
Copyright © 2013 Elsevier

A copy can be downloaded for personal non-commercial research or study, without prior permission or charge

The content must not be changed in any way or reproduced in any format or medium without the formal permission of the copyright holder(s)

http://eprints.gla.ac.uk/83835/

Deposited on: 29 July 2013
Production and temperature sensitivity of long chain alkenones in the cultured haptophyte *Pseudoisochrysis paradoxa*

Susanna Theroux, Jaime Toney, Linda Amaral-Zettler, Yongsong Huang

PII: S0146-6380(13)00156-3
DOI: http://dx.doi.org/10.1016/j.orggeochem.2013.07.006
Reference: OG 2980

To appear in: *Organic Geochemistry*

Received Date: 26 March 2013
Revised Date: 15 July 2013
Accepted Date: 18 July 2013

Please cite this article as: Theroux, S., Toney, J., Amaral-Zettler, L., Huang, Y., Production and temperature sensitivity of long chain alkenones in the cultured haptophyte *Pseudoisochrysis paradoxa*, *Organic Geochemistry* (2013), doi: http://dx.doi.org/10.1016/j.orggeochem.2013.07.006

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.
Production and temperature sensitivity of long chain alkenones in the cultured haptophyte *Pseudoisochrysis paradoxa*

Susanna Theroux\textsuperscript{a,b,1}, Jaime Toney\textsuperscript{a,2}, Linda Amaral-Zettler\textsuperscript{a,b,c}, Yongsong Huang\textsuperscript{a,*}

\textsuperscript{a} Department of Geological Sciences, Brown University, 324 Brook St., Providence RI 02912, USA

\textsuperscript{b} Josephine Bay Paul Center, Marine Biological Laboratory, 7 MBL Street, Woods Hole, MA 02540, USA

\textsuperscript{c} Department of Ecology and Evolutionary Biology, Brown University, 80 Waterman Street, Providence, RI 02912, USA

* Corresponding author. Tel. (401) 863-3822.  
E mail address: yongsong_huang@brown.edu (Yongsong Huang).

\textsuperscript{1} Present address: DOE Joint Genome Institute, 2800 Mitchell Drive, Walnut Creek, CA 94598, USA.

\textsuperscript{2} Present address: School of Geographical and Earth Sciences, University of Glasgow, Glasgow G12 8QQ, UK.
ABSTRACT

The alkenone unsaturation index ($U_{37}^K$ or $U_{37}^{K'}$) serves as a critical tool for reconstructing temperature in marine environments. Lacustrine haptophyte algae are genetically distinct from their ubiquitous and well studied marine counterparts, and the unknown species-specific genetic imprints on long chain alkenone production by lacustrine species have hindered the widespread application of the $U_{37}^K$ temperature proxy to lake sediment records. The haptophyte *Pseudoisochrysis paradoxa* produces alkenones but its $U_{37}^K$ calibration has never been determined. It has an alkenone fingerprint abundant in tetraunsaturated alkenones, a hallmark of lacustrine environments. We present here the first calibration of the $U_{37}^K$ index to temperature for a culture of *P. paradoxa*. We found that the $U_{37}^K$ index accurately captured the alkenone response to temperature whereas the $U_{37}^{K'}$ index failed to do so, with $U_{37}^{K'}$ values below 0.08 projecting to two different temperature values. Our results add a fifth species-specific $U_{37}^K$ calibration and provide another line of evidence that different haptophyte species require different $U_{37}^K$ calibrations. The findings also highlight the necessary inclusion of the $C_{37:4}$ alkenone when reconstructing temperatures from *P. paradoxa*-derived alkenone records.

**Keywords:** Alkenones, haptophytes, paleolimnology, $U_{37}^K$
1. Introduction

For over two decades, long chain alkenones have been used to reconstruct sea surface temperature from marine sediments. Haptophyte algae in the order Isochrysidales are the exclusive producers of these lipids, an extremely specific biomarker group found throughout open ocean, coastal, and lacustrine environments.

The paleotemperature proxy $U_{37}^K$ incorporates the abundance of the di-$(C_{37:2})$, tri-$(C_{37:3})$ and tetra-$(C_{37:4})$ unsaturated alkenones as a proxy for water temperature (Brassell et al., 1986), with greater proportions of the more unsaturated alkenones occurring at lower temperature (Marlowe, 1984; Brassell et al., 1986). A modified proxy $U_{37}^K$ (Prahl and Wakeham, 1987; Prahl et al., 1988) has been widely applied to marine sediments where the tetraunsaturated alkenone is largely absent. The cosmopolitan marine species *Emiliania huxleyi* and *Gephyrocapsa oceanica* are responsible for the majority of alkenone production in marine waters (Conte et al., 1994), allowing the universal application of the marine $U_{37}^K$ calibration (Volkman et al., 1980, 1985, 1995; Prahl and Wakeham, 1987; Muller et al., 1988; Sikes and Volkman, 1993; Sikes et al., 1997; Conte et al., 2006).

Alkenones are also found in lake sediments around the world (Cranwell, 1985; Volkman et al., 1988; Fulford-Smith and Sikes, 1996; Li et al., 1996; Wang and Zheng, 1998; Zink et al., 2001; Chu et al., 2005; D’Andrea and Huang, 2005; Pearson et al., 2008; Theroux et al., 2010; Toney et al., 2010; 2011). Those in lakes have been shown to reflect mean annual air temperature (Chu et al., 2005) and in situ lake water temperature (Toney et al., 2010; D’Andrea et al., 2011). However, there is no universal $U_{37}^K$ calibration applicable to all lake sediment records, largely as the result
of the genetic diversity of lacustrine haptophytes and resulting differences in alkenone biosynthetic pathways (Theroux et al., 2010). Identification of haptophyte species via alkenone fingerprint has been difficult, as many haptophyte species have similar alkenone profiles (Marlowe et al., 1984, Theroux et al., 2010). Using the ratio of \( \text{C}_{37}/\text{C}_{38} \) alkenones for species differentiation (Prahl et al., 1988) was found to be inconsistent across haptophyte species (Theroux et al., 2010). DNA sequencing has allowed more accurate identification of alkenone-producing haptophytes in lake environments (Coolen et al., 2004; D’Andrea et al., 2005; Theroux et al., 2010) and a close relative of the brackish water haptophyte \( \text{Pseudoisochrysis paradoxa} \) has been identified in lakes in North America and the Tibetan Plateau in China (Theroux et al., 2010). The definition of a \( \text{P. paradoxa} \text{U}^{K}_{37} \) calibration would therefore expand our reference dataset of species-specific calibrations and allow temperature reconstruction from sediments with alkenones derived from \( \text{P. paradoxa} \).

Originally isolated from the brackish York River Estuary in Chesapeake Bay, Virginia, USA, \( \text{P. paradoxa} \) has never been formally described (\textit{nomen nudum}; Jordan et al., 2004). It was reported to have alkenones (Marlowe et al., 1984), although its \( \text{U}^{K}_{37} \) temperature calibration was not determined. The 18S ribosomal RNA (rRNA) gene sequence for \( \text{P. paradoxa} \text{CCMP715} \text{ (CCAP 949/1, Genbank AM490999; Medlin et al., 2008) is 99\% identical to that of the coastal/lacustrine alkenone-producing haptophytes } \text{Isochrysis galbana} \text{CCMP1323 (CCAP 927/1; Genbank HM149540) and Chrysotila lamellosa ALGO HAP17 (CCAP 818/1; Genbank AM490998). Similarly, marine species } \text{E. huxleyi} \text{ and } \text{G. oceanica} \text{ are identical at the 18S rRNA level, highlighting the difficulty in distinguishing haptophyte species through 18S rRNA gene sequences alone. Until a formal
description of *P. paradoxa* confirms otherwise, we will refer to culture CCMP715 by its given name of *P. paradoxa*.

The global extent of *P. paradoxa* populations is unknown; if it ecologically resembles its close relative *I. galbana*, it can survive in a wide range of fresh, brackish and marine environments and contribute to their alkenone sediment records (Volkman et al., 1980; Marlowe et al., 1990; Versteegh et al., 2001; Liu et al., 2009).

Only a few species of haptophytes have been grown in culture to determine their $U^{37}$ calibration: ubiquitous marine species *Emiliania huxleyi* (Prahl et al., 1988; Volkman et al., 1995; Conte et al., 1998; Prahl et al., 2003) and *Gephyrocapsa oceanica* (Sikes and Volkman, 1993; Volkman et al., 1995; Sawada et al., 1996; Conte et al., 1998) and lacustrine/brackish species *I. galbana* (Versteegh et al., 2001) and *Chrysotila lamellosa* (Sun et al., 2007). Given the diversity of haptophytes in brackish environments (Theroux et al., 2010) and the need for a better understanding of brackish haptophyte ecology, we grew *P. paradoxa* at a variety of temperatures to determine its alkenone unsaturation-temperature relationship.

2. Methods
2.1. *Pseudoisochrysis paradoxa* cultures

Cultures of *Pseudoisochrysis paradoxa* (CCMP715, also known as CCAP 949/1, CCAPVA12, UTEX 1988) were from the Provasoli-Guillard National Center for Marine Algae and Microbiota. We grew them in 0.2 μm filter-sterilized seawater amended with f/2 nutrients (Guillard, 1975) in full spectrum light on a 24:0 h light:dark cycle. We verified that the medium was alkenone-free. We grew the batch cultures in triplicate volumes of 50 ml at 5, 10, 15, 21 and 24 °C. We initiated
cultures at an equal cell concentration of 8000 cell/ml using an inoculum from a
culture acclimatized to a given temperature for 2 weeks. We monitored cell
concentration for 3 weeks using haemocytometer counts to ensure that the cultures
remained in the exponential phase. After 3 weeks, the cultures were harvested. One of
the three 5 °C cultures was discarded because it failed to grow.

2.2. Lipid analysis

Culture material was filtered onto precombusted 47 mm glass fiber filters
(Whatman, Piscataway, NJ), immediately frozen at -20 °C and then freeze-dried
overnight (Labconco, Kansas City, MO). We extracted the filters using 3 x 20 minute
bursts of sonication in 50 ml dichloromethane (DCM) and ran the total lipid extracts
using an Agilent 6890 Plus gas chromatograph flame ionization detector (GC-FID)
instrument for detection and quantification of alkenones using an internal C_{36} n-alkane
standard and an external alkenone standard of known U^K_{37} temperature value to ensure
analytical precision (< 0.1 °C proxy-derived temperature). A Varian VF200 60 m
fused silica GC column (60 m × 250 µm width × 0.10 µm film thickness) was used as
follows: 100 °C (1 min) to 200 °C (held 1 min) at 20 °C/min, then at 4 °C/min to 320
°C (held 5 min).

3. Results

3.1. Cultures

Triplicate cultures of *P. paradoxa* behaved similarly at each temperature
regime (Table 1), with growth rate fluctuating only by 0.01 division/day. Growth rate
and alkenone concentration per cell displayed an inverse relationship (Fig. 1), with
growth rate highest at 21 °C (1.01 division/day) and lowest at 5 °C (0.48
division/day) and alkenone concentration highest at 5 °C (1.961 pg/cell) and lowest at
21 °C (0.042 pg/cell). Final cell count was highest at 21 °C and lowest at 5 °C (Table
1). The C$_{37:4}$ alkenone comprised almost 40% of the C$_{37}$ alkenones in the 5 °C culture
(Fig. 3), although at all temperatures C$_{37:3}$ was the dominant alkenone (Fig. 4).

3.2. U$^{K}_{37}$ and U$^{K'}_{37}$ calibrations

We calculated both polynomial and linear U$^{K}_{37}$ and U$^{K'}_{37}$ calibrations for the
cultures and plotted them vs. growth temperature (Fig. 2). For both U$^{K}_{37}$ and U$^{K'}_{37}$, the
polynomial equation had a better fit to the alkenone unsaturation data. The U$^{K}_{37}$
calibration ($U^{K}_{37} = 0.012T^2-0.0142T-0.2935$, R$^2$ 0.98, root mean squared error, RMSE
2.26) was more robust than the U$^{K'}_{37}$ calibration ($U^{K'}_{37} = 0.001T^2-0.0256T+0.1754$, R$^2$
0.89, RMSE 2.38) and, most importantly, the U$^{K'}_{37}$ temperature calibration failed to
reconstruct temperature < 15 °C (Fig. 2). Below 15 °C, the cultures had increasing
U$^{K'}_{37}$ values with decreasing temperature, such that most U$^{K'}_{37}$ values afforded two
temperature values. The linear U$^{K}_{37}$ calibration ($U^{K}_{37} = 0.0226T-0.5149$, R$^2$ 0.91,
RMSE 2.16) also had a better fit than the linear U$^{K'}_{37}$ calibration ($U^{K'}_{37} = 0.0047T-
0.0071$, R$^2$ 0.39, RMSE 10.27).

The linear U$^{K}_{37}$ calibration for had a similar slope to the in situ calibration
from Lake George, ND (0.0226 vs. 0.0169; Fig. 5; Toney et al., 2012), and clustered
with other lake-based calibrations, apart from the marine calibrations. The linear U$^{K'}_{37}$
calibration was also distinct from reported U$^{K'}_{37}$ calibrations from other haptophyte
cultures and environmental samples (Fig. 6) and had a y intercept and slope (0.0047) closest to that of *I. galbana* (0.009; Versteegh et al., 2001).

4. Discussion

4.1. Growth stage and alkenone production

The effect of growth rate on haptophyte $U_{37}^{K}$ and $U_{37}^{K'}$ values is unclear (Conte et al., 1995; Epstein et al., 1998; Popp et al., 1998). Studies have shown that differences in alkenone indices can exist between batch methods and continuous culture methods (Popp et al., 1998) although it is debated as to which of the two methods more accurately replicates conditions in the natural environment. Growth phase has also been shown to influence alkenone unsaturation in batch culture (Conte et al., 1998; Epstein et al., 1998), although continuous culture, and therefore constant growth state, imparted no change on $U_{37}^{K}$ values (Popp et al., 1998). We harvested all our culture samples during exponential growth phase to control this variation.

The cultures demonstrated higher alkenone concentration per cell at the slowest growth rate in the 5 °C culture (Table 1, Fig. 1). This agrees with observations in culture for *E. huxleyi* and *G. oceanica* (Conte et al., 1998). However, low growth temperature often corresponds to low growth rate, so it is unclear whether or not low growth temperature alone would result in enhanced alkenone accumulation. Alkenones are believed to serve as an energy storage molecule in haptophytes (Epstein et al., 2001; Eltgroth et al., 2005) and the concentration per cell increases during stationary growth phase and decreases after cultures are placed in the dark (Epstein et al., 2001; Eltgroth et al., 2005). The accumulation of alkenones at low temperature and low growth rate, as seen here for *P. paradoxa*, may be the result
of photosynthetic energy input exceeding cell capacity for growth and division
(Roessler, 1990).

In batch culture experiments, $U_{37}^K$ decreases under nutrient stress and
increases under prolonged darkness (Versteegh et al., 2001; Prahl et al., 2006), both
conditions that may result in slower growth rate. We used a 24:0 h light to dark
regime to eliminate alkenone metabolism during darkness. Cultures of various strains
of *E. huxleyi* grown in 12:12 or 0:24 light to dark regimes exhibited contrasting
increases or decreases in $U_{37}^K$ values depending on light regime (Epstein et al., 2001,
Versteegh et al., 2001). The fluctuations in were 0.013 to 0.029 units, lower than our
$U_{37}^K$ standard deviation (Table 1), so we do not believe the 24 h light regime exerted a
significant change in $U_{37}^K$ and $U_{37}^{K'}$ values.

4.2. Comparison with other species

*P. paradoxa* alkenones resembled other lacustrine haptophyte alkenone
profiles via a high abundance of the $C_{37:4}$ alkenone (Cranwell, 1985; Li et al., 1996;
Zink et al., 2001). Like its close relative *I. galbana CCMP1323*, *P. paradoxa* had a
predominant $C_{37:3}$ alkenone and absence of the $C_{38}$ Me ketone (Fig. 3), as also
observed in the original Marlowe et al. (1984) alkenone description. In our *P.
paradoxa* 15 °C culture, $C_{37:3}$ alkenone comprised 70% of the total $C_{37}$ alkenones, very
similar to the Marlowe et al. (1984) value of 68.4%.

Sediments and water samples from Lake George, North Dakota (Toney et al.,
2010), as well as Lake BrayaSø in Greenland (D’Andrea and Huang, 2005; D’Andrea
et al., 2011) and Ace Lake, Antarctica (Coolen et al., 2004) contain alkenone
signatures with dominant $C_{37:4}$. Previously, $C_{37:4}$ ($C_{37:4}/C_{37:4}+C_{37:3}+C_{37:2}$) as a % value was proposed as a paleosalinity proxy (Roselle-Melé et al., 1994; 2002; Schulz et al., 2000; Sikes and Sicre, 2002; Bendle and Rosell-Melé, 2004), although the lack of correlation between salinity and $C_{37:4}$ alkenone % in a global array of lake systems (Chu et al., 2005; Mercer et al., 2005; Theroux et al., 2010; Toney et al., 2010) instead suggests that the relationship between $C_{37:4}$ and salinity is a result of haptophyte community shifts along a salinity or temperature gradient (Harada et al., 2003, 2008).

$C_{37:4}$ can range from 8-53% in *C. lamellosa* (Rontani et al., 2004) and 0-34% in *I. galbana* (Marlowe et al., 1984), and up to 96% in a series of lakes in China (Chu et al., 2005), with *P. paradoxa* salinity values fitting this range, with values between 6% at 24 °C and 40% at 5 °C (Table 1, Fig. 4). The alkenone concentration was also within the range observed for *I. galbana* (0.0098-0.61 pg/cell; Versteegh et al., 2001). The highest value for *P. paradoxa* (1.96 pg/cell) was close to the range observed in an *Isochrysis* culture (1.8 pg/cell; Marlowe, 1984).

Although the haptophytes *P. paradoxa, I. galbana* and *C. lamellosa* found in brackish waters have similar alkenone signatures and cellular concentration, they have different $U_{37}^K$ and $U_{37}^{K'}$ calibrations (Figs. 5, 6). Studies have shown that geographically isolated strains of the same species of haptophyte, *E. huxleyi* and *G. oceanica*, have different patterns of alkenone unsaturation with temperature (Conte et al., 1995, 2006; Volkman et al., 2005). Therefore, it is no surprise that different but closely related haptophytes will also possess different $U_{37}^K$ calibrations. The slopes of $U_{37}^K$ calibrations across brackish haptophyte species are similar (Fig. 5), suggesting that the temperature dependence of alkenone unsaturation is consistent, but the
determining factor for the $U^{37}_{\text{K}}$ calibration y intercept is still unknown. Although the $P.\ paradoxa\ U^{37}_{\text{K}}$ and $U^{37}_{\text{K'}}$ calibrations were distinct from marine haptophyte calibrations from $G.\ oceanica$ and $E.\ huxleyi$, non-linear calibrations have been suggested for cultures of $G.\ oceanica$ (Volkman et al., 1995) and a global marine surface water calibration (Conte et al., 2006).

4.3. Application to the natural environment

Lake George, ND, harbors two alkenone producing haptophytes (Theroux et al., 2010; Toney et al., 2011), one closely related to $P.\ paradoxa$ and one related to an uncultured Ace Lake, Antarctica haptophyte (Volkman et al., 1988; Fulford-Smith and Sikes, 1996; Coolen et al., 2004). The downcore alkenone distribution in Lake George is dominated by $C_{37:4}$ (Toney et al., 2010) and the distribution in an enrichment culture of Lake George haptophytes was also $C_{37:4}$-dominant (Toney et al., 2012). In contrast, $P.\ paradoxa$ cultures had abundant $C_{37:4}$ but dominant $C_{37:3}$ (Fig. 4). In cultures of $P.\ paradoxa$ and Lake George haptophytes, the $U^{37}_{\text{K}}$ calibration had a more robust relationship to temperature than the $U^{37}_{\text{K'}}$ calibration (Toney et al., 2012), a result of the abundant $C_{37:4}$ in both. The similarity in slope between the linear $P.\ paradoxa$ calibration and the Lake George in situ calibration (0.0226 and 0.0169, respectively) suggests a significant alkenone contribution to Lake George alkenones by $P.\ paradoxa$-like haptophyte (Fig. 5). The offset between the Lake George in situ $U^{37}_{\text{K}}$ calibration and the $P.\ paradoxa$ culture-based calibration may be explained by a missing contribution from the second alkenone-producer in Lake George, the subject of a current study (Theroux, 2012). These results emphasize the utility of in situ
calibrations for reconstructing relative temperature fluctuations, but highlight the fact that in situ calibrations are a composite of alkenone contributions that may be derived from multiple haptophytes. Accurate absolute temperature reconstruction requires not only an accurate $U_{37}^K$ calibration, but also knowledge of the contributing haptophyte species and their fluctuation in abundance back through time, something that can be discerned using preserved paleoDNA (Coolen et al., 2009).

5. Conclusions

The application of the $U_{37}^K$ paleotemperature proxy to lake sediments depends upon a robust $U_{37}^K$ calibration. As evidenced by studies to date, lakes are not uniform in their alkenone-producing haptophyte populations, thereby complicating the use of a universal lacustrine $U_{37}^K$ proxy calibration. Our study is one of few to cultivate an individual haptophyte species at various temperatures to calibrate the $U_{37}^K$-temperature relationship. *P. paradoxa* has proved to be distinct in its alkenone distribution vs. temperature, with a calibration equation significantly different from its close relative *I. galbana* CCMP1323 and other brackish haptophytes. The similarity between the Lake George in situ calibration and the *P. paradoxa* calibration reported in this study gives credence to the applicability of in situ $U_{37}^K$ calibrations.

The failure of the *P. paradoxa* $U_{37}^K$ calibration to reconstruct temperature < 15 °C highlights the importance of incorporating $C_{37:4}$ alkenones for temperature reconstruction when *P. paradoxa* is the likely alkenone contributor, and such a rubric may extend to all $C_{37:4}$-abundant alkenone producers. As with all alkenone-based temperature reconstructions, it is important to verify the continuity of alkenone
distributions throughout downcore sediments in order to apply a single calibration to the temperature reconstruction. We anticipate future studies comparing both in situ and culture-based $U_{37}^K$ calibrations to further resolve species-specific modes of alkenone production in lake environments.

Acknowledgements

This work was supported by a National Science Foundation award to Y.H. (EAR-1122749) and L.A.-Z. (EAR-1124192), a Brown SEED fund to Y.H. and L.A.-Z., and an American Association of University Women dissertation fellowship to S. We thank three anonymous reviewers for helpful comments.

Associate Editor – J. K. Volkman

References


Harada, N., Sato, M., Sakamoto, T., 2008. Freshwater impacts recorded in
tetraunsaturated alkenones and alkenone sea surface temperatures from the
Okhotsk Sea across millennial-scale cycles. Paleoceanography 23,

of alkenones synthesized by a bloom of Emiliania huxleyi in the Bering Sea.

haptophytes. Micropaleontology 50, 55-79.


diversity of freshwater alpine Lake Puma Yumco on the Tibetan Plateau.
Geomicrobiology Journal 26, 131-145.

and alkyl alkenoates and the fossil coccolith record of marine sediments.
Chemical Geology 88, 349-375.

Marlowe, I.T., Green J.C., Neal, A.C., Brassell, S.C., Eglinton, G., Course, P.A.,
1984. Long-chain, n-C37-C39 alkenones in the Prymnesiophyceae. Distribution of
alkenones and other lipids and their taxonomic significance. British
Phycological Journal 19, 203-216.

and implications for selectivity of phytoplankton extinctions across the K/T

Mercer, J.L., Zhao, M.X., Colman, S.M., 2005. Seasonal variations of alkenones and
in the Chesapeake Bay water column. Estuarine Coastal and Shelf Science

63, 675-682.


Table Captions

Table 1
Average growth rate, cell concentration and alkenone concentration for cultures of *P. paradoxa*.

Fig. Captions

Fig. 1. Alkenone cell concentration vs. growth rate for *P. paradoxa* cultures (error bars represent standard deviation).

Fig. 2. (Left) *P. paradoxa* $U_{37}^K$ calibration; (Right) *P. paradoxa* $U_{37}^C$ calibration. Both linear and polynomial calibrations are provided.

Fig. 3. Gas chromatogram of *P. paradoxa* grown at 10 °C. Inset: Photomicrograph of *P. paradoxa* culture. Scale bar 5 µm.

Fig. 4. Average alkenone concentration for *P. paradoxa* cultures at different temperatures. Error bars represent standard deviation.

Fig. 5. Comparison of *P. paradoxa* $U_{37}^K$ calibration with other species and lake-based calibrations. References: German Lakes, Zink et al. (2001); Lake George in situ, Toney et al. (2010); Lake BrayaSø, D'Andrea et al. (2011); marine *E. huxleyi*, Prahl et al. (1988); *P. paradoxa*, this paper; polar marine waters, Sikes and Volkman (1993);
Lake George in situ 2008, Toney et al., (2012); *C. lamellosa*, Sun et al. (2007);


**Fig. 6.** Comparison of *P. paradoxa* $^{137}$ calibration with other species and lake-based calibrations. References: *P. paradoxa*, this paper; German lakes, Zink et al. (2001); Chinese lakes, Chu et al. (2005); Chinese freshwater/brackish lakes, Chu et al. (2005); Chinese saline lakes, Chu et al. (2005); *C. lamellosa*, Sun et al. (2007); Global marine, Prahl and Wakeham (1987); *I. galbana*, Versteegh et al. (2001); marine *G. oceanica* GO1, Sawada et al. (1996); marine *G. oceanica* JB01, Volkman et al. (1995).
Table 1

Editors note: The headings in the top horizontal column should not be in bold and the SD entries should not be in italics.
Fig. 1. 

\[ y = -2.578 \ln(x) + 0.0427 \]

\[ R^2 = 0.99 \]

Editor's note: the titles on the x & y axes should not be in bold.
Ed: Same comment about axes as above

Fig. 2.
Fig. 3.
Ed: Again axes titles not in bold

**Fig. 4.**
Fig. 5.

Axes titles not in bold
Axes titles not in bold

**Fig. 6.**
Highlights

> Cultured *Pseudoisochrysis paradoxa* contained an abundant, but not dominant, C\textsubscript{37:4} alkenone.
> *P. paradoxa* U\textsuperscript{K}\textsubscript{37} calibration matched lake U\textsuperscript{K}\textsubscript{37} calibrations and was distinct from marine calibrations.
> The U\textsuperscript{K}\textsubscript{37} calibration had a superior fit than the U\textsuperscript{K'}\textsubscript{37} calibration.
> C\textsubscript{37:4} should be incorporated for reconstructing temperature from *P. paradoxa* alkenone records.