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doi:10.1016/j.gca.2004.06.004

## Hydrogen isotope ratios of recent lacustrine sedimentary n-alkanes record modern climate variability

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(Received December 29, 2003; accepted in revised form June 14, 2004)

**Abstract**—Hydrogen isotope ratios were measured on n-alkanes (n-C<sub>12</sub> to n-C<sub>31</sub>) extracted from recent lake surface sediments along a N-S European transect to test if modern climate variability is recorded in these biomarkers.  $\delta D$  values of the n-alkanes are compared to  $\delta D$  values of meteoric water from the IAEA-GNIP database spanning a range from  $-119\text{‰}$  in northern Sweden to  $-41\text{‰}$  in southern Italy, to lake water  $\delta D$  values, and to mean annual temperatures, varying between  $-2.0^\circ\text{C}$  in the north and  $13.7^\circ\text{C}$  in the south.

$\delta D$  values of the short-chained n-alkanes n-C<sub>12</sub> to n-C<sub>20</sub>, excluding algal derived n-C<sub>17</sub> and n-C<sub>19</sub>, are higher in the north and lower in the south. The isotopic fractionation  $\epsilon$  for hydrogen between meteoric water and the short-chained n-alkanes is increasing from N to S by more than  $100\text{‰}$  and is significantly correlated to mean annual temperature for n-C<sub>16</sub> and n-C<sub>18</sub>. This suggests that these n-alkanes may originate from a different source in the northern lakes, possibly due to petroleum contamination, or are synthesized using a different biochemical pathway.

The n-C<sub>17</sub> and n-C<sub>19</sub> alkanes of algal origin, the n-C<sub>21</sub> and n-C<sub>23</sub> alkanes originating from water plants, and the long-chain n-alkanes n-C<sub>25</sub>, n-C<sub>27</sub>, n-C<sub>29</sub>, and n-C<sub>31</sub> of terrestrial origin, clearly correlate with  $\delta D$  values of meteoric water, lake water, and mean annual temperature, indicating that they excellently record the  $\delta D$  value of meteoric water. The mean hydrogen isotope fractionation  $\epsilon_{C_{17}/w}$  of  $-157\text{‰}$  (SD = 13) between n-C<sub>17</sub> and meteoric water is fairly constant over the wide range of different climates and lake environments, suggesting only minor influence of environmental factors on this biochemical fractionation. This suggests that  $\delta D$  values of n-C<sub>17</sub> are suitable to reconstruct the isotopic composition of source water. The mean fractionation between the long-chain n-alkanes and water is  $-128\text{‰}$  (SD = 12). The mean difference of  $31\text{‰}$  between both  $\epsilon$  values is likely due to evaporative enrichment of deuterium in the leaf water. If this is the only influence on the enrichment, the difference between the  $\delta D$  values of terrestrial and aquatic compounds might be suitable to reconstruct terrestrial evapotranspiration of the lake environment. Copyright © 2004 Elsevier Ltd

### 1. INTRODUCTION

Compound-specific hydrogen isotope ratios of organic compounds are emerging as a new paleoclimatic and paleohydrological proxy (Andersen et al., 2001; Sauer et al., 2001; Huang et al., 2002; Yang and Huang, 2003; Sachse et al., 2004). The use of this technique on compounds originating from specific groups of organisms, so-called biomarkers, opens new perspectives to trace changes in various compartments of the water cycle, and hence, climate variability in the geological past. The hydrogen isotope composition of environmental water is dependent on climatic conditions such as temperature, evaporation, and precipitation (Craig, 1961; Craig and Gordon, 1965; Gonfiantini, 1986; Gat, 1996). Current knowledge suggests that the fractionation of H isotopes in biosynthesis is constant and mostly controlled by the biochemical pathway used (Sessions et al., 1999). Therefore  $\delta D$  ratios of biomarkers have the potential to record changes in the isotopic composition of the H source. This is well known for  $\delta D$  values from cellulose of tree rings (Epstein et al., 1976; Yapp and Epstein, 1982; Sternberg, 1988; Feng and Epstein, 1995), and also for bulk plant lipids (Sternberg, 1988). For n-alkanes from algae, a fractionation ( $\epsilon_{n\text{-alkane}/\text{water}}$ ) of  $-160\text{‰}$ , and for sterols  $-201\text{‰}$ , has been

reported (Sessions et al., 1999; Sauer et al., 2001). Moreover, n-alkanes from terrestrial higher-plants are more enriched in deuterium, resulting in an  $\epsilon_{n\text{-alkane}/\text{water}}$  value of about  $-117\text{‰}$  for C3 plants (Estep and Hoering, 1980; Sternberg et al., 1984; Chikaraishi and Naraoka, 2003), since leaf water in plants, the H source for biosynthesis, is usually enriched by 20 to  $80\text{‰}$  relative to soil water (Ziegler, 1989).

Lake sediments are excellent climate archives (e.g., Allen et al., 1999) for recording regional climate changes on the continents, and are therefore, the prime target for biomarker  $\delta D$  analysis. Evidentially, palmitic acid extracted from recent lake sediments throughout North America records the  $\delta D$  of the source water (Huang et al., 2002). Other more specific biomarkers, such as several sterols, also record the  $\delta D$  value of the source water (Sauer et al., 2001). However, as carboxylic acids are only minor compounds in older sediments, those biomarkers are not always suitable for paleoclimatic reconstruction. Therefore, n-alkanes, which are present in considerable amounts even in paleozoic sediments, do not contain exchangeable H (Schimmelmann et al., 1999), are relatively easy to extract and purify, and should be reliable compounds in terms of  $\delta D$  analysis.

The n-alkanes from recent sediments cover a wider range of  $\delta D$  values (Sauer et al., 2001) due to different biological sources. The occurrence of the short-chain even-carbon-numbered n-C<sub>12</sub> to n-C<sub>22</sub> alkanes in sediments from different en-

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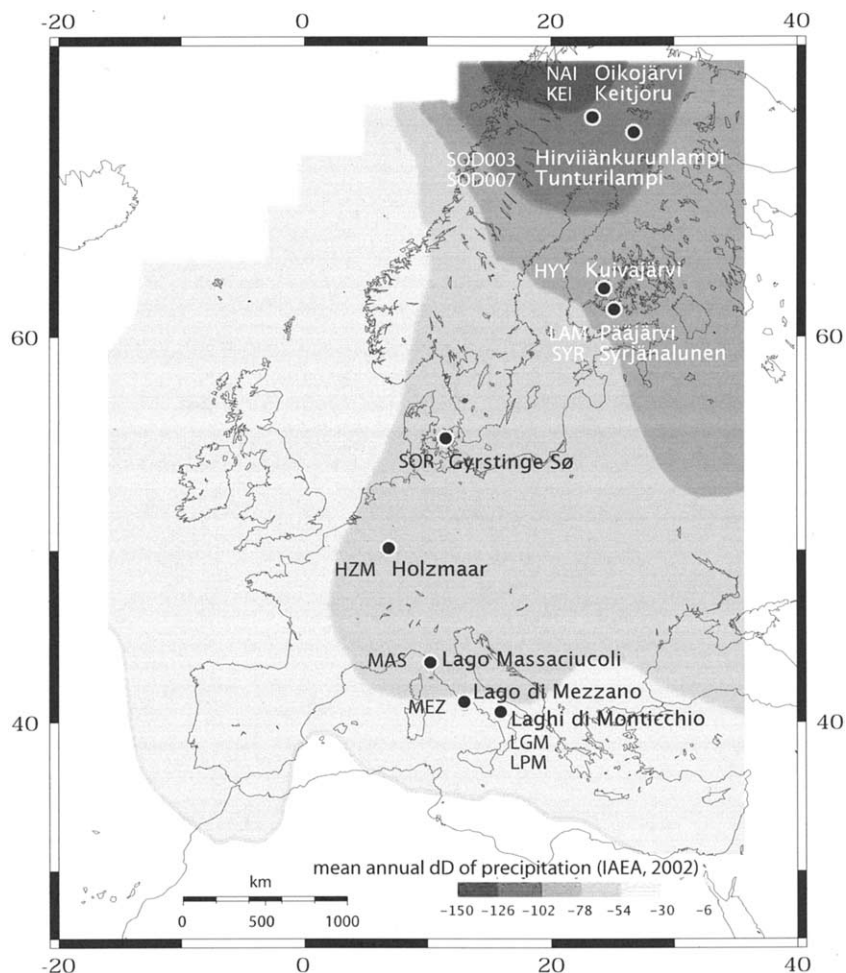


Fig. 1. Location of the 13 sampled lakes in Europe and the mean annual  $\delta D$  value of precipitation for Europe (taken from the IAEA-GNIP database; IAEA, 2001).

vironments is generally attributed to bacteria (Han and Calvin, 1969; Grimalt and Albaiges, 1987). Short-chained n-alkanes, especially n-C<sub>17</sub>, are generally considered as indicators for input by algae and photosynthetic bacteria (Han et al., 1968; Han and Calvin, 1969; Cranwell et al., 1987; Meyers, 2003), n-alkanes with 21, 23, and/or 25 carbon atoms are derived from submerged aquatic plants (Ficken et al., 2000), whereas the n-C<sub>25</sub> to n-C<sub>31</sub> alkanes originate from the leaf waxes of land plants (Eglinton and Hamilton, 1967; Cranwell et al., 1987). The n-C<sub>23</sub>, n-C<sub>25</sub>, and n-C<sub>31</sub> alkanes can also be derived from *sphagnum* species (Baas et al., 2000). Consequently, the  $\delta D$  values of different n-alkanes should yield information on the origin of hydrogen in the bacteria, algae and plants.

First reconstructions of paleohydrological conditions using  $\delta D$  values from sedimentary n-alkanes of various ages up to the Permian show promising results (Xie et al., 2000; Andersen et al., 2001; Sauer et al., 2001; Sachse et al., 2004). So far a systematic approach comparing recent sedimentary n-alkane  $\delta D$  values with climate data are still lacking. Therefore, we sampled surface sediments from 13 lakes along a N-S climatic gradient from Northern Finland to Southern Italy to test if the climatic gradient is recorded in n-alkane  $\delta D$  values and to

present a solid base for the application of n-alkane  $\delta D$  values as a new paleohydrological proxy.

## 2. MATERIALS AND METHODS

### 2.1. Sample Sites and Field Sampling

All sampled lakes (Fig. 1) are small groundwater-fed lakes with a relatively small catchment area (Table 1) and low human impact, except Pääjärvi Lake in Finland, which is significantly bigger, and Lago di Massaciucoli, which is subject to temporal saltwater influx from the Mediterranean Sea. Six lakes are oligotrophic, four lakes eutrophic, one lake is mesotrophic, and one is oligo/mesotrophic. For an overview of basic limnological parameters see Table 2. The sites cover a mean annual temperature range from  $-2.0^{\circ}\text{C}$  (Naimakka, northern Sweden) to  $13.7^{\circ}\text{C}$  (Monticchio, southern Italy). Temperature and evaporation are the main factors controlling the  $\delta D$  value of the meteoric water, source water for the lakes (Gonfiantini, 1986). The resulting mean annual  $\delta D$  value of meteoric water on the sites covers a range from  $-119.0\text{‰}$  vs. VSMOW in northern Sweden/Finland (mean annual  $\delta D$  from 1992 to 1995 in Naimakka, Sweden) to  $-36.6\text{‰}$  vs. VSMOW in southern Italy (mean annual  $\delta D$  from 2000 in Bari; IAEA, 2001 Fig. 1).

Sediments were sampled in August and September 2002 using a gravity corer (HTH-Teknik, Luleå, Sweden) operated from a dismountable raft. The new sediment covering roughly the last year (usually the

Table 1. Location and basic geographic and meteorological data of the sampled lakes.

Sample code	Lake	Geographic location		Altitude [m asl]	Water depth [m]	Lake area [km <sup>2</sup> ]	Catchement area [km <sup>2</sup> ]	Mean annual temperature [°C]	Mean annual precipitation [mm]
NAI	Oikojärvi (FIN)	68°50'55"N	21°10'50"E	463	8.4	1.5	4.5	-2.0	450
KEI	Keitjoru (SWE)	68°40'7"N	21°30'58"E	428	2.0	0.01	1.5	-2.0	450
SOD003	Hirviänkurunlampi (FIN)	67°22'44"N	26°51'3"E	178	4.7	0.011	>50	-0.3	529
SOD007	Tunturilampi (FIN)	67°21'23"N	27°10'6"E	293	10.7	0.36	8.4	-0.3	529
HYY	Kiuvjärvi (FIN)	61°50'55"N	24°16'46"E	149	13.3	0.84	30	3.5	640
SYR	Syrjänalunen (FIN)	61°11'37"N	25°8'29"E	156	8.5	0.3	n.d.	n.a.	n.a.
LAM	Pääjärvi (FIN)	61°3'36"N	25°5'55"E	151	44.9	13.42	244	3.6	619
SOR	Grystinge (DK)	55°33'11"N	11°41'42"E	30	8.2	2.64	n.d.	8.6	636
HZM	Holzmaar (GER)	50°7'10"N	6°52'44"E	436	24.0	0.058	2	10.5	1042
MAS	Lago di Massaciucoli (ITA)	43°50'N	10°18'49"E	18	2.0	7	n.d.	14.1	906
MEZ	Lago di Mezzano (ITA)	42°36'46"N	11°46'9"E	466	30.4	0.445	0.907	13.1	1030
LGM	Lago Grande di Monticchio (ITA)	40°55'58"N	15°36'10"E	674	36.7	0.405	2.37	13.7	815
LPM	Lago Piccolo di Monticchio (ITA)	40°55'55"N	15°36'48"E	685	36.0	0.08	2.37	13.7	815

Mean annual temperature data and mean annual precipitation data from NAI, KEI: personal communication D. Hammarlund; SOD: Finnish Meteorological Institute (personal communication T. Laurilla); HYY: Helsinki University (personal communication T. Vesala); LAM: Lammi Biological Station (personal communication L. Arvola); SOR: wetter.com for Copenhagen; HZM: Manderscheid station, Deutscher Wetterdienst; MAS: worldclimate.com for Pisa; MEZ: data from Valentano (Ramrath, 1997); LGM, LPM: Watts et al. (1996). Longitude, latitude and altitude (map datum WGS 84) were determined on-site using a handheld GPS. Water depth (e.g. of the sampling site) was determined using an echosounder.

upper 1 to 2 cm), was collected up to four times in the deepest part of the lake, as determined from depth maps and echo-sounding. In Lake Pääjärvi, the eastern basin, whose maximum depth is 44 m, was sampled. Water temperature, pH, oxygen saturation and redox profiles were determined on-site using a Multiprobe (YSI Inc., Yellow Springs, Ohio, U.S.A.), ensuring comparability of the lakes. Water samples were collected using a water sampler (Hydro-Bios Apparatebau GmbH, Kiel, Germany) and a pump from the high-productivity zone of the lake (usually 1–2 m). Additionally the inflows and outflows of the lakes, if existent, were sampled. All water samples (1 L) were filtered through a 0.45 µm GF/F filter. The GF/F filters were frozen and later used for chlorophyll a concentration measurement. For this purpose filters were ground and dissolved in acetone, the extinction of the extract was measured using a UV/VIS spectrometer (Helios gamma, ThermoSpectronic, Madison, WI, USA) following the procedure from Jeffrey and Humphrey (1975).

Phosphorus concentration was determined on-site using a field photometer (PhotoLab S12, WTW GmbH & Co. KG, Weilheim, Germany). Since most of the lakes are situated close to a permanent research station, basic meteorological data such as temperature and

precipitation exist for the last 10 yr. Long-term precipitation isotope data are taken from the IAEA GNIP database (IAEA, 2001), accessible on the internet. Additionally the Online Isotope Precipitation Calculator (OIPC, accessible at <http://www.es.usc.edu/~gbowen/Isomaps.html>) using the IAEA database and interpolation algorithms described in Bowen and Revenaugh (2003) was used for sites where no δD values exist. Calculated values were compared with available data and showed virtually no differences (Table 3).

## 2.2. Analysis of Water Samples for δ<sup>18</sup>O and δD Values

Water samples were analyzed for their δ<sup>18</sup>O and δD values using a modified thermal conversion/elemental analyzer system connected to an isotope ratio mass spectrometer (IRMS) (TC/EA + Delta<sup>plus</sup>XL, Finnigan MAT, Bremen, Germany). The average standard deviation (2σ) was 0.5‰ for δD values and below 0.1‰ for δ<sup>18</sup>O values. A detailed description of the method is in preparation (W.A. Brand, personal communication).

Table 2. Limnological data of the sampled lakes as determined on-site (for chlorophyll a concentration, see 1.1).

Sample code	Secci depth [m]	Water T surface [°C]	DO saturation		pH surface	Chl a conc. above thermocline		PO4-P [µg/l]	Trophic state	Dominating vegetation
			below thermocline [%]	TDS surface [µS]		[µg/l]	[µg/l]			
NAI	3.65	16.5	73	27	6.82	3.6	<50	oligotrophic	<i>Betula Alba</i>	
KEI	2.00	15.0	93	14	6.25	0.3	<50	oligotrophic	<i>Betula Nana. Carex, sphagnum</i>	
SOD003	3.87	15.7	92	31	6.86	2.9	<50	oligotrophic	<i>Pinus</i>	
SOD007	2.95	16.3	50	15	7.28	0.7	<50	oligotrophic	<i>Pinus, Betula</i>	
HYY	1.56	21.1	44	28.7	6.57	5	<50	mesotrophic	<i>Pinus, Spruce, some Betula</i>	
SYR	4.35	20.6	92	51	7.67	0.7	<50	oligotrophic	<i>Pinus, some Spruce</i>	
LAM	1.77	23.3	50	93	7.18	3.4	n.d.	mesotrophic	<i>Pinus, Spruce, some Betula</i>	
SOR	0.70	22.1	92	420	8.64	63.4	60	eutrophic	<i>Fagus sylvatica</i>	
HZM	1.40	20.3	10	246	8.5	4.9	50	eutrophic	<i>Fagus sylvatica, Spruce, few Betula</i>	
MAS	0.30	25.5	n.d.	4250	8.3	7.3	<50	eutrophic	<i>Quercus, Alnus</i>	
MEZ	9.96	22.7	88	197	7.77	0.1	<50	oligotrophic	<i>Alnus, Quercus</i>	
LGM	1.40	22.5	20	440	7.25	6.8	<50	eutrophic	<i>Fagus sylvatica, Alnus</i>	
LPM	3.50	20.6	n.d.	350	7.72	2.5	<50	oligo/meso	<i>Fagus sylvatica, Alnus</i>	

T = temperature; DO = dissolved oxygen; TDS = total dissolved solids; chl a = chlorophyll a; PO4-P = phosphorous (as PO<sub>4</sub> species).

Table 3. Isotopic composition of meteoric water at the lake sites.

Sample code	$\delta\text{D}$ meteoric water (IAEA)		$\delta\text{D}$ meteoric water (OIPC)		95% CI		$\delta^{18}\text{O}$ meteoric water (OIPC)		95% CI		$\delta\text{D}$ inflow		$\delta^{18}\text{O}$ inflow		$\delta\text{D}$ lake water		$\delta^{18}\text{O}$ lake water	
NAI	-119.0	1)	-114.9	9.2	-15.43	1.12	-93.7	0.4	-12.04	0.16	-94.9	0.6	-12.5	0.0				
KEI	-119.0	1)	-114.1	8.6	-15.32	1.06	-100.1	0.1	-13.75	0.07	-96.6	0.6	-13.62	0.04				
SOD003	n.a.		-103.9	8.0	-14.04	1.17	-109.6	1.0	-14.55	0.01	-103.7	0.2	-13.92	0.02				
SOD007	n.a.		-105.2	8.1	-14.23	1.19	-108.8	0.4	-14.98	0.05	n.d.	n.d.	-12.29	0.05				
HYY	n.a.		-92.9	7.9	-12.85	1.12	-	-	-	-	-81.1	0.6	-10.59	0.00				
SYR	n.a.		-91.4	7.0	-12.38	1.02	-	-	-	-	-86.5	0.3	-11.95	0.06				
LAM	n.a.		-91.3	7.0	-12.36	1.02	-	-	-	-	-73.7	0.0	-9.55	0.14				
SOR	-70.7	2)	-67.1	4.5	-9.45	0.61	-55.2	0.5	-7.79	0.05	-46.4	0.7	-6.40	0.08				
HZM	-52.1	3)	-60.1	3.1	-8.67	0.51	-54.5	0.7	-8.48	0.09	-47.1	0.6	-6.44	0.07				
MAS	-47.0	4)	-41.6	6.4	-6.47	0.71	-	-	-	-	-6.3	0.6	-0.24	0.05				
MEZ	-40.1	5)	-45.7	6.3	-7.04	0.69	-36.8	0.3	-6.29	0.17	-3.5	0.6	0.65	0.11				
LGM	-36.6	6)	-47.3	5.2	-7.23	0.56	-	-	-	-	-13.5	0.2	-0.92	0.10				
LPM	-36.6	6)	-47.3	5.2	-7.23	0.56	-	-	-	-	-32.6	0.6	-3.99	0.09				

IAEA-GNIP data (IAEA, 2001) correspond to the following sites: (1) Naimakka (1992–1995) about 1 km from KEI site; (2) Taastrup (1965–1971); (3) Koblenz (1974–1975); (4) Pisa (1992–1995); (5) Ancona (2000); (6) Bari (9–12/2000). Note: except for Naimakka (KEI, NAI site), the IAEA sites are sampling site, the differences in IAEA and OIPC data are due to the different geography (altitude, distance to the sea etc.). OIPC  $\delta\text{D}$  and  $\delta^{18}\text{O}$  meteoric water data and 95% confidence interval (CI) were calculated using the Online Isotopes in Pre (OIPC) using the interpolation algorithms described in Bowen and Revenaugh (2003) Lake and inflow water  $\delta\text{D}$  and  $\delta^{18}\text{O}$  values were measured. All  $\delta$  values given in ‰ vs. VSMOW.

### 2.3. Sample Preparation, Biomarker Identification, and Quantification

Sediment samples were ground and freeze-dried. Soluble organic matter was extracted using an accelerated solvent extractor (ASE200, Dionex Corp., Sunnyvale, USA) with a dichloromethane/methanol mixture (10:1) at 100°C and 2000 psi for 15 min in 2 cycles. Depending on the amount of organic C in the sample, 2 to 7 g of sample were used for extraction. Where sufficient amount of sediment was available, the extraction procedure was performed on two to three independent samples.

The total extract was separated on a silica-gel column into 3 fractions: aliphatics (solvent: hexane), aromatics (solvent: chloroform), and other compounds (solvent: methanol). Compounds of the aliphatic fraction were identified and quantified using a GC-FID (TraceGC, ThermoElectron, Rodano, Italy) equipped with a DB5ms column (30m, ID:0.32 mm, film thickness: 0.5  $\mu\text{m}$ , Agilent, Palo Alto, USA) by comparison to an external n-alkane standard mixture.

### 2.4. Gas Chromatography Temperature Conversion Isotope Ratio Monitoring Mass Spectrometry (GC-TC-IRMS) for Analysis of $\delta\text{D}$ Values on the n-Alkanes

One  $\mu\text{L}$  of the hexane dissolved aliphatic fraction was injected into a HP5890 GC (Agilent Technologies, Palo Alto, USA), equipped with a DB5ms column (30m, ID:0.32 mm, film thickness: 0.5  $\mu\text{m}$ , Agilent). The injector was operated at 280°C in splitless mode. The oven was maintained for 2 min at 60°C then heated with 6°C/min to 320°C and held for 10 min at the final temperature. The column flow was held constant at 1.7 mL/min throughout the run. One fraction of the separated compounds was transferred to an ion-trap mass spectrometer (GCQ, ThermoElectron, San Jose, USA) to monitor possible coelution of the n-alkanes with other substances. If coelution was evident in the MS spectrum, the  $\delta\text{D}$  value was not used for interpretation. The other fraction was transferred to a high-temperature conversion oven operated at 1425°C (Burgoyne and Hayes, 1998; Hilker et al., 1999) and quantitatively converted to  $\text{H}_2$  that was introduced into an isotope ratio mass spectrometer (IRMS) (Delta<sup>plus</sup>XL, Finnigan MAT, Bremen, Germany) for compound-specific analysis of  $\delta\text{D}$  values. Each sample was independently measured three times. All  $\delta\text{D}$  values were normalized to the VSMOW scale using a mixture of n-alkanes (n-C<sub>10</sub> to n-C<sub>32</sub>). The  $\delta\text{D}$  values of the n-alkanes in the standard mixture were calibrated against international reference substances (NBS-22, IAEA-OH22) using the offline TC/EA technique. After the measurement of no more than two samples (6 GC runs), the

standard mixture was measured independently three times. If necessary a drift correction was applied.

Achieved precision expressed as the average standard deviation for all peaks from the samples was 7‰ (n  $\approx$  2700, 180 runs with  $\sim$ 15 peaks, depending on sample), for the standard mixtures of 4‰ (n = 3520, 160 runs with 22 peaks each). The  $\text{H}_3^+$  factor was determined once a day and stayed constant at 5.9 (SD = 0.26; n = 23) during the three-week measurement campaign.

### 2.5. Calculation of the Isotopic Fractionation $\epsilon$

The isotopic difference between the  $\delta\text{D}$  value of the lake water and the  $\delta\text{D}$  value of the n-alkanes was calculated using Eqn. 1.

$$\epsilon_{\text{alkane/water}} = 1000 \cdot \left( \frac{\delta D_{\text{alkane}} + 1000}{\delta D_{\text{water}} + 1000} - 1 \right) \quad (1)$$

## 3. RESULTS AND DISCUSSION

### 3.1. n-Alkane Concentrations

In general, the total concentration of extractable n-alkanes is slightly higher in the lakes from southern Europe (up to 0.5 mg/g TOC) compared to the northern lakes (0.2 mg/g TOC as a minimum), indicating a higher organic input in the south (Fig. 2). The concentration of the short-chain n-alkanes n-C<sub>17</sub> and n-C<sub>19</sub> of aquatic origin is highest in the eutrophic lakes (up to 0.4 mg/g TOC for n-C<sub>17</sub> in HZM). Most prominent n-alkanes in nearly all lake sediments are the long chain n-alkanes n-C<sub>25</sub> to n-C<sub>31</sub> of terrestrial origin, expressed through the high average chain length (ACL) values varying between 21.2 and 27.6 (Fig. 2). A clear odd-over-even carbon number predominance was found in all sediments with carbon predominance indices (CPI) between 1.4 and 6.6 (Fig. 2), typical for a biological origin. The CPI increases slightly from north to south, suggesting together with the longer vegetation period, higher input of organic material and higher productivity in the southern lakes, although dilution with other sedimentary components and different preservation regimes may also influence the TOC contents.



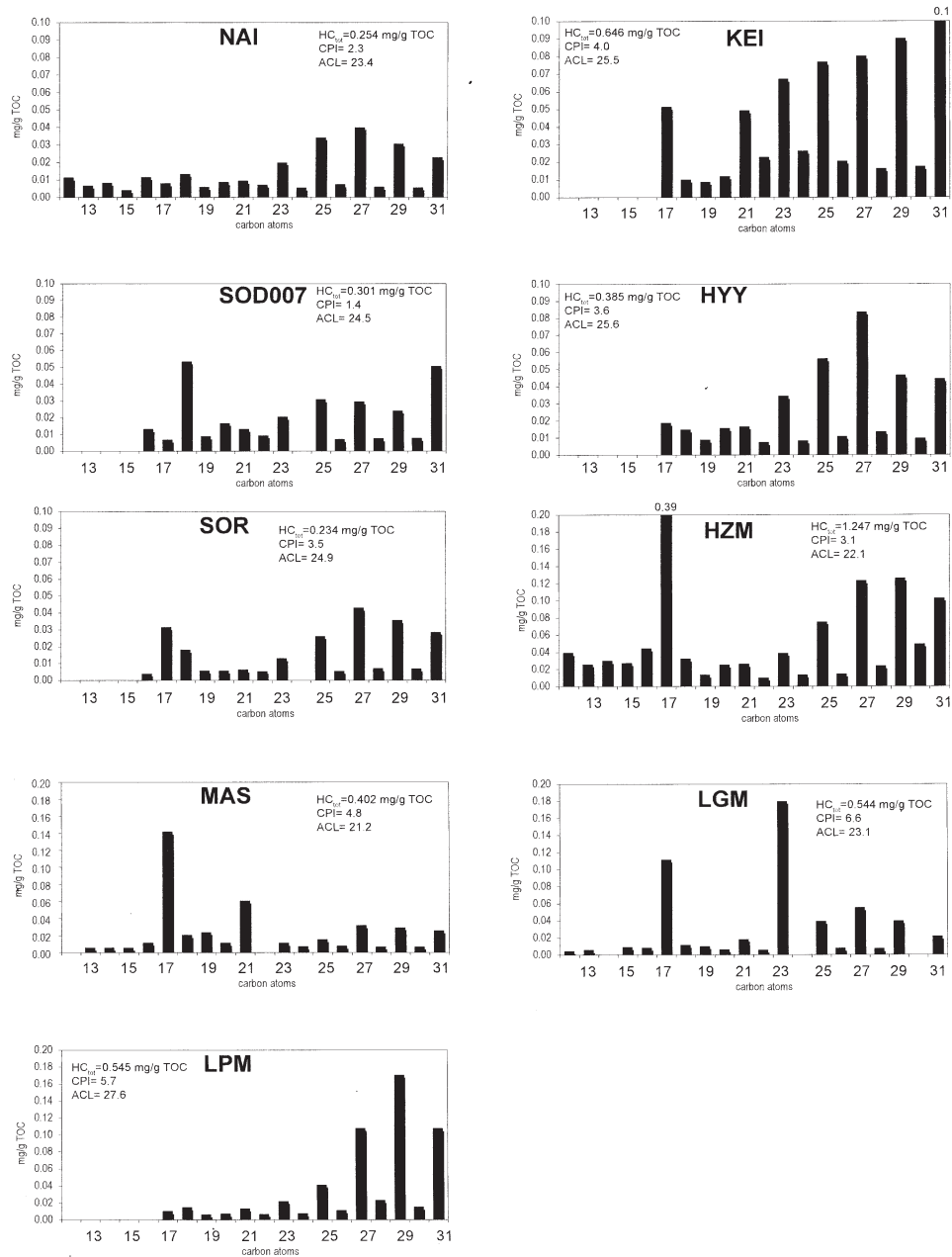


Fig. 2. Distribution, CPI (Carbon Preference Index =  $\sum_{\text{odd}} \text{Cn} / \sum_{\text{even}} \text{Cn}$ ) and ACL (Average Chain Length =  $(\sum \text{Cn} * n) / \sum \text{Cn}$ ) indices and total concentration (HC<sub>tot</sub>) of the n-alkanes from nine of the sampled lakes. Note that the scale of the y-axis (concentration) is doubled for the southern lakes (HZM, MAS, LGM, LPM) because of higher concentration of n-alkanes.

### 3.2. δD and δ<sup>18</sup>O Values of Water

The δD and δ<sup>18</sup>O values of the lake inflow water, if an inflow is present, are up to 14‰ heavier for δD and up to 1.5‰ heavier for δ<sup>18</sup>O relative to the calculated OIPC values (Fig. 3). Considering the confidence interval of the calculated OIPC data, the inflow water δ values are within the 95% limit or slightly heavier. Due to evaporation and temperature effects, a heavier summer δ value would be expected. The northernmost lake NAI represents a notable exception, since measured δD values are 21‰ and δ<sup>18</sup>O values 3.4‰ heavier than the calcu-

lated meteoric water. Since there is no dense vegetation cover around the small stream, and summer 2002 was exceptionally warm and dry in Scandinavia, evaporation already affects the contributing stream. However, the isotopic data for the inflowing waters from the seven lakes where those data exist suggest that the main water source is the meteoric water. Evaporation only seems to be of minor influence.

δD and δ<sup>18</sup>O values of the lake water from the high productivity zone (in general at 1–2 m depth) are up to 20‰ heavier for hydrogen and up to 3‰ heavier for oxygen than the calculated OIPC values, except the three Italian lakes MAS, MEZ,

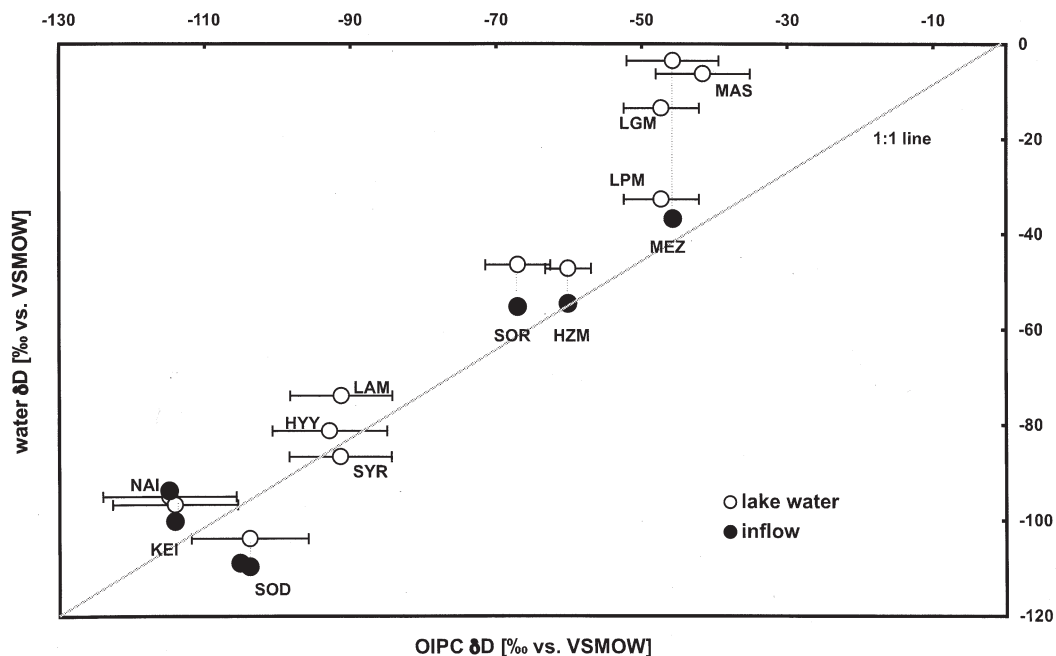


Fig. 3. Comparison between lake water  $\delta D$  values (open circles: high-productivity zone, usually in 1 to 2 m depth; filled circles: inflows) and meteoric water  $\delta D$  values, calculated using OIPC (see text). Dotted lines show the enrichment/depletion of inflow water vs. lake water. Error bars for the  $\delta D$  of OIPC data constitute the calculated 95% confidence interval and are also applicable for the inflow data. Error bars (standard deviation) for  $\delta D$  lake water lie within the points.

and LGM (Fig. 3). Nearly half of the variation still lies within the 95% confidence interval of the calculated OIPC data (Table 3). Furthermore, it must be acknowledged that the isotope ratios of the lake water are only a one-point measurement in summer, when evaporation very likely will enrich deuterium in the water. However, since the organisms in the water column will incorporate hydrogen throughout the growing season and probably show most productivity in spring and late summer, the summer  $\delta D$  water value will be heavier than the average  $\delta D$  water value for the growing season. We therefore assume the OIPC data to be a mean annual value also valid for the lake water.

For the Italian lakes MEZ and LGM a stronger evaporation effect is visible. The lake water is 33 to 35‰ heavier in  $\delta D$  than the calculated precipitation value (Fig. 3). Lake MAS, also in Italy, is a special case because it lies within several kilometers of the Mediterranean coast, and temporal influx from isotopically heavier seawater occurs. The isotopic ratios of these three Italian lakes do not plot on the Global Meteoric Water Line, but on an evaporation line, whereas all other lake waters do. In the case of the three Italian lakes, an underestimation of the isotopic fractionation  $\epsilon$  would be the case if we rely on the OIPC data. Despite this deviation in three southern lakes, the OIPC data have been used as the hypothetical mean annual  $\delta D$  composition of the lake water.

### 3.3. $\delta D$ Values of the n-Alkanes

In general the  $\delta D$  values of n-alkanes from one sample can differ by more than 100‰. The main reasons for this variation are different biological sources of these n-alkanes, using dif-

ferent water sources for biosynthesis. Using  $\delta D$  values, four main n-alkane groups can be distinguished:

1. Even carbon numbered short-chain n-alkanes ( $n-C_{12}$ ,  $n-C_{14}$ ,  $n-C_{16}$ ,  $n-C_{18}$ ,  $n-C_{20}$ ) and  $n-C_{13}$  and  $n-C_{15}$ .
2. Odd carbon numbered n-alkanes of medium-chain length ( $n-C_{17}$ ,  $n-C_{19}$ ,  $n-C_{21}$ ,  $n-C_{23}$ ).
3. Even carbon numbered medium to long-chain n-alkanes ( $n-C_{22}$ ,  $n-C_{24}$ ,  $n-C_{26}$ ,  $n-C_{28}$ ,  $n-C_{30}$ ).
4. Odd carbon numbered long-chain n-alkanes ( $n-C_{25}$  to  $n-C_{31}$ ).

In the following we will discuss the variations of the  $\delta D$  values of those n-alkane groups along the transect in detail.

#### 3.3.1. $\delta D$ values of the even carbon numbered short-chain n-alkanes ( $n-C_{12}$ , $n-C_{14}$ , $n-C_{16}$ , $n-C_{18}$ , $n-C_{20}$ ), $n-C_{13}$ , and $n-C_{15}$ .

$\delta D$  values for  $n-C_{16}$  and  $n-C_{18}$  could be determined on only five lake samples along the transect due to overall low concentration of these substances (Table 4). They usually are the heaviest measured  $\delta D$  values of the sample. Interestingly,  $\delta D$  values of the compounds are lighter in the south than in the north. The  $\delta D$  value for  $n-C_{18}$  is significantly lighter than the other alkanes in the sediments from SOR and LGM sites, probably indicating a different H source for  $n-C_{18}$  in these two eutrophic lakes.  $\delta D$  values from  $n-C_{16}$  and  $n-C_{18}$  correlate positively with latitude and negatively with mean annual temperature and meteoric water  $\delta D$  values (Table 4; Fig. 4), although the number of samples is limited. The other short-chained substances seem to follow this trend, but statistical evaluation is not possible due to the limited amount of

Table 4.  $\delta D$  values [ $\text{‰}$  vs. VSMOW] and statistical data (p-value and the correlation coefficient r) on the measured n-alkanes.

Sample code	n-C12	SD	n-C13	SD	n-C14	SD	n-C15	SD	n-C16	SD	n-C17	SD	n-C18	SD	n-C19	SD	n-C20	SD	n-C21	SD	
NAI	-146	12			-120	1			-114	10	-259	6	-131	2							
KEI																				-245	4
SOD003	-142	4			-122	3			-100	3			-142	1							
HYY											-229	6	-135	13						-244	8
SYR																					
LAM																					
SOR									-148	n.d.	-219	1	-238	4	-241	2	-145	5	-213	2	
HZM	-138	2	-140	10	-126	10	-148	15	-170	5	-208	0	-158	14							
MAS											-208	3			-201	7				-235	6
MEZ											-198	0									
LGM							-130	2			-175	0	-245	10	-201	5				-194	5
LPM																				-180	5
correlation vs. latitude	p	r	p	r	p	r	p	r	p	r	p	r	p	r	p	r	p	r	p	r	
	0.26	-0.92	-	-	0.2	0.95	-	-	<b>0.006</b>	<b>0.97</b>	<b>0.002</b>	<b>-0.93</b>	<b>0.05</b>	<b>0.76</b>	0.11	-0.97	-	-	0.08	-0.76	
mean annual temperature	0.22	0.94	-	-	0.16	-0.97	-	-	<b>0.015</b>	<b>-0.95</b>	<b>0.004</b>	<b>0.91</b>	<b>0.04</b>	<b>-0.78</b>	<b>0.04</b>	<b>0.99</b>	-	-	0.12	0.70	
$\delta D$ precipitation (OIPC)	0.18	0.96	-	-	0.12	-0.98	-	-	<b>0.023</b>	<b>-0.93</b>	<b>0.007</b>	<b>0.89</b>	<b>0.03</b>	<b>-0.8</b>	0.2	0.96	-	-	0.15	0.74	
$\delta D$ lake water	0.39	0.82	-	-	0.33	-0.87	-	-	<b>0.048</b>	<b>-0.95</b>	<b>0.015</b>	<b>0.85</b>	<b>0.03</b>	<b>-0.85</b>	0.11	0.98	-	-	0.23	0.58	

Bold values indicate a significant correlation within the 95% confidence interval ( $p < 0.05$ ). SD = standard deviation ( $2\sigma$ ).

Table 4. (Continued)

Sample code	n-C22	SD	n-C23	SD	n-C24	SD	n-C25	SD	n-C26	SD	n-C27	SD	n-C28	SD	n-C29	SD	n-C30	SD	n-C31	SD	
NAI											-209	6			-221	2					
KEI	-231	11	-234	2	-214	14	-234	4	-222	n.d.	-229	5			-230	5				-230	4
SOD003			-235	7							-210	11			-215	9					
SOD007	-268	9	-268	5							-220	3			-227	5					
HYY			-218	2			-206	3			-202	1			-205	4				-209	3
SYR			-207	1			-202	1							-207	2					
LAM			-272	12			-205	4			-213	6			-205	4					
SOR	-217	4			-177		8	-193	1	-177	2		-183	4	-201	2	-181	5	-208	0	
HZM			-225	6			-180	8			-184	10			-198	1				-198	5
MAS											-129	17			-159	7					
MEZ			-164	11			-153	13			-163	4			-167	3					
LGM															-180	3				-185	16
LPM							-168	5			-177	2	-170	3	-169	2					
correlation vs. latitude	p	r	p	r	p	r	p	r	p	r	p	r	p	r	p	r	p	r	p	r	
	0.54	-0.66	0.05	-0.70	-	-	<b>0.000074</b>	<b>-0.97</b>	-	-	<b>0.001</b>	<b>-0.87</b>	-	-	<b>0.000001</b>	<b>-0.95</b>	-	-	<b>0.006</b>	<b>-0.97</b>	
mean annual temperature	0.59	0.61	0.07	0.66	-	-	<b>0.00008</b>	<b>0.97</b>	-	-	<b>0.0005</b>	<b>0.89</b>	-	-	<b>0.00001</b>	<b>0.94</b>	-	-	<b>0.01</b>	<b>0.96</b>	
$\delta D$ precipitation (OIPC)	-	-	0.50	0.71	-	-	<b>0.00006</b>	<b>0.97</b>	-	-	<b>0.0003</b>	<b>0.9</b>	-	-	<b>0.000001</b>	<b>0.95</b>	-	-	<b>0.016</b>	<b>0.94</b>	
$\delta D$ lake water	-	-	0.11	0.66	-	-	<b>0.0003</b>	<b>0.95</b>	-	-	<b>0.0005</b>	<b>0.91</b>	-	-	<b>0.000010</b>	<b>0.93</b>	-	-	<b>0.02</b>	<b>0.92</b>	

Bold values indicate a significant correlation within the 95% confidence interval ( $p < 0.05$ ). SD = standard deviation ( $2\sigma$ ).

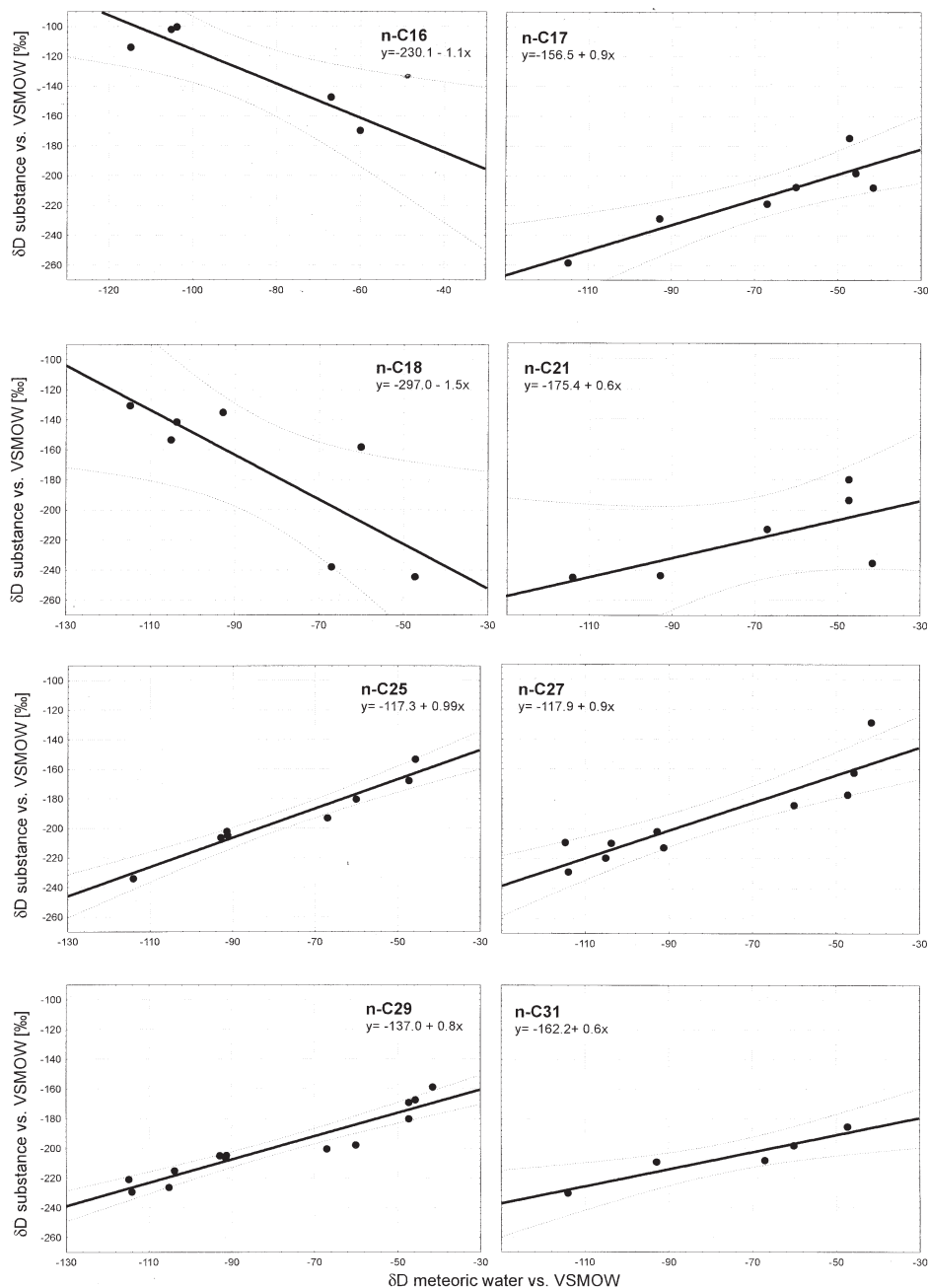


Fig. 4.  $\delta\text{D}$  values of the n-C<sub>16</sub>, n-C<sub>17</sub>, n-C<sub>18</sub>, n-C<sub>21</sub>, n-C<sub>25</sub>, n-C<sub>27</sub>, n-C<sub>29</sub>, and n-C<sub>31</sub> alkane vs. the meteoric water  $\delta\text{D}$  value (calculated using OIPC; Bowen and Revenaugh, 2003). Dotted lines show the 95% confidence interval of the regression line.

data.  $\epsilon_{\text{substance/water}}$  is increasing from the north to the south for those short-chained n-alkanes.  $\epsilon_{\text{C}_{16}/\text{w}}$  and  $\epsilon_{\text{C}_{18}/\text{w}}$  show a statistically significant inverse relationship with meteoric water  $\delta\text{D}$  and  $\delta\text{D}$  lake water values, and correlate with temperature (Table 5; Fig. 5). This is surprising since n-C<sub>16</sub> and n-C<sub>18</sub> are together with n-C<sub>17</sub>, widely used as indicators for algal input. However, both n-alkane  $\delta\text{D}$  values significantly differ from n-C<sub>17</sub>  $\delta\text{D}$  values.

Our results suggest that  $\delta\text{D}$  values of n-C<sub>16</sub> and n-C<sub>18</sub> are independent of the meteoric water  $\delta\text{D}$  value and show an inverse relationship with mean annual temperature. The frac-

tionation between water and n-C<sub>16</sub> and n-C<sub>18</sub> appears to be larger in areas with higher temperatures. To our knowledge such a relationship has not been reported until now.

Such large differences between the  $\delta\text{D}$  values of n-C<sub>16</sub>, n-C<sub>18</sub>, and n-C<sub>17</sub> have not been observed in paleozoic samples or oils (Li et al., 2001).

Several explanations might be considered:

1. Dependence of  $\epsilon_{\text{substance/water}}$  on growth rate. Since  $\epsilon$  is smaller in the north (shorter vegetation period, less nutrient



Table 5.  $\epsilon_{\text{substance/water}}$  values and 2σ standard deviation (SD) for each site and mean values along the transect. Bold values indicate constant  $\epsilon_{\text{substance/water}}$  values along the transect.

Sample code	C12		C13	C14	C15	C16	C17	C18	C19	C20	C21	C22	C23	C24	C25	C26	C27	C28	C29	C30	C31
	$\epsilon_{\text{substance/water}}$	[% vs. VSMOW]																			
NAI	-35	1	-6	-162	-18	-148	-132	-136	-113	-135	-122	-107	-120	-130	-131						
KEI	-43	4	-21	-42	-42	-148	-132	-136	-113	-135	-122	-130	-130	-130	-124						
SOD003		3		-54	-54	-166	-182	-182	-147	-125	-122	-128	-118	-118	-128						
SOD007				-47	-47	-166	-182	-182	-147	-125	-122	-128	-136	-136	-128						
HYY				-150	-150	-166	-182	-182	-147	-125	-122	-128	-136	-136	-128						
SYR				-163	-163	-166	-182	-182	-147	-125	-122	-128	-136	-136	-128						
LAM				-174	-174	-166	-182	-182	-147	-125	-122	-128	-136	-136	-128						
SOR				-160	-160	-166	-182	-182	-147	-125	-122	-128	-136	-136	-128						
HZM				-134	-134	-162	-182	-182	-147	-125	-122	-128	-136	-136	-128						
MAS				-207	-207	-162	-182	-182	-147	-125	-122	-128	-136	-136	-128						
MEZ				-157	-157	-162	-182	-182	-147	-125	-122	-128	-136	-136	-128						
LGM				-134	-134	-162	-182	-182	-147	-125	-122	-128	-136	-136	-128						
LPM				-207	-207	-162	-182	-182	-147	-125	-122	-128	-136	-136	-128						
Mean	-54	-39	-32	-90	-94	-172	-157	-94	-172	-53	-161	-158	-153	-115	-126	-120	-122	-130	-122	-141	-10
SD	26	58	34	5	74	13	12	74	13	44	22	25	28	3	7	2	14	8	8	3	10

supply) than in the south for these possibly nonphotosynthetic bacteria derived compounds, a fractionation dependent on growth rate is possible. Sessions et al. (1999) observed a wider range of  $\epsilon$  values for lipids in actively growing organisms than in plants. However, growth-rate dependency of  $\epsilon$  for fatty acids of a methane-oxidizing bacteria could not be confirmed (Sessions et al., 2002).

2. Different sources for these compounds in the north and in the south. Since  $\epsilon_{\text{substance/water}}$  values in the south are closer to  $\epsilon$  values of n-C<sub>17</sub>, they could share the same algal source in the southern lakes. The high δD values in the north could be related to methane-oxidizing bacteria, since Sessions et al. (2002) predict δD values of fatty acids produced by methanotrophs to be between -50 to -170‰. However, it is unlikely that in the sediments of the oligotrophic Scandinavian lakes significant amounts of methane are produced. Another possible source for the isotopically light compounds in the north could be fossil hydrogen, possibly introduced by petroleum contamination. But contamination seems unlikely for the Scandinavian lakes (NAI, SOD), since population density is very low, and most lakes are several kilometers away from rarely used roads. It is also unlikely for Lake Grystinge (SOR), used for drinking water storage with restricted access. Also petroleum contribution should not only affect the short-chained alkanes, but all alkanes, which is not evident. Fossil oil or shale samples lack the clear odd-over-even carbon number predomination expressed in the high CPI indices for the analyzed samples.

It is unlikely that transport by wind over long distances accumulates significant amounts of those alkanes in the most remote areas, but not in more densely populated areas of southern Italy. The only lakes where motorboat traffic was allowed (LAM and MAS) do not contain sufficient amounts of short-chain alkanes and do not show lower than average CPI indices. However, a possible mixing of petroleum derived alkanes with biologically derived alkanes cannot be ruled out, a way to test this hypothesis would be compound-specific radiocarbon analysis to determine the amount of fossil (“dead”) carbon. Further research is necessary to clarify the origin of H in the short-chained n-alkanes with even carbon numbers, and if different fractionations of the hydrogen isotopes occur in heterotrophs compared to autotrophic organisms.

3.3.2. δD values of the n-C<sub>17</sub>, n-C<sub>19</sub>, n-C<sub>21</sub>, n-C<sub>23</sub> alkanes

These substances, especially n-C<sub>17</sub>, are of particular interest since they are widely used as a paleoclimatic proxy. δD values of the n-C<sub>17</sub> alkane were determined in the samples from eight lakes. The other samples either contained no n-C<sub>17</sub>, or the substance could not properly be separated on the GC column. However, n-C<sub>17</sub> shows a clear negative correlation with latitude (Table 4; Fig. 6) and a positive one with mean annual temperature. δD values of n-C<sub>17</sub> strongly correlate to the lake water δD value and to the δD value of meteoric water with an intercept of -156.5 (Fig. 4), suggesting that the meteoric water is the H source for n-C<sub>17</sub>.  $\epsilon_{\text{C17/W}}$  values from the lakes range from -134 to -174‰ with the mean at -157‰ (Table 5), about the same value as observed in laboratory experiments (Sessions et al., 1999).  $\epsilon_{\text{C17/W}}$  is constant along the N-S

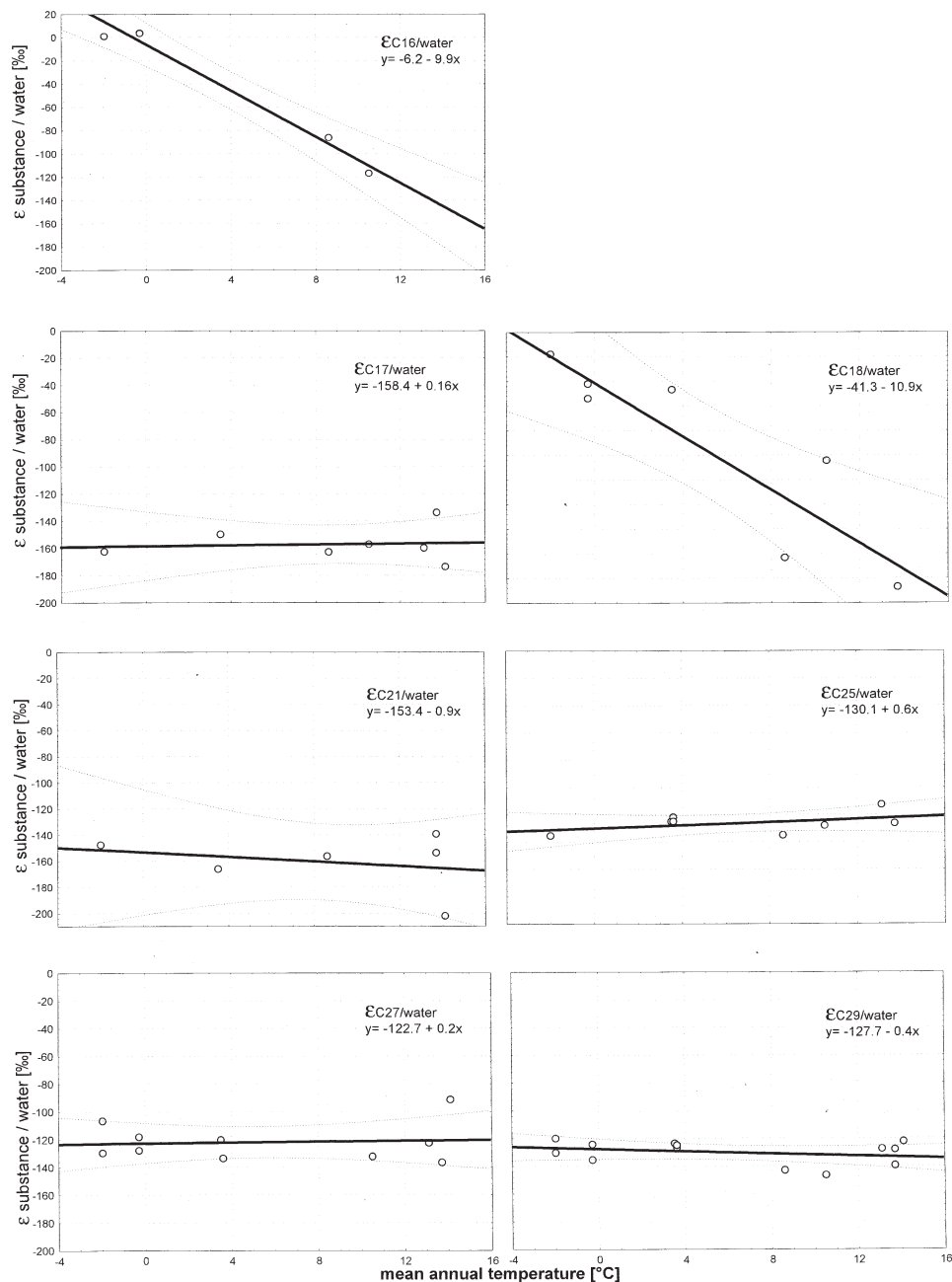


Fig. 5.  $\epsilon_{\text{substance/water}}$  values calculated for the n-C<sub>16</sub>, n-C<sub>17</sub>, n-C<sub>18</sub>, n-C<sub>21</sub>, n-C<sub>25</sub>, n-C<sub>27</sub>, and the n-C<sub>29</sub> alkanes plotted vs. mean annual temperature on the lake sites. Note: only the relationships for n-C<sub>16</sub> and n-C<sub>18</sub> are statistically significant (n-C<sub>16</sub>:  $p = 0.002$ ,  $r = -0.99$ ; n-C<sub>18</sub>:  $p = 0.006$ ,  $r = -0.90$ ).

transect (Fig. 5), covering different climates and lakes of different trophic state, supporting the hypothesis that the fractionation of hydrogen isotopes during incorporation into organic matter is only dependent on the biochemical pathway used and does not depend on environmental parameters. These results demonstrate that the n-C<sub>17</sub> alkane excellently records the lake water  $\delta\text{D}$  value and can be used to reconstruct the  $\delta\text{D}$  value of lake water (see also Fig. 6).

n-C<sub>21</sub> and n-C<sub>23</sub> are considered to be derived from aquatic plants living in the water (Ficken et al., 2000).  $\delta\text{D}$  values are often, but not always similar, to n-C<sub>17</sub> with more scatter

(Table 4). They are lighter in the north than in the south, following the same trend as n-C<sub>17</sub>, but except for n-C<sub>19</sub> (although just 3 samples provided enough substance and no colution), do not correlate significantly with latitude, temperature, or water  $\delta\text{D}$  values. This might be due to the higher standard deviations of  $\delta\text{D}$  values of n-C<sub>19</sub>, n-C<sub>21</sub>, and n-C<sub>23</sub> relative to n-C<sub>17</sub>, since they often sit on a hump of unresolved compounds in the chromatogram.

Different water plants use water from different depths, characterized by different  $\delta\text{D}$  values, or they might have different evapotranspiration rates. n-C<sub>21</sub> and n-C<sub>23</sub>  $\delta\text{D}$  values seem to

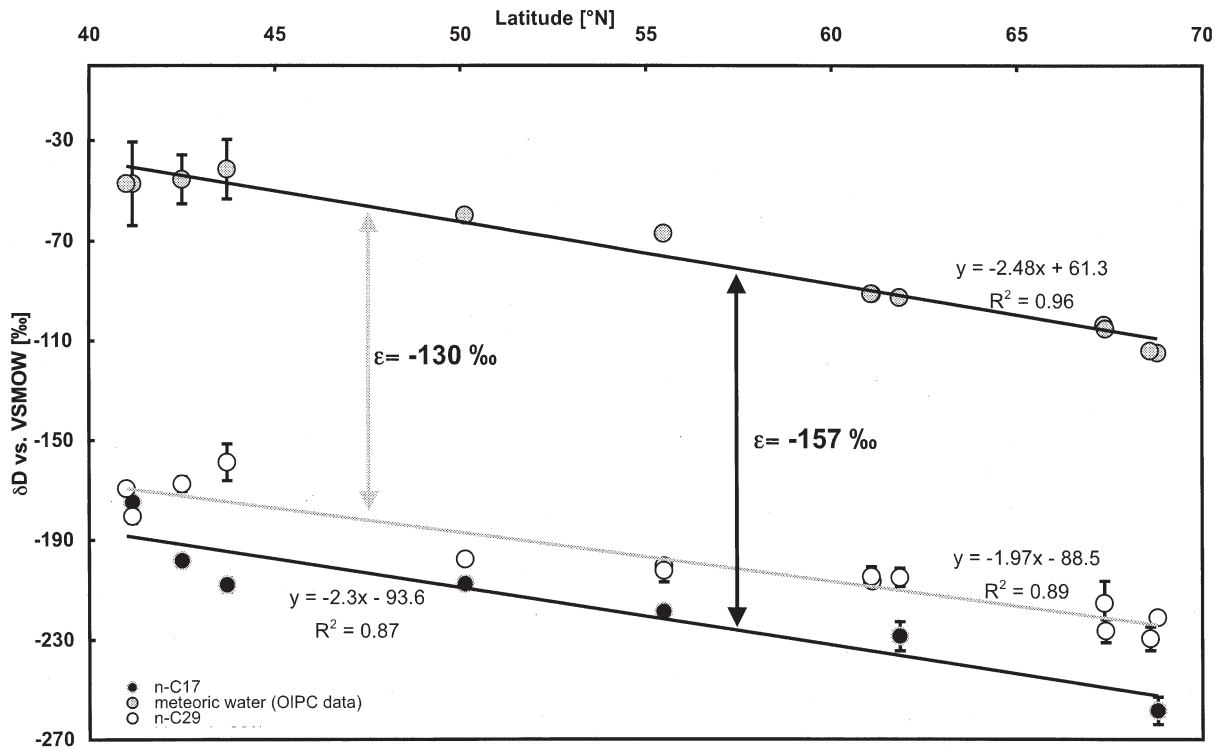


Fig. 6. Variation of meteoric water δD values, δD values and the isotopic fractionation ε for the aquatic n-C<sub>17</sub> and the terrestrial n-C<sub>29</sub> alkane along the N-S European transect.

record the isotopic composition of lake water, but for reconstruction of the lake water δD value, n-C<sub>17</sub> is much more recommended.

3.3.3. δD values of the even carbon numbered medium to long-chain n-alkanes (n-C<sub>22</sub>, n-C<sub>24</sub>, n-C<sub>26</sub>, n-C<sub>28</sub>, n-C<sub>30</sub>)

Since the substances are present in very low concentrations, only a few samples yielded enough substance to measure δD values. Interpretation of their origin is, therefore, speculative. However, the even carbon numbered substances seem to be related in δD values to their following odd carbon numbered neighbors, being in general some per mill heavier.

δD values of n-C<sub>22</sub> were only measured in 3 samples (KEI, SOD007 and SOR) and seem to be related to n-C<sub>21</sub> and n-C<sub>23</sub>, usually being several per mil heavier (Table 4).

n-C<sub>24</sub> (only measured in KEI and SOR) is ~20‰ heavier than n-C<sub>25</sub>, n-C<sub>26</sub>, n-C<sub>28</sub>, and n-C<sub>30</sub> are also 10 to 20‰ heavier than n-C<sub>27</sub>, n-C<sub>29</sub>, and n-C<sub>31</sub> in the same sample.

Overall it seems that the source of n-C<sub>22</sub> is the same source as for n-C<sub>21</sub> and n-C<sub>23</sub>, and n-C<sub>24</sub> to n-C<sub>30</sub> share the same source as their odd carbon numbered neighbors n-C<sub>25</sub> to n-C<sub>31</sub>, but involving some enrichment mechanism, resulting in 10 to 20‰ heavier δD values. Since this enrichment is not reported in plants (Chikaraishi and Naraoka, 2003), it is likely that these alkanes are produced during microbial induced degradation or recycling processes of the odd-carbon numbered alkanes taking place in the sediment. However, it is also possible that a different biological origin, for instance grass, which would be

subject to different amounts of evapotranspiration than trees, is the reason for this slight enrichment.

3.3.4. δD values of the odd carbon numbered long-chain n-alkanes (n-C<sub>25</sub> to n-C<sub>31</sub>)

These four substances, commonly considered as indicators for higher terrestrial plant input, are the most abundant aliphatic compounds in all lake sediments sampled here, only surpassed by n-C<sub>17</sub> in some samples. All of these biomarkers show similar δD values in a given sample. They negatively correlate with latitude and positively with mean annual temperature and δD values of meteoric water and lake water (Table 4; Fig. 4). Substances are isotopically lighter in the north and ~70‰ heavier in the south (Fig. 6).

ε<sub>C<sub>29</sub>/W</sub> ranges for all 12 lakes between -120 to -147‰ with the mean at -130‰ (Table 5). This is 27‰ heavier than the average ε<sub>C<sub>17</sub>/W</sub>. Several studies have confirmed that leaf water in plants is enriched by 20 to 80‰ relative to soil water due to evapotranspiration processes in the leaf (Ziegler, 1989). Furthermore Chikaraishi & Naraoka (2003) report an ε<sub>water</sub> value for the long-chain n-alkanes from several C3 plants of -117‰, consistent with our data.

The long-chained odd carbon numbered n-alkanes record the meteoric water δD value. They are, in general, ~30‰ heavier than n-C<sub>17</sub> (Fig. 6), due to evaporation processes in the leaves of the plants. If we consider that the aquatic and terrestrial n-alkanes share the same H source, meteoric water, the difference between δD values of both substances could serve as a proxy for evapotranspiration of the lake ecosystem. The mean

difference between the terrestrial and the aquatic  $\delta D$  values is highest for the three largest lakes MAS, LAM, and NAI in terms of lake area and catchment area with values between 40 and 70‰. In these lakes higher input of allochthonous biomass from distant areas with different isotopic composition of meteoric water can lead to higher differences of both  $\delta D$  values. The difference ranges around 10 to 20‰ for the other lakes, explainable by the enrichment of leaf water due to evaporation processes in the leaf. For the lakes LGM and LPM, which are at the same site and should experience the same amount of evapotranspiration, it is virtually identical ( $\sim 9\%$ ). However, since we have no evapotranspiration measurements from the sites, we cannot completely explain the differences between the  $\delta D$  values of the terrestrial vs. aquatic substances and confirm that it is indeed a marker of the evapotranspiration of the lake ecosystem. n-Alkanes originate from the epicuticular waxes of leaves, developed shortly after the unfolding of the leaf in spring (Gulz, 1994), when evapotranspiration is not yet very strong. However, the n-alkane composition of the wax is changing throughout the year, differing from tree species (Piasentier et al., 2000). If most of the n-alkanes are produced in spring, we would not see a stronger enrichment relative to the aquatic n-alkanes for sites with higher evapotranspiration in summer. This would explain the rather constant difference of 30‰ along the transect.

#### 4. CONCLUSIONS

Hydrogen isotope ratios of recent lacustrine sedimentary n-alkanes clearly record the isotopic composition of the meteoric source water in lakes from different climates. In particular n-C<sub>17</sub> and n-C<sub>25</sub>, n-C<sub>27</sub>, n-C<sub>29</sub>, and n-C<sub>31</sub> can be used to reconstruct the source water  $\delta D$  value. The constant fractionation  $\epsilon_{C_{17/W}}$  of about  $-157\%$  along the transect supports the hypothesis fractionation of H during biosynthesis is independent of environmental parameters.

The enrichment of  $\sim 30\%$  of the terrestrial long-chain n-alkanes to the aquatic n-C<sub>17</sub> is likely due to evaporation processes in plant leaves. The variation in the difference between terrestrial and aquatic n-alkanes might be influenced by input of allochthonous biomass of distant origin on the sites. Since it is not clear if the leaf wax n-alkanes are synthesized *de novo* year-round, further investigation involving evapotranspiration measurements and study of possible seasonal variations in leaf-wax n-alkane  $\delta D$  values will clarify if this difference can serve as a valuable proxy for the evapotranspiration of the lake ecosystem.

The hydrogen source of the short-chain n-alkanes n-C<sub>12</sub> to n-C<sub>20</sub> (excluding n-C<sub>17</sub> and n-C<sub>19</sub>) is probably not the meteoric water pool. Nonphotosynthetic bacteria synthesizing these compounds or a contribution of fossil hydrogen (petroleum contamination) might be responsible. Further research on the differences of H isotope fractionation in autotrophic vs. heterotrophic organisms is necessary. Compound-specific radiocarbon measurements on the short-chained alkanes could help clarify their origin.

Our results demonstrate that hydrogen isotope ratios of recent sedimentary n-alkanes excellently record the  $\delta D$  value of the lake water (Fig. 6), and hence, enable climatic reconstruction. Moreover, the use of terrestrial and aquatic compounds might be used as a proxy for evapotranspiration of the lake

environment. n-Alkanes are abundant in sediments and relatively easy to purify, so the application of compound-specific hydrogen isotope measurements to samples from the geological past opens new possibilities for the reconstruction of changes in the water cycle.

*Acknowledgments*—We would like to thank Steffen Rühlow and Carsten Sachse (MPI-BGC Jena) for the help while sampling, and Bernd Schlöffel and Frank Voigt (MPI-BGC Jena) for construction of the raft. We are grateful to Gerhard Daut (University of Jena) and Jörg Negendank (GFZ Potsdam) for providing sampling equipment. Dan Hammarlund (University of Lund), Tuomas Laurilla (Finnish Meteorological Institute, Sodankylä), Lauri Arvola (Lammi Biological Station), Timo Vesala (Helsinki University), Niels-Otto Jensen (Risø National Laboratory), G. Schleser and A. Lücke (KFZ Jülich), Jörg Negendank and Cathrin Brüchmann (GFZ Potsdam), and Günther Seufert (JRC, San Rossore) are acknowledged for providing help with selection of the lakes, on-site support, and data on the lakes. We thank W.A. Brand, R.A. Werner, and H. Geilmann (MPI-BGC Jena) for measurement of  $\delta D$  and  $\delta^{18}O$  values of the lake water. We would like to thank Alex L. Sessions, two anonymous reviewers, and the Associate Editor Jeffrey S. Seewald for their helpful comments on the original manuscript. This study is supported by DFG grants G1262/3, G1262/8, and DFG Priority Program (SPP) 1054.

*Associate editor:* J. Seewald

#### REFERENCES

- Allen J. R. M., Brandt U., Brauer A., Hubberten H. W., Huntley B., Keller J., Kraml M., Mackensen A., Mingram J., Negendank J. F. W., Nowaczyk N. R., Oberhansli H., Watts W. A., Wulf S., and Zolitschka B. (1999) Rapid environmental changes in southern Europe during the last glacial period. *Nature* **400**, 740–743.
- Andersen N., Paul H. A., Bernasconi S. M., McKenzie J. A., Behrens A., Schaeffer P., and Albrecht P. (2001) Large and rapid climate variability during the Messinian salinity crisis: Evidence from deuterium concentrations of individual biomarkers. *Geol.* **29**, 799–802.
- Baas M., Pancost R., van Geel B., and Damste J. S. S. (2000) A comparative study of lipids in Sphagnum species. *Organic Geochemistry* **31**, 535–541.
- Bowen G. and Revenaugh J. (2003) Interpolating the isotopic composition of modern meteoric precipitation. *Water Resources Research* **39**, 9-1-9-13.
- Burgoyne T. W. and Hayes J. M. (1998) Quantitative production of H-2 by pyrolysis of gas chromatographic effluents. *Analytical Chemistry* **70**, 5136–5141.
- Chikaraishi Y. and Naraoka H. (2003) Compound-specific delta D-delta C-13 analyses of n-alkanes extracted from terrestrial and aquatic plants. *Phytochemistry* **63**, 361–371.
- Craig H. (1961) Isotopic variations in meteoric waters. *Science* **133**, 1702–1703.
- Craig H. and Gordon L. (1965) Deuterium and oxygen 18 variations in the ocean and the marine atmosphere. In *Proceedings of a Conference on Stable Isotopes in Oceanographic Studies and Paleotemperatures* (ed. E. Tongioli), pp. 9–130, CNR-Laboratorio di Geologia Nucleare.
- Cranwell P. A., Eglinton G., and Robinson N. (1987) Lipids of aquatic organisms as potential contributors to lacustrine sediments. 2. *Organic Geochemistry* **11**, 513–527.
- Eglinton G. and Hamilton R. J. (1967) Leaf epicuticular waxes. *Science* **156**, 1322–1334.
- Epstein S., Yapp C. J., and Hall J. H. (1976) Determination of D-H ratio of non-exchangeable hydrogen in cellulose extracted from aquatic and land plants. *Earth and Planetary Science Letters* **30**, 241–251.
- Estep M. F. and Hoering T. C. (1980) Biogeochemistry of the stable hydrogen isotopes. *Geochim. Cosmochim. Acta* **44**, 1197–1206.
- Feng X. H. and Epstein S. (1995) Climatic temperature records in delta-D data from tree-rings. *Geochim. Cosmochim. Acta* **59**, 3029–3037.

- Ficken K. J., Li B., Swain D. L., and Eglinton G. (2000) An n-alkane proxy for the sedimentary input of submerged/floating freshwater aquatic macrophytes. *Organic Geochemistry* **3**, 745–749.
- Gat J. R. (1996) Oxygen and hydrogen isotopes in the hydrologic cycle. *Annu. Review of Earth and Planetary Sciences* **24**, 225–262.
- Gonfiantini R. (1986) Environmental isotopes in lake studies. In *Handbook of Environmental Isotope Geochemistry* (ed. P. Fritz and J. C. Fontes), pp. 113–168, Elsevier.
- Grimalt J. O. and Albaiges J. (1987) Sources and occurrence of C<sub>12</sub>–C<sub>22</sub> n-alkane distributions with even carbon-number preference in sedimentary environments. *Geochim. Cosmochim. Acta* **51**, 1379–1384.
- Gulz P. G. (1994) Epicuticular leaf waxes in the evolution of the plant kingdom. *J. of Plant Physiology* **143**, 453–464.
- Han J. and Calvin M. (1969) Hydrocarbon distribution of algae and bacteria and microbiological activity in sediments. *Proceedings of the National Academy of Science of the United States of America* **64**, 436–443.
- Han J., McCarthy E., Van Hoesen W., Calvin M., and Bradley W. (1968) Organic geochemical studies, ii. a preliminary report on the distribution of aliphatic hydrocarbons in algae, in bacteria and in a recent lake sediment. *Proceedings of the National Academy of Sciences of the United States of America* **59**, 29–33.
- Hilkert A. W., Douthitt C. B., Schlüter H. J., and Brand W. A. (1999) Isotope ratio monitoring gas chromatography/mass spectrometry of D/H by high temperature conversion isotope ratio mass spectrometry. *Rapid Communications in Mass Spectrometry* **13**, 1226–1230.
- Huang Y., Shuman B., Wang Y., and Webb T. (2002) Hydrogen isotope ratios of palmitic acid in lacustrine sediments record late Quaternary climate variations. *Geol.* **30**, 1103–1106.
- IAEA (2001) GNIP Maps and Animations. International Atomic Energy Agency. Accessible at <http://isohis.iaea.org>.
- Jeffrey S. W. and Humphrey G. F. (1975) New spectrophotometric equations for determining chlorophylls a, b, c1 and c2 in higher-plants, algae and natural phytoplankton. *Biochimie Und Physiologie Der Pflanzen* **167**, 191–194.
- Li M. W., Huang Y. S., Obermajer M., Jiang C. Q., Snowdon L. R., and Fowler M. G. (2001) Hydrogen isotopic compositions of individual alkanes as a new approach to petroleum correlation: case studies from the Western Canada sedimentary basin. *Organic Geochemistry* **32**, 1387–1399.
- Meyers P. A. (2003) Applications of organic geochemistry to paleolimnological reconstructions: a summary of examples from the Laurentian Great Lakes. *Organic Geochemistry* **34**, 261–289.
- Piasentier E., Bovolenta S., and Malossini F. (2000) The n-alkane concentrations in buds and leaves of browsed broadleaf trees. *J. of Agricultural Science* **135**, 311–320.
- Ramrath A. (1997) Laminierte sedimente des Lago di Mezzano (Latium, Italien) - limnogeologie und rekonstruktion von umweltbedingungen der letzten 34.000 jahre. Ph.D. thesis, Universität Potsdam.
- Sachse D., Radke J., Gaupp R., Schwark L., Lüniger G., and Gleixner G. (2004) Reconstruction of palaeohydrological conditions in a lagoon during the 2nd Zechstein cycle through simultaneous use of  $\delta$ D values of individual n-alkanes and  $\delta$ 18O and  $\delta$ 13C values of carbonates, revised version. *Int. J. Earth Sci.* **93**, 554–564.
- Sauer P. E., Eglinton T. I., Hayes J. M., Schimmelmann A., and Sessions A. L. (2001) Compound-specific D/H ratios of lipid biomarkers from sediments as a proxy for environmental and climatic conditions. *Geochim. Cosmochim. Acta* **65**, 213–222.
- Schimmelmann A., Lewan M. D., and Wintsch R. P. (1999) D/H isotope ratios of kerogen, bitumen, oil, and water in hydrous pyrolysis of source rocks containing kerogen types I, II, IIS, and III. *Geochim. Cosmochim. Acta* **63**, 3751–3766.
- Sessions A. L., Burgoyne T. W., Schimmelmann A., and Hayes J. M. (1999) Fractionation of hydrogen isotopes in lipid biosynthesis. *Organic Geochemistry* **30**, 1193–1200.
- Sessions A. L., Jahnke L. L., Schimmelmann A., and Hayes J. M. (2002) Hydrogen isotope fractionation in lipids of the methane-oxidizing bacterium *Methylococcus capsulatus*. *Geochim. Cosmochim. Acta* **66**, 3955–3969.
- Sternberg L., Deniro M. J., and Ajie H. (1984) Stable hydrogen isotope ratios of saponifiable lipids and cellulose nitrate from cam, C-3 and C-4 plants. *Phytochemistry* **23**, 2475–2477.
- Sternberg L. D. L. (1988) D/H ratios of environmental water recorded by D/H ratios of plant lipids. *Nature* **333**, 59–61.
- Watts W. A., Allen J. R. M., Huntley B., and Fritz S. C. (1996) Vegetation history and climate of the last 15,000 years at Laghi di Monticchio, southern Italy. *Quaternary Science Rev.* **15**, 113–132.
- Xie S., Nott C., Avsejs L., Volders F., Maddy D., Chambers F., Gledhill A., Carter J., and Evershed R. (2000) Palaeoclimate records in compound-specific  $\delta$ D values of a lipid biomarker in ombrotrophic peat. *Organic Geochemistry* **31**, 1053–1057.
- Yang H. and Huang Y. S. (2003) Preservation of lipid hydrogen isotope ratios in Miocene lacustrine sediments and plant fossils at Clarkia, northern Idaho, USA. *Organic Geochemistry* **34**, 413–423.
- Yapp C. J. and Epstein S. (1982) Climatic significance of the hydrogen isotope ratios in tree cellulose. *Nature* **297**, 636–639.
- Ziegler H. (1989) Hydrogen isotope fractionation in plant tissues. In *Stable Isotopes in Ecological Research* (ed. P. Rundel et al.), pp. 105–123, Springer-Verlag.