



## An interlaboratory study of TEX<sub>86</sub> and BIT analysis using high-performance liquid chromatography–mass spectrometry

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[1] Recently, two new proxies based on the distribution of glycerol dialkyl glycerol tetraethers (GDGTs) were proposed, i.e., the TEX<sub>86</sub> proxy for sea surface temperature reconstructions and the BIT index for reconstructing soil organic matter input to the ocean. In this study, fifteen laboratories participated in a round robin study of two sediment extracts with a range of TEX<sub>86</sub> and BIT values to test the analytical reproducibility and repeatability in analyzing these proxies. For TEX<sub>86</sub> the repeatability, indicating intralaboratory variation, was 0.028 and 0.017 for the two sediment extracts or  $\pm 1-2^{\circ}\text{C}$  when translated to temperature. The reproducibility, indicating among-laboratory variation, of TEX<sub>86</sub> measurements was substantially higher, i.e., 0.050 and 0.067 or  $\pm 3-4^{\circ}\text{C}$  when translated to temperature. The latter values are

higher than those obtained in round robin studies of Mg/Ca and U<sub>37</sub><sup>k'</sup> paleothermometers, suggesting the need to primarily improve compatibility between labs. The repeatability of BIT measurements for the sediment with substantial amounts of soil organic matter input was relatively small, 0.029, but reproducibility was large, 0.410. This large variance could not be attributed to specific equipment used or a particular data treatment. We suggest that this may be caused by the large difference in the molecular weight in the GDGTs used in the BIT index, i.e., crenarchaeol versus the branched GDGTs. Potentially, this difference gives rise to variable responses in the different mass spectrometers used. Calibration using authentic standards is needed to establish compatibility between labs performing BIT measurements.

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## 1. Introduction

[2] Reconstruction of ancient seawater temperatures is of considerable importance in understanding past climate changes. Over the last decades several temperature proxies have been developed and used to reconstruct past seawater temperatures on the basis of inorganic or organic fossil remains. Two of the most popular tools are presently the Mg/Ca ratio of planktonic foraminifera [Nürnberg *et al.*, 1996; Elderfield and Ganssen, 2000] and the U<sub>37</sub><sup>K'</sup> ratio based on long-chain C<sub>37</sub> alkenones derived from haptophyte algae [Brassell *et al.*, 1986; Prahl and Wakeham, 1987].

[3] Recently, a second organic seawater temperature proxy based on archaeal glycerol dibiphytanyl glycerol tetraether (GDGT) lipids, the TEX<sub>86</sub>, was proposed [Schouten *et al.*, 2002]. These lipids are biosynthesized by marine Crenarchaeota which are ubiquitous in marine environments and are among the dominant prokaryotes in today's oceans [Karner *et al.*, 2001; Herndl *et al.*, 2005]. Marine Crenarchaeota biosynthesize different types of GDGTs, i.e., GDGTs containing 0 to 3 cyclopentyl moieties (GDGT-0 to GDGT-3; see structures in Figure 1) and crenarchaeol which, in addition to four cyclopentyl moieties, has a cyclohexyl moiety (GDGT-4). Finally, they also biosynthesize small quantities of a crenarchaeol regio-isomer (GDGT-4'). A study of marine surface sediments showed that higher overlying sea surface temperatures result in an increase in the relative amounts of GDGTs with two or more cyclopentyl moieties. The TEX<sub>86</sub> ratio

was proposed as a means to quantify the relative abundance of GDGTs [Schouten *et al.*, 2002]:

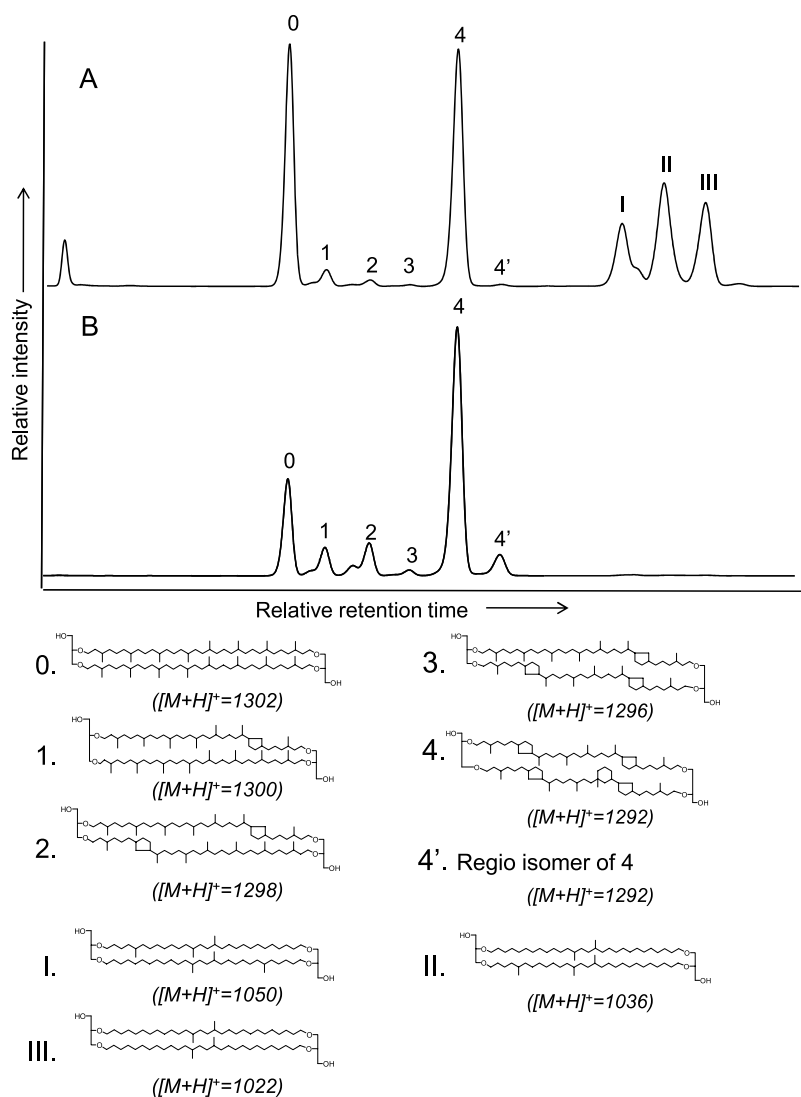
$$\text{TEX}_{86} = \frac{[\text{GDGT} - 2] + [\text{GDGT} - 3] + [\text{GDGT} - 4']}{[\text{GDGT} - 1] + [\text{GDGT} - 2] + [\text{GDGT} - 3] + [\text{GDGT} - 4']} \quad (1)$$

The TEX<sub>86</sub> has recently been calibrated with annual mean sea surface temperature using marine sediment core tops with the following resulting equation [Kim *et al.*, 2008]:

$$T = -10.78 + 56.2 * \text{TEX}_{86} (r^2 = 0.935, n = 223) \quad (2)$$

Studies have shown that this proxy can be analyzed in a range of sediments up to 120 My old and applied to the reconstruction of ancient sea surface water temperatures [e.g., Schouten *et al.*, 2003; Forster *et al.*, 2007]. TEX<sub>86</sub> values in modern sediments range typically from 0.3 to 0.7 [e.g., Kim *et al.*, 2008], while in ancient sediments they can be as high as 0.96 [e.g., Forster *et al.*, 2007].

[4] In addition to archaeal GDGTs, bacterial GDGTs with nonisoprenoidal carbon skeletons also are encountered frequently in marine sediments (GDGT-I to GDGT-III, Figure 1). Several studies have now shown that they are especially abundant in soils and peats [Weijers *et al.*, 2006] and progressively decrease in concentration from coastal sediments to open marine sediments, suggesting a terrestrial origin [Hopmans *et al.*, 2004; Herfort *et al.*, 2006; Kim *et al.*, 2006]. Hopmans *et al.* [2004] proposed the BIT index to quantify the relative abundance of these bacterial GDGTs ver-



**Figure 1.** HPLC base peak chromatogram of interlaboratory standards (a) S1 and (b) S2. Numbers in italics with the structures of GDGTs indicate the masses of the  $[M+H]^+$  ions of the GDGTs. Samples were run at the Royal NIOZ under conditions described by Schouten *et al.* [2007].

sus crenarchaeol as a proxy for the input of terrestrial organic matter into marine sediments:

$$\text{BIT} = \frac{[\text{GDGT - I}] + [\text{GDGT - II}] + [\text{GDGT - III}]}{[\text{GDGT - 4}] + [\text{GDGT - I}] + [\text{GDGT - II}] + [\text{GDGT - III}]} \quad (3)$$

Several studies have now shown that this proxy can be applied to trace the relative importance of soil organic matter in coastal marine environments [e.g., Herfort *et al.*, 2006; Kim *et al.*, 2006; Huguet *et al.*, 2007; Walsh *et al.*, 2008]. Furthermore, Weijers *et al.* [2006] found that high input of soil organic matter in marine sediments can potentially bias TEX<sub>86</sub> values as it can also contain GDGTs 1–3. They recommended simultaneous reporting

of BIT indices in order to monitor for this effect. BIT values can range from 0.01 in open marine sediments up to 1 in some soils [e.g., Hopmans *et al.*, 2004; Kim *et al.*, 2006; Huguet *et al.*, 2007; Walsh *et al.*, 2008].

[5] A prerequisite for the wider application of these proxies is the robustness and analytical reproducibility of their analysis. This is especially important with these proxies as they are analyzed by high-performance liquid chromatography (HPLC) coupled to mass spectrometry (MS) [Hopmans *et al.*, 2000; Schouten *et al.*, 2007; Escala *et al.*, 2007], a technique that was, until recently, not commonly used in many paleoceanographic and organic geochemical laboratories. A

**Table 1.** HPLC/MS Methods Used by Participants in the Round Robin Study<sup>a</sup>

Lab	HPLC Column	HPLC Gradient	MS Type	MS Method	Integration
1	Prevail Cyano	Hex:IPA	Ion trap	Mass scanning	[M+H] <sup>+</sup> ions
2	Prevail Cyano	Hex:IPA	Single quad	SIM	[M+H] <sup>+</sup> ions
4	Prevail Cyano	Hex:IPA	Single quad	SIM	[M+H] <sup>+</sup> ions
5	Teknokroma Cyano	Hex:IPA	Ion trap	na	[M+H] <sup>+</sup> ions
6	Prevail Cyano	Hex:IPA	Single quad	SIM	[M+H] <sup>+</sup> + [M+H+1] <sup>+</sup> ions
7	Prevail Cyano	Hex:IPA	Ion trap	na	Mass ranges
11	Prevail Cyano	Hex:IPA	Ion trap	na	[M+H] <sup>+</sup> ions
12	Prevail Cyano	Hex:IPA	Ion trap	na	[M+H] <sup>+</sup> ions
13	Prevail Cyano	Hex:IPA	Single quad	SIM	[M+H] <sup>+</sup> ions
14	Prevail Cyano	Hex:IPA	Single quad	SIM	[M+H] <sup>+</sup> ions
15	Prevail Cyano	Hex:IPA	Single quad	SIM	[M+H] <sup>+</sup> ions
16	Prevail Cyano	Hex:IPA	TOF	Mass scanning	[M+H] <sup>+</sup> ions
17	Prevail Cyano	Hex:IPA	Single quad	SIM	[M+H] <sup>+</sup> ions
18	Prevail Cyano	Hex:IPA	Ion trap	na	Mass ranges
19	Prevail Cyano	Hex:IPA	Single quad	SIM	[M+H] <sup>+</sup> ions
20	Prevail Cyano	Hex:IPA	Single quad	Mass scanning	[M+H] <sup>+</sup> ions

<sup>a</sup>Hex, hexane; IPA, isopropanol; SIM, selected ion monitoring; TOF, time of flight; na, not applicable.

common procedure to establish the robustness and reproducibility of an analytical method is a round robin study, as has been done for the U<sub>37</sub><sup>K'</sup> ratio of long-chain C<sub>37</sub> alkenones [Rosell-Melé *et al.*, 2001] and for the Mg/Ca ratio of (foraminiferal) carbonates [Rosenthal *et al.*, 2004; Greaves *et al.*, 2008]. To assess the reproducibility of the HPLC/MS technique for TEX<sub>86</sub> and BIT analysis, we performed an anonymous round robin study on filtered polar fractions obtained from extracts of two sediments, following the general outline and methods as in previous paleoceanographic proxy round robin studies by Rosell-Melé *et al.* [2001] and Rosenthal *et al.* [2004].

## 2. Materials and Methods

[6] A general invitation was sent to a large number of laboratories to participate in an anonymous round robin study, to which 21 labs responded positively. To assess systematic errors in TEX<sub>86</sub> and BIT analysis these labs received two vials, each containing 1 mg of a polar fraction of a sediment extract labeled S1 and S2, prepared at the NIOZ Royal Netherlands Institute for Sea Research. Labs were requested to analyze the samples when their HPLC/MS set up was performing well according to their criteria and to inject sufficient enough amounts to be above the limit of quantification [cf. Schouten *et al.*, 2007]. The vials were distributed by the end of August 2007 and results reported here are those of the fifteen labs which reported their results before 1 January 2008. One lab (14) reported their results for S1 after this deadline. Their results are included

in Tables 1–4 but are not considered further in this study.

### 2.1. Sediment Origin and Extraction Procedure

[7] The standards comprised filtered polar fractions of sediment extracts labeled S1 and S2. Sediment S1 was derived from a piston core taken

**Table 2.** Reported Results of TEX<sub>86</sub> Analysis<sup>a</sup>

Lab	TEX <sub>86</sub> S1	SD	n	TEX <sub>86</sub> S2	SD	n
1	0.423	0.014	10	0.697	0.007	5
2	0.401	0.009	16	0.701	0.010	10
4	<i>0.479<sup>b</sup></i>	<i>0.007</i>	5	0.745	0.007	7
5	<i>0.472<sup>b</sup></i>	<i>0.036<sup>c</sup></i>	6	<i>0.675</i>	<i>0.044<sup>c</sup></i>	6
6	0.414	0.008	15	0.713	0.004	16
7	<i>0.348<sup>b</sup></i>	<i>0.018</i>	3	0.660	0.006	3
11	0.410	0.004	3	0.718	0.004	3
12	0.393	0.013	5	0.694	0.005	5
13	0.420	0.012	8	0.699	0.007	8
14 <sup>d</sup>	0.84	0.02	4			
15	0.414	0.016	4	0.673	0.008	6
16	0.410	0.007	5	0.711	0.003	5
17	0.433	0.008	6	0.697	0.004	6
18	0.378	0.005	5	0.666	0.007	5
19	<i>0.480<sup>b</sup></i>	<i>0.003</i>	7	0.739	0.004	7
20	0.381		2	0.680	0.004	3

<sup>a</sup> Values in italics were rejected and not further considered in the statistical treatment of the data.

<sup>b</sup> Outliers based on visual inspection of normal probability plots of laboratory means.

<sup>c</sup> Outliers based on visual inspection of chi-squared probability plots of laboratory variances.

<sup>d</sup> Result submitted after passing of the deadline and not included further in this study.



**Table 3.** Reported Results of BIT Index Analysis<sup>a</sup>

Lab	BIT S1	SD	n	BIT S2	SD	n
1	<i>0.489</i>	<i>0.069<sup>b</sup></i>	6	0.016	0.0005	4
2	0.582	0.013	16	0.017	0.0005	4
4	0.739	0.006	5	0.040	0.0008	7
5	<i>0.652</i>	<i>0.065<sup>b</sup></i>	6	<i>0.118<sup>c</sup></i>	<i>0.0184</i>	6
6	0.455	0.008	15	0.015	0.0005	16
7	0.668	0.008	3	0.035	0.0020	3
11	0.338	0.014	3	<i>0.009<sup>c</sup></i>	<i>0.0028</i>	3
12	0.658	0.002	5	0.029	0.0021	5
13	0.595	0.010	8	0.019	0.0005	8
14 <sup>d</sup>	0.34	0.01	4			
15	0.821	0.011	4	<i>0.055<sup>c</sup></i>	<i>0.0094<sup>b</sup></i>	6
16	0.447	0.014	5	0.012	0.0008	5
17	0.664	0.005	6	0.031	0.0014	6
18	0.250	0.011	5	0.012	0.0012	5
19	<i>0.476<sup>c</sup></i>	<i>0.031<sup>b</sup></i>	7	0.025	0.0023	7
20	0.626	0.015	3	<i>0.030</i>	<i>0.0070<sup>b</sup></i>	3

<sup>a</sup> Values in italics were rejected and not further considered in the statistical treatment of the data.

<sup>b</sup> Outliers based on visual inspection of chi-squared probability plots of laboratory variances.

<sup>c</sup> Outliers based on visual inspection of normal probability plots of laboratory means.

<sup>d</sup> Result submitted after passing of the deadline and not included further in this study.

in the Drammensfjord, Norway (D2-H; 59 40.11 N, 10 23.76 E; water depth 113 m; sediment depth 746–797 cm). Sediment S2 was derived from a gravity core (TY92–310G; 16 03 N, 52 71 E; 880 m water depth; 0–42 cm depth) taken in the Arabian Sea. The reason to choose these two sediments is that they were expected to cover a large range of TEX<sub>86</sub> (temperate versus tropical) and BIT (coastal versus open ocean) values.

[8] The sediments were freeze-dried and Soxhlet extracted for 24 h using a mixture of dichloromethane (DCM) and methanol (7:1, v/v). The combined extracts were separated over a column filled with alumina oxide into an apolar and polar fraction using hexane: DCM (9:1, v/v) and DCM:methanol (1:1, v/v), respectively. The resulting pooled polar fraction was condensed by rotary evaporation and further dried under a stream of nitrogen. The polar fraction was weighed and dissolved in hexane/isopropanol (99:1, v/v) in a concentration of 2 mg/ml. Aliquots of 1 mg were filtered using a PTFE 0.4 μm filter, dried under a stream of nitrogen and distributed to the different labs.

## 2.2. TEX<sub>86</sub> and BIT Analysis

[9] All labs used HPLC/Atmospheric Pressure Chemical Ionization (APCI)/MS to analyze GDGTs. The HPLC methods used by the different labs are listed in Table 1 and generally followed that of Schouten *et al.* [2007], i.e., a cyano column with a hexane-isopropanol gradient as the mobile phase. Injected sample sizes ranged from 3 to 300 μg of filtered polar fraction. Base peak chromatograms of HPLC/MS analyses of S1 and S2 are shown in Figure 1.

## 2.3. Statistical Analysis

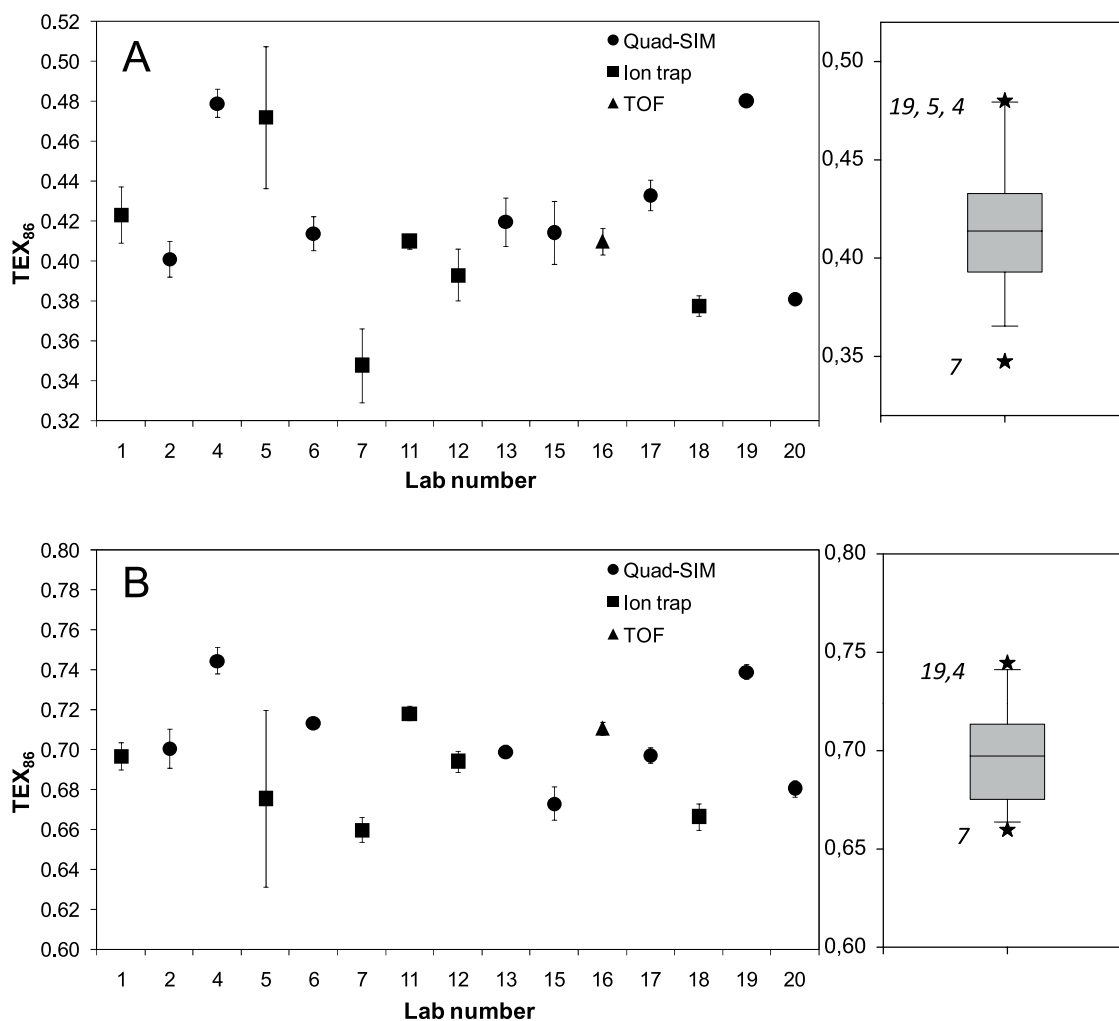
[10] Statistical analysis was based on the international standard ISO 5725 for interlaboratory tests [International Organization for Standardization, 1986]. Repeatability (r) and reproducibility (R)

**Table 4.** Reported Results of TEX<sub>86</sub> and BIT Index Analysis After Adopting a Common Integration Style<sup>a</sup>

Lab	TEX <sub>86</sub> S1	BIT S1	TEX <sub>86</sub> S2	BIT S2
1	0.423 (0.000)	0.489 (0.000)	0.697 (0.000)	0.016 (0.000)
2	0.401 (0.000)	0.581 (0.000)	0.701 (0.000)	0.017 (0.000)
4	0.436 (0.043)	0.721 (0.018)	0.745 (0.000)	0.031 (0.009)
5				
6	0.439 (0.025)	0.459 (0.004)	0.713 (0.000)	0.013 (0.002)
7	0.348 (0.000)	0.674 (0.006)	0.662 (0.002)	0.030 (0.006)
11	0.413 (0.004)	0.337 (0.002)	0.717 (0.001)	0.009 (0.000)
12	0.390 (0.003)	0.636 (0.022)	0.691 (0.003)	0.022 (0.007)
13	0.414 (0.006)	0.584 (0.010)	0.703 (0.004)	0.013 (0.006)
14 <sup>b</sup>		0.590 (0.250)		
15	0.408 (0.006)	0.811 (0.011)	0.704 (0.031)	0.039 (0.016)
16	0.410 (0.000)	0.447 (0.000)	0.711 (0.000)	0.012 (0.000)
17	0.432 (0.001)	0.665 (0.001)	0.698 (0.001)	0.030 (0.001)
18	0.378 (0.000)	0.250 (0.000)	0.666 (0.000)	0.012 (0.000)
19	0.479 (0.001)	0.453 (0.031)	0.738 (0.001)	0.022 (0.003)
20				

<sup>a</sup> Numbers in parentheses indicate absolute difference with previously reported values in Tables 2 and 3.

<sup>b</sup> Result submitted after passing of the deadline and not included further in this study.



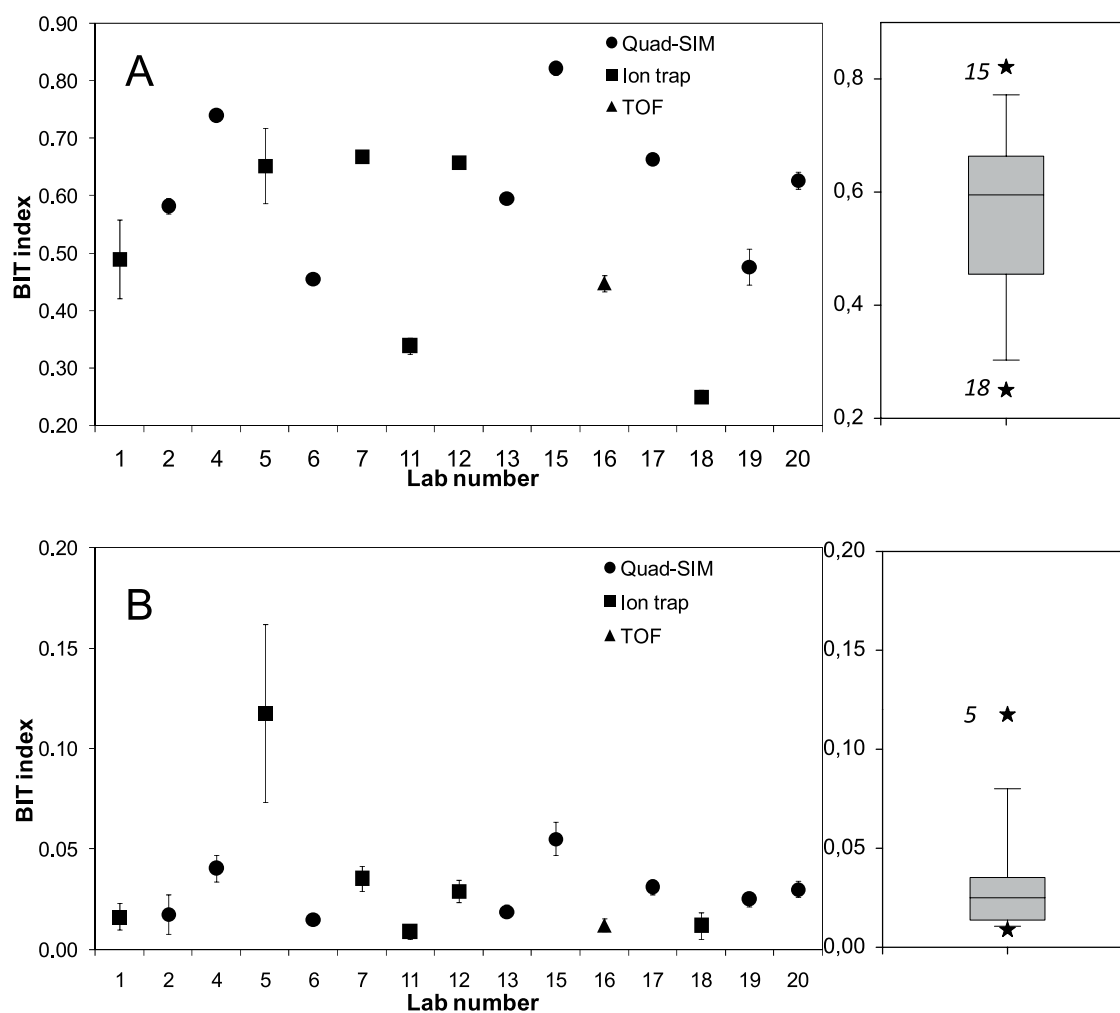
**Figure 2.** Graph of reported average TEX<sub>86</sub> values of individual labs for (a) sample S1 from Arabian Sea sediment and (b) sample S2 from Drammensfjord sediment. Error bars indicate standard deviations (SD) of measurements. Next to the graphs, box plots are shown for each sample. Box indicates lower 25% and upper 75% percentile, and bars indicate lower 10% and upper 90% percentile. Numbers in box plot indicate lab numbers.

values were estimated. The repeatability  $r$  should be interpreted as the value below which the difference between two single test results obtained by the same method on identical test material under the same test conditions (same operator, same apparatus, same laboratory and within a short interval of time) may be expected to lie with a probability of 95%. The reproducibility  $R$  should be interpreted as the value below which the difference between two single test results obtained by the same method on identical test material but under different test conditions (different operators, different apparatus, different laboratory and not necessarily within a short interval of time) may be expected to lie with a probability of 95%. Under these definitions, all laboratories are considered to be using the “same method,” and  $R$  refers to interlaboratory results, while  $r$  refers to intralabor-

atory results. Outlying data and labs were detected by visual inspection of normal probability plots of laboratory means, chi-square probability plots of laboratory variances and Bartlett’s test for homogeneity of variances.

### 3. Results and Discussion

[11] The results discussed here of the anonymous round robin study of two sediment extracts, labeled S1 and S2, are based on the fifteen labs which reported their results before the deadline of 1 January 2008. The results of the TEX<sub>86</sub> and BIT analyses of the different labs are listed in Tables 2 and 3 and plotted in Figures 2 and 3, while the methods used are summarized in Table 1. All labs used almost identical LC conditions (solvent gradients, column



**Figure 3.** Graph of reported average BIT values of individual labs for (a) sample S1 from Arabian Sea sediment and (b) sample S2 from Drammensfjord sediment. Error bars indicate standard deviations (SD) of measurements. Next to the graphs, box plots are shown for each sample. Box indicates lower 25% and upper 75% percentile, and bars indicate lower 10% and upper 90% percentile. Numbers in box plot indicate lab numbers.

type) but a variety of mass spectrometry techniques, i.e., eight labs used quadrupole MS, six labs used ion trap MS and one lab used time-of-flight MS (TOF). Note that most labs analyzed the samples within 1–2 days and thus standard deviations listed do not represent long-term reproducibility. Furthermore, since labs received “ready-to-inject” polar fractions, the results do not allow evaluation of the effects of individual sample work up procedures as was done for the  $U_{37}^{K7}$  ratio of long-chain  $C_{37}$  alkenones [Rosell-Melé *et al.*, 2001] and for the Mg/Ca ratio of (foraminiferal) carbonates [Rosenthal *et al.*, 2004].

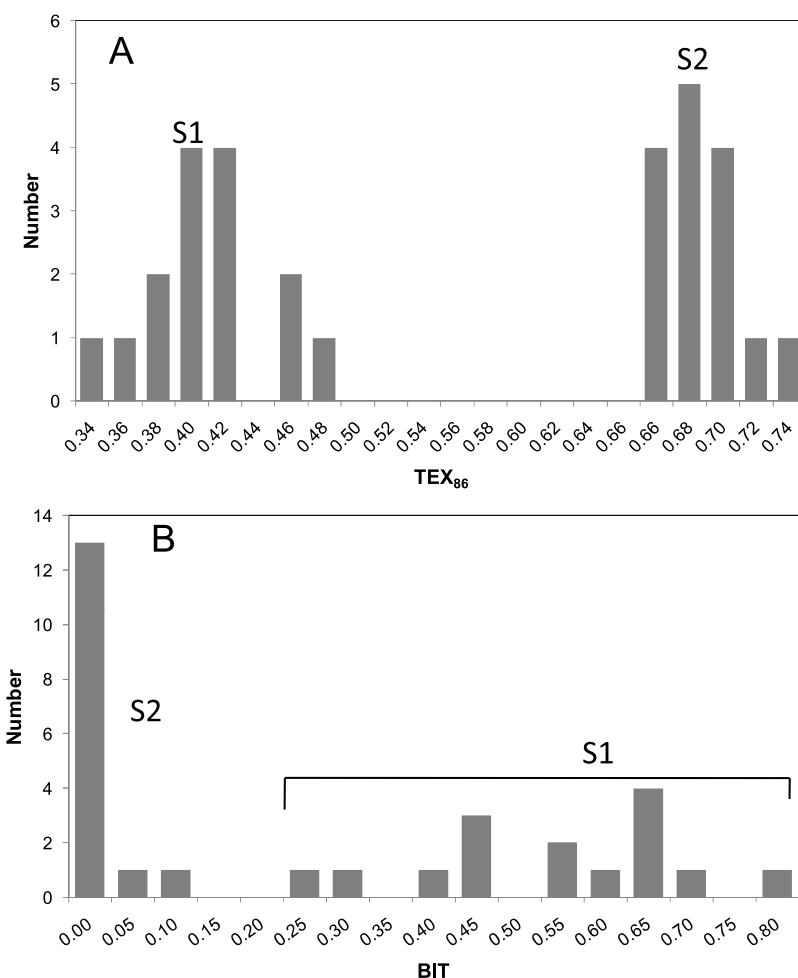
### 3.1. TEX<sub>86</sub> Analysis

[12] The results of the TEX<sub>86</sub> analysis are listed in Table 2 and shown in Figure 2. In Figure 4a we

plotted the distribution of TEX<sub>86</sub> values for both samples S1 and S2. The results have a reasonably Gaussian-like distribution with a broader range for sample S1. We then statistically identified (see section 2.3) four outliers for S1 (labs 4,5,7,19) and one outlier for S2 (lab 5) which were removed from subsequent statistical treatment. These anomalous results cannot be attributed to a particular mass spectrometric technique since the outliers were from two labs using a quadrupole MS and two labs using an ion trap MS (Table 1 and Figure 2).

[13] The estimated repeatability for TEX<sub>86</sub>, after removal of the outliers, was 0.028 and 0.017 for S1 and S2, respectively (Table 5). The reproducibility, however, was slightly higher for S2, i.e., 0.067 compared to 0.050 for S1. However, the variance estimate for S1 was made after removal of four



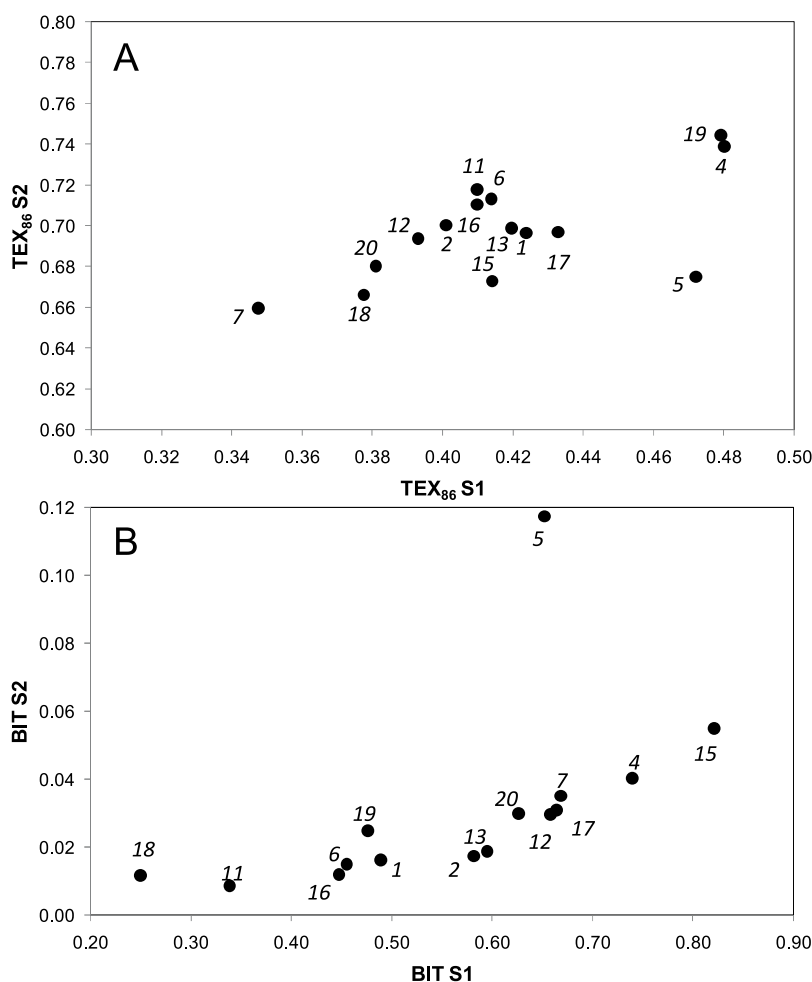


**Figure 4.** Distribution of (a) TEX<sub>86</sub> and (b) BIT values.

outliers. Removal of only the most severe outlier (lab 5) would have resulted in a reproducibility of 0.092. If we convert these TEX<sub>86</sub> values to temperatures [Kim *et al.*, 2008] then the repeatability of TEX<sub>86</sub> analysis corresponds to 1.9 and 1.1°C for S1 and S2, respectively, while the reproducibility corresponds to 3.3 and 4.5°C for S1 and S2, respectively (Table 5). The better repeatability and, when taking account of the number of outliers removed from S1, reproducibility of sample S2 likely is due to the higher abundances of the minor GDGTs, GDGTs 1–3 and GDGT-4', relative to GDGT-0 and crenarchaeol (GDGT-4). This is likely to have enabled a more reliable quantification, as amounts were not only above the limit of detection but also above the limit of quantification which is likely to be an order of magnitude higher for TEX<sub>86</sub> analysis [cf. Schouten *et al.*, 2007].

[14] To investigate potential causes for outliers and differences between labs, we plotted the TEX<sub>86</sub> values of S1 against S2 (Figure 5a). This reveals

that, in general, there is a tendency toward some systematic difference. For example, outliers in TEX<sub>86</sub> measurements of S1 also tend to be outliers in TEX<sub>86</sub> measurements of S2. This suggests that the differences between labs are not caused by inhomogeneity between individual vials of the standards. Another potential cause for the differences may be the “integration style” used, i.e., which criteria were used to define peak starts and ends. The latter can be important because coelutions occur between the GDGTs of interest and other minor isomers. Therefore, labs were asked to reintegrate the peak areas in their chromatogram according to a prescribed format and preferably by a person not aware of the previous results. Twelve labs reported the results of this exercise which showed that with only a few exceptions, the changes in TEX<sub>86</sub> were relatively minor (Table 4) and unlikely to account for the observed differences. The results of lab 5 are, for both samples, outliers. Examination of their LC/MS equipment



**Figure 5.** Crossplots of (a) TEX<sub>86</sub> and (b) BIT index for sample S1 against S2. Numbers in italics indicate lab numbers.

revealed that the cone of the nebulizer was not well aligned with the ion source, and the inner surfaces of the interface had some chemical residues. This highlights the fact that maintenance of the APCI interface is of prime importance to obtain consistent and robust results.

[15] The results obtained for TEX<sub>86</sub> analysis compared reasonably well to those obtained for other paleothermometers, especially considering the relatively recent development of the proxy. *Rosell-Melé et al.* [2001] found for U<sub>37</sub><sup>k'</sup> analyses of several sediments a repeatability of 1.6°C, but their reproducibility of 2.1°C was substantially better than obtained in our study. *Rosenthal et al.* [2004] reported a repeatability of 1–2°C and a reproducibility of 2–3°C for Mg/Ca analysis of foraminifera, also numbers that are similar to our study. These estimates also already contained biases induced by work up procedures, something which is not applicable in our study. In fact, the reproducibility

of standard mixtures, which does not include biases by sample work up, is even better at 0.5 and 1.3°C for Mg/Ca and U<sub>37</sub><sup>k'</sup>, respectively. Thus, our interlaboratory study suggests that repeatability (*r*) of TEX<sub>86</sub> temperatures is similar to those of other paleothermometers but that the reproducibility (*R*) among labs is significantly higher. Hence, there is a need to improve reproducibility between labs using standards or calibrations. It also should be noted, however, that a large number of the participating labs had relatively little experience in analyzing GDGTs using HPLC/APCI/MS at this point. Presumably, the robustness of these analyses will improve with increasing experience.

### 3.2. BIT Analysis

[16] The results of the analysis of samples S1 and S2 for BIT are displayed in Table 3 and Figures 3 and 4. Sample S2 is from an open marine sediment with a small contribution of soil organic matter,

**Table 5.** Summary Statistics of All Measurements Made by the Different Laboratories

	Mean <sup>a</sup>	Mean	Median	Mode	SD <sup>a</sup>	SD	r	R	%RSD <sub>r</sub>	%RSD <sub>R</sub>
TEX <sub>86</sub> S1	0.420	0.409	0.410	0.380	0.034	0.017	0.028	0.050	6.8	12
TEX <sub>86</sub> S2	0.702	0.704	0.701	0.690	0.026	0.023	0.017	0.067	2.4	9.5
BIT S1	0.554	0.558	0.583	0.738	0.131	0.138	0.029	0.410	5.2	74
BIT S2	0.032	0.022	0.018	0.040	0.027	0.009	0.004	0.028	16	127

<sup>a</sup>Including outliers.

and thus values are nearly all below 0.1 (Figure 3b and Table 3). On the basis of the Bartlett's test four outliers were removed (labs 5,11,15,20) but the variability remained inhomogeneous even after removal of these four outliers. The repeatability was 0.004, while the reproducibility was much larger at 0.028. Sample S1 is from a Norwegian fjord, which likely contains substantial amounts of soil organic carbon [e.g., *Huguet et al.*, 2007]. Indeed substantially higher BIT indices were measured for this sample than for S2. However, a large spread in BIT values ranging from 0.25 to 0.82 (on a scale from 0 to 1; Figure 3a and Table 3) and a broad nonuniform distribution were found (Figure 4b), quite different from that observed for the TEX<sub>86</sub> measurements. For BIT measurements of S1, the repeatability estimate is 0.029 while the reproducibility estimate is high at 0.410 even after removing three outliers on the basis of Bartlett's test (Table 5).

[17] The large reproducibility estimate for sample S1 and the inhomogeneity in variances between different BIT measurements is striking. It suggests that the BIT index can be determined by most labs fairly reproducibly but that there are considerable differences between labs. This suggests that there is some major underlying problem in determining the BIT index which is not apparent for TEX<sub>86</sub> analysis, even though both parameters are measured in a single analysis. A similar reintegration exercise was performed for the BIT measurements as with the TEX<sub>86</sub> measurements but again this did not result in substantial changes in the reported results (Table 4). Plotting the results of BIT measurements of S1 against S2 shows that the differences are systematic (Figure 5b) and thus again cannot be due to inhomogeneity between the distributed vials. Furthermore, there is no particular distinction in BIT values based on the type of mass spectrometer used (Figure 3), nor do similar clusters form among laboratories as those found for the TEX<sub>86</sub> results (Figure 5a).

[18] There may be several reasons for this large spread in BIT indices. First, branched GDGTs have a later elution time. Most chromatographic pro-

grams made use of a hexane-isopropanol gradient and thus, depending on the elution time, a varying percentage of isopropanol may have been present in the APCI chamber during the ionization of the branched GDGTs compared to the amount present during the ionization of crenarchaeol (GDGT-4). This may have given rise to differences in the ionization efficiency of the GDGTs in the APCI and thus variation in the BIT index may depend on the chromatographic behavior of the GDGTs on the LC column. However, at the NIOZ lab similar BIT values were obtained for S1 despite variations in retention time of up to 5 min or when using an isocratic elution program, suggesting that varying isopropanol concentrations does not have a major effect on the indices measured. Second, and likely more importantly, there is a large mass difference between branched GDGTs ( $m/z$  1022–1050) and crenarchaeol ( $m/z$  1292). Thus, the BIT index will be more affected by the mass calibration and tuning of the mass spectrometer used, in contrast to the TEX<sub>86</sub>, where mass differences of the GDGTs used are much smaller ( $m/z$  1300 to  $m/z$  1292). This difference does not depend on the type of mass spectrometer (Figure 3). To solve this problem unequivocally, mixtures of authentic standards of crenarchaeol and a branched GDGT in known ratios are required, something which needs to be considered in future round robin studies. Until then, it is clear that the BIT index can only be used as a crude qualitative measure for the relative input of soil organic matter in coastal systems. The results also have consequences for assessing biases in TEX<sub>86</sub> using an absolute BIT value [cf. *Weijers et al.*, 2006]. Instead, it may be possible to assess this bias by correlating BIT values with TEX<sub>86</sub> values, i.e., large changes in soil organic matter input, and thus in the BIT index, will likely lead to changes in the TEX<sub>86</sub>.

#### 4. Conclusions

[19] An anonymous interlaboratory study of TEX<sub>86</sub> and BIT analysis of two sediment extracts was



carried out by fifteen different laboratories around the world and revealed relatively large variances between the different labs, especially for BIT analysis. Repeatability of TEX<sub>86</sub> analysis was, in terms of temperature, similar to the work-up and analytical repeatability of other paleothermometers ( $\pm 1$ – $2^\circ\text{C}$ ) but the reproducibility between labs was larger ( $\pm 3$ – $4^\circ\text{C}$ ), indicating the need for improved analytical protocols. Paleotemperature reconstructions based on TEX<sub>86</sub> therefore are likely to perform as well as other proxies for determining magnitudes and rates of climatic changes, based on the generally good laboratory repeatability. The poor reproducibility will only impact the reconstruction of absolute temperatures. For BIT values the reproducibility was large (0.410), potentially because of differences in mass calibration and tuning of the mass spectrometers used. Our results suggest that there is a clear need for further round robin studies which should include the use of mixtures of authentic standards, constraining the effects of mass calibrations and tuning set ups, evaluation of sample work up procedures and the monitoring of long-term reproducibility.

## References

- Brassell, S. C., G. Eglinton, I. T. Marlowe, U. Pflaumann, and M. Sarnthein (1986), Molecular stratigraphy: A new tool for climatic assessment, *Nature*, *320*, 129–133, doi:10.1038/320129a0.
- Elderfield, H., and G. Ganssen (2000), Past temperature and  $\delta^{18}\text{O}$  of surface ocean waters inferred from foraminiferal Mg/Ca ratios, *Nature*, *405*, 442–445, doi:10.1038/35013033.
- Escala, M., A. Rosell-Melé, and P. Masqué (2007), Rapid screening of glycerol dialkyl glycerol tetraethers in continental Eurasia samples using HPLC/APCI-ion trap mass spectrometry, *Org. Geochem.*, *38*, 161–164, doi:10.1016/j.orggeochem.2006.08.013.
- Forster, A., S. Schouten, K. Moriya, P. A. Wilson, and J. S. Sinninghe Damsté (2007), Tropical warming and intermittent cooling during the Cenomanian/Turonian oceanic anoxic event 2: Sea surface temperature records from the equatorial Atlantic, *Paleoceanography*, *22*, PA1219, doi:10.1029/2006PA001349.
- Greaves, M., et al. (2008), Interlaboratory comparison study of calibration standards for foraminiferal Mg/Ca thermometry, *Geochem. Geophys. Geosyst.*, *9*, Q08010, doi:10.1029/2008GC001974.
- Herfort, L., S. Schouten, J. P. Boon, M. Woltering, M. Baas, J. W. H. Weijers, and J. S. Sinninghe Damsté (2006), Characterization of transport and deposition of terrestrial organic matter in the southern North Sea using the BIT index, *Limnol. Oceanogr.*, *51*, 2196–2205.
- Herndl, G. J., T. Reinthaler, E. Teira, H. van Aken, C. Veth, A. Pernthaler, and J. Pernthaler (2005), Contribution of Archaea to total prokaryotic production in the deep Atlantic Ocean, *Appl. Environ. Microbiol.*, *71*, 2303–2309, doi:10.1128/AEM.71.5.2303-2309.2005.
- Hopmans, E. C., S. Schouten, R. D. Pancost, M. T. J. Van Der Merr, and J. S. Sinninghe Damsté (2000), Analysis of intact tetraether lipids in archaeal cell material and sediments using high performance liquid chromatography/atmospheric pressure ionization mass spectrometry, *Rapid Commun. Mass Spectrom.*, *14*, 585–589, doi:10.1002/(SICI)1097-0231(20000415)14:7<585::AID-RCM913>3.0.CO;2-N.
- Hopmans, E. C., J. W. H. Weijers, E. Schefuß, L. Herfort, J. S. Sinninghe Damsté, and S. Schouten (2004), A novel proxy for terrestrial organic matter in sediments based on branched and isoprenoid tetraether lipids, *Earth Planet. Sci. Lett.*, *224*, 107–116, doi:10.1016/j.epsl.2004.05.012.
- Huguet, C., R. H. Smittenberg, W. Boer, J. S. Sinninghe Damsté, and S. Schouten (2007), Twentieth century proxy records of temperature and soil organic matter input in the Drammensfjord, southern Norway, *Org. Geochem.*, *38*, 1838–1849, doi:10.1016/j.orggeochem.2007.06.015.
- International Organization for Standardization (1986), Determination of repeatability and reproducibility for a standard test method by interlaboratory tests, *ISO 5725*, 2nd ed., Geneva, Switzerland.
- Karner, M., E. F. DeLong, and D. M. Karl (2001), Archaeal dominance in the mesopelagic zone of the Pacific Ocean, *Nature*, *409*, 507–510, doi:10.1038/35054051.
- Kim, J.-H., S. Schouten, R. Buscail, W. Ludwig, J. Bonnin, J. S. Sinninghe Damsté, and F. Bourrin (2006), Origin and distribution of terrestrial organic matter in the NW Mediterranean (Gulf of Lions): Exploring the newly developed BIT index, *Geochem. Geophys. Geosyst.*, *7*, Q11017, doi:10.1029/2006GC001306.
- Kim, J.-H., S. Schouten, E. C. Hopmans, B. Donner, and J. S. Sinninghe Damsté (2008), Global sediment core-top calibration of the TEX<sub>86</sub> paleothermometer in the ocean, *Geochim. Cosmochim. Acta*, *72*, 1154–1173, doi:10.1016/j.gca.2007.12.010.
- Nürnberg, D., J. Bijma, and C. Hemleben (1996), Assessing the reliability of magnesium in foraminiferal calcite as a proxy for water mass temperatures, *Geochim. Cosmochim. Acta*, *60*, 803–814, doi:10.1016/0016-7037(95)00446-7.
- Prahl, F. G., and S. G. Wakeham (1987), Calibration of unsaturation patterns in long-chain ketone compositions for paleotemperature assessment, *Nature*, *330*, 367–369, doi:10.1038/330367a0.
- Rosell-Melé, A., et al. (2001), Precision of the current methods to measure the alkenone proxy  $\text{U}_{37}^K$  and absolute alkenone abundance in sediments: Results of an interlaboratory comparison study, *Geochem. Geophys. Geosyst.*, *2*(7), 1046, doi:10.1029/2000GC000141.
- Rosenthal, Y., et al. (2004), Interlaboratory comparison study of Mg/Ca and Sr/Ca measurements in planktonic foraminifera for paleoceanographic research, *Geochem. Geophys. Geosyst.*, *5*, Q04D09, doi:10.1029/2003GC000650.
- Schouten, S., E. C. Hopmans, and J. S. Sinninghe Damsté (2002), Distributional variations in marine crenarchaeotal membrane lipids: A new organic proxy for reconstructing ancient sea water temperatures?, *Earth Planet. Sci. Lett.*, *204*, 265–274, doi:10.1016/S0012-821X(02)00979-2.
- Schouten, S., E. C. Hopmans, M. M. M. Kuypers, Y. Van Breugel, A. Forster, and J. S. Sinninghe Damsté (2003), Extremely high sea water temperatures at low latitudes during the middle Cretaceous as revealed by archaeal membrane lipids, *Geology*, *31*, 1069–1072, doi:10.1130/G19876.1.
- Schouten, S., C. Huguet, E. C. Hopmans, M. V. M. Kienhuis, and J. S. Sinninghe Damsté (2007), Improved analytical methodology and constraints on analysis of the TEX<sub>86</sub> paleothermometer by high performance liquid chromatography/



- atmospheric pressure chemical ionization-mass spectrometry, *Anal. Chem.*, *79*, 2940–2944, doi:10.1021/ac062339v.
- Walsh, E. M., A. E. Ingalls, and R. G. Keil (2008), Sources and transport of terrestrial organic matter in Vancouver Island fjords and the Vancouver-Washington Margin: A multiproxy approach using  $\delta^{13}\text{C}_{\text{org}}$ , lignin phenols, and the ether lipid BIT index, *Limnol. Oceanogr.*, *53*, 1054–1063.
- Weijers, J. W. H., S. Schouten, O. C. Spaargaren, and J. S. Sinninghe Damsté (2006), Occurrence and distribution of tetraether membrane in soils: Implications for the use of the BIT index and the TEX<sub>86</sub> SST proxy, *Org. Geochem.*, *37*, 1680–1693, doi:10.1016/j.orggeochem.2006.07.018.