## Molecular Markers as tools

**Applications:**

- Productivity
- (Paleo) Environmental conditions
- Temperature
- $CO_2$ concentration
- Salinity
- Benthic and photic zone oxicity/anoxicity
- Marine vs riverine vs eolian input
- Ecosystem studies
- Climate change
- Paleoclimate (“Molecular stratigraphy”).

**Molecular stratigraphic tools:**

- Paleothermometers (alkenone unsaturation).
- Paleobarometers (alkenone, chlorin $\delta^{13}C$).
- Paleochronometers (molecular $^{14}C$).
- Paleotracers (e.g., leaf waxes in dust).

## Organic "Paleothermometers": Long-chain ketones (alkenones) as SST indicators

**Reading list**


- Brassell S.C., Eglinton G., Marlowe I.T., Pflaumann U. and Sarnthein M. (1986) Molecular Stratigraphy: A new tool for climatic assessment. *Nature*, **320**, 129-133..
- F.G. Prahl and Wakeham S.G. (1987) Calibration of unsaturation patterns in long-chain ketone compositions for paleotemperature assessment. *Nature*, **330**, 367-369.
- Marlowe I.T., Brassell S.C., Eglinton G. and Green J.C. (1990) Long-chain alkenones and alkylalkenoates and the fossil coccolith record of marine sediments. Chem. Geol. 88, 349-375.
- Brassell S.C. (1993) Applications of biomarkers for delineating marine paleoclimatic fluctuations during thr Pleistocene. In Organic Geochemistry (Eds. M.H. Engel & S.A. Macko). pp. 699-738, Plenum Press, New York.
- Muller P.J., Kirst G., Ruhland G., von Storch I. And Rosell-Mele (1998) Calibration of the alkenone paleotemperature index UK37’ based on core-tops from the eastern southern South Atlantic and the global ocean (60°N-60°S). Geochmi. Cosmochim. Acta 62, 1757-1772.
- Proceedings of a workshop on alkenone-based paleoceanographic indicators (www.g-cubed.org, 2000)

## The alkenone story - birth of a novel paleoceanographic tool

**1978**
- *Boon et al., 1978*
- First identification in sediments
- DSDP core from Walvis Ridge, SW Africa
- Technique: field desorption-MS of total lipid extract and TLC fractions
- Identified as ketones with elemental compostion of $C_{37}H_{70}O$ (m/z 530) and $C_{38}H_{72}O$ (m/z 544)

**1980**
- *de Leeuw et al. 1980*
- Confirmation of structure as $C_{37}$-$C_{39}$ methyl and ethyl ketones

*Volkman et al. 1980*
- Identification of same compounds in *Emiliania huxleyi*
- Feeding experiments reveal conservative behavior on passage through gut of zooplankton and excretion as fecal pellets
- Identification of associated compounds ($C_{31}$-$C_{38}$ odd-chain alkenes) in *E. huxleyi*
- Formed throughout growth cycle of *E. huxleyi*
- Proposed as markers for *E. hux.*

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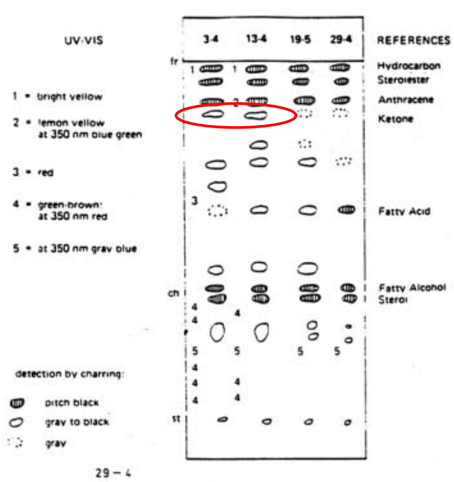
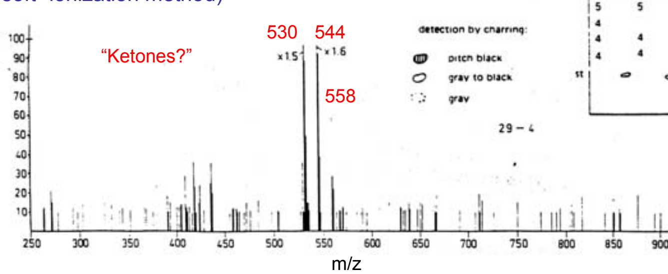
Walvis Ridge sediments,
SE Atlantic
Boon et al., 1978

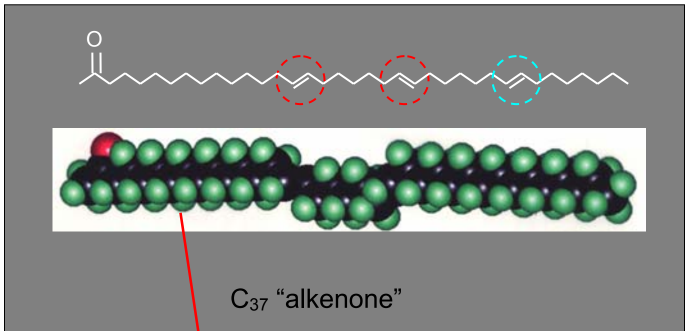
Thin-layer chromatography

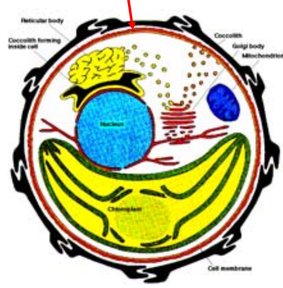


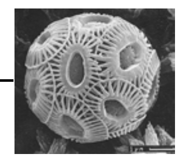

Field desorption-MS of TLE
("soft" ionization method)



## Alkenones – Magical Molecules!



Photomicrograph of E. Huxleyi

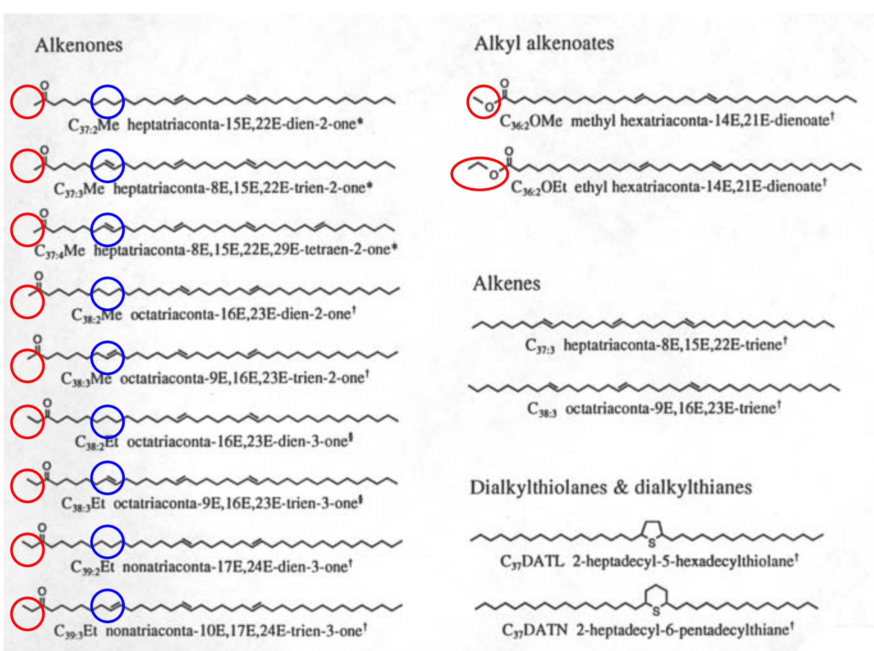


Table II. *n*-Alkenones occurring in prymnesiophycean algae and marine sediments, in GC elution order (OV-1)

| Designation[1] | RRT[2] | Abbreviation[3] | Name[4] | Structure (see Fig. 1) | *n* | R |
|---|---|---|---|---|---|---|
| A | 0·99 | $C_{37:4}$ Me | heptatriaconta-8,15,22,29-tetraen-2-one[5] | III | 4 | $CH_3$ |
| B | 1·00 | $C_{37:3}$ Me | heptatriaconta-8,15,22-trien-2-one | II | 4 | $CH_3$ |
| C | 1·01 | $C_{37:2}$ Me | heptatriaconta-15,22-dien-2-one | I | 4 | $CH_3$ |
| D | 1·06 | $C_{38:4}$ Et | octatriaconta-9,16,23,30-tetraen-3-one[5] | III | 4 | $C_2H_5$ |
| E | 1·06 | $C_{38:4}$ Me | octatriaconta-9,16,23,30-tetraen-2-one[5] | III | 5 | $CH_3$ |
| F | 1·07 | $C_{38:3}$ Et | octatriaconta-9,16,23-trien-3-one | II | 4 | $C_2H_5$ |
| G | 1·08 | $C_{38:3}$ Me | octatriaconta-9,16,23-trien-2-one | II | 5 | $CH_3$ |
| H | 1·09 | $C_{38:2}$ Et | octatriaconta-16,23-dien-3-one | I | 4 | $C_2H_5$ |
| J | 1·09 | $C_{38:2}$ Me | octatriaconta-16,23-dien-2-one | I | 5 | $CH_3$ |
| K | 1·14 | $C_{39:3}$ Et | nonatriaconta-10,17,24-trien-3-one | II | 5 | $C_2H_5$ |
| L | 1·17 | $C_{39:2}$ Et | nonatriaconta-17,24-dien-3-one | I | 5 | $C_2H_5$ |

[1] See Table III in text.
[2] Relative Retention Time: Retention time relative to that for compound B (OV-1 column); see Materials and Methods for conditions.
[3] Gives C no., no. of double bonds, alkyl substituent.
[4] See text for assignments of double bond positions.
[5] Novel compounds.

15 C cultures,
12h/12h light/dark cycle
Harvested during exponential growth phase

Table III. Concentrations of *n*-alkenones detected in species of the Prymnesiophyceae expressed as percentages of the total

| | Alkenones (see Table II) | Species and Plymouth no. | | | | | |
|---|---|---|---|---|---|---|---|
| | | *Emiliania huxleyi* (92d) | *Chrysotila lamellosa* (353) | *Chrysotila lamellosa* (528) | *Isochrysis galbana* (I) | *Isochrysis* sp. (507) | *Isochrysis* sp. (506a) |
| A | $C_{37:4}$ Me | 4·4 | 29·9 | 36·5 | 19·9 | 9·6 | — |
| B | $C_{37:3}$ Me | 47·4 | 54·1 | 49·4 | 56·7 | 68·4 | 74·1 |
| C | $C_{37:2}$ Me | 16·1 | 3·9 | 1·9 | 2·6 | 9·8 | 17·0 |
| D | $C_{38:4}$ Et | 0·8[1] | — | — | — | — | — |
| E | $C_{38:4}$ Me | | — | — | — | — | — |
| F | $C_{38:3}$ Et | 12·6 | 6·1[1] | 8·4[1] | 10·3[1] | 5·3[1] | 5·3[1] |
| G | $C_{38:3}$ Me | 3·2 | | | | | |
| H | $C_{38:2}$ Et | 10·7 | 3·4[1] | 1·9[1] | 4·3[1] | 0·5[1] | 2·2[1] |
| J | $C_{38:2}$ Me | 2·7 | | | | | |
| K | $C_{39:3}$ Et | 1·6 | 2·6 | 1·9 | 6·2 | 6·4 | 1·1 |
| L | $C_{39:2}$ Et | 0·5 | — | — | — | — | 0·3 |
| | Total | 100·0 | 100·0 | 100·0 | 100·0 | 100·0 | 100·0 |

[1] $C_{38}$ isomers not resolved on the GC.

# *Emiliania huxleyi*

### *Affiliation and Evolution*

- Class: Haptophyta (Prymnesiophyta)
- Order: Isochrysidales
- Family: Gephyrocapsaceae
- *E. huxleyi* first appeared during late Pleistocene (ca. 250ka)

### *Distribution and Abundance*

- Cosmopolitan eurythermal species (sub-polar to equatorial regions)
- Often found in high concentrations (up to $5x10^3$ $l^{-1}$)
- Occasional development of dense blooms
- Most widespread extant coccolithophoric species
- Dominant in transitional and subarctic floral zones
- Isochrysis/Chrysotila limited to coastal environments
- *E. huxleyi* considered to be the dominant source of alkenones in the open ocean
- Predominant in the upper 200m of the water column in the subarctic Pacific ocean
- Constitutes between 40-87% and 40-67% of coccoliths in surface sediments in the North Atlantic and Pacific oceans respectively

**Some Definitions:**

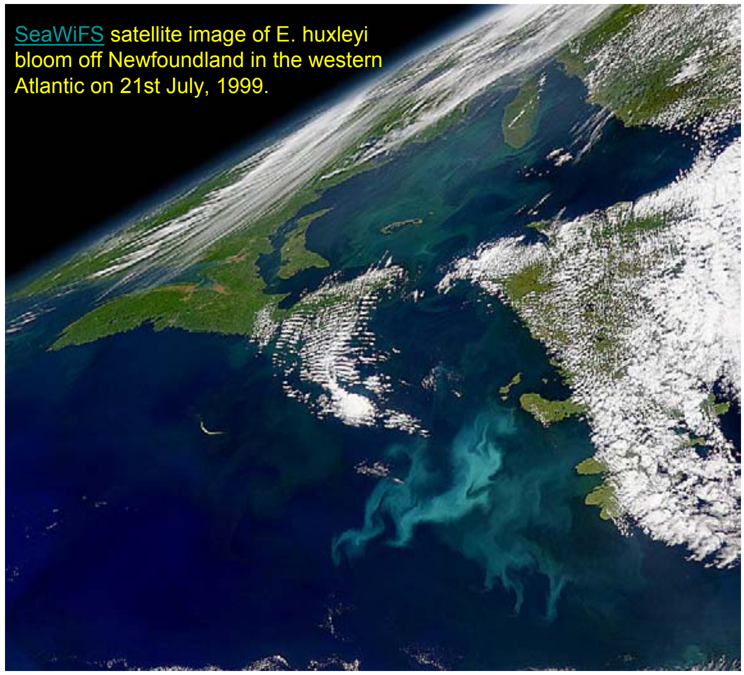
*Class:*
- A taxonomic group containing one or more orders.

*Order:*
- A taxonomic group containing one or more families.

*Family:*
- A taxonomic group containing one or more genera.

*Genus (pl. Genera):*
- The second most specific taxonomic level, includes closely related species. Interbreeding between organisms within the same genus can occur.

*Species:*
- A taxonomic category subordinate to a genus (or subgenus) composed of individuals possessing common characters distinguishing them from other categories of individuals of the same taxonomic level. In taxonomic nomenclature, species are designated by the genus name followed by a Latin or Latinised adjective or noun.
- A taxonomic group whose members can interbreed.




# Emiliania huxleyi

*Morphology and Composition*
- 2 distinct morphotypes
- warm water form and cold water form

*Alkenone characteristics*
- long chain-length ($C_{37}$-$C_{39}$)
- spacing of positions of unsaturation (C-7 not C-2 and C-3)
- double-bond configuration (i.e. *E* not *Z*)
- major components of living cell carbon (5-11%)

Other features
- Co-occurring methyl and ethyl alkenoates
- $C_{31}$-$C_{37}$ odd carbon number alkenes
- Carotenoid: 19'-hexanoyloxyfucoxanthin
- Unusual water-soluble acidic polysaccharide

## Long-chain ketones

- Recognized in three genera of prymnesiophycean algae
- Emiliania
- Chrysotila
- Isochrysis

*Biosynthesis and biological role*

- Algae biosynthesize alkenones from $CO_2$ via a $C_{36}$ alkenoic acid precursor (Volkman et al., 1980)
- Precise biological role not known
- Believed to be membrane fluidity regulators (lipid bilayer)
- "margerine vs butter" analogy

*Occurrence*

- Identified in sediments from a wide variety of depositional environments (see table)
- Also identified in freshwater (lacustrine) sediments
- Occur in POM in Atlantic and Pacific oceans
- Found in remote marine aerosols collected on New Zealand (introduced into the atmosphere by bubble bursting - Sicre et al., 1990)

## The alkenone story - birth of a novel paleoceanographic tool

**1984**

- *Marlowe et al 1984*
- Alkenones found to be common to Prymnesiophyceae
- Alkyl alkenoates found as associated related compounds
- Chemotaxonomic value confirmed
- Degree of unsaturation related to growth temperature

**1985**

- *Cranwell et al. 1985*
- Alkenones identified in freshwater lake sediments**1986**
- *Farrimond et al. 1986*
- Alkenones reported in Cretaceous black shales
- Demonstrates additional biological precursor for alkenones pre-dates appearance of *E. huxleyi*

**1986**

- *Brassell et al., 1986*
- Relationship in degree of unsaturation and d18O observed
- Proposal as a molecular marker for sea-surface temperature
- Introduction of parameter, Uk37
- Correlation between latitude, SST and Uk37 in Quaternary sediments
- Introduction to the concept of ***molecular stratigraphy***

Farrimond et al. 1986

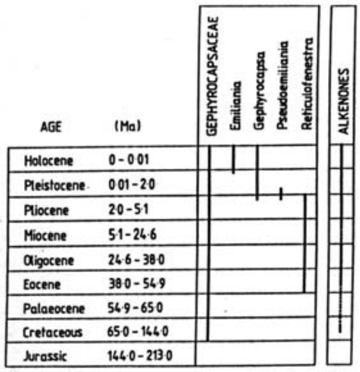
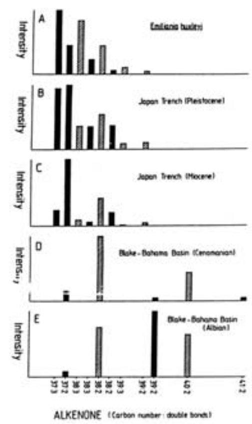
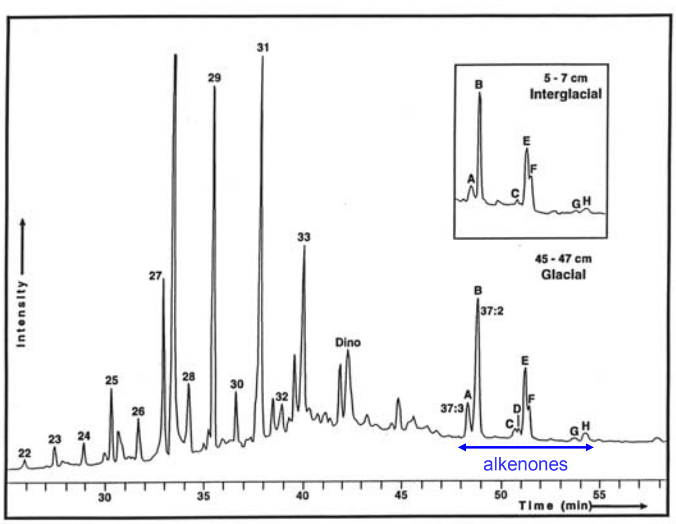
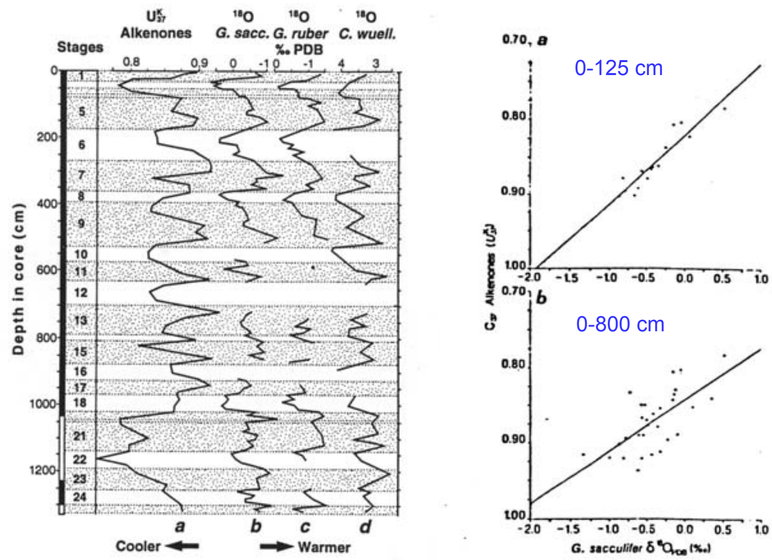



Fig. 5. Geological age ranges of genera belonging to the family Gephyrocapsaceae, and the reported sedimentary occurrence of long-chain alkenones. (Modified from Marlowe *et al.*, 1985). The dotted lines reflect age ranges where alkenones have not been reported from sediments.



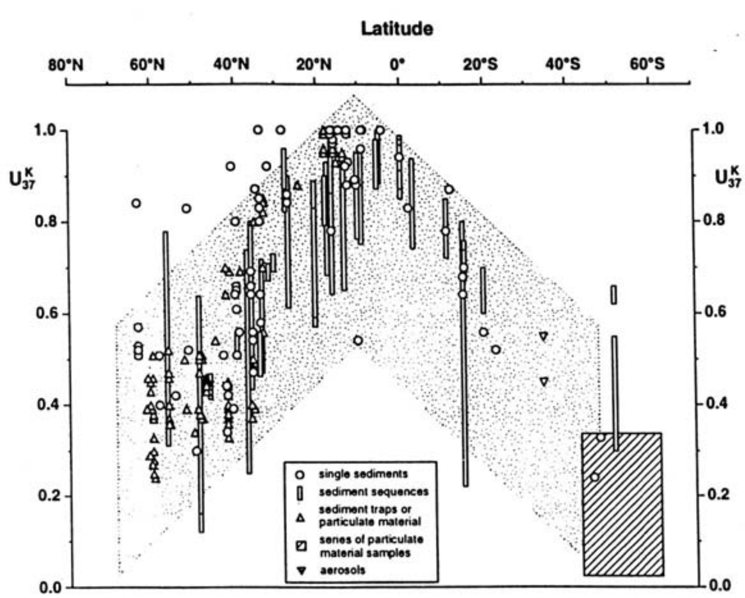

Gas chromatograph of TLE of Kane Gap sediments






Brassell et al., 1986

Latitudinal variations in $U^K_{37}$ values of sediments and particulate samples



## The alkenone story - birth of a novel paleoceanographic tool

**1987**

*Prahl and Wakeham, 1987*

- Calibration of Uk37’ w.r.t. SST for natural POM populations (sinking and suspended) in Atlantic and Pacific oceans

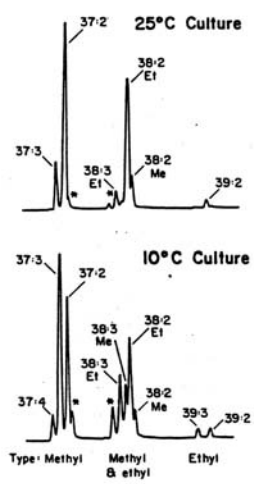
**1988**

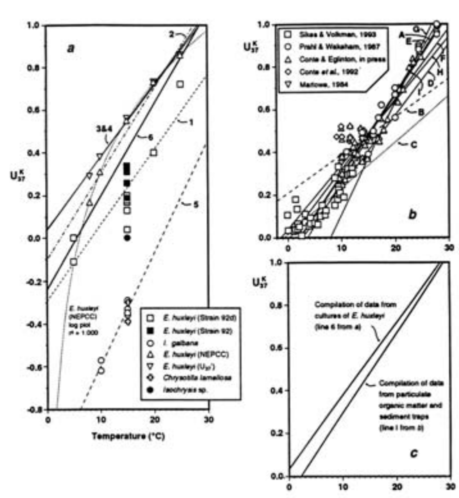
*Prahl et al, 1988*

- Calibration of Uk37 vs laboratory cultures of E. huxleyi (commonly accepted calibration)
- Confirm systematic changes in
- degree of unsaturation
- overall chain length distribution
- proportion in alkyl alkenoates/alkenones

*Rechka and Maxwell, 1988*

- Complete structural assignment of alkenones
- Found to be unusual all *E* (trans-) configuration
- Refractory nature postulated to be related to unusual double-bond configuration




Prahl & Wakeham, 1988

## Alkenone Unsaturation as an Indicator of SST

**Fundamental relationship**

- A <u>decrease</u> in temperature leads to an <u>increase</u> in the degree of unsaturation
- Initial ratio:

$$U^K_{37} = [C37:2]-[C37:4]/[C37:2+C37:3+C37:4]$$

(Brassell et al., 1986)

- Modified to:

$$U^K_{37}' = [C37:2]/[C37:2 + C37:3]$$

(Prahl and Wakeham, 1987)

- *Ratio can be measured very precisely (GC-FID)*

**Calibration**

- Most commonly used:

$U^K_{37}' = 0.033T + 0.043$ (Prahl and Wakeham, 1987)

$U^K_{37}' = 0.033T + 0.044$ (core-top calibration of Muller et al.).

- Accuracy of SST estimation: ± 1°C (in open ocean, temperate and sub-polar waters)

## Measurement of Alkenone Unsaturation

*Conventional method*

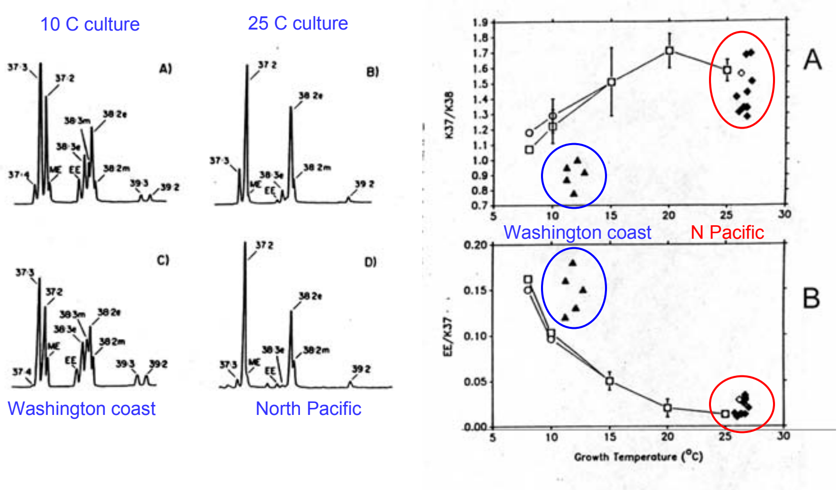
- Solvent extraction
- Column chromatography or Thin layer chromatography
- Gas Chromatography

*Purification methods*

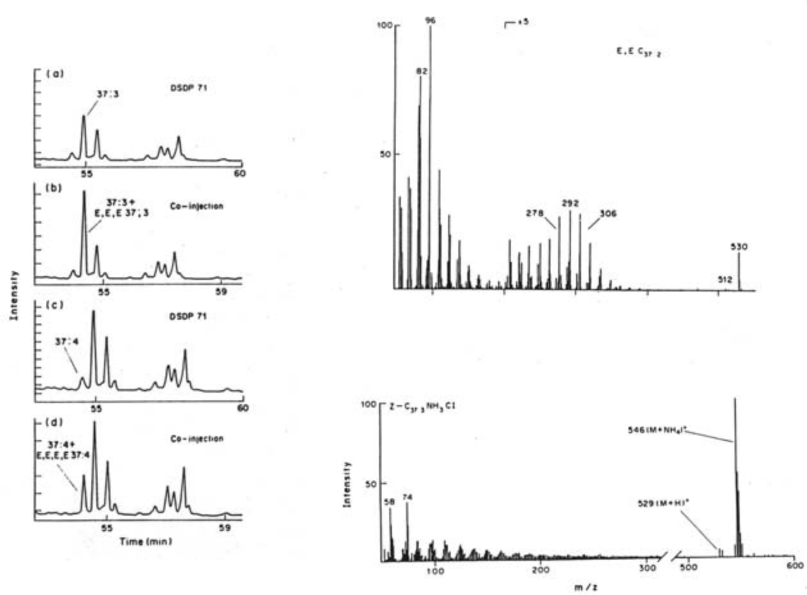
- Silylation
- Transesterification
- Solid phase extraction

*Novel detection methods*

- Short-column gas chromatography-CI mass spectrometry
- DEI-HRMS (DT-MS)
- GC/TOF-MS
- GCxGC



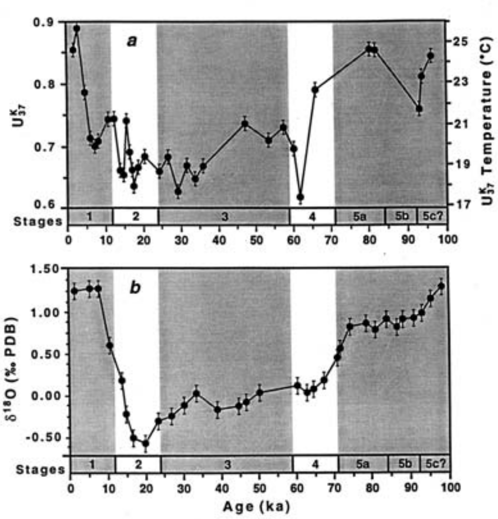

Prahl et al., 1988




Rechka & Maxwell, 1988

## The alkenone story - birth of a novel paleoceanographic tool

- **1989**
- *Poynter et al. 1989*
- Analysis of "stacked" core records confirmed Uk37 vs d18O relationship
- **1990**
- *Marlowe et al. (1990)*
- Micropaleontological and molecular data suggests genera belonging to family Gephyrocapsaceae were all potential sources of alkenones in sediments deposited since Eocene (45Ma). Cretaceous samples - ancestors of this family
- *McCaffrey et al. (1990)*
- Alkenone Uk37 found to record short-term climatic varations (El Nino events) in Peru margin sediments over last 300yrs.
- *Jasper and Hayes (1990)*
- $\delta$13C of alkenones used for reconstruction of pCO2 over last 70kyr from quaternary sediments (Pygmy basin, Gulf of Mexico) - correspondence with Vostok ice core record.




Molecular stratigraphy of Pigmy Basin sediments, Gulf of Mexico (Jasper and Gagosian, 198?)

## The alkenone story - birth of a novel paleoceanographic tool

**1992**

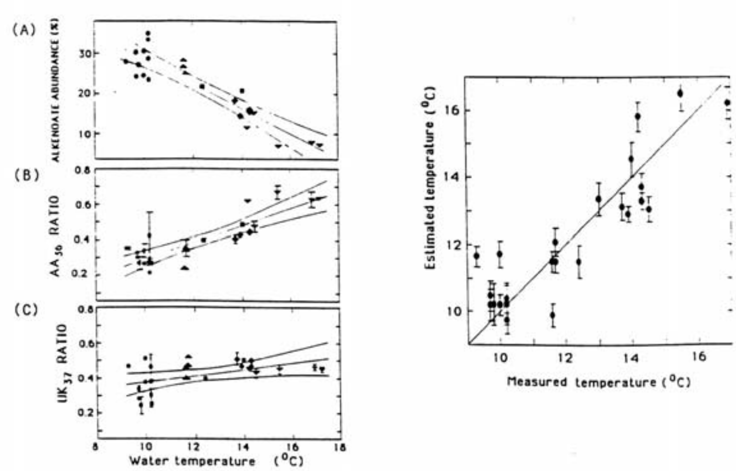
*Conte et al. (1992)*
- Calibration of alkenone and alkyl alkenoate distributions in Eastern North Atlantic (high latitude, cold water).
- Assessment of diagenetic alteration in water column and in sediments indicates SST signature preserved, despite significant compound loss
- Definition of new parameter based on alkyl alkanoate abundance, “AA36”

*Kennedy and Brassell, (1992)*
- Annual climatic variations over 20th century interpreted from Uk37 in Santa Barbara basin laminated sediments

*Freeman and Wakeham, (1992)*
- Analysis of Uk37 in Black Sea sediments indicates a different calibration required.
- Different d13C values for C37:4 relative to C37:2 and C37:3 - different sources?

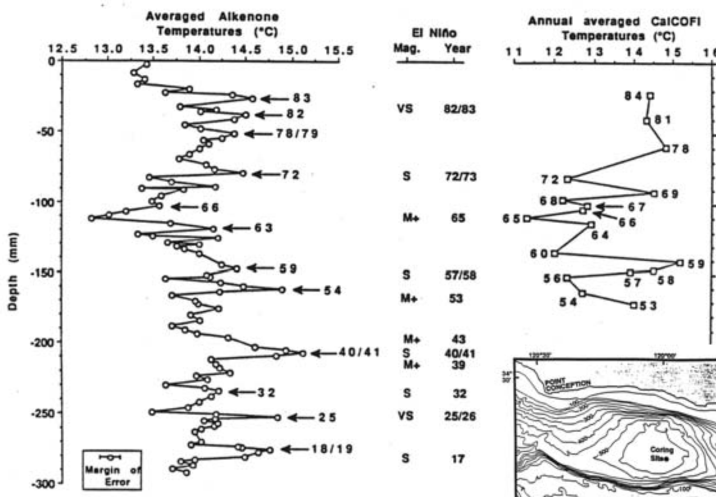
*Eglinton et al. (1992)*
- High resolution Uk37 record produced through automated sample processing and analysis.

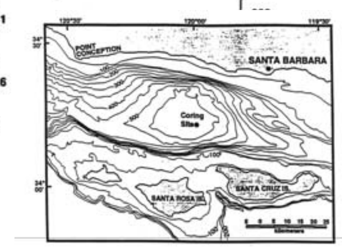
## Water column-based SST calibration




Conte et al., 1992

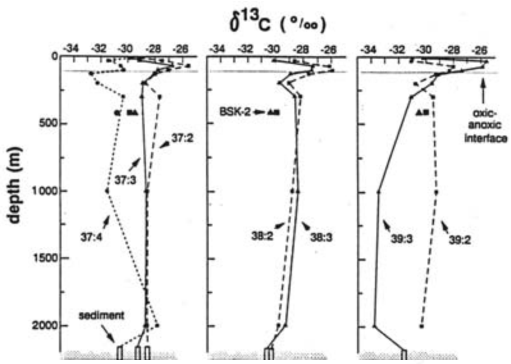
# Alkenone-based SST records of El Nino

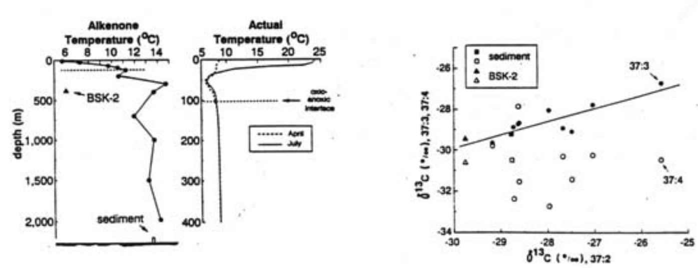



Kennedy & Brassell, 1992

Alkenones in the Black Sea
(Freeman and Wakeham, 1992)



## The alkenone story - birth of a novel paleoceanographic tool

**1993**
- *Jasper and Hayes, (1993)*
- d13C of alkenones used to estimated fraction of marine carbon in Quaternary sediments.
- *Rostek et al. (1993)*
- Application of coupled Uk37 and d18O records to estimate salinity.
- *Sikes and Volkman (1993)*
- Extension of Uk37 temperature calibration below 11 deg C.

**1995**
- *Volkman et al. (1995)*
- Identification of alkenones in *Gephyrocapsa oceanica*.

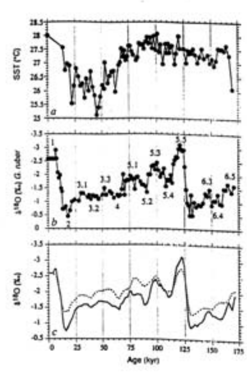
**1998**

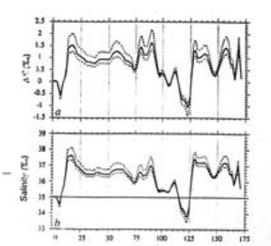
*Muller et al. (1998)*
- "Global" core top Uk37 calibration.

**1999**
- *Sachs et al. (1999)*
- Very high resolution Uk37 record for NW Atlantic across MIS-3.

## Reconstructing sea surface temperature and salinity using alkenone and $\delta^{18}O$ records

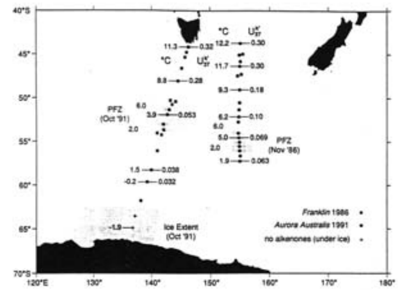
Rostek et al. (1993)

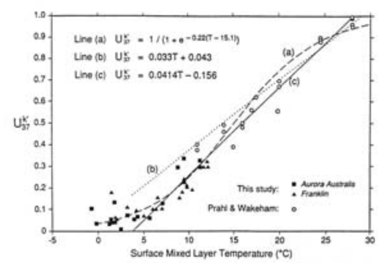



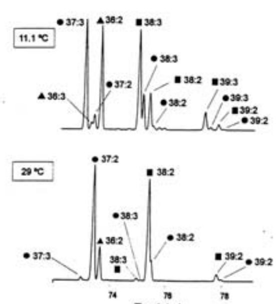
Calibration of alkenone unsaturation ratios for paleotemperature estimation in cold polar waters

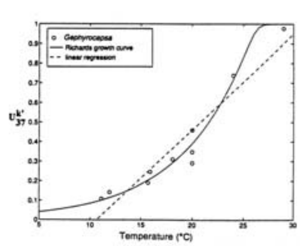
Sikes & Volkman (1993)

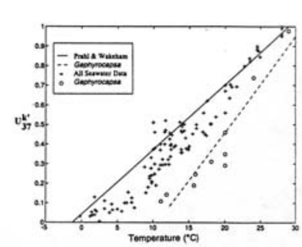



Alkenones in *Gephyrocapsa oceanica*

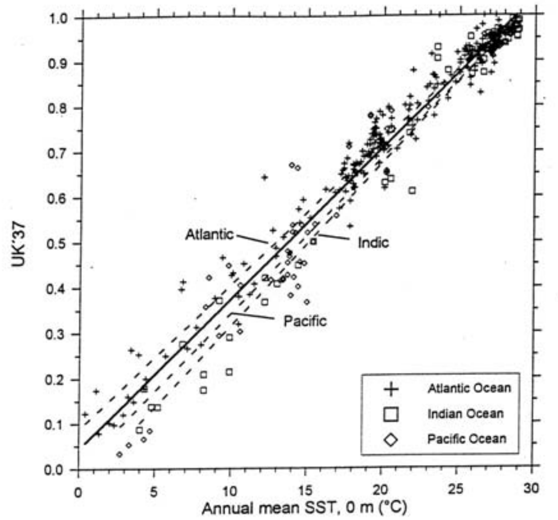


## Global core-top calibration of $U^{K}_{37}$' vs SST

(Muller et al., 1998)




## The alkenone story - birth of a novel paleoceanographic tool

**2000**
- *Benthien and Muller 2000*
- Evidence for lateral transport of alkenones.

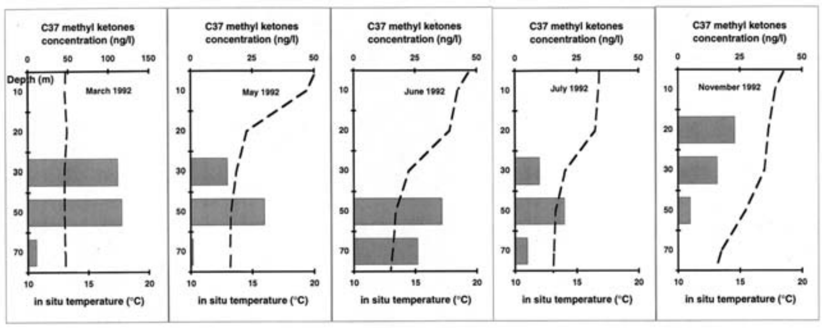
**2001**
- *Zink et al.*
- Temperature relationship observed in alkenones from feshwater lakes

**2001**
- *Xu et al.*
- Identification of a novel ($C_{36:2}$) alkenone in Black Sea sediments

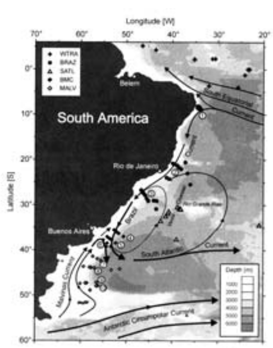
**2002**
- *Ohkouchi et al. (2002)*
- Temporal offsets observed between alkenones and planktonic foraminifera in a marine sediment drift.

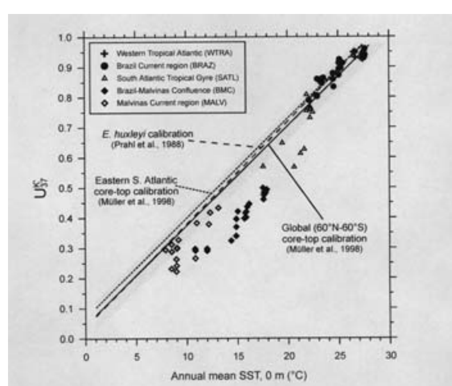
- **The future?**

## Seasonal variations in depth of alkenone production



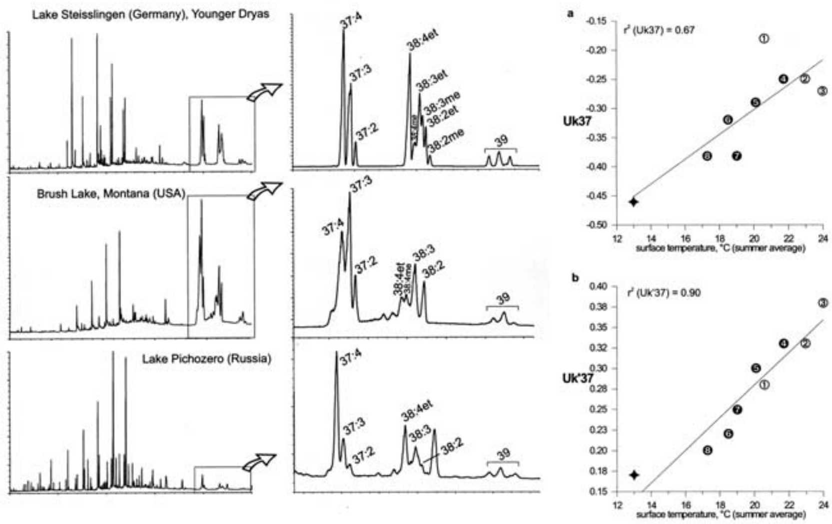

## Lateral transport of alkenones to the Argentine Basin




Benthien & Muller, 2000

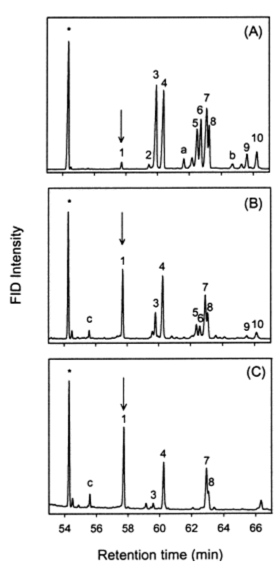
## Alkenones in freshwater lakes

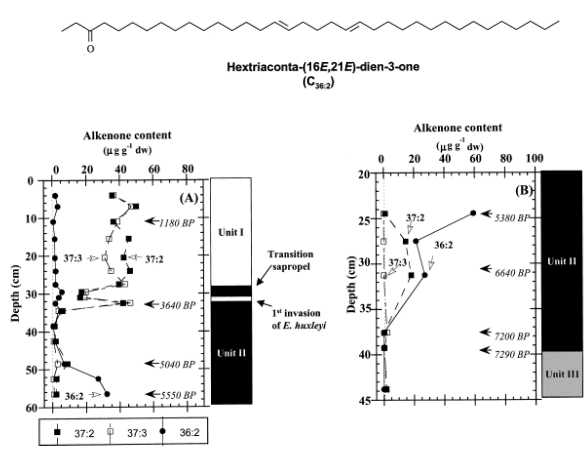



Zink et al. 2001

## A novel alkenone in Black Sea sapropel (Unit II)

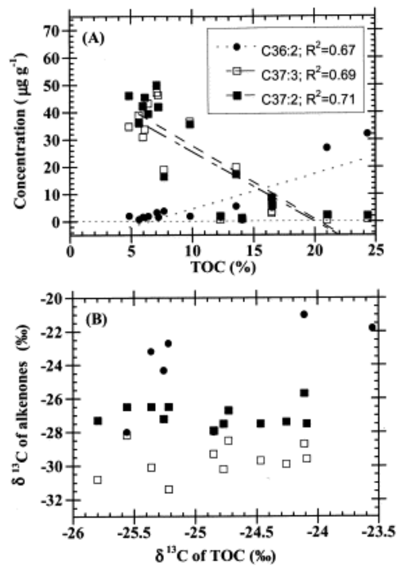
(Xu et al., 2001)



# A novel alkenone in Black Sea sapropel (Unit II)

(Xu et al., 2001)



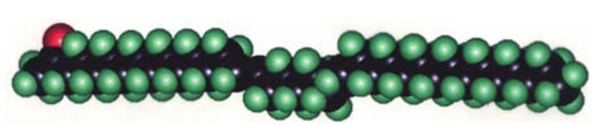

# Coupled molecular and microfossil $^{14}$C measurements

Premise:

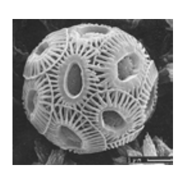
- Marine algal biomarker compounds (e.g., alkenones) and planktonic forams both encode surface ocean-derived signatures (incl. $^{14}$C content of DIC).
- Age discrepancies must therefore indicate different subsequent fates.
- Marine organic matter is predominantly associated with the fine fraction of sediments – prone to resuspension and redistribution.
- Foraminiferal tests are coarse, sand-sized particles – less susceptible to redistribution by bottom currents.

Approach:

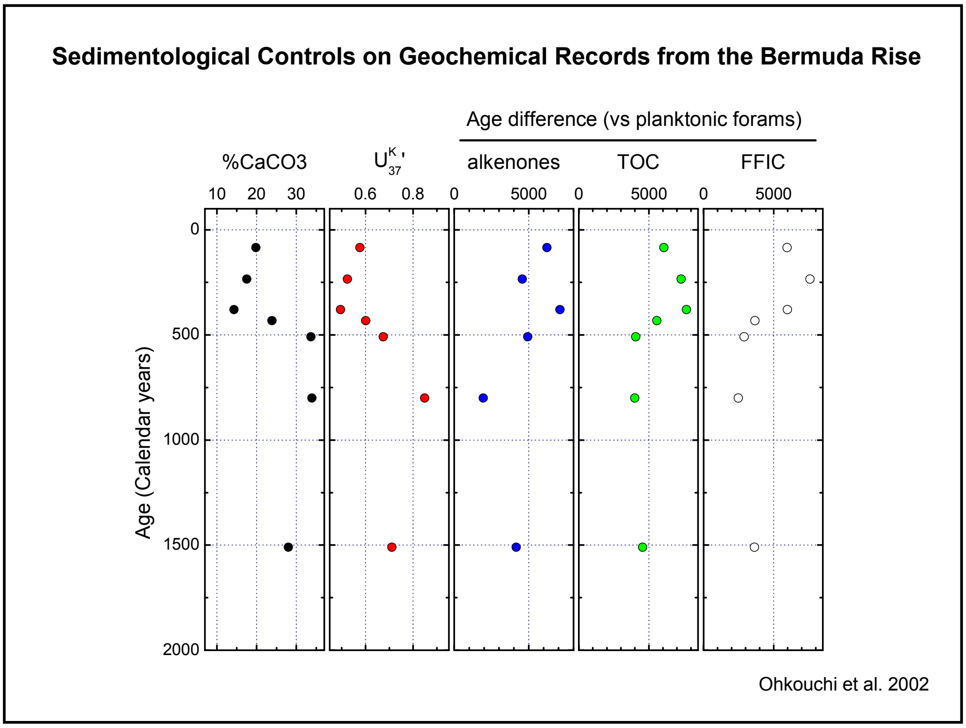
- Use $^{14}$C relationships between planktonic foraminifera, algal biomarkers (e.g., alkenones), and bulk OC isolated from the same sediment intervals as a tool to examine sedimentological processes (lateral transport, bioturbation).

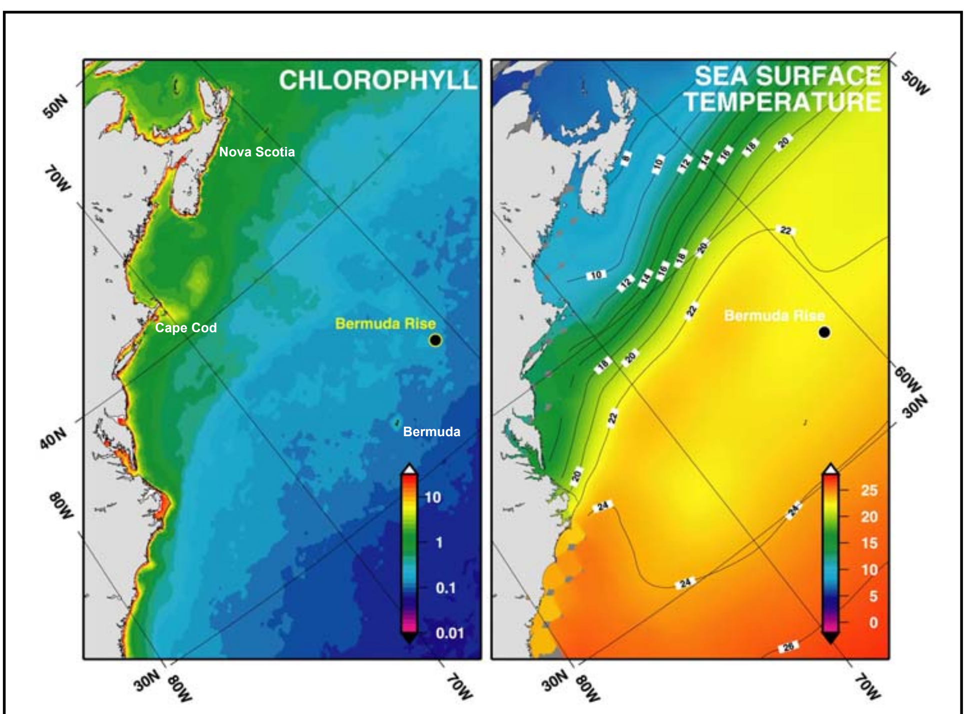


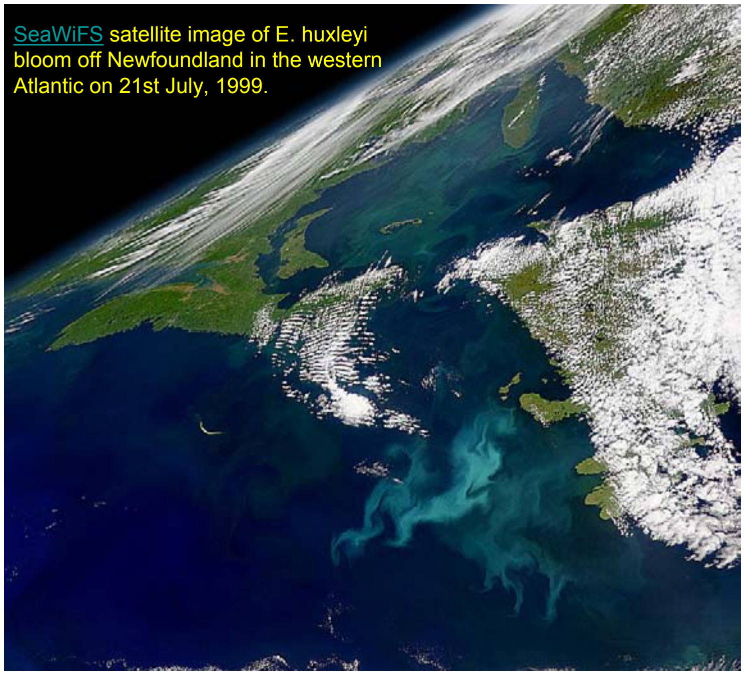
$C_{37}$ "alkenone"

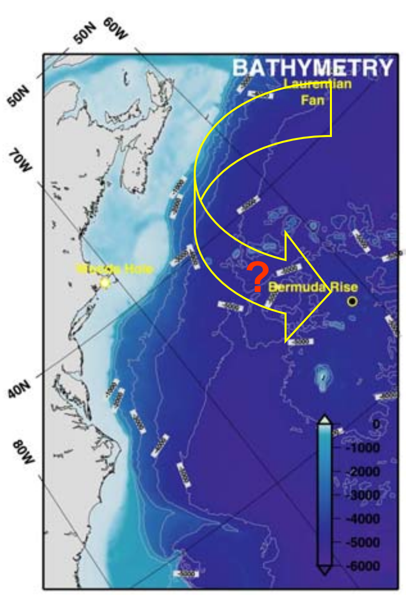


E. Huxleyi


Sedimentological Controls on Geochemical Records from the Bermuda Rise
Age difference (vs planktonic forams)
%CaCO3
$U^{K'}_{37}$
alkenones
TOC
FFIC
Age (Calendar years)
Ohkouchi et al. 2002

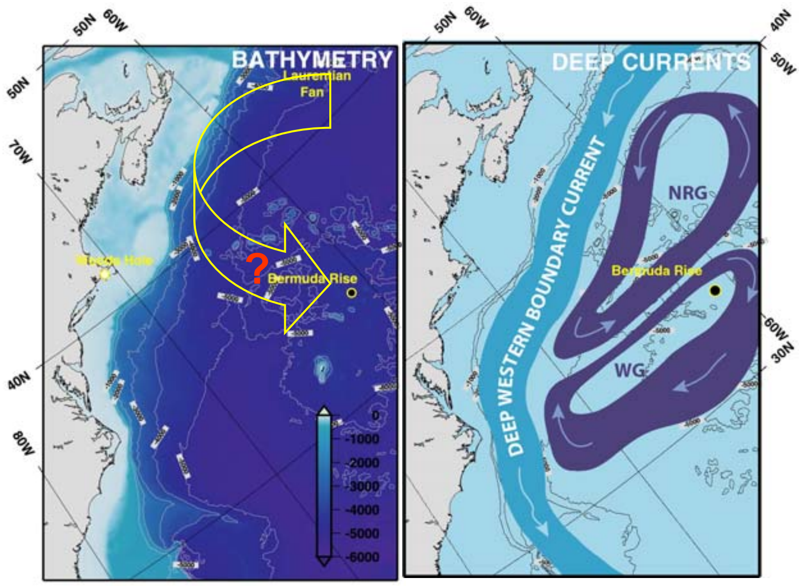

CHLOROPHYLL
Nova Scotia
Cape Cod
Bermuda Rise
Bermuda
SEA SURFACE TEMPERATURE
Bermuda Rise



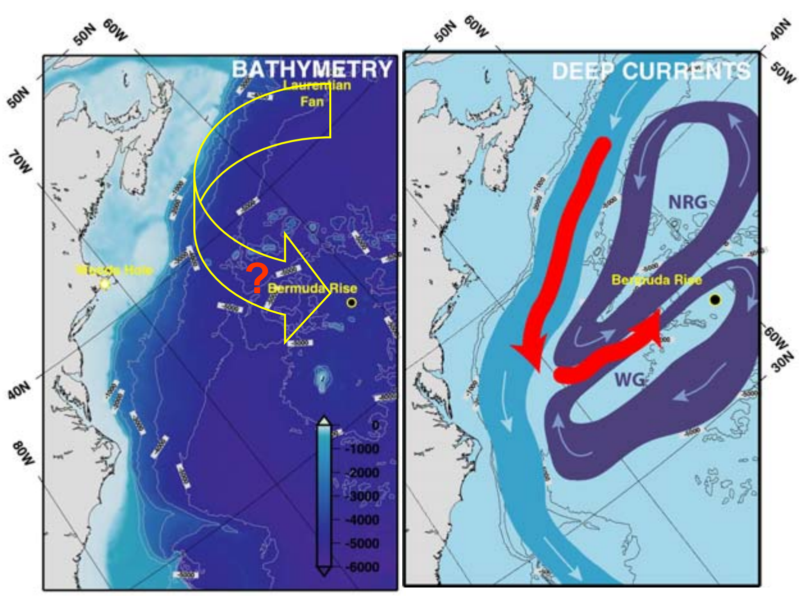



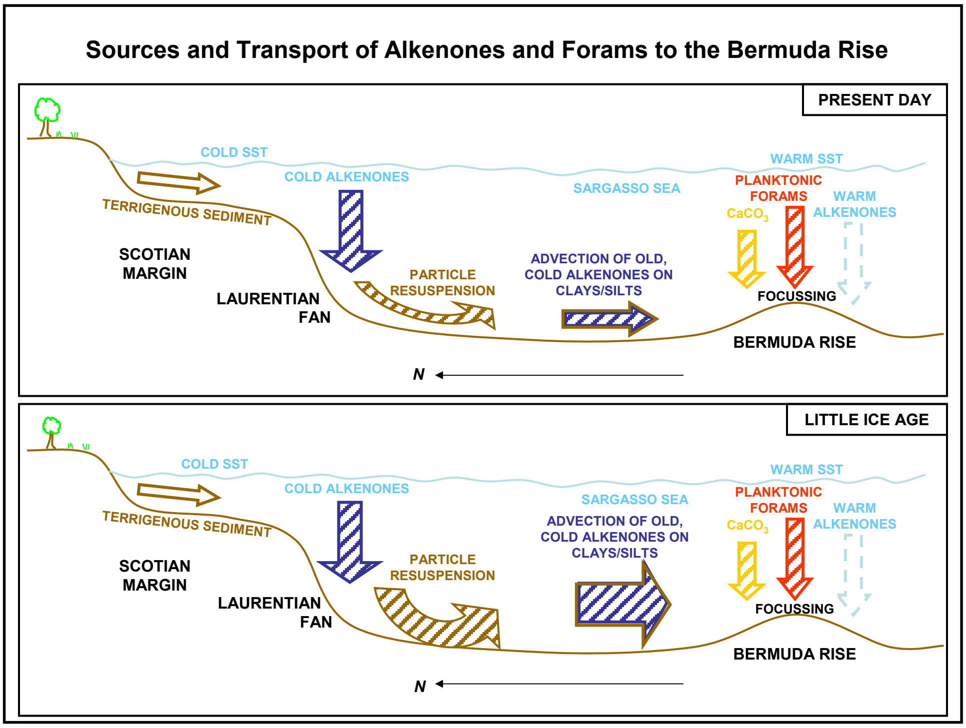

Long-range transport of organic matter (and alkenones) from the Scotian Margin to the Bermuda Rise?

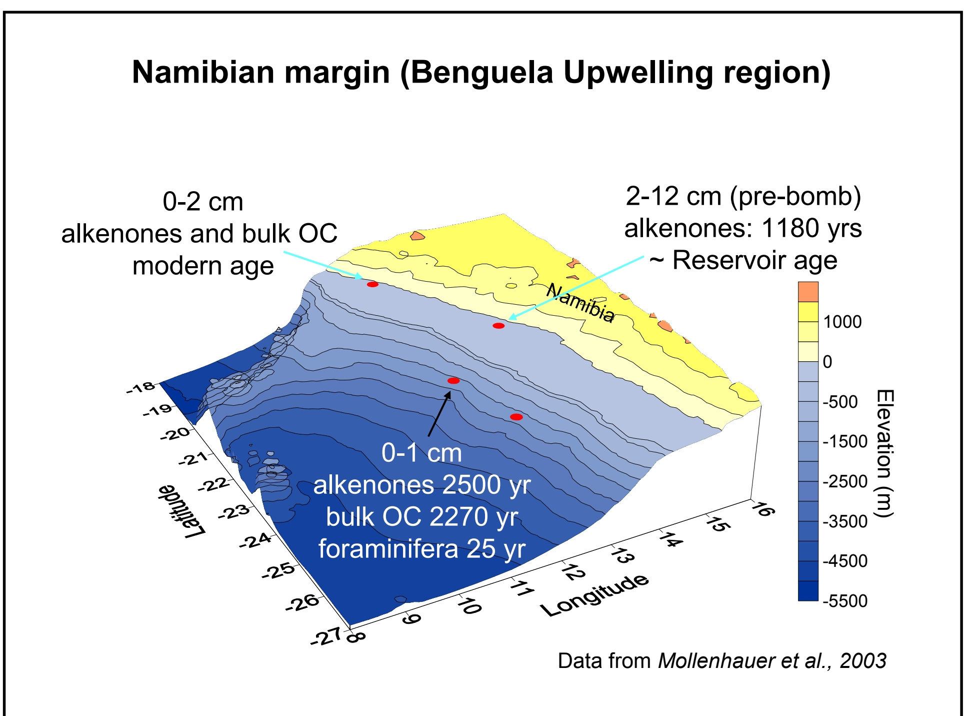
Lateral transport of organic matter to the Bermuda Rise



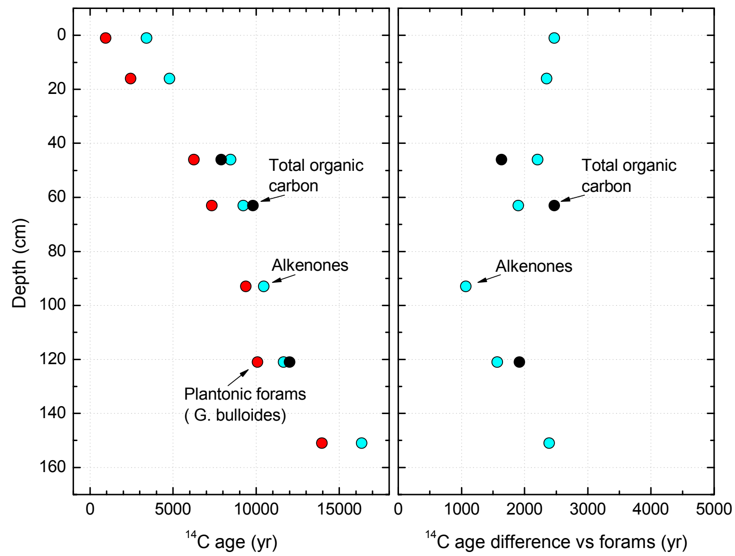

Lateral transport of organic matter to the Bermuda Rise




Sources and Transport of Alkenones and Forams to the Bermuda Rise
PRESENT DAY
COLD SST
COLD ALKENONES
TERRIGENOUS SEDIMENT
SCOTIAN MARGIN
LAURENTIAN FAN
PARTICLE RESUSPENSION
SARGASSO SEA
ADVECTION OF OLD, COLD ALKENONES ON CLAYS/SILTS
WARM SST
PLANKTONIC FORAMS
$CaCO_3$
WARM ALKENONES
FOCUSSING
BERMUDA RISE
N
LITTLE ICE AGE
COLD SST
COLD ALKENONES
TERRIGENOUS SEDIMENT
SCOTIAN MARGIN
LAURENTIAN FAN
PARTICLE RESUSPENSION
SARGASSO SEA
ADVECTION OF OLD, COLD ALKENONES ON CLAYS/SILTS
WARM SST
PLANKTONIC FORAMS
$CaCO_3$
WARM ALKENONES
FOCUSSING
BERMUDA RISE
N

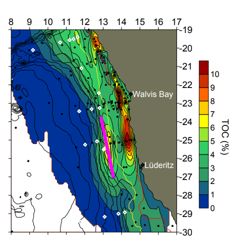

Namibian margin (Benguela Upwelling region)
0-2 cm
alkenones and bulk OC
modern age
2-12 cm (pre-bomb)
alkenones: 1180 yrs
~ Reservoir age
Namibia
0-1 cm
alkenones 2500 yr
bulk OC 2270 yr
foraminifera 25 yr
Latitude
-18
-19
-20
-21
-22
-23
-24
-25
-26
-27
Longitude
8
9
10
11
12
13
14
15
16
Elevation (m)
1000
0
-500
-1500
-2500
-3500
-4500
-5500
Data from *Mollenhauer et al., 2003*

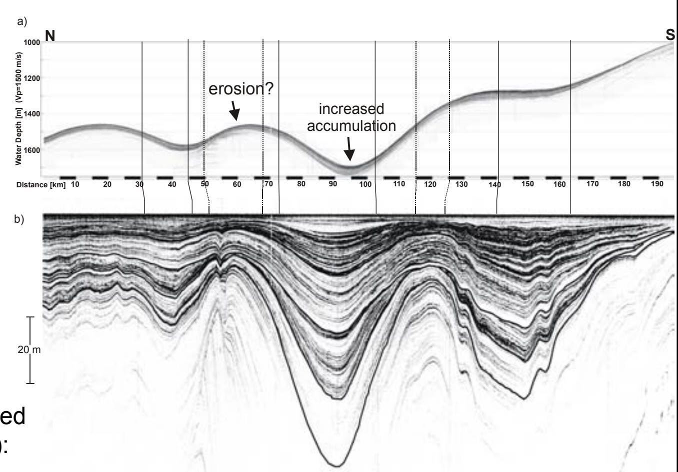
## $^{14}C$ ages of Namibian margin sedimentary components (core 226660-5)




Mollenhauer et al., 2003

## Accumulation maximum on upper continental slope



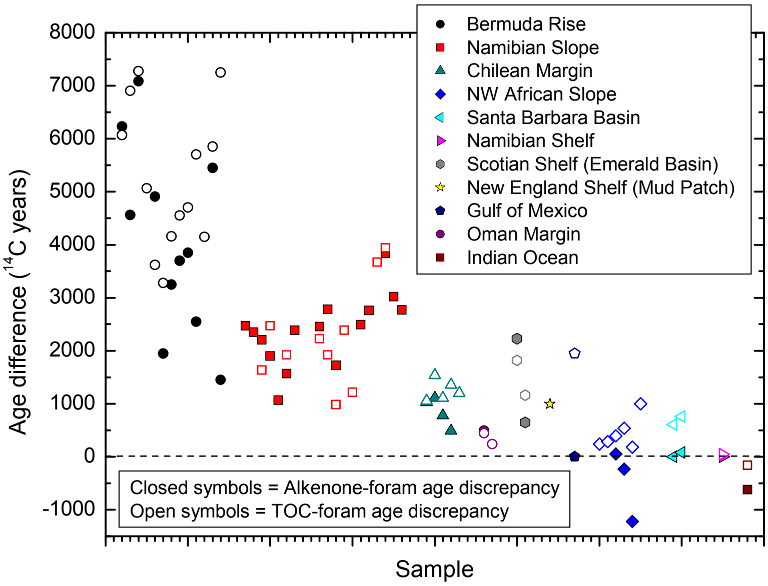




Sites of increased accumulation in protected locations (depocenters): Implication of current controlled sedimentation

Seismic data processing: André Janke

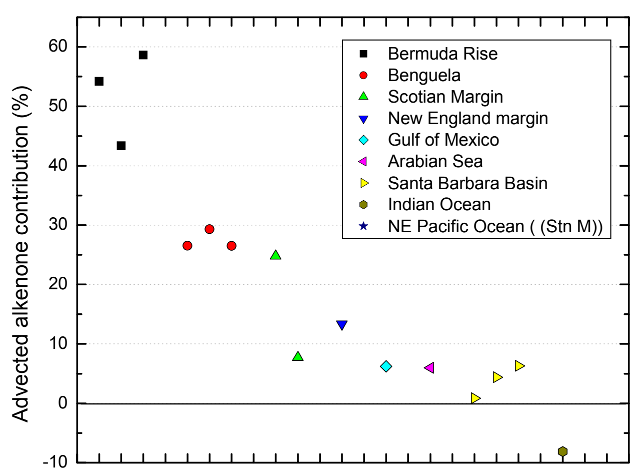
*Mollenhauer et al., 2002*

## $^{14}C$ age discrepancies between organic matter and calcareous microfossils

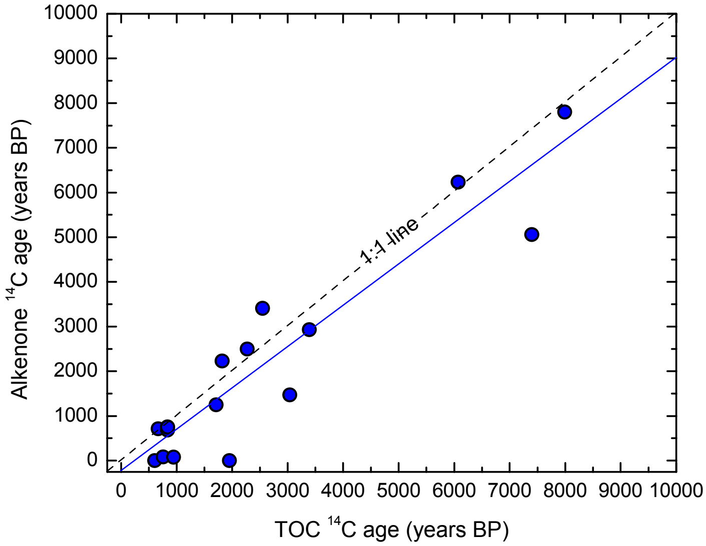



## Estimates of advective alkenone contributions

**Assumptions:**
$\Delta^{14}C$ of indigenous alkenones = $\Delta^{14}C$ of forams
$\Delta^{14}C$ of advected alkenones = -1000 permil (infinite $^{14}C$ age)

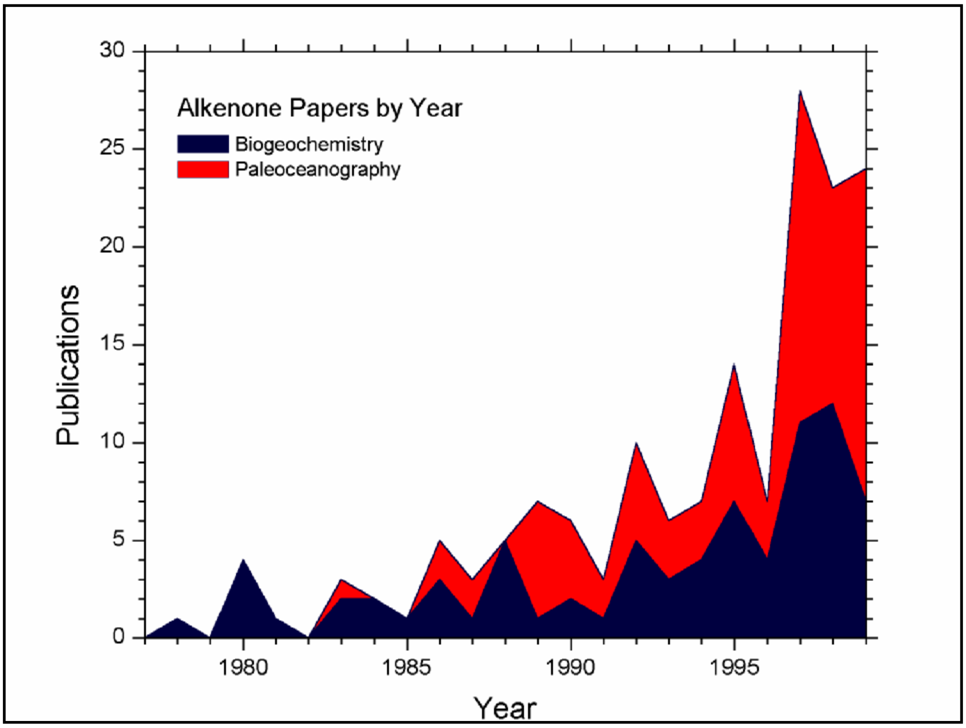


## Comparison of alkenone and TOC $^{14}C$ ages in surficial (< 3 cm) marine sediments




# Important Remaining Questions

- How are alkenones biosynthesized and what their physiological role?.
- What are the spatial and temporal productivity patterns for alkenone producers.
- Coastal vs. open ocean
- Vertical distribution in the water column
- Time-periods pre-dating *E. hux.*
- What are the reaction pathways by which alkenones are degraded?
- Is the ketone group or the unsaturation the initial site of attack?
- Influences of oxic v anoxic conditions.
- Importance of sediment redistribution processes on alkenone/molecular records.
- Lateral advection (drift deposits).
- Differential bioturbation.


Alkenone Papers by Year
Biogeochemistry
Paleoceanography
Publications
Year
30
25
20
15
10
5
0
1980
1985
1990
1995