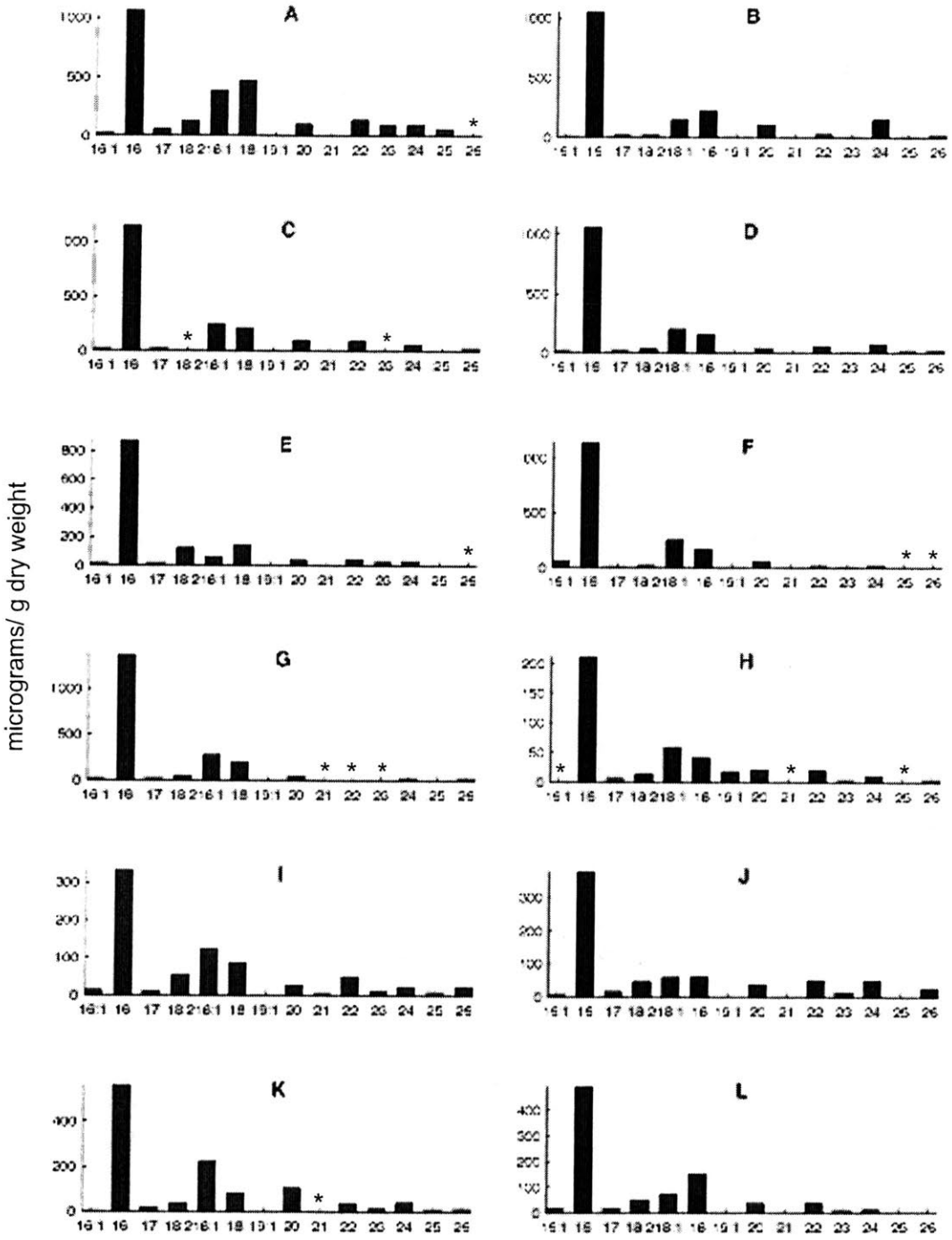

Figure 3-2: Fatty Acid distributions: Numbers along the x-axes denote the the carbon number of the n-alkanoic acid and the degrees of unsaturation associated with it. For example 18:2 is c18 fatty acid with 2 degrees of unsaturation. The letters above each plot corresponds with the species ID identified in Table 2.1. The asterisks mark a coelution of the given compound.

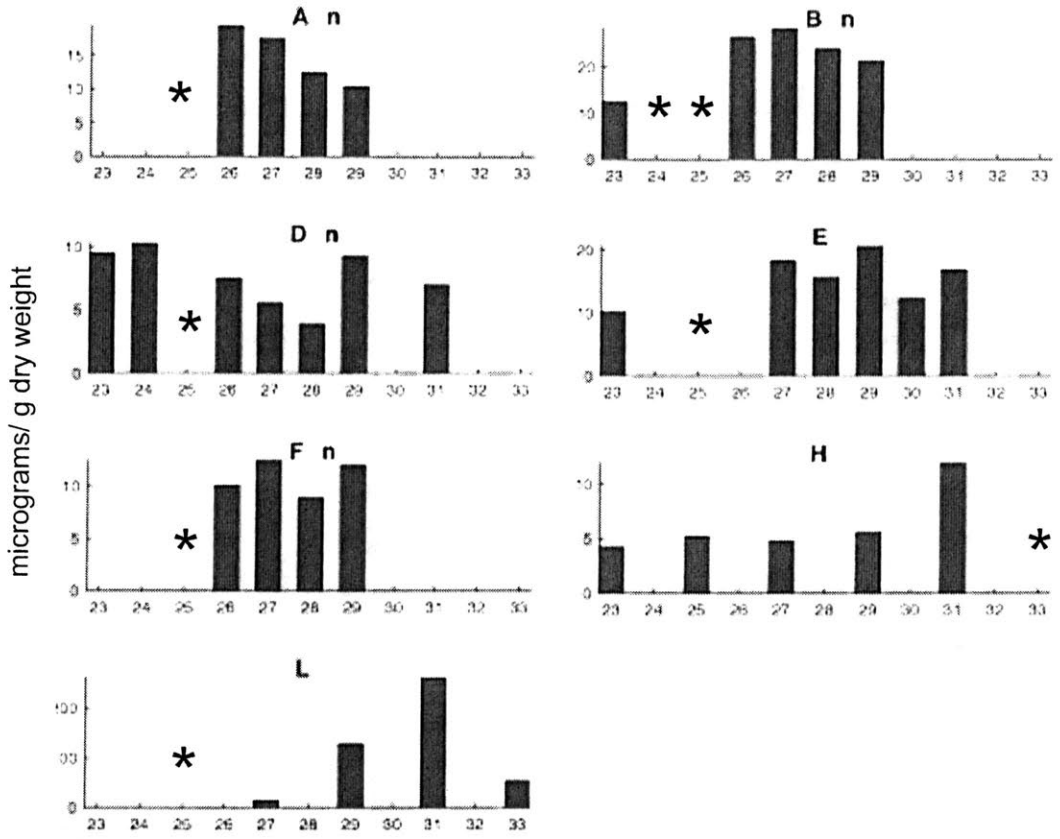

Figure 3-3: *n*-Alkane distributions for seven species: Numbers along the x-axes denote the carbon number of the n-alkanes. The letters above each plot correspond with the species ID identified in Table 2.1 and the 'n' indicates the concentration measures are taken from the neutral fraction of the saponified TLE. The asterisks mark a coelution of the given compound.

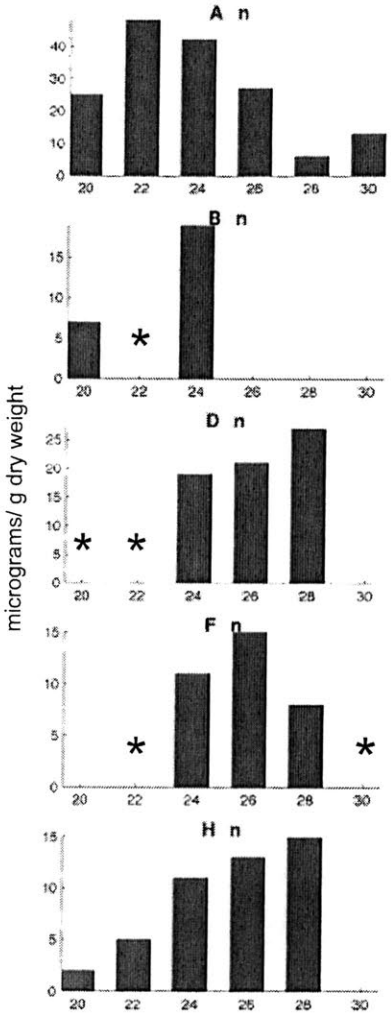

Figure 3-4: n-Alkanol distributions for five neutral fractions: Numbers along the x-axes denote the carbon number of the n-alkanols The letters above each plot correspond with the species ID identified in Table 2.1 and the 'n' indicates the concentration measures are taken from the neutral fractions. The asterisks mark a coelution of the given compound.

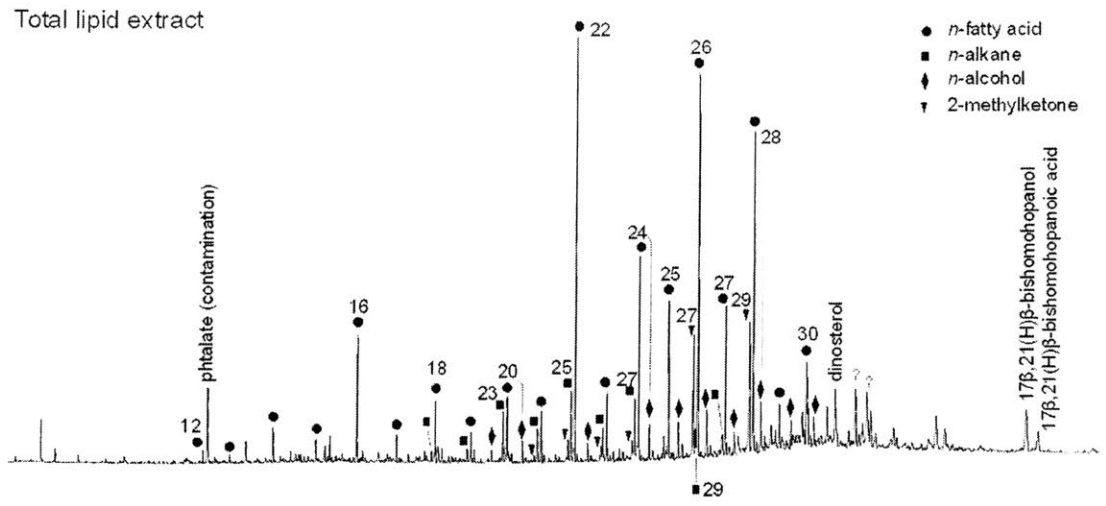

Figure 3-5: Gas chromatogram of the total lipid extract of sediment of El Junco (15 cm depth). Numbers refer to carbon chain lengths. (Source: Smittenberg, unpublished. 2004.)

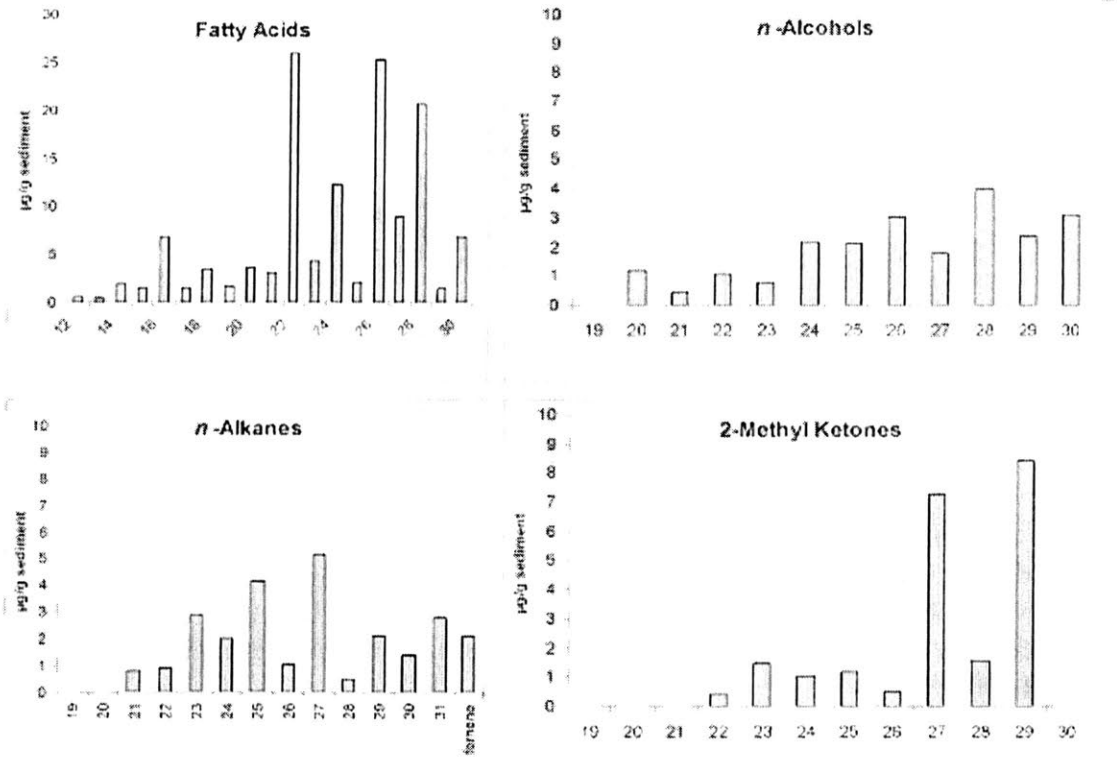

Figure 3-6: Concentrations and Distributions of Compounds in Saponified Sediment Extract. (Source: Smittenberg, unpublished. 2004.)

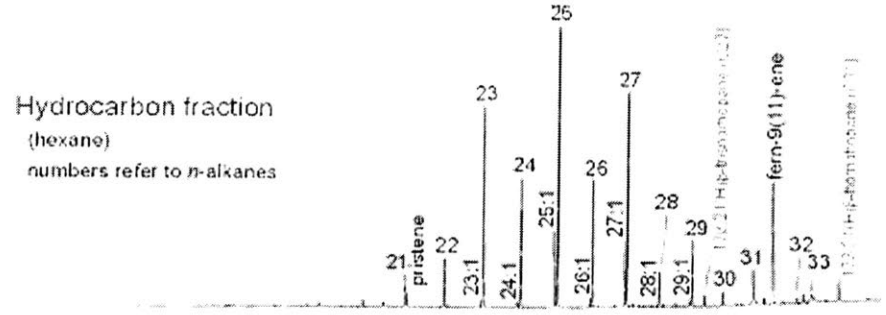

Figure 3-7: The is the hydrocarbon fraction of the sediment total lipid extract. Numbers correspond with n-alkanes.

Figure 3-8: Gas chromatogram of hydrocarbon fraction from the saponified total lipid extract of the sediment of El Junco (15 cm depth). (Source: Smittenberg, unpublished. 2004.)

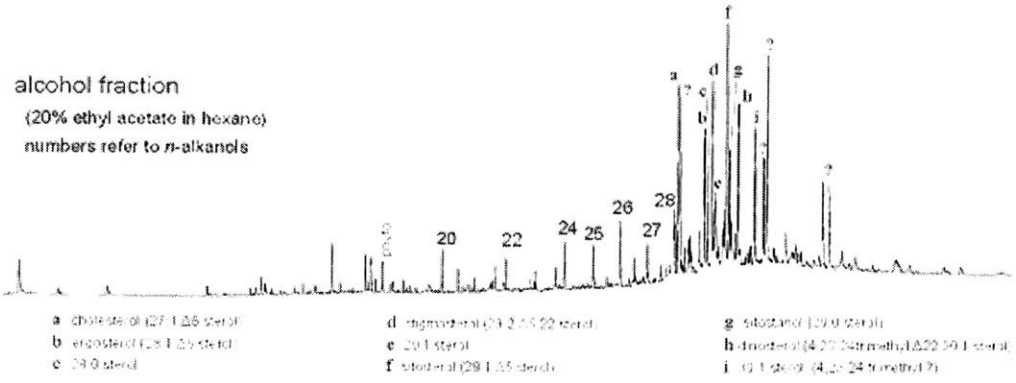

Figure 3-9: Gas chromatogram of alcohol fraction from the saponified total lipid extract of the sediment of El Junco (15 cm depth). Numbers correspond with n-alkanols. Letters designate sterols. (Source: Smittenberg, unpublished. 2004.)

Chapter 4

Discussion

The first step in identification of candidates for D/H biomarker analysis is establishing distinctions between aquatic and terrestrial plant lipid inputs, as well as plant and non-plant inputs. Aquatic plant lipids will reflect the historical water balance of the lake, while terrestrial plant lipids will reflect the background D/H ratio produced by historical precipitation and cloud condensation. A comparison of lipid distributions in samples of prevalent plant species inputs with those in the sediment and the implications of these distributions follows. The goal of this comparison is to elucidate the origin of various components of the sediment TLE.

4.1 Fernene: An Indicator for Terrestrial Plant Input

A photograph of the El Junco crater lake published in Nature in 1968 shows the crater walls surrounding the lake crowded with the tree fern *Cyathea weatherbyana* (F) [6]. The lipid analysis of the leaves of this fern show a great abundance of the triterpenoidal hydrocarbon, fern-9(11)-ene. The sediment shows an abundance of the same compound in its hydrocarbon fraction. Fernene has only been isolated in terrestrial ferns and other closely related species [18]. Fern-9(11)-ene was not detected in the floating water fern, *A. microphylla* or the gully fern, *T. Krausii.* Its

detection in sea sediments has led some researchers to purport microbiota are also a source of fernenes [21]. However, no biogenic source has been found to support this. As such, fernenes in sea sediments could be transported by aerosols in a similar way as long-chain n-alkanes. It appears reasonable, in the case of the El Junco catchment, to assume that *C. weatherbyana* (F) is the source of fernene sedimentary input and thereby that terrestrial plant make some significant contributions to the lipid distribution preserved in the sediment record.

4.2 Observations on Fatty Acid Distributions

The most complete data set for any of the chemical classes is that for the fatty acids. Their abundance made peaks easy to identify and quantify. As such the information that can be gleaned from fatty acids is probably the most accurate information provided in this study. In this section, we discuss sources of high C_{16} and C_{18} fatty acid concentrations in samples, the viability of palmitic acid as a biomarker, and the potential reasons for disparities in the fatty acids distributions of plant and sediment data.

4.2.1 C_{16} and C_{18} Fatty Acid Abundances

High or variable content of saturated C_{16} and C_{18} fatty acids in some previous analyses of vascular plant samples has been attributed to bacterial contamination of samples [3]. Bacterial compounds are known to contain high concentrations of these saturated fatty acids and as such, this seems to be a reasonable explanation for unexpectedly high concentrations of these compounds in lipid extracts. The comparison of TLE and Rinse chromatograms from the twelve plant species tested here, however, indicates that the palmitic and stearic acids originate from the plant sample and not contaminants. Figure 4.2.1 shows the TLE and rinse fraction chromatograms for *C. weatherbyana* (F). The TLE shows the highest measured concentration of palmitic acid in any of the samples and moderately high levels of glue contamination. The rinse fraction shows very high levels of glue contamination (as would be expected

from any contaminant) and very low levels of C_{16} and C_{18}. Other samples show a similar pattern. Samples with very high glue or paper contamination show nearly imperceptible abundances of fatty acids the in rinse fraction. Samples with very low levels of paper and glue contamination show the highest relative abundances of fatty acids in the rinse fraction. Qualitatively, aquatic plants appear to show slightly higher rinse fraction abundances of unsaturated C_{16} and C_{18} fatty acids, indicating a possible bacterial component. Regardless it is clear that both aquatic and terrestrial plants, and not their contaminants, produce significant amounts of these saturated fatty acids.

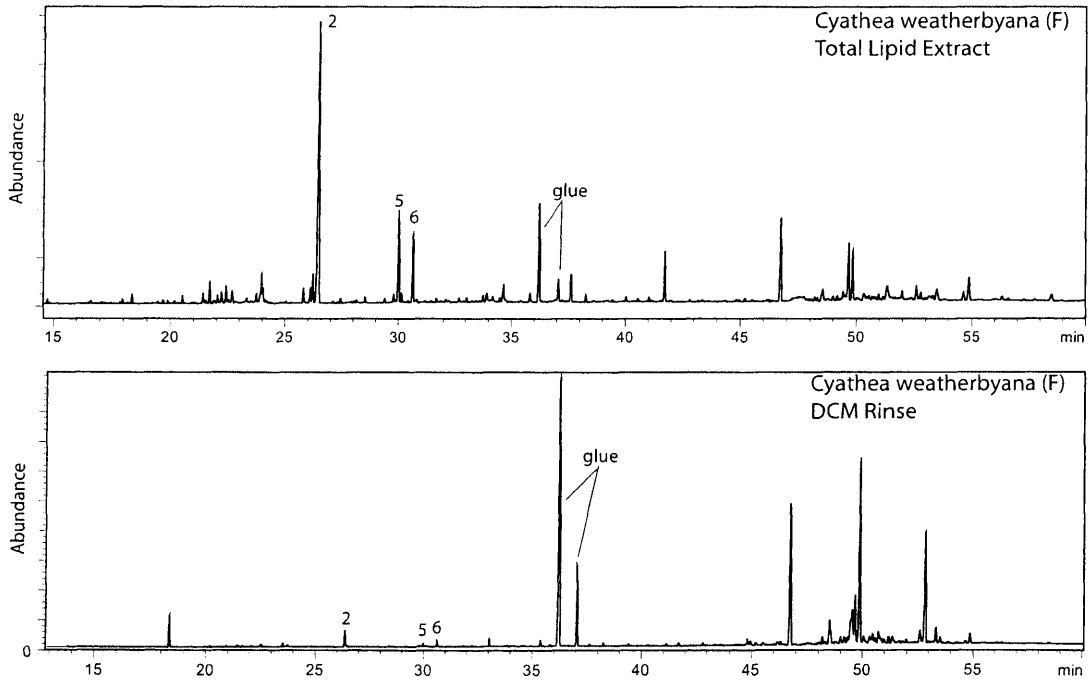

Figure 4-1: A comparison of lipid abundances in TLE and rinse fractions for *C. weatherbyana*, the sample with the highest concentration of palmitic acid. Numbers correspond to those in Table ??, (2) designating palmitic acid and (6) stearic acid.

4.2.2 Disparity in Fatty Acid Distributions in Plants and Sediment

The lack of correspondence found between fatty acid distributions of plant samples and the sediment— in particular, the dominance of C_{22}, and low concentrations of C_{16} and C_{18} fatty acids in the sediment extracts— has several possible explanations. One is that a high preferential production of long chain length fatty acids from non-plant sources exists. These could be bacterial or phytoplanktonic or other biogenic sources. Existence of a significant dinosterol concentration in the sediment lipid extract indicates there are important non-plant sources for sedimentary input. Regardless, such sources would have to be extremely abundant to overwhelm C_{16} fatty acid input and no literature supports this hypothesis. A more likely explanation is the preferential degradation of C_{16} and C_{18} fatty acids by chemical or biologic processes before or after plant input to the lake occurs. Palmitic and stearic acid are known energy sources for a variety of heterotrophs, including many microbial organisms. Additional evidence suggests that much of the C_{12}-C_{18} fatty acid material from terrestrial sources in broken down before deposition in the lake [9]. This possibility is further discussed in the following section.

4.2.3 Palmitic Acid as Potential Biomarker

The terrific abundance and ubiquity of palmitic acid across species gives it distinct advantages and disadvantages over other compounds as a potential biomarker for precipitation reconstruction. Its abundance makes it easier to measure accurately and its ubiquity limits its susceptibility to the entry and exit of species into and out of the catchment with periodic ecological changes. However, given the large concentrations present in terrestrial and aquatic plants alike, coupled with the knowledge of a significant terrestrial plant derived component in sediment extracts, fatty acids seem an unreliable indicator for past water balance in the El Junco lake. Some previous research on fatty acid sources in lacustrine sediments may contradict this reasoning, though. Cranwell (1982) cites rapid diagenesis of C_{16} and C_{18} fatty acids in terrestrial

detritus prior to deposition as an explanation for evidence supporting the attribution of C_{12}–C_{18} fatty acids in lacustrine sediments to aquatic sources. In a study of 33 North American lakes, Huang et al (2002) [15] found δD values of palmitic acid in surface sediments of lakes reflect the contemporary water balance of the lake and compared downcore δD values of one pond with pollen-inferred temperature variations and found a correlation. By the reasoning of these researchers, unlike the hydrocarbons that comprise waxy leaf surfaces, fatty acids reside completely within the interior of plant leaves. As such, while terrestrial-derived hydrocarbons are carried by the wind and deposited in the lake, fatty acids from terrestrial sources never reach the lake basin due to inaccessibility in living plants and rapid degradation in terrestrial plant detritus. While this argument may prove true for the El Junco lake, it is vulnerable to the specifics of the hydrological and depositional environment. Most of the lakes in the Huang study, for example, are North American glacial lakes in moist, temperate climate settings. El Junco is a crater lake with comparatively steep catchment boundaries, in a desert climate. In such an environment, it is not obvious the same assumptions about pre-depositional diagenesis and terrestrial run-off hold true. As such, palmitic acid is most likely not the ideal biomarker for precipitation reconstruction.

4.3 Parsing Terrestrial and Aquatic Inputs

In order to glean more information from the results about terrestrial versus aquatic inputs to the lake sediments, the next two sections discuss in further detail the lipid distributions in two chemical classes. The n-alkanes are well-known for their varying distributions between higher and lower levels of plant life [9]. The knowledge of these differences can be applied to give clues as to the relative levels of aquatic and terrestrial plant inputs. Sterols, on the other hand, offer compound specific tools for distinguishing aquatic and terrestrial sources.

4.3.1 Variations in n-Alkane Distributions

The most clear difference observed between terrestrial and aquatic plant lipids may be that observed in the n-alkane distributions. Of the nine plant species for which n-alkane distributions could be reliably measured, three were submerged or floating aquatic plants, two were emergent (rooted in the lake sediment) aquatic plants and four were terrestrial. The averaged abundance distributions for each of these three groups in comparison to the sediment distribution are shown in Figure ??. The major trends, as specified in the previous chapter, are a strong bimodal distribution peaking around C_{31} in terrestrial plants and weak bimodal or unimodal distribution peaking around C_{25}(inferred)–C_{27} and C_{29}, for submerged and emergent aquatic plants, respectively.

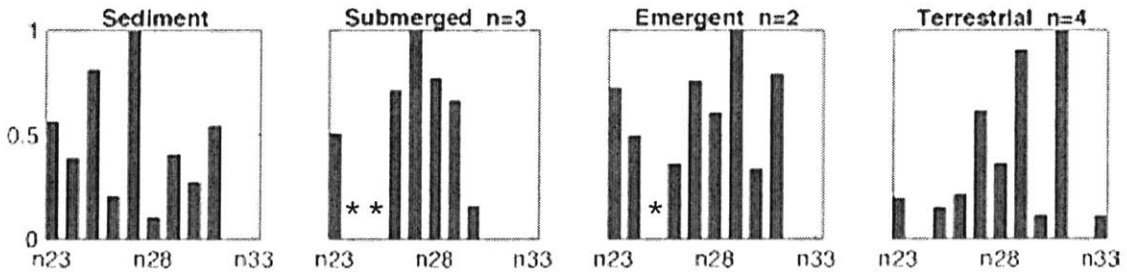

Figure 4-2: Comparison of distributions of n-alkanes in the sediment and three plant groups (as classified in Ficken et al.(2000) [12]). The n indicates the number of species included in distribution.

The n-Alkane Proxy for Aquatic Plants

Ficken et al. (2000)[12] suggests a proxy for gaging submerged plant contribution to lake sediment:

$$P_{aq} = \frac{C_{23} + C_{25}}{C_{23} + C_{25} + C_{29} + C_{31}}$$

The proxy was formulated through a similar comparison study of plant and sediment samples taken in and around lakes on Mt. Kenya. The study found that values of P_{aq} averaged at 0.69 for submerged and floating species, 0.25 for emergent species

(those rooted in lake sediments) and 0.09 for terrestrial species. The value of P_{aq} for the El Junco sediment is 0.59, suggesting a strong aquatic plant input. The coelution of C_{25} peaks made the quantitative comparison of P_{aq} values for the El Junco catchment plants with the P_{aq} values found in the Ficken study infeasible. On a qualitative level, however, the plant data presented supports the validity of the proxy, as it shows the migration of the peak abundance from mid-chain length species in submerged plants to long chain length species in terrestrial plants.

4.3.2 Variation in Sterol Distributions

Sterols require more complicated purification and control methods than *n*-alkyl lipids in order to be used as accurate measures for D/H ratio precipitation reconstructions [20, 15]. However, sterol analysis offers an opportunity for less ambiguous, compound-specific investigations than fatty acids or *n*-alkanes. Algal sterols in both lake and marine sediments have been used to reconstruct D/H water balance with an accuracy of ±10 ‰[20]. Identification of aquatic plant sterols in El Junco sediments could be used similarly. While the most abundant sterols were often the same across plant groups, cholesterol, for example, was only observed in the submerged aquatic plants. Klink et al. (1992) showed that the distribution of 4-methyl sterols in the abundant submerged water plant *Utricularia neglecta*, a species very similar to *U. foliosa* (A), matched up with the distribution in lake sediments [16]. While these similar compound distributions have not been identified in El Junco lake sediments or plant samples, closer investigation and further identification of sterols are still needed to preclude the possibility of their existence. Additionally, the dinosterol peak observed in the total lipid extract and alcohol fraction of the sediment sample implies a dinoflagellate source for sediment input [26].

4.4 Other Observed Anomalies

The high prevalence of C_{27} and C_{29} ketones in the sediment data is mysterious, considering the virtually non-existent ketone content of the plant samples. The lack of

identified ketone peaks in the chromatograms of the plant extracts does not necessarily indicate these compounds are not present. Rather, given the crowded nature of the plant sample chromatograms and the relatively low abundance of ketones in the sediment, these compounds may have been overlooked or coeluted with larger peaks in this preliminary analysis. Further fractionation or closer inspection of the data may reveal their presence. Likewise the *n*-alkanol distribution of the plant samples was strongly bimodal in comparison to the weak bimodal distribution of the sediment alcohol fraction, but the inferior peaks may just have been undetectable without further fractionation of the samples. A number of large peaks, especially for late-eluting peaks, remain unidentified in both the plant and sediment chromatograms. Further mass-spectrographic research is needed to determine the significance of these compounds.

Chapter 5

Conclusions

5.1 On Biomarkers

While a set of biomarkers ideally suited for precipitation reconstruction was not explicitly identified through this work, several useful facts were uncovered about sedimentary input to the El Junco lake.

5.1.1 Fernene and Terrestrial Plant Input

Significant concentrations of fernene were found exclusively in the El Junco lake sediment and the terrestrial tree fern, *C. weatherbyana*, indicating two important results. First, terrestrial plants in the catchment of the crater lake make a significant contribution to sedimentary lipids. This fact may seem trivial, but it is an important one to recognize in the selection of a biomarker D/H ratio analysis for precipitation reconstruction. Lipids that are present in high concentrations in both terrestrial and aquatic plants will not likely be good candidates for hydrogen isotope analysis. Second, fern-9(11)-ene, may be a good biomarker for measurement of environmental background D/H ratios. As the water balance of the greater hydrological system (i.e. in this crater lake the D/H ratio of incoming precipitation) will vary with time, the background D/H ratio will need to be reconstructed as well to accurately reproduce past evaporation-precipitation trends.

5.1.2 *n*-Alkanes and Aquatic Plant Input

The mid-chain length abundant distribution of sedimentary *n*-alkanes suggests that submerged or floating plants are a major source of lipids in the lake sediments. The aggregate distributions of the n-alkanes in three groups of plants examined: submerged, emergent(aquatic, but rooted), and terrestrial confirm the migration of the *n*-alkane peaks from mid-chain length (C_{25}) to long-chain length (C_{29}) as between submerged and terrestrial growth settings. The inferred prevalence of aquatic plant lipids in the sediment indicates that the potential for finding good biomarker candidates is considerable in further examinations of the data or future studies using fresh plant samples.

5.1.3 The Ubiquity of Palmitic Acid

The unsaturated C_{16} fatty acid appears to be a major component in all plant species examined in this study, both terrestrial and aquatic. While some researchers have suggested high palmitic acid content in total lipid extracts from vascular plants is evidence of sample contamination [3], comparison of the dominance of C_{16} fatty acid peaks in the total lipid extract versus the rinse fractions in the data strongly suggests that this is not the case. Additionally, a comparison of palmitic acid abundances across plant groups shows there is not a correlation between growth environment (aquatic versus terrestrial) and C_{16} fatty acid concentration. Instead palmitic acid appears to be a dominant component in all of the examined El Junco catchment plant total lipid extracts. Further investigation could indicate the low palmitic acid content in rinse fractions indicates that *n*-alkanoic acids reside primarily inside plant leaves, while hydrocarbons are concentrated near the surface and that preferential degradation of C_{16} and C_{18} fatty acids within the soil and plant detritus of the El Junco catchment results in primarily aquatic contributions to sedimentary palmitic acid content. However, no evidence for this hypothesis is implicit in this study and as such palmitic acid does not appear to be an ideal biomarker for D/H reconstruction.

5.1.4 Preferential Degradation and Non-Plant Inputs

Several disparities were observed between lipid distributions of plant and sediment samples. The striking difference between plant and sediment concentrations of C_{16} and C_{18} saturated fatty acids is most likely the result of preferential degradation of the compounds between plant production and deposition. Some non-plant sources, such as a large dinosterol peak, were observed in the TLE and alcohol fractions of the sediment data, indicating microbial inputs in addition to those from vascular plants. However, many similarities between identity and distribution of *n*-alkyl lipids and sterols overall suggests a significant contribution of sedimentary lipids from the El Junco catchment plant life.

5.2 On Obtaining Quality Plant Lipid Data

5.2.1 Controlling Sample Quality

Contamination from newspaper, glue and forty years of handling obviously increased the uncertainties in the analyses presented here. While it was impossible to avoid contamination of the samples used for this study, observations made in the comparison between rinse fractions and total lipid extracts of the twelve plant samples suggest two especially important techniques for collecting plant samples for future studies. First, newspaper should never be used in the preservation process of plant samples intended for lipid analysis, as it contaminates samples with *n*-alkanes and dehydroabietic acid. Pressing samples between sheets of old newspaper is the standard practice of botanists in the collection of herbarium samples. At least one other researcher has observed the contamination that can result from this technique. Aquatic plants seem particularly vulnerable to such contamination. Similarly, it is important to wash aquatic plants thoroughly with water immediately after collection to remove any bacterial debris that may contribute surplus C_{16} and C_{18} fatty acids.

5.2.2 Research Techniques

Two mistakes in the methods of the study diminished the quality of the data. First, the 5α-cholestane standard coleuted with another abundant compound, the C_{24} fatty acid TMS ether. If a test had been run prior to addition of the standard to all TLEs, to ensure coelution would not be a problem, the margin of error in quantitation would have been reduced substantially. The TLE chromatograms for many of the plant samples were extremely crowded. Even in the saponified TLE acid and neutral fractions coelution was still a big problem. Further fractionation of samples would have been helpful for more effective identification and quantitaion of chromatogram peaks.

DCM rinse fractions were reserved for contaminant control observations in this study, but even with fresh samples that should contain little contamination, rinses could be the key to very useful information about the lipids from terrestrial plants that are most likely to reach the lake sediment. Higher hydrocarbon abundances in the rinse fraction relative to fatty acids (as seen in the *Psidum galapageum* rinse–see Appendix A) and relatively high fernene may support the validity use of fatty acids as aquatic biomarker or confirm their incompatibility with such a role in this catchment.

5.3 Suggestions for Further Study and Closing Thoughts

5.3.1 The Quest for Viable Aquatic Biomarkers

While this study did not identify the ideal aquatic plant biomarker for precipitation reconstruction of the El Junco crater lake basin, it provides a crucial first step in the identification of such biomarkers in future investigations. Contamination threw an unfortunate additional variable into this study that can be avoided in future studies with fresh, carefully collected samples. An upcoming expedition to San Cristobal in late 2004 should provide the opportunity to acquire such samples. Both the correlations and disparities observed in the preliminary plant-sediment lipid comparison provide

valuable hints as to the best places to begin with the second round of biomarker identification efforts.

5.3.2 The Big Picture

In the face of uncertainty about the effects of anthropogenic climate change on local environments, paleoclimatology is a valuable tool. Where theories and models conflict about the dynamics of El Niño, information about past climate behavior can be a litmus test for reasonable predictions. In the case of the El Junco lake sediment record, past precipitation reconstructions can provide information about future consequences of global warming on an particularly climate-sensitive area of the world. Not only will such knowledge contribute to the understanding of a complex climate system, it can help guide policies and set priorities to protect vulnerable populations and ecosystems from harm.

Bibliography

[1] Hiroyuki Ageta, Kenji Iwata, and Shinsaku Natori. A fern constituent, fernene: A triterpenoid hydrocarbon of a new type. *Tetrahedron Letters*, (22):1447–1450, July 1963.

[2] R.J. Allan, J. Lindesay, and D. Parker. *El Niño, Southern Oscillation and climatic variability.* Csiro, Australia, 1996.

[3] Marianne Baas, Rich Pancost, Bas van Geel, and Jaap S. Sinninghe Damsté. A comparative study of lipids in *sphagnum* species. *Organic Geochemistry*, 31(6):535–541, June 2000.

[4] A. C. Clement, R. Seager, and M. A. Cane. Orbital controls on the El Niño/southern oscillation and the tropical climate. *Paleoceanography*, 14(4):441–456, August 1999.

[5] Paul Colinvaux. Re: Galapagos plant samples. email.

[6] Paul A. Colinvaux. Reconnaissance and chemistry of the lakes and bogs if the galapagos islands. *Nature*, 219:590–594, August 1968.

[7] Paul A Colinvaux. Climate and the galapagos islands. *Nature*, 240:17–20, November 1972.

[8] Paul A. Colinvaux and Eileen K. Schofield. Historical ecology in the galapagos islands: I. a holocene pollen record from the el junco lake, isla san cristobal. *Journal of Ecology*, 64(3):989–1012, November 1974.

[9] P. A. Cranwell. Lipids of aquatic sediments and sedimenting particulates. *Progress in Lipid Research*, 21:271–308, 1982.

[10] J. W. De Leeuw, N. L. Frewin, P. F. van Bergen, J. S. Sinninghe Damsté, and M. E. Collinson. Organic carbon as a palaeoenvironmental indicator in the marine realm. In Dan W. J. Bosence and Peter A. Allison, editors, *Marine Palaeoenvironmental Analysis fron Fossils*, number 83 in Geological Society Special Publication, chapter 4, pages 43–72. The Geologic Society Publishing House, Bath, United Kingdom, 1995.

[11] Alexey V. Fedrov and S. George Philander. Is El Niño changing? *Science*, 288:1997–2002, June 2000.

[12] K. J. Ficken, B. Li, D. L. Swain, and G. Eglinton. An n–alkane proxy for sedimentary input of submerged/floating freshwater aquatic macrophytes. *Organic Geochemistry*, 31(7–8):745–749, 2000.

[13] K. J. Ficken, F. A. Street-Perrott, R. A. Perrott, D. L. Swain, D. O. Olago, and G. Eglinton. Galcial/interglacial variations in carbon cycling revealed by molecular and isotope stratigraphy of lake nkunga, mt. kenya, east africa. *Organic Geochemistry*, 29(5–7):1701–1719, 1998.

[14] José-Luis Giner, Laurent Wünsche, Robert A. Andersen, and Carl Djerassi. Dinoflagellates cyclize squalene oxide to lanosterol. *Biochemical Systematics and Ecology*, 19(2):143–145, July 1991.

[15] Yongsong Huang, Bryan Shuman, Yi Wang, and Thompson Webb III. Hydrogen isotope ratios of palmitic acid in lacustrine sediments record late quaternary climate variations. *Geology*, 30(12):1103–1106, December 2002.

[16] G. Klink, F. Dreier, A. Buchs, and F. O. Gülačar. A new source for 4-methyl sterols in freshwater sediments. *Organic Geochemistry*, 18(6):757–763, 1992.

[17] Athanasios Koutavas, Jean Lynch-Stieglitz, Thomas M. Marchitto Jr., and Julian P. Waschs. El Niño-like pattern in ice age tropical pacific sea surface temperature. *Science*, 297:226–230, July 2002.

[18] K. Nishimoto, M. Ito, and S. Natori. The structures of arundoin, cylindrin and fernenol. *Tetrahedron*, 24:735–752, 1968.

[19] National Oceanic and Climate Diagnostics Center Atmospheric Administration. http://www.cdc.noaa.gov/enso/. website.

[20] Peter E. Sauer, Timothy I. Eglinton, John M. Hayes, Arndt Schimmelmann, and Alex L Sessions. Compound-specific d/h ratios of lipid biomarkers from sediments as a proxy for environmental and climatic conditions. *Geochimica et Cosmochimica Acta*, 65(2):213–222, 2001.

[21] Bernd R.T. Simoneit. Cyclic triterpenoids of the geosphere. In R. B. Johns, editor, *Biological markers in the sedimentary record*, Geological Society Special Publication, chapter 2, pages 43–99. Elsevier, Amsterdam, 1986.

[22] Rienk Smittenberg. Unpublished Data and Figures, 2004.

[23] Leonel da Silveria Lobo Sternberg. D/h ratios of environmental water recorded by e/h ratios of plant lipids. *Nature*, 333:59–61, May 1988.

[24] D. Z. Sun. A possible effect of an increase in the warm-pool sst on the magnitude of El Niño warming. *Journal of Climate*, 16:185–205, 2003.

[25] A. Timmermann, J. Oberhuber, A. Bacher, M. Esch, M. Latif, and E. Roeckner. Increased El Niño frequency in a climate model forced by future greenhouse warming. *Nature*, 398:694–696, April 1999.

[26] John K. Volkman, Robert B. Gagosian, and Stuart G. Wakeham. Free and esterified sterols of the marine dinoflagellate *Gonyaulax polygramma*. *G-Animal's Journal*, 19(6):457–465, 1984.

[27] John K. Volkman and Daniel G. Holdsworth. Determination of resin acids by gas chromotography and high-performance liquid chromatography in paper mill effluent, river waters and sediments from the upper Derwent Estuary, Tasmania. *Journal of Chromatography*, 643:209–219, 1993.