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Biomarker evidence for the co-occurrence of three races (A, B and L) of *Botryococcus braunii* in El Junco Lake, Galápagos

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Abstract

In spite of the fact that individual races of *Botryococcus braunii* are widely distributed in lakes, reports of the co-existence of different chemical races are rare. Here we report biomarker evidence for the co-occurrence of three different races of *B. braunii* (A, B and L) in the water column and sediments of El Junco Lake, a freshwater crater lake at 700 m elevation on San Cristóbal Island, Galápagos. We also show the existence of lycopadienes and lycopatrienes in suspended particles and sediments.

Biomarker indicators of the three races were: *cis* and *trans* C_{25}–C_{31} *n*-alkadienes and a C_{29} triene for race A, a series of $C_{34}H_{58}$ botryococcenes for race B, and a $C_{40}H_{78}$ hydrocarbon, *trans*,*trans*-lycopadiene accompanied by minor amounts of isomers for race L. Epoxides derived from *n*-alkadienes and *trans*,*trans*-lycopadiene, specific to races A and L respectively, were also detected. Several previously unreported lycopadienes and lycopatrienes were identified in both the water column and sediment, and are believed to represent by-products and intermediates, respectively, of *trans*,*trans*-lycopadiene synthesis.

Botryococcene and lycopadiene concentrations reached 16.7 and 0.8 mg/g dry sediment in near-surface sediments, and varied over more than three orders of magnitude, while *n*-alkadienes were present in trace quantities. Pyrolysates from the kerogen fraction of sediment contained lycopadiene and alkadiene related lipids along with chemically resistant polymers of *B. braunii* cell walls. Apparently *n*-alkadienes, botryococcenes and lycopadienes and -trienes can survive in oxic sediments for several decades, and the down core variation in these lipids likely reflects changes in lake hydrology, alternately favoring the bloom or near-extinction of *B. braunii* race B.

1. Introduction

The green alga *Botryococcus braunii* is a colonial member of the Trebouxiophyceae (as recently revised by Senousy et al. (2004)), characterized by high concentrations of hydrocarbons and unusual

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lipids (Metzger and Largeau, 1999, 2005). Widely distributed in freshwater lakes and ponds, it also inhabits brackish and ephemeral lakes. Since the pioneering works of Swain and Gilby (1964) and Maxwell et al. (1968), the hydrocarbons from *B. braunii* have been extensively studied and three types have been identified: (i) long straight chain odd-carbon numbered hydrocarbons (mono- to tetra-unsaturated) from C_{23} to C_{33}, typically alkadienes with formula C_nH_{2n-2} (e.g. **I** and **II**, Appendix); (ii) branched triterpenoids from C_{30} to C_{37} with generalized formula C_nH_{2n-10}, termed botryococcenes (e.g. **III** and **IV**, Appendix); and (iii) an acyclic tetraterpenoid, lycopadiene, typically $C_{40}H_{78}$ (**V**, Appendix) (see Metzger and Largeau, 1999 and references therein). The absence of clear morphological differences between the strains producing the different types of hydrocarbons led to their classification into three chemical races: A, B and L with reference to alkadienes, botryococcenes and lycopadiene, respectively (Metzger et al., 1991).

Various other biomarkers can also be diagnostic of *B. braunii*, for example, polymethylsqualene (Huang and Poulter, 1989), cyclobotryococcenes (David et al., 1988; Huang et al., 1988, 1995), and botryolins (tetramethylsqualene triethers) (Metzger et al., 2002) which are indicative of *B. braunii* race B. Botryals (C_{52-64} α-branched, α-unsaturated fatty aldehydes) and botryococcoid ethers, ether lipids derived from alkadienes, are characteristic of race A (Metzger and Casadevall, 1991). Lycopanerols, tetraterpenoid ether lipids derived from lycopadiene (Rager and Metzger, 2000; Metzger et al., 2003), can indicate the presence of race L. Terpenoid diepoxides derived from tetramethylsqualene and lycopadiene are characteristic of races B and L, respectively (Metzger, 1999). Aliphatic polyaldehydes of very high molecular weight occur in the three races of *B. braunii* (Metzger et al., 1993, 2007; Berthéas et al., 1999) which likely originate from the self condensation of a α,ω-C_{32} dialdehyde (Metzger et al., 1993) and represent the precursors of algaenans, the resistant material present in algal cell walls.

Some of these compounds and their diagenetic products have been identified in sediments. For example, many botryococcenes, cyclic or acyclic, together with botryococcanes (e.g. Moldowan and Seifert, 1980; Brassell et al., 1985; Huang et al., 1995, 1999) and tetramethylsqualanes (Summons et al., 2002) identified in lacustrine sediments and crude oils are the signature of *B. braunii* race B. C_{27}, C_{29}, C_{31} alkadienes and trienes were present in the pyrolysate of an elastic organic substance from the periphery of Lake Balkash, and derived from *B. braunii* race A (Gatellier et al., 1993). C_{40} monoaromatic lycopane derivatives are indicative of *B. braunii* race L (Derenne et al., 1994; Adam et al., 2006). Furthermore, macrocyclic alkanes (C_{15}–C_{34}) and their methylated derivatives (C_{17}–C_{26}) were recently discovered in Torbanites and crude oils and thought to originate from the diagenesis of *B. braunii* algaenan (Audino et al., 2001a,b, 2002), a chemically resistant material present in the cell walls of algae of the three races of the alga.

Despite the fact that individual races of *B. braunii* are ubiquitous in lakes, reports of their co-existence are unusual. Races A and B were found to co-exist in Devilbend Reservoir, Australia (Wake and Hillen, 1981) and in Overjuyo Lake, Bolivia (Metzger et al., 1988, 1989). Races B and L co-existed in Khao Kho Hong, Thailand (Metzger and Casadevall, 1987; Metzger et al., 1988) and in Yamoussoukro Lake, Ivory Coast (Metzger et al., 1988, 1990). Co-existence in these aforementioned lakes was established from a small number of cultures grown on Petri dishes (Devilbend Reservoir excepted). In a Miocene lacustrine formation, Grice et al. (1998) discovered sulfur-containing alkadiene, botryococcene and lycopadiene-derived biomarkers and inferred the co-occurrence of the three *B. braunii* races. However, in no case were the *B. braunii* lipids isolated *in situ* from lake waters or contemporary sediments, a more definitive means of establishing the variety and relative abundance of the *B. braunii* races present.

The existence of *B. braunii* in the geologic past is notably evidenced by the presence of botryococcene derivatives in sediments and petroleum (Moldowan and Seifert, 1980; Brassell et al., 1985; McKirdy et al., 1986; Huang et al., 1995; Grice et al., 1998; Smittenberg et al., 2005). However, these biomarkers are only related to race B since races A and L do not synthesize botryococcene-related lipids. Quantifying the contribution to sedimentary organic matter from the A and L races is more difficult to achieve on a molecular basis. Lycopadiene derivatives from the L race can be used as diagnostic markers (Metzger and Casadevall, 1987; Metzger et al., 1991). However, lycopadiene itself has not been identified in sediments (Adam et al., 2006), and lycopane, the reduced counterpart, is not solely derived from lycopadiene (e.g. Freeman et al., 1994; Sinninghe Damsté et al., 2003). Nevertheless, some

mono- and di-unsaturated lycopane-related hydrocarbons and monocyclic aromatic lycopane derivatives identified in some Tertiary sediments and in their pyrolysis products were considered diagnostic of the L race (Derenne et al., 1994; Grice et al., 1998; Fuhrmann et al., 2004; Adam et al., 2006). Moreover, straight chain alkanes, typically *n*-C_{27}, *n*-C_{29} and *n*-C_{31}, late diagenetic products of alkadienes and trienes from race A, are also produced by terrestrial higher plants. Nevertheless, in certain settings, early diagenetic products of alkadienes and trienes confirm the presence of the race A as shown in a Coorongite (a recent algal deposit) on the shores of Lake Balkash (Gatellier et al., 1993).

Here, we report biomarker evidence for the coexistence of all three races (A, B and L) of *B. braunii* in a contemporary lake and the presence of authentic lycopadienes and of some biosynthetic derivatives in the lake sediment.

2. Site and methods

2.1. Site

The Galápagos Islands are located roughly 1000 km due west of Ecuador. They are of recent volcanic origin and contain many natural basins in craters and hollows dammed by lava flows. Their climate is semi-arid to arid. The only permanent freshwater lake in the Galápagos is Laguna El Junco (0.30°S, 91.00°W), which is located at 700 m above sea level in the highlands of San Cristóbal (Chatham), the easternmost island in the Galápagos archipelago. Dr. Paul A. Colinvaux conducted a limnological survey and cored the sediments of El Junco in July 1966 (Colinvaux, 1968, 1972). The lake itself is contained within the caldera (collapsed cone) of an extinct volcano and is about 300 m wide. It is generally accepted that the lake has been in existence since the end of the last northern ice age. The name El Junco is Spanish for sedge, which is present along the banks of the lake. It is filled by rainwater and therefore its depth varies with the seasons. It is thus at its deepest in the rainy season (October to May), with the highest rainfall usually in April. An overflow channel 3 m deep and 2 m wide is evident in the lowest part of the rim, but no water has been observed to flow through it (Colinvaux, 1972; Sachs, observations, September 2004; M. Steinitz-Kannan, personal communication). Apart from this overflow and possible seepage through the rim, the lake basin is closed (endorheic).

2.2. Samples

In September 2004 we collected suspended particles in El Junco Lake by filtration (0.7 μm pore diameter) and net tow (30 μm pore diameter) along with several 75 cm long sediment cores. Two of those cores were analyzed for this study: EJ7-MW1 sampled at 3 cm intervals, and EJ5-MW2 sampled at 10 cm intervals.

El Junco Lake was 6 m deep in September 2004. It was well mixed as evidenced by a near-isothermal structure (19.3 °C at 1 m, 18.6 °C at 5.5 m), and was well oxygenated from top to bottom (93% O_2 saturation at 1 m and 89% O_2 saturation at 5.5 m). A sample of 250 ml of lake water was filtered through 47 mm diameter Whatman GF/F filters (0.7 μm pore diameter) at water depths of 0, 1, 2, 3, 4 and 5 m for the collection of suspended particles.

2.3. Lipid extraction and separation

Particulate and sediment samples (about 0.8–1 g) were freeze-dried prior to extraction with organic solvents. An internal standard containing 20 μg of C_{37} *n*-alkane, C_{21} *n*-alkanol and C_{21} *n*-fatty acid was added to sediment samples. Extractions were performed on a Dionex ASE-200 pressurized fluid extractor with dichloromethane (DCM) and methanol (MeOH) (9:1) at 1500 psi and 150 °C. Neutral lipids were separated from fatty acids on an aminopropyl cartridge-style SPE column (Burdick & Jackson, size 500 mg/4 ml) by eluting with DCM/isopropyl alcohol (3:1), followed by 4% acetic acid in diethyl ether to recover fatty acids (not discussed here). Neutral lipids were fractionated by column chromatography with 5% water-deactivated silica gel. Hydrocarbons were eluted with hexane, wax esters with 10% ethyl acetate (EtOAc) in hexane and sterols/alcohols with dichloromethane. Hydrocarbons were further fractionated on activated silica gel to separate botryococcenes from other hydrocarbons by elution with hexane.

Lipids were extracted from the netted particles after filtration onto GF/F glass fiber filters (125 mm diameter × 0.7 μm pore diameter) and freeze drying.

All hydrocarbon fractions were analyzed by gas chromatography–mass spectrometry (GC–MS) to positively identify each lipid and determine its purity, then by gas chromatography with flame ionization detection (GC-FID) to determine its concentration by comparing the peak areas of biomarkers to that of the internal standard. Agilent 6890 gas

chromatographs were operated with programmable temperature vaporization (PTV) inlets, 60 m Varian Chrompac CP-Sil 5 capillary columns with 0.32 mm i.d. and 0.25 μm film thickness, and helium as carrier gas. The temperature program for hydrocarbons was 80–150 °C at 10 °C/min, then to 325 °C at 4 °C/min, and a 13 min isothermal period. The temperature program for hydrocarbon fractions following kerogen pyrolysis was 100–300 °C at 4 °C/min.

2.4. Separation and identification of botryococcenes III and IV

In order to positively identify the botryococcenes present in the sediment, a large aliquot of the botryococcene fraction from the 60–70 cm interval of core EJ5-MW2 was purified by high performance liquid chromatography (HPLC) using a Waters 600E instrument fitted with a differential Waters 2414 refractometer thermostated at 30 °C. The crude mixture was fractionated into four subfractions, on a 5 μm XTerra™ MS C_{18} column (4.6 × 250 mm) connected to a Waters 410 differential refractometer by repeated injections (20 μl, 10% in $CHCl_3$) and elution with acetonitrile at a flow rate of 2 ml/min. Sub-fraction #2 was analysed by GC–MS and 1D and 2D ^{1}H and ^{13}C nuclear magnetic resonance spectroscopy (NMR) and shown to contain primarily two C_{34} botryococcenes, **III** and **IV** (structures are given in the Appendix).

2.5. Identification of trans,trans-lycopadiene V

Lycopadiene standard was obtained from the culture of a strain (Madras 2) originating from Kulavai Lake in the Madras region in India (Metzger et al., 1997). Identification of **V** in Lake El Junco was performed by comparison of mass spectra and GC co-injection.

2.6. Hydrolysis of sediment and off-line pyrolysis

Two solvent extracted sediment samples (0–10 cm and 60–70 cm; each ca. 1 g) were treated with base hydrolysis in 60 ml 1 N KOH in MeOH/H_2O (8:2) for 1 h under reflux. After filtration on FH type filters (Millipore; 0.45 μm pore size), the residues were washed with 100 ml MeOH, then with water until a neutral pH was reached, and subsequently hydrolyzed with 60 ml aqueous 4 N HCl for 6 h under reflux. The final residues, washed with MeOH and water as above, were pyrolyzed at 400 °C (1 h) under a helium flow of 15 ml min^{-1}. Subsequent fractionation of the pyrolysates (trapped at −15 °C) was performed using alumina column chromatography and successively eluting with heptane, toluene and MeOH (Largeau et al., 1986).

2.7. Dimethyldisulfide derivatization of unsaturated hydrocarbons from pyrolysis

Dimethyldisulfide (DMDS; 200 μl) and 20 μl of a solution of iodine (60 mg) in 1 ml diethyl ether were added to a 200 μl solution of heptane containing ca. 2 mg of the hydrocarbons from the pyrolysis of kerogen in the 60–70 cm interval of sediment. After 48 h at 50 °C, the reaction was stopped by the addition of 200 μl aqueous sodium thiosulfate (15% w/v). The heptane solution was decanted and the aqueous phase was re-extracted twice with 200 μl heptane. The solvent of the combined organic phases was evaporated and the crude mixture analyzed using GC–MS.

2.8. Hydrogenation

Hydrogenation of an aliquot of the hydrocarbon fraction from the 0–1 cm sediment interval was performed in heptane in the presence of a rhodium catalyst (Rh/C, 5%) at 70 °C and 20 atm pressure for 1 h, after which the reaction mixture was centrifuged, the supernatant concentrated under a stream of nitrogen, and its contents analyzed by GC–MS.

3. Results and discussion

3.1. Biomarkers of B. braunii in suspended particles of El Junco Lake

3.1.1. Hydrocarbons

Suspended particulate samples appeared pale yellow on GF/F filters through which 250 ml of lake water had been filtered. The hydrocarbon distributions at all depths were very similar, presumably as a result of the shallow depth (6 m) and well mixed nature of the water column. The total ion chromatogram (TIC) of the hydrocarbon fraction from a depth of 1 m is shown in Fig. 1a. Straight chain hydrocarbons from C_{18} to C_{31} and lycopadienes predominate the chromatogram. The straight chain hydrocarbons are dominated by alkadienes and a triene typical of *B. braunii* race A: a series of odd carbon numbered C_{25}–C_{31} doublets for *cis*/*trans* iso-

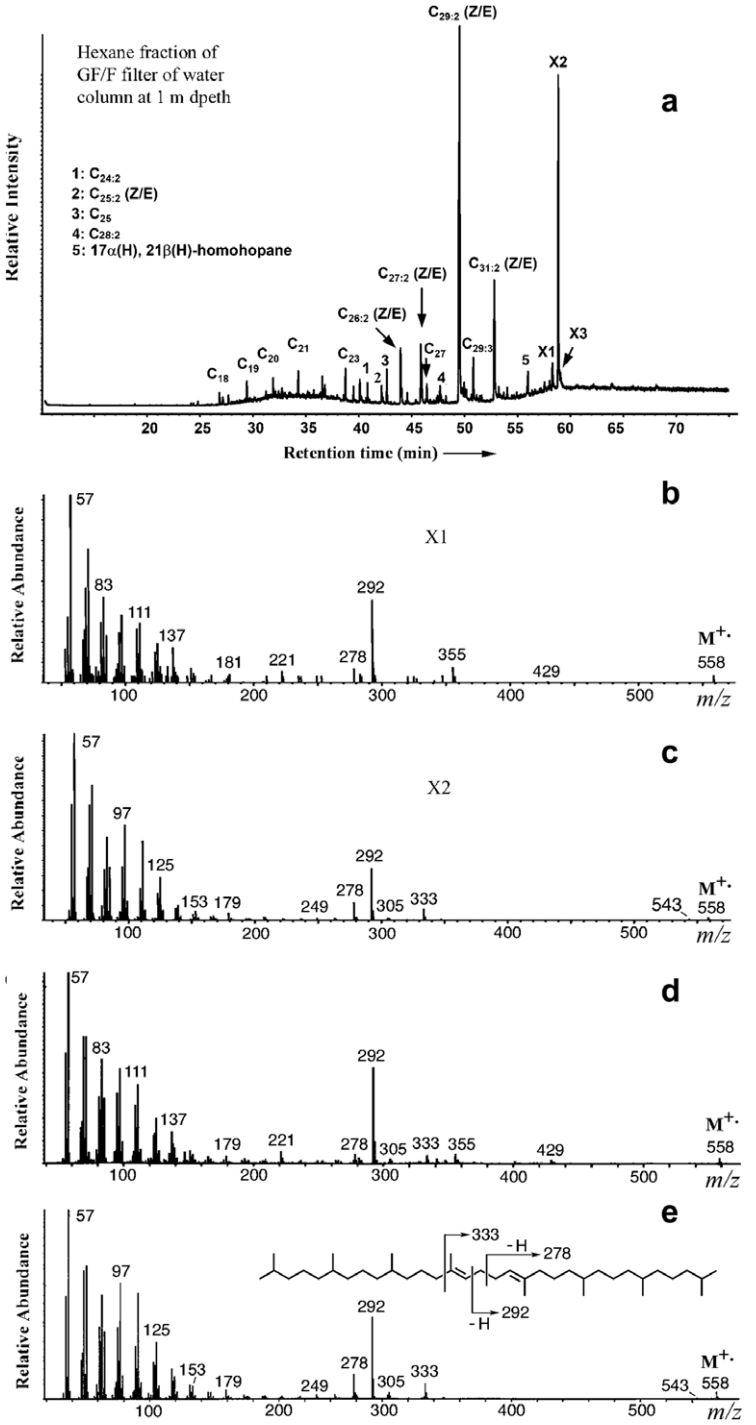

Fig. 1. Total ion chromatogram and mass spectra of hydrocarbons in suspended particles (>0.7 μm) from 1 m depth: (a) Total ion chromatogram showing the presence of alkadienes and lycopadienes; (b) and (c) are the mass spectra of two main isomers of lycopadienes present in the chromatogram; (d) and (e) are the mass spectra of the two main lycopadiene isomers obtained from a culture of race L (strain Madras 2), with the structure in (d) unknown, and that in (e) being *trans*,*trans*-lycopadiene.

mers, exhibiting a terminal double bond and a mid-chain unsaturation at ω9 of *Z* and *E* stereochemistry, with C_{29} dienes (structures **I** and **II**, respectively, see Appendix) predominating, and a C_{29} triene. Two main hydrocarbon profiles are generally found in strains of this race: either a mixture of *cis* dienes

and triene(s) or a mixture of *cis*/*trans* dienes but without the triene (Metzger et al., 1985a, 1986, 1989, 1997). An origin from *B. braunii* race A also seems plausible for three doublets of unusual C_{24}, C_{26} and C_{28} dienes, likely as *cis*/*trans* isomers (Fig. 1a). Indeed, small quantities of C_{26} and C_{28} alkadienes were previously identified in the culture of a Bolivian strain of *B. braunii* race A (Metzger, 1993). These observations suggest that at least two different populations of race A are present in the lake.

Saturated hydrocarbons were comprised of straight chain alkanes ranging from C_{18} to C_{27} (Fig. 1a) showing an odd over even predominance.

The hydrocarbon fraction contained three lycopadiene isomers (Fig. 1a). The mass spectra of the two primary isomers are shown in Fig. 1b–c. The predominant lycopadiene appears to be *trans*,-*trans*-lycopadiene **V**, i.e. 2,6(*R*),10(*R*),14,19,23(*R*),27(*R*),31-octamethyldotriaconta-14(*E*), 18(*E*)-diene (X2, Fig. 1c), based on co-injection with an authentic standard obtained from an L-race culture (Metzger et al., 1997) and comparison of the mass spectra (Fig. 1e). A minor isomer was also detected (X1, Fig. 1b) and had a very similar mass spectrum (Fig. 1b and d, respectively). The same isomer has previously been identified in two strains from Kulavai Lake, India (Metzger et al., 1997). Re-analyses of lycopadienes isolated from the two Kulavai Lake strains indicated that they too contained three lycopadiene isomers and not two as previously reported. The third isomer is also present in trace quantities in El Junco suspended particles, visible as a small shoulder (X3) on peak X2 in the TIC. Given the low abundance and poor separation of this third isomer, only the characteristic ion of *m*/*z* 292 was detected in its mass spectrum with no indication of the molecular ion.

No botryococcenes were detected in suspended particles from El Junco Lake. Some small peaks in the TIC between $C_{27:2}$ and $C_{29:2}$ (Fig. 1a) may have mass spectra with similar fragmentation patterns to the botryococcenes but with the low abundances no molecular or characteristic ions were confirmed.

3.1.2. Epoxide biomarkers

Suspended particles from 0, 1, 2, 3, 4 and 5 m water depth all contained epoxides, as deduced from GC–MS analysis of the hexane/EtOAc fractions (9:1 v/v). Four monoepoxides and one diepoxide were detected, all previously identified in cultures of *B. braunii* (Metzger and Casadevall, 1989; Delahais and Metzger, 1997; Metzger, 1999). The TIC of a sample collected at 3 m (Fig. 2a) is dominated by a C_{40} monoepoxide (**VIII**), which derives from *trans*,*trans*-lycopadiene (Fig. 2c), a biomarker for *B. braunii* race L, and is accompanied by smaller quantities of diepoxy-lycopane (**IX**) (Fig. 2d). In addition, three monoepoxides with mid-chain epoxide groups and derived from A-race specific *n*-alkadienes (**VII**) were identified by co-injections with authentic standards isolated from a culture of the Austin strain (Metzger and Casadevall, 1989) and mass spectral comparisons (Fig. 2b). The C_{29} epoxide was the most abundant compound of the three followed by the C_{27} and C_{31} epoxides (Fig. 2a).

To date, the presence of epoxides derived from lycopadiene and epoxy-*n*-alkenes has not been reported in wild populations of *B. braunii* even though they are important intermediates in the biosynthesis of common lipids in all three races (Metzger and Casadevall, 1992; Metzger, 1999) such as **XV** and **XVI** in races A and L, respectively (Metzger and Largeau, 2005, and references therein).

3.2. Biomarkers of Botryococcus braunii in large (>30 μm) suspended particles

The net tow (30 μm pore diameter) contained primarily copepod zooplankton in addition to some unidentified black particles. Copepods were red in color and typically ca. 100 μm in diameter. They are tentatively identified as Calanoid copepods resembling *Notodiaptomus amazonicus*, although the hooked fifth leg of the El Junco males was considerably larger than in *N. amazonicus*, and other features support them as being an endemic species (Dr. Miriam Steinitz-Kannan, personal communication).

The hydrocarbon fraction from the net tow contained biomarkers from all three races (A, B and L) of *B. braunii* and represents, to the best of our knowledge, the first report of their co-existence in a lake (Fig. 3a). There were small concentrations of straight chain *n*-alkanes ranging from C_{21} to C_{33}, suggesting two different sources: (1) long chain *n*-alkanes (from C_{27} to C_{33}) from higher plants with an odd over even predominance and (2) short chain *n*-alkanes (from C_{21} to C_{25}) accompanied by corresponding *n*-alkenes ($C_{23:1}$ and $C_{25:1}$) most likely from algae. Except for the *n*-alkanes and *n*-alkenes, cholesta-3,5-diene, and fernene, the hydrocarbons in the >30 μm suspended particles can be attributed to the three races of *B. braunii*.

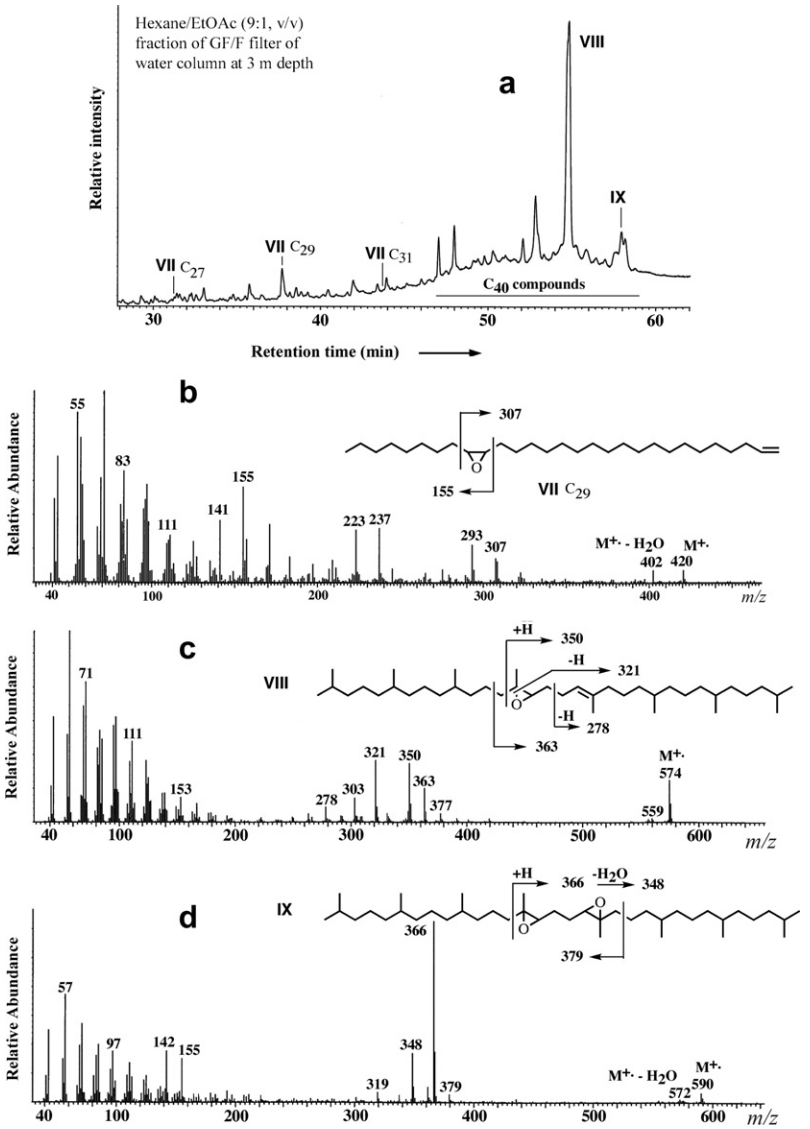

Fig. 2. Total ion chromatogram and mass spectra of the hexane/ethyl acetate (9:1 v/v) fraction from suspended particles (>0.7 μm) collected at 3 m depth: (a) total ion chromatogram of the fraction containing epoxides. (b–d) Mass spectra of the C_{29} monoepoxide (**VII**) and the C_{40} mono- and di-epoxides (**VIII** and **IX**, respectively).

The distribution of alkadienes and alkatrienes in the large (>30 μm) suspended particles was nearly identical to that in the small (>0.7 μm) suspended particles. They were characterized by odd carbon numbered alkadienes from C_{25} to C_{31} predominated by *cis*/*trans* (*Z*/*E*) isomers of $C_{29:2}$ **I** and **II**, respectively (Fig. 3a). An appreciable amount of even carbon numbered dienes, mainly C_{24} and C_{26}, were also present in the net tow (large particles), as was the $C_{29:3}$ triene (Fig. 3a).

The pattern of lycopadienes in the net tow (large particles) was the same as in the >0.7 μm suspended particles, containing three isomers dominated by the *trans*,*trans*-lycopadiene. But the relative abundance of lycopadienes to alkadienes was much smaller in the large particles than in the >0.7 μm suspended particles.

In contrast to the suspended particles, the net tow (>30 μm) contained significant quantities of botryococcenes (Fig. 3a) comprised primarily of six C_{34} isomers (Fig. 3b). The two primary isomers (Fig. 3b) were tentatively identified as botryococcenes **III** and **IV** on the basis of their mass spectra and their co-elution with authentic standards (see Section 3.4.2).

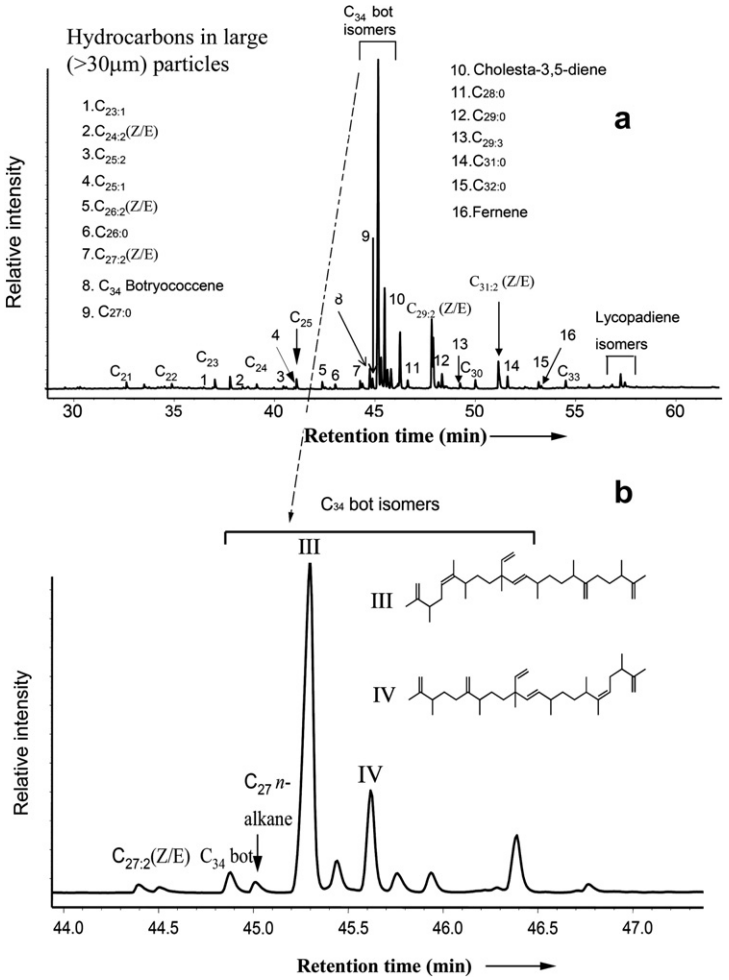

Fig. 3. Total ion chromatogram and mass spectral information of hydrocarbons from net tow (>30 μm) suspended particles: (a) total ion chromatogram demonstrating the co-existence of alkadienes, botryococcenes and lycopadienes; (b) expanded portion of the TIC containing the botryococcenes and showing the structures of two most abundant isomers.

C_{34} botryococcene **III** was found to be especially abundant in wild algae of a small pool in Paquemar, Martinique (Metzger et al., 1985a,b) and further identified in the cultures of numerous strains from different lakes (e.g. Metzger et al., 1988; Sato et al., 2003). C_{34} botryococcene **IV** was recently reported in a strain from Japan (Okada et al., 1997).

Different relative abundances of the chemical markers for the three races (alkadienes, botryococcenes and lycopadienes) could derive from variations in the populations of each race in a lake as well as from differences in the hydrocarbon concentration in each race. Ecological surveys of phytoplankton in lakes generally group all *B. braunii* algae into a single population, resulting in a paucity of data on the distribution of individual races (e.g. Green, 1976; Huszar and Reynolds, 1997; Murugavel and Pandian, 2000). On the other hand, the lipid content of *B. braunii* varies significantly from race to race, from strain to strain in a given race, and as a function of physiological state within a strain (see Metzger and Largeau, 1999, 2005, and references therein).

Thus, depending on their geographical origin, algae of race B generally contain ca. 30–40% of dry biomass as hydrocarbons, those of race A contain 1–61% of the dry biomass as hydrocarbons, averaging ca. 10%, and those of race L contain 0.1–8% of dry wt as hydrocarbons (e.g. Metzger et al., 1985a, 1990; Metzger and Largeau, 1999).

Moreover, it must be stressed that high lipid concentrations cause the algae to float, perhaps leading to an enrichment of the lipid rich B race in a near-surface net tow. The existence of enduring blooms

of *B. braunii* race B in the surface waters of Darwin River Reservoir, Australia (Wake and Hillen, 1980, 1981; Townsend, 2001), supports this hypothesis.

High abundances of *B. braunii* lipids in the net tow from El Junco Lake can be rationalized on the basis that those algae are a major food source for copepods (Dr. Miriam Steinitz-Kannan, personal communication; Thompson and Irvine, 1997).

3.3. B. braunii lipids in El Junco surface sediments

A 75 cm long sediment core (EJ7-MW1) was sectioned into 1 cm intervals, extracted and analyzed for biomarkers. The sediment was dark brown mud when dried. The total ion chromatogram of the hydrocarbon fraction from 0 to 1 cm is shown in Fig. 4a.

The hydrocarbon distribution in surface sediments was similar to that in the large (>30 μm) particles with the following exceptions: (1) an increased proportion of lycopadienes; (2) a decreased proportion of C_{34} botryococcenes; (3) an increased proportion of long chain *n*-alkanes, presumably from higher plant waxes; and (4) no indication of alkadienes or alkatrienes (Fig. 4a). The straight chain *n*-alkanes and alkenes had the same pattern as in the large (>30 μm) suspended particles, ranging from C_{21} to C_{33} and exhibiting doublets of $C_{23:1}/C_{23:0}$, $C_{25:1}/C_{25:0}$ and $C_{27:1}/C_{27:0}$. The concentration of $C_{25:1}$ and $C_{25:0}$ were 5.2 and 26.3 μg/g dry sediment, respectively (Table 1).

High concentrations (827 μg/g of dry sediment) of lycopadienes ($C_{40}H_{78}$) were present in the surface sediment, accompanied by lower concentrations of a $C_{40}H_{76}$ series (Fig. 4a and b). To our knowledge this represents the first report of multiple lycopadienes in sediments.

With the exception of two strains of race L originating from Kulavai Lake (Madras region, India; Metzger et al., 1997) such high abundances of lycopadiene isomers in living race L algae have not been reported, and low hydrocarbon contents of the Madras strains prevented structural determination of the second lycopadiene.

The high abundance of unsaturated lycopane type C_{40} hydrocarbons in El Junco sediment permitted positive identification of at least 13 lycopadiene and lycopatriene isomers, none of the latter having been detected in the suspended particles (1–13; Fig. 4b). The mass spectra of the primary lycopadiene isomers (6–9; Fig. 4c–f) each contained the molecular ion *m*/*z* of 558 and likely differed in the location of the double bonds. Their combined concentration reached 768.8 μg/g of dried sediment (Fig. 4b). We expect that compound X1 in the suspended particles (Fig. 1a) may have been comprised of the same two co-eluting lycopadiene isomers we observe in the surface sediment (6–7; Fig. 4b).

Though they have not previously been reported in *B. braunii*, lycopatriene isomers (10–13 in Fig. 4b) were identified based on the molecular ion *m*/*z* 556. Mass spectra and co-injection standards established structure **VI** for peak 11 in Fig. 4b. Furthermore, the existence of a lycopane skeleton for all lycopadienes and lycopatrienes was confirmed by the synthesis of two lycopane diastereoisomers with identical mass spectra (Fig. 5e and f) upon hydrogenation of an aliquot of compounds **I–XIII** with rhodium catalyst (Fig. 5a). The formation of lycopane diastereomers, as also observed in the catalytic hydrogenation of a *trans*,*trans*-lycopadiene standard (Fig. 5b), is related to the existence of asymmetric centers bearing a methyl group at positions 6, 10, 23 and 27, all with the same (*R*) configuration (Metzger and Casadevall, 1987). The only published report of lycopatriene we found was from archaea isolated from a deep sea hydrothermal vent (Lattuati et al., 1998). Subsequently we have looked for and found very low concentrations of a lycopatriene, believed to be structure **VI** based on its mass spectrum, in an L race *B. braunii* strain (Kulavai).

We hypothesize that lycopatrienes are intermediates in lycopadiene synthesis via carotenoid reduction and that the enzyme catalyzing the reduction of lycopatrienes to form lycopadienes is efficient enough that only trace amounts of lycopatriene accumulate in the cell.

In El Junco surface sediment, three major lycopadiene isomers were present in similar proportions (accounting for 6 and 7 in Fig. 4b as one isomer because they coelute), while in the suspended particles (both >0.7 μm and 30 μm fractions) the *trans*, *trans* isomer was most abundant. The latter distribution is analogous to that observed for living L race *B. braunii* such as the strains from Madras, India (Metzger et al., 1997). Differences in the lycopadiene distributions in particles and sediments beg the question whether the two lycopadienes (6/7 and 9; Fig. 4b) come from isomerization of a *trans*,-*trans* isomer (structure **V**; peak 8 in Fig. 4b) in the surface sediment or whether they are true metabolites of *B. braunii*. Similar lycopadiene distributions in El Junco suspended particles (large and small) and the Madras strains likely result from a living algal source.

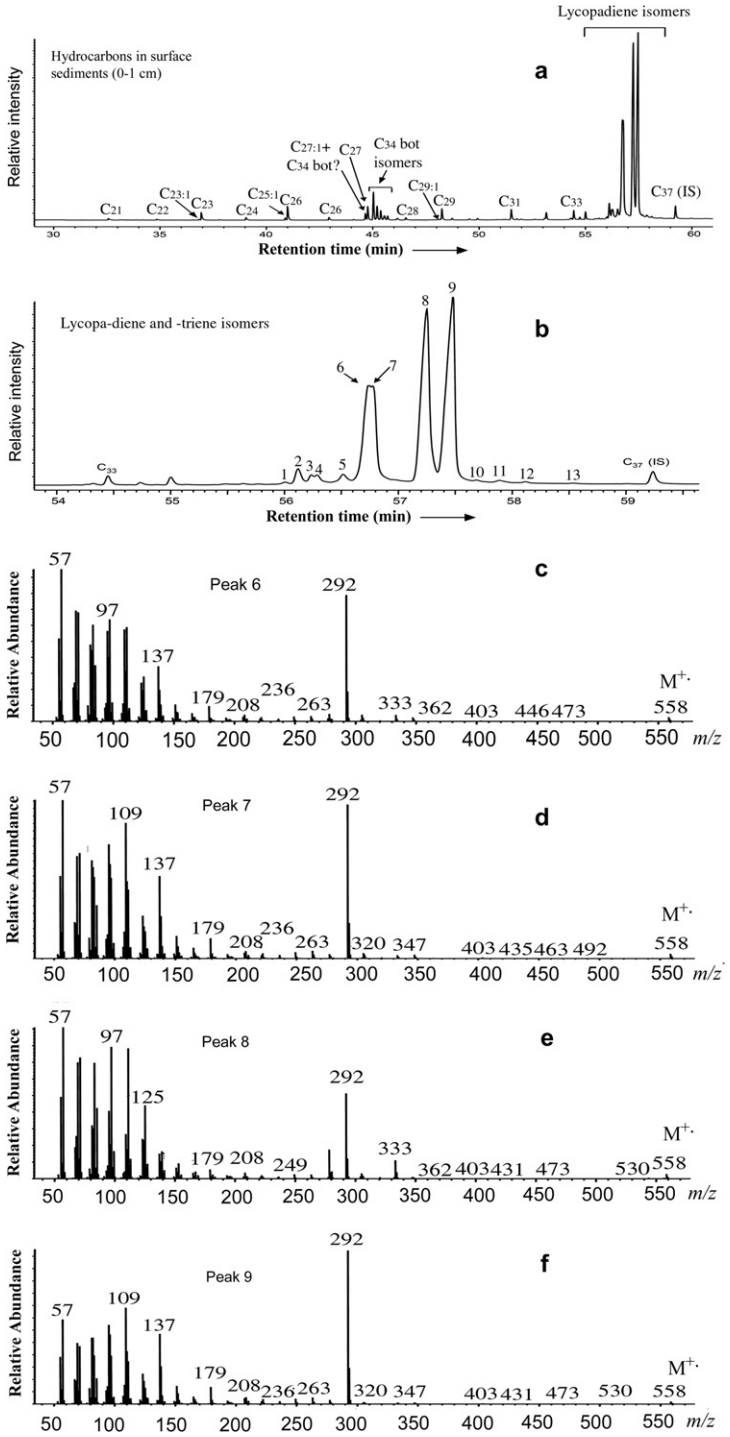

Fig. 4. Total ion chromatogram and mass spectra of hydrocarbons in surface sediment (0–1 cm): (a) Total ion chromatogram showing the presence of alkadienes and lycopadienes; (b) Expanded portion of the TIC containing the lycopadienes and lycopatrienes. Mass spectra of the most abundant lycopadiene isomers are shown in (c–f). IS = internal standard.

One hypothesis for the differing ratios of lycopadienes in El Junco surface sediments relative to suspended particles and *B. braunii* cultures is that the *trans*, *trans* isomer **V** (peak 8 in Fig. 4b) is synthesized primarily during the exponential growth phase (Metzger et al., 1990) and its relative abun-

Table 1
Abundance of biomarkers from *B. braunii*, other algae and higher plants at selected depths in the upper 76 cm of El Junco sediment

Depth (cm)	Age (year ago)	Biomarker concentration (μg/g dried sediment)						
		$C_{23:1}$	$C_{23:0}$	$C_{25:1}$	$C_{25:0}$	$C_{31:0}$	BOT	LP
0–1	3	2.6	19.7	5.2	26.3	17.5	86.0	827.6
3–4	17	2.8	22.5	4.9	29.0	19.9	12.7	140.4
6–7	30	2.7	21.0	4.1	26.8	18.5	8.9	133.9
9–10	56	3.2	23.2	5.3	27.8	18.6	5.1	52.7
12–13	93	2.6	21.7	4.1	29.9	18.3	3.1	42.0
15–16	125	2.9	22.6	5.9	36.6	19.9	4.0	30.4
21–22	183	3.0	31.3	9.8	43.1	26.5	81.4	200.4
36–37	306	11.3	79.2	75.5	46.1	26.8	133.8	48.2
45–46	363	7.2	32.9	36.7	30.9	23.3	576.5	63.2
48–49	379	5.9	24.6	24.6	28.9	24.8	12649.0	67.5
54–55	408	6.6	22.0	27.0	26.6	26.4	16714.5	81.5
69–70	448	3.9	22.5	13.5	22.8	19.3	9412.8	101.2
75–76	460	5.2	24.1	20.5	26.3	22.4	7986.2	51.8

BOT = Total amount of all isomers of botryococcenes (C_{34}).
LP = Total amount of all isomers of lycopadienes and lycopatrienes.
Ages are estimated based on ^{210}Pb dating and ^{14}C dating.
Bot (botryococcene) = total amount of all isomers of C_{34} botryococcenes (structures of two main isomers (**III**, **IV**) are given in Appendix); LP (lycopadienes and lycopatrienes) = total amount of lycopadienes (**V**) and lycopatrienes (**VI**); $C_{25:1} = C_{25}$ *n*-alkene and $C_{25:0} = C_{25}$ *n*-alkane. Ages are estimated from ^{14}C dating of total organic matter and ^{210}Pb dating (Conroy and Overpeck, personal communication).

dance in a population results from a balance between its production and its loss to ether lipid syntheses (e.g., compound **XVI**) (Rager and Metzger, 2000). One possibility is that the concentration of the *trans*, *trans* isomer **V** decreases in senescent algae, eventually approaching the concentrations of the two other isomers (peaks 6/7 and 9 in Fig. 4b), in which case the high relative abundance of the two isomers (6/7 and 9 in Fig. 4b) may therefore indicate that race L was senescent at the time of deposition.

Botryococcenes were also present in surface sediment, reaching 78.7 μg/g of dried sediment (Table 1). All botryococcenes observed were C_{34} homologues. As with the lycopadienes, hydrogenation of the main C_{34} botryococcene isomer in the surface sediment produced two botryococcane diastereomers (Fig. 5a, c and d). Though the distribution of C_{34} botryococcene isomers in the surface sediment was nearly identical to that in the >30 μm suspended particles, their abundance relative to lycopadienes was much lower than in either size class of suspended particles (Fig. 1a and Fig. 4a; Table 1).

There was scant evidence for the presence of alkadienes or alkatrienes in the surface sediment. Trace quantities (<10 ng/g) of *E* and *Z* isomers of C_{27}, C_{29} and C_{31} alkadienes were detected in extracted ion chromatograms, but no C_{29} alkatriene was detected.

Based on the hydrocarbon yield of the three races of *B. braunii*, and the lipid distribution found in the surface sediment of El Junco Lake, we estimate that the L race population exceeded that of the B race, which exceeded that of the A race over the last few years.

3.4. *B. braunii* lipids in El Junco Lake sediment

3.4.1. *The 36–37 cm interval*

B. braunii lipids in the 36–37 cm interval of sediment had a similar distribution to those in the surface (0–1 cm) sediment characterized by alkene/alkane doublets, botryococcenes and lycopadienes (Fig. 6a). One unique characteristic was the clear presence of alkadiene peaks (Fig. 6a–c). The *Z* and *E* isomers of the C_{29} alkadiene (molecular ions at *m*/*z* 404) eluted prior to the $C_{29:1}/C_{29:0}$ alkene/alkane doublet and the *Z* isomer was more abundant (Fig. 6a–c). Although neither of the $C_{27:2}$ alkadiene isomers were identifiable in the TIC, and only one isomer of the $C_{31:2}$ alkadiene (*m*/*z* 432) was observed, extracted ion chromatography supports the existence of both *Z* and *E* isomers of all three alkadiene homologues ($C_{27:2}$, $C_{29:2}$, $C_{31:2}$) (Fig. 6a), making this the first reported co-occurrence of hydrocarbon biomarkers from all three *B. braunii* races (alkadienes, botryococcenes and lycopadienes) in the same lake sediment.

The ratios of alkenes to alkanes in the 36–37 cm depth interval were much higher than in the surface (0–1 cm) sediments, especially for the $C_{25:1}/C_{25:0}$

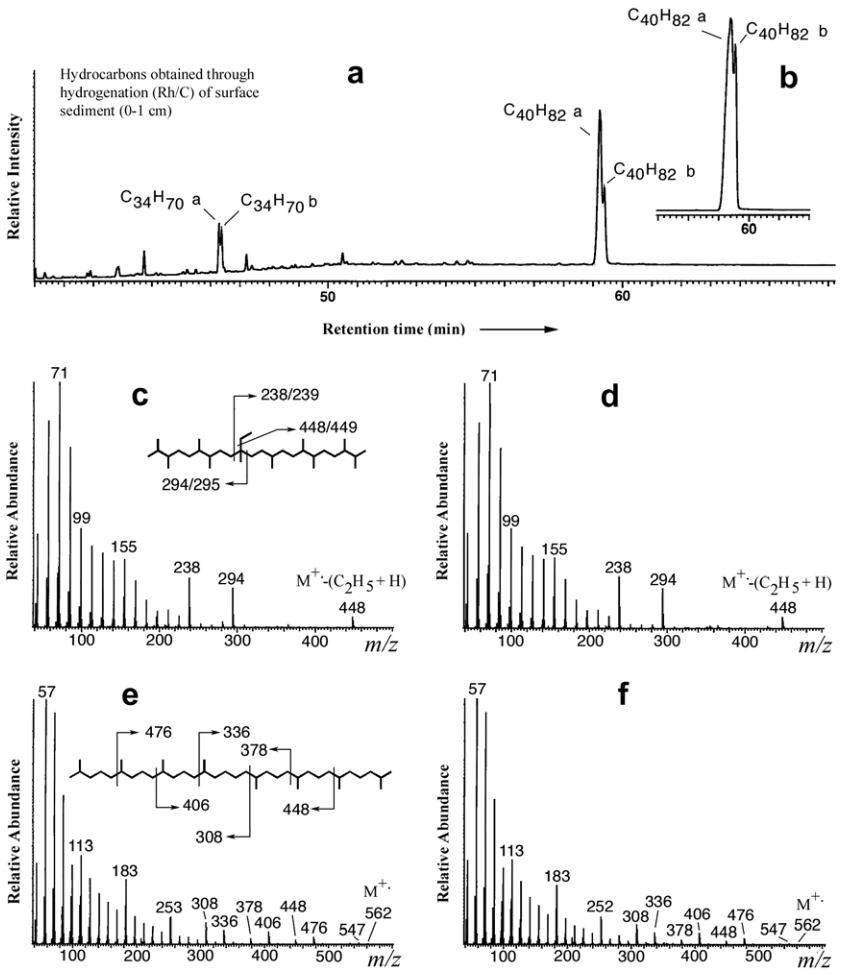

Fig. 5. Products of hydrocarbon hydrogenation with a rhodium catalyst. Total ion chromatogram (a) and mass spectra (c–f) of the hydrogenated hydrocarbons from surface sediment. Total ion chromatogram of the mixture of lycopane diastereomers (b) obtained by hydrogenation of the *trans*,*trans*-lycopadiene standard. (c–d) Mass spectra of botryococcane diastereomers $C_{34}H_{70}$ a and $C_{34}H_{70}$ b, respectively. (e–f) Mass spectra of lycopane diastereomers $C_{40}H_{82}$ a and $C_{40}H_{82}$ b, respectively.

and $C_{27:1}/C_{27:0}$ pairs, likely the result of environmental conditions favorable to the growth of other algae (Gelpi et al., 1970; Zhang et al., 2004).

Only once before has the co-occurrence of *n*-alkenes and *n*-alkadienes been reported in lacustrine sediments, the long chain mono-, di- and tri-unsaturated alkenes found in an African crater lake (de Mesmay et al., 2007).

The botryococcenes at 36–37 cm had a concentration of 134 μg/g sediment (Table 1) and were all C_{34} homologues. Though in higher abundance than in the surface sediment, their distribution of isomers was similar.

Lycopadienes in the 36–37 cm sediment interval consisted of the same three isomers as in the 0–1 cm surface sediment and suspended particles on GF/F filter (>0.7 μm) (Fig. 6a vs. Fig. 3a) but were characterized by a higher relative abundance of the *trans*,*trans* isomer compared to those in 0–1 cm surface sediment (Fig. 6a vs. Fig. 4a).

3.4.2. The 69–70 cm interval

An extremely high concentration of botryococcenes, 9400 μg/g of sediment, was observed in the 69–70 cm interval of sediment (Fig. 7a; Table 1). The high absolute and relative abundance of botryococcenes (to alkadienes and lycopadienes) suggests that a large population of the B race of *B. braunii* existed during the deposition of that sediment ~448 year ago and that the B race population exceeded both the A and L race populations.

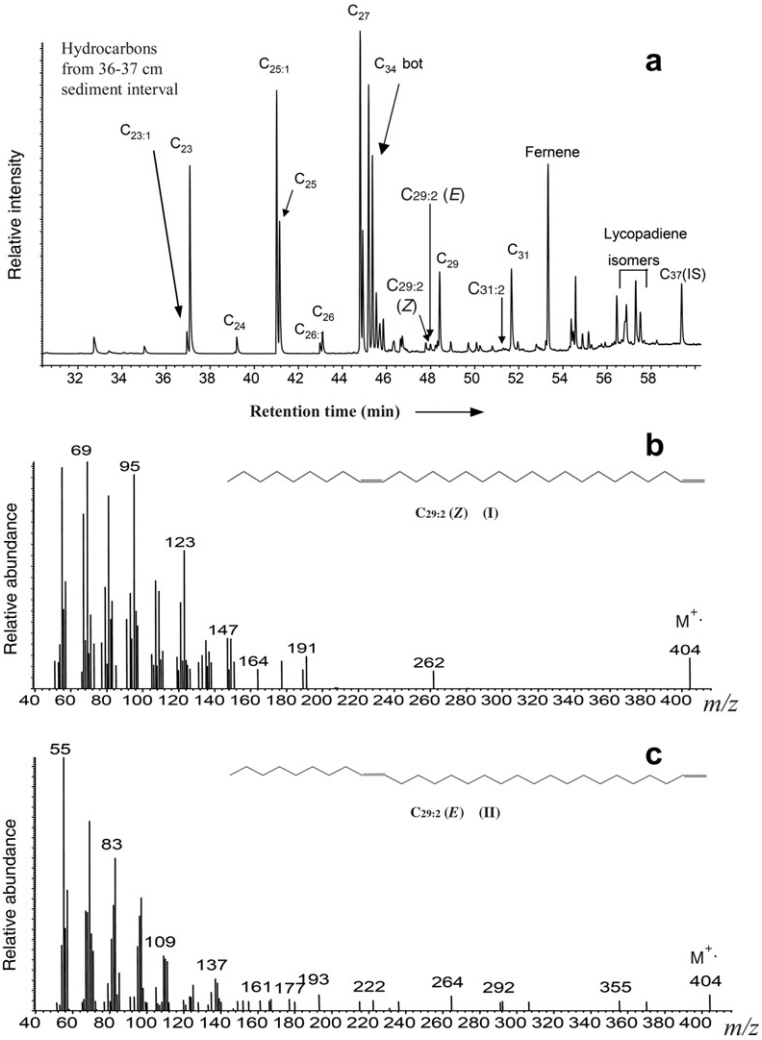

Fig. 6. Total ion chromatogram and mass spectra of hydrocarbons from the 36–37 cm interval of sediment: (a) Total ion chromatogram showing the co-occurrence of botryococcenes and lycopadienes as well as alkadienes in trace quantities. (b–c) Mass spectra of *Z* and *E* isomers of the C_{29} alkadiene. IS = internal standard.

The high abundance of botryococcenes in this sediment interval allowed structural characterization by 1D and 2D ^{1}H and ^{13}C NMR spectroscopies. After elution from silica gel with hexane (Fig. 7b), the hydrocarbons were separated into four sub-fractions by HPLC. Sub-fraction 2 contained the two C_{34} botryococcenes **III** and **IV**, based on NMR spectra (Metzger et al., 1985b; Okada et al., 1997). NMR spectroscopy was not performed on other fractions due to insufficient quantities of pure C_{34} isomers.

Lycopadienes in the 69–70 cm interval had the same triple isomer pattern as in the suspended particles and the 36–37 cm sediments. But compared to the surface (0–1 cm), sediments contained a higher relative abundance of the *trans*,*trans* isomer (**V**, Appendix; Fig. 7a vs. Fig. 2a; Table 1), implying that the L race may not have been entirely senescent at the time of deposition.

As was the case in the 0–1 cm sediment interval, alkadienes were only detectable by extracted ion chromatography. Compared to both size classes of suspended particles, the down core abundance of alkadienes was significantly lower than that of lycopadienes, and alkadiene epoxides were undetectable. In contrast mono- and di-epoxides of lycopadiene were present throughout the core (in the 10% EtOAc fraction). Considering the fact that alkadiene epoxides and lycopadiene (mono- or di-) epoxides were present in the >0.7 μm suspended particles it is puzzling that no alkadiene epoxides were found in the

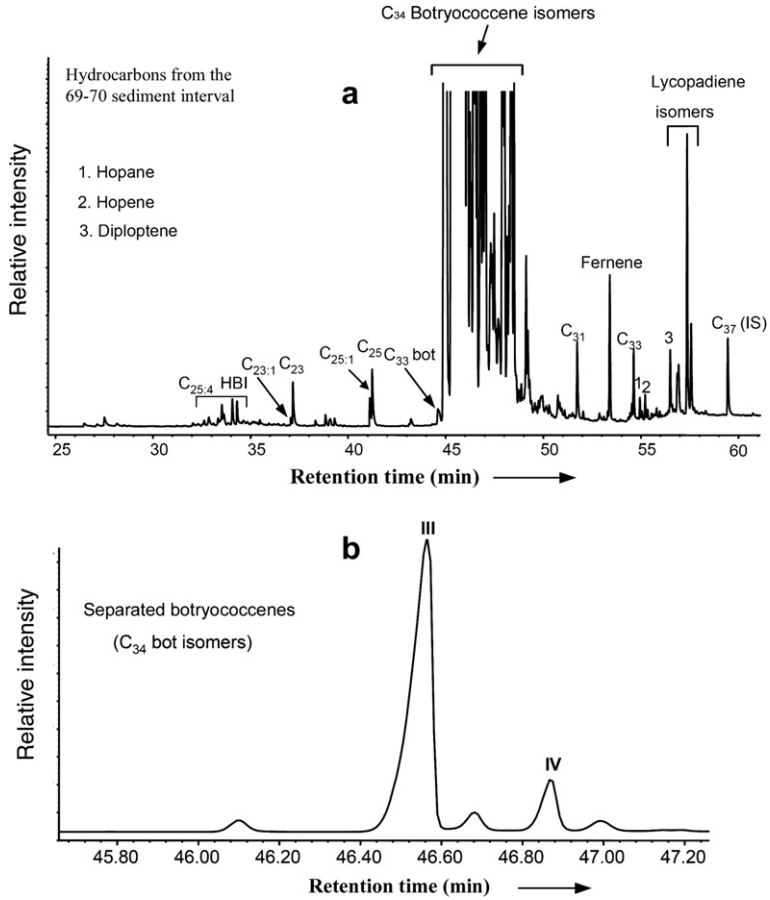

Fig. 7. Total ion chromatogram and mass spectral information of hydrocarbons from the 69–70 cm interval of sediment: (a) Total ion chromatogram showing the presence of alkadienes and lycopadienes. (b) Expanded portion of the botryococcene portion of the TIC. IS = internal standard.

sediment. One possibility is that they were quantitatively metabolized into ether lipids (e.g. **XV**; Metzger and Casadevall, 1992) or degraded into other metabolites during the earliest stages of diagenesis, with terpenoid hydrocarbons, botryococcenes and lycopadienes likely exhibiting a higher resistance to bacterial degradation (Ratledge, 1978).

Straight chain hydrocarbons at 69–70 cm had a distribution similar to that in the 0–1 cm interval, consisting of relatively low concentrations of C_{29} to C_{33} *n*-alkanes from higher plants (Eglinton et al., 1962), and relatively high concentrations of C_{23} to C_{27} alkene/alkane doublets from aquatic algae (Gelpi et al., 1970; Zhang et al., 2004) (Table 1).

3.5. Pyrolysis products of kerogen

Kerogen (i.e., insoluble organic matter) samples dating back to the Ordovician contain evidence of *B. braunii* (e.g. Cane and Albion, 1973; Cane, 1977). It is believed that selective preservation of highly aliphatic biomacromolecules in the cell wall of *B. braunii*, the so called algaenans, occurs (e.g. Largeau et al., 1984, 1986) along with polymerization of *B. braunii* lipids during early diagenesis under oxic conditions (Dubreuil et al., 1989; Gatellier et al., 1993). In order to evaluate the *B. braunii* signature in El Junco lake kerogen, we subjected two previously solvent extracted sediment samples (from 0 to 10 cm and 60 to 70 cm) to base and acid treatment, and pyrolyzed the residues (Larter and Horsfield, 1993).

The pyrolysates were fractionated by alumina column chromatography, yielding a hydrocarbon fraction (heptane), a mid-polarity fraction (toluene) and a polar fraction (MeOH). Only the hydrocarbon fractions were analyzed in the present study and they accounted for ca. 9% of the kerogens and exhibited complex total ion chromatograms (Fig. 8a and b). A series of *n*-alkanes/*n*-alk-1-enes

from C_{15} to C_{32} was observed with the maximal abundance at C_{19}/C_{20}, accompanied by other *n*-alkenes and *n*-alkadienes eluting before the *n*-alk-1-enes (Fig. 8a and b). In contrast to the 0–10 cm kerogen, hydrocarbons from the 60–70 cm kerogen were characterized by a large relative concentration of *n*-$C_{29:2}$ diene, *cis*-1,20-*n*-nonacosadiene **I** (Fig. 8b). A small shoulder on the latter peak was the *trans*-isomer **II**. Both isomers were identified based on mass chromatograms of tetra(methylthio)nonacosane derivatives formed by adduction with dimethyldisulfide.

The isoprenoid distribution in the kerogen samples consisted of C_{27} and C_{29} norhopenes, a series of C_{16} to C_{21} compounds containing saturated and mono-unsaturated C_{16}, C_{20} and C_{21} isoprenoids but dominated by trimethyl-2,6,10-pentadecane and prist-1-ene and a series of 20 tetraterpenes that had similar abundances (Fig. 8a and b).

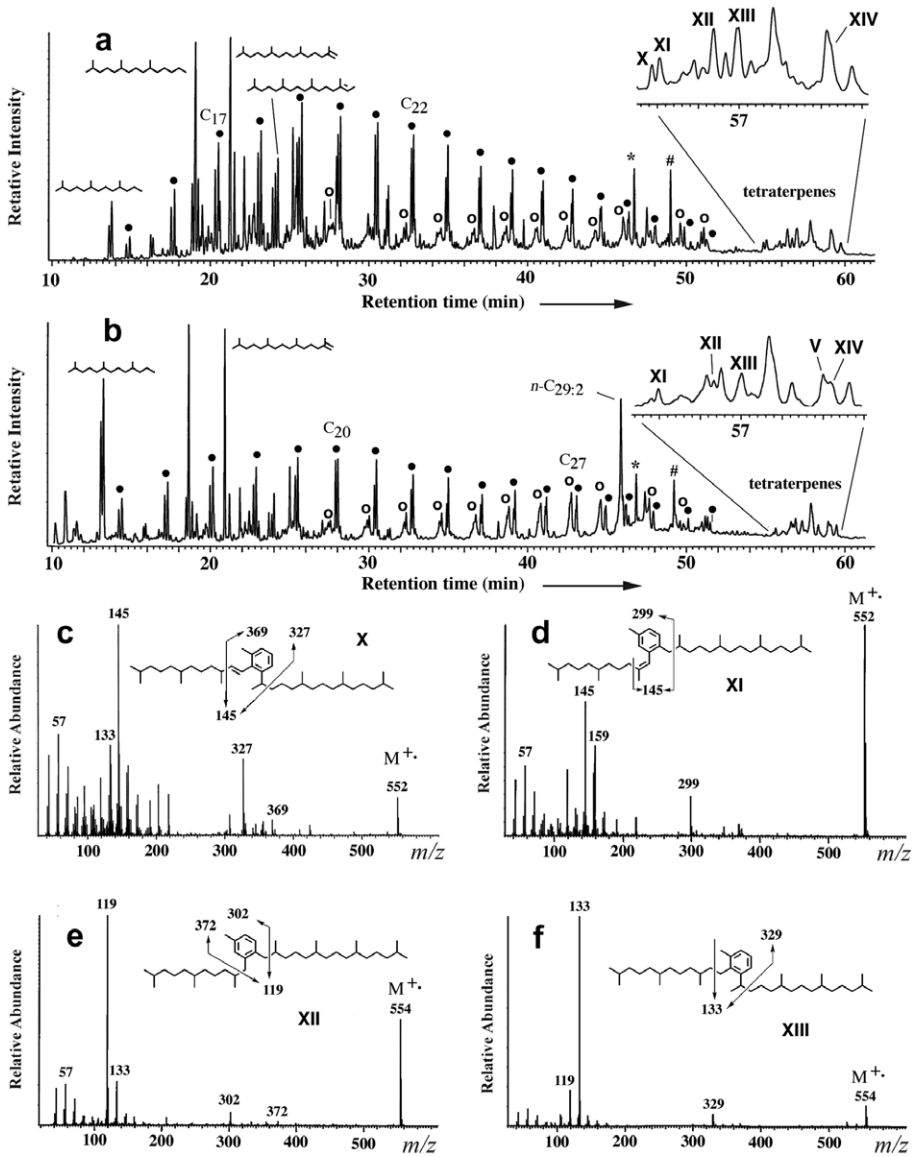

Fig. 8. Total ion chromatogram and mass spectra of the hydrocarbon fractions from off-line 400 °C pyrolysates of the residues obtained from two hydrolyzed sediments: 0–10 cm interval (a) and 60–70 cm interval (b). Filled circles: *n*-alk-1-ene/*n*-alkane doublets. Open circles: clusters of *n*-alkadienes and *n*-alkenes with mid-chain unsaturation. ∗: 22,29,30-trisnor-hop-17(21)-ene, #: 30-norhop-17(21)-ene. (c–f) Mass spectra of monoaromatic lycopane derivatives **X**–**XIII** in the pyrolysates of El Junco kerogen fractions.

The mass spectra of the tetraterpenes were characterized by molecular ions $[M]^+$ at *m/z* 558 ($C_{40}H_{78}$), 554 ($C_{40}H_{74}$), or 552 ($C_{40}H_{72}$). A peak with *m/z* 558 was found to correspond to *trans,trans*-lycopadiene ($C_{40}H_{78}$, **V**) based on co-injection of an authentic standard. Though clearly present at 60–70 cm (Fig. 8b), this compound could not be confirmed in the 0–10 cm kerogen pyrolysate (Fig. 8a).

Published mass spectra (Derenne et al., 1990; Adam et al., 2006) further suggest that three monoaromatic lycopane derivatives, $C_{40}H_{74}$ (**X**–**XII**), occurred in the pyrolysates (Fig. 8a and b). Compounds **XII** (mass spectrum in Fig. 8e) and **XIV** were formerly identified in the pyrolysate of an algaenan isolated from *B. braunii* race L (Derenne et al., 1990), then in the pyrolysate of a Tertiary Torbanite (Derenne et al., 1994) and, together with **XIII** (mass spectrum in Fig. 8f), in an extract from a sample of Messel oil shale (Adam et al., 2006). In addition, two $C_{40}H_{72}$ compounds were tentatively identified as **X** and **XI** based on their mass spectra. They are hypothesized to exhibit the same carbon skeleton as **XIII** and **XII**, respectively, but contain an additional unsaturation conjugated with the aromatic ring, as deduced from the presence of an ion at *m/z* 145 (Fig. 8c and d). (The stereochemistry of the non-aromatic unsaturations in **X** and **XI** shown in the Appendix is arbitrary.)

The presence of a series of straight chain hydrocarbons consisting of alkanes, alk-1-enes, alk-9-enes, and alka-1,ω9-dienes strongly suggests that the kerogen fractions were derived at least in part from the algaenans of the three races of *B. braunii*. The algaenans of races A, B and L share a basic building block formed by the condensation–polymerization of a *n*-C_{32} di-unsaturated α,ω-dialdehyde. During pyrolysis these algaenans release similar series of alkanes, alkenes and alkadienes to those observed in the El Junco kerogens (Gelin et al., 1994; Berthéas et al., 1999; Metzger et al., 2007, and references therein).

The presence of appreciable quantities of C_{40} lycopane derivatives in the kerogens, and of C_{29} alkadienes in the 60–70 cm interval of sediment, are presumed to be the signatures of races L and A. In an oxic depositional setting like El Junco Lake, a substantial fraction of algal lipids (such as the A race alkadienes) is likely to be polymerized, as previously observed in a Lake Balkash Coorongite (organic deposit of *Botryococcus*) (Gatellier et al., 1993). Furthermore, the monoaromatic lycopanes **X**–**XIV** could originate from the cyclization/aromatization of cross linked and insoluble tetraterpenoid ether lipids from L race *B. braunii* (e.g. lycopanerol **XVI**, Appendix; Rager and Metzger, 2000) during pyrolysis. These pyrolytic data thus suggest that *B. braunii* derived biopolymers contribute to the formation of contemporary lacustrine sediments.

3.6. Botryococcene variations as environmental indicators

A time scale for the sediment core was developed from ^{14}C and ^{210}Pb dating by Dr. Jonathan Overpeck and Jessica Conroy at the University of Arizona (personal communication). The ages of sediment intervals we analyzed, adopted from their chronology, are shown in Table 1.

Biomarker concentrations in the upper 76 cm of El Junco sediment are shown in Table 1. The concentrations of alkadienes are not listed because their abundance was too low to accurately determine. The concentration of the C_{31} *n*-alkane, which has a higher plant source (Eglinton et al., 1962), was nearly constant through the core. Vegetation in the El Junco watershed atop a volcanic crater is relatively sparse and probably did not vary much in extent during the last 460 years. By contrast, alkenes such as *n*-$C_{25:1}$, which are believed to derive from aquatic microalgae (Gelpi et al., 1970), exhibited large down core variations (Table 1).

Of all *B. braunii* specific lipids, the botryococcenes displayed the largest down core variation in concentration. Total botryococcene concentrations varied by almost four orders of magnitude, from 3.1 at 12–13 cm to 16,700 μg/g at 54–55 cm (Table 1). More generally, in samples from the upper 22 cm of the core, total botryococcene concentrations were less than 100 μg/g. They were between 100 and 1000 μg/g in samples from 36 to 46 cm and they were greater than 1000 μg/g in samples from 48 to 76 cm.

The concentration of a trace organic constituent of sediment from a small endorheic lake is influenced by three factors: the flux of that compound to the sediment surface, the contemporaneous flux of other sedimentary components, and the preservation (or loss) rate of that compound and other constituents in the sediment. In the case of botryococcenes we also have to consider the per cell concentration in a population of B race *B. braunii* as a fourth factor that could influence down core botryococcene concentrations.

The total flux of sediment has been relatively constant in the lake over the last 10,000 years based

on a linear interpolation of time between numerous radiocarbon dates in the upper 3.5 m of sediment (Conroy and Overpeck, personal communications). Furthermore, other sedimentary lipids, such as lycopadienes, have very different down core concentration profiles, and the concentration of the C_{31} *n*-alkane from higher plants was nearly constant down core at 21.7 ± 3.5 μg/g. Changes in the flux of other sedimentary components through time are therefore not likely to have caused the down core changes in botryococcene concentration. Similar arguments are valid against the notion that changes in the preservation rate of botryococcenes through time caused the down core changes in its concentration. It is expected that other hydrocarbons, such as the alkanes, alkenes, alkadienes and lycopadienes would have similar preservation potentials (or degradation constants), at least within an order of magnitude. Yet the botryococcene variations span almost four orders of magnitude while those of the other *B. braunii* hydrocarbons span one order of magnitude. Lastly, the concentration of botryococcenes in B race *B. braunii* has been observed to vary over just one order of magnitude (Metzger and Largeau, 2005), eliminating within cell variability as the primary cause of the down core botryococcene changes. We thus conclude that most of the down core variations in botryococcene concentration reflect temporal changes in the abundance of B race *B. braunii* living in El Junco Lake.

The environmental conditions favoring the predominance of *B. braunii* in lakes are not well known, and there is almost no information on the environmental factors favoring one race of *B. braunii* over another; but nutrient concentrations in the lake are likely to play an important role in dictating the lake ecology. In a small closed basin lake known to experience large changes in volume associated with the El Niño-Southern Oscillation, it is not difficult to imagine substantial dilution of nutrients during El Niño events, when the lake level and volume rise, and substantial concentration of nutrients during La Niña when the lake level and volume fall (Steinitz-Kannan et al., 1998).

4. Conclusions

Lipid biomarkers contained in suspended particles and sediments from El Junco Lake, Galápagos demonstrate for the first time that all three races of the green alga *Botryococcus braunii* (A, B and L) have co-existed intermittently during the last 460 years. In some sediment intervals biomarkers from *B. braunii* constitute more than 90% of the solvent extractable lipids.

A series of *cis*/*trans* *n*-alkadienes, C_{34} botryococcene isomers, and *trans*,*trans*-lycopadiene, characteristic of races A, B and L, respectively, were identified in the water column and sediments deposited during the last 460 years. Moreover, characteristic epoxides of races A and L, derived from *n*-alkadienes and *trans*,*trans*-lycopadiene, respectively, were detected in the water column and sediments, demonstrating that these highly reactive compounds can survive in sediments for centuries under well oxygenated water.

Also reported for the first time are lycopadienes in lacustrine sediments, and several new lycopadiene ($C_{40}H_{78}$) and lycopatriene ($C_{40}H_{76}$) isomers. The new lycopadiene isomers may be byproducts of *trans*,*trans*-lycopadiene synthesis. The lycopatrienes, on the other hand, may be intermediates in the synthesis of *trans*,*trans*-lycopa-14(*E*),18(*E*)-diene that result from successive reductions of an acyclic carotenoid precursor.

Analysis of kerogen pyrolysates from the sediment indicated that algaenans and other cross linked substances derived from *B. braunii* probably contain ether lipids biosynthetically related to lycopadiene and alkadienes.

The abundance of botryococcenes in the upper 76 cm of sediment, representing the last 460 years of deposition, varied by over three orders of magnitude from 3 to 16,700 μg/g sediment. These changes most likely reflect changes, in the population of *B. braunii* race B in the lake, that were caused by changing hydrologic conditions.

Acknowledgements

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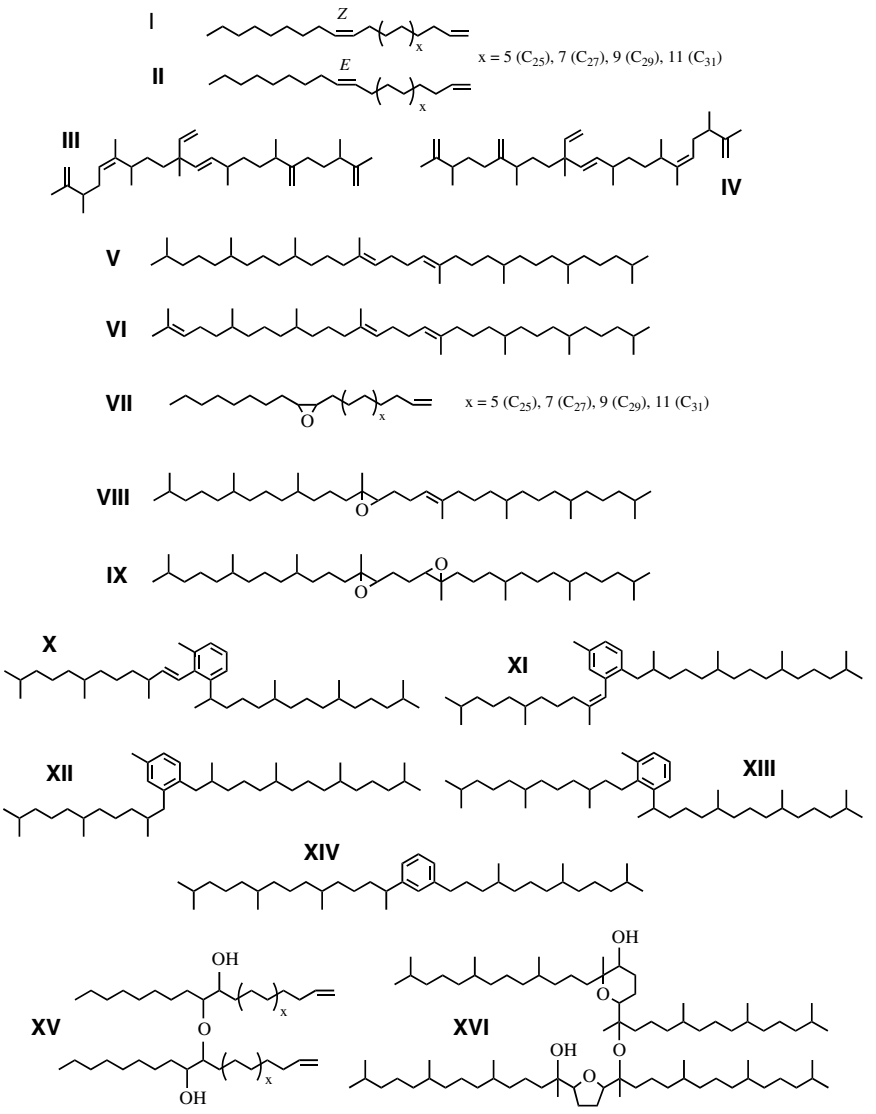
Appendix



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