

**INVESTIGATIONS OF THE ISOTOPIC COMPOSITION OF ALGAL LIPIDS:  
IMPLICATIONS FOR SOURCE IDENTIFICATION AND BIOSYNTHETIC PATHWAYS.**

ANDREW REVILL<sup>a</sup>, JOHN VOLKMAN<sup>a</sup>, DANIEL HOLDSWORTH<sup>a</sup> AND STEVEN ROWLAND<sup>b</sup>

a. CSIRO Marine Research, PO Box 1538, Hobart, TAS 7001

b. Department of Environmental Science, University of Plymouth, Plymouth, Devon, UK

The recent development of compound specific isotope ratio mass spectrometry has led to the technique becoming widely utilised in a variety of studies to determine the sources of organic matter in seawater and recent and ancient sediments from the isotopic composition of specific lipid biomarkers. These studies have generally exploited relatively large isotopic differences between C3 and C4 plants, microalgae and bacteria. At the present time, a limiting factor in the interpretation of stable carbon isotope composition of individual compounds is our incomplete understanding of the relationship between aqueous CO<sub>2</sub> concentrations [CO<sub>2</sub>aq] and the isotopic composition of the POC and lipid classes in microalgae. Also, very few data are available about possible differences in isotopic fractionation between microalgal species.

The relationship between [CO<sub>2</sub>aq] and the <sup>13</sup>C of POC (i.e. carbon fixation) in marine environments has been reviewed by Hayes<sup>1</sup>. The majority of microalgae rely on passive diffusion of CO<sub>2</sub> through the cell membrane which has an associated isotopic fractionation such that external CO<sub>2</sub> concentrations may influence the <sup>13</sup>C of the algal biomass. For example, phytoplankton from cold high latitude waters are typically depleted in <sup>13</sup>C due to elevated CO<sub>2</sub> concentrations caused by increased solubility at low temperatures (reviewed by Sackett<sup>2</sup>). The converse of this has been the suggestion that physical isolation of algae, for example in sea ice where CO<sub>2</sub> re-equilibrium is limited, will cause an increase in <sup>13</sup>C utilisation<sup>3,4</sup>. There are also numerous possibilities for further fractionation during lipid biosynthesis which could be influenced by environmental conditions affecting the growth rate of the alga. To investigate this further, we have grown various algae under different growth regimes to investigate the effect on lipid isotopic composition. This talk will outline the results from two sets of experiments.

The cosmopolitan coccolithophore *Emiliania huxleyi* was cultured under a wide variety of CO<sub>2</sub> concentrations extending beyond those normally found in nature, to investigate the different degrees of fractionation between lipid classes and the consistency of these differences with changing [CO<sub>2</sub>aq]. The results indicate that when compared to POC, different lipids show different levels of isotopic offset (phytol 2‰, fatty acids 2-4‰, alkenones 5‰, sterol 8‰) and that these differences are consistent across the range of CO<sub>2</sub> concentrations (Fig. 1). However, the results also indicate that some compounds such as palmitic acid (16:0) do not follow the same trend, which may be a result of its involvement in different lipid pools. The higher than expected offset for the long-chain unsaturated ketones (alkenones) has important implications as to how the isotopic composition of these compounds in the sediment record might be used as an indicator of paleo CO<sub>2</sub> levels (Jasper and Hayes<sup>5</sup>).

A second alga, the diatom *Rhizosolenia setigera*, is of interest as it produces unusual compounds known as 'highly branched isoprenoid alkenes' (HBIs; Fig. 2). The concentration of these C<sub>25</sub> and C<sub>30</sub> hydrocarbons in the algal cell has previously been shown to be affected by salinity and temperature. In this study we show, using compound specific isotope analyses, that under certain growth conditions there is a possible change in the biosynthetic pathway for these compounds. It appears that phytol, the side-chain of chlorophyll a, is made *via* conventional mevalonic acid (MVA) route in the chloroplast at both growth temperatures (18 and 25 °C) investigated. However, the C<sub>25</sub> HBIs at both temperatures (and C<sub>30</sub> HBIs at 18 °C) are made by MVA and non-MVA routes. The C<sub>30</sub> HBIs at 25°C have a significantly different isotope composition suggesting that they are synthesised *via* a non-MVA route or different combination of MVA and non-MVA isoprene units.

#### Acknowledgements

We would like to thank Dr Sue Blackburn, Dion Frampton and Claudia Grahl for help with cultures and useful discussions.

1. Hayes, J. M. (1993) *Marine Geology* **113**, 111-125.

2. Sackett, W. M. (1991) *Mar. Chem.* **34**, 153-156.

3. Rau, G. H., Sullivan, C. W., and Gordon, L. I. (1991) *Mar. Chem.* **35**, 355-369.

4. Revill, A. T., Volkman, J. K., O'Leary, T., Summons, R. E., Boreham, C. J., Banks, M. R., and Denwer, K. (1994) *Geochimica et Cosmochimica Acta* **58**, 3803-3822.