Currently Funded Research Projects

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Carbon Isotopic Fractionation in Marine Microalgae (NSF-OCE 0094637)

My main research program at UH has been the study of carbon isotopic fractionation in marine microalgae. Interest in this work stems from the assumption that carbon isotopic fractionation during photosynthesis in some way reflects environmental conditions at the time of organic matter formation. In particular, it has been assumed that the degree of fractionation is positively correlated with the concentration of dissolved CO$_2$ and thus carbon isotopic measurements of marine sedimentary organic matter may be used as indicators of past variations in the concentration of atmospheric carbon dioxide. Although ice core records of CO$_2$ and temperature variability have established linkages between climate change and atmospheric carbon dioxide (CO$_2$) and these studies have allowed greater understanding of paleoclimate records, ice cores are limited spatially to high latitude terrestrial environments and temporally to the last ~0.5 my. Extension of atmospheric CO$_2$ records to more ancient times through the development of a geologic proxy for CO$_2$ is a major objective of paleoclimate studies. Although carbon isotopic analyses of marine organic matter have been viewed as a promising CO$_2$ proxy, our laboratory studies have demonstrated that microalgal growth rates and cell geometry in addition to CO$_2$(aq) concentration affect carbon isotopic fractionation in marine microalgae. Specifically we have investigated in laboratory chemostat cultures how environmental variables ([CO$_2$(aq)] and $\delta^{13}$CCO$_2$) and cellular properties (microalgal growth rate, cell geometry, active DIC accumulation, and calcification) influence the carbon isotopic fractionation.

In field studies, cell geometry can be quantitatively constrained only when the source of the phytoplankton carbon analyzed is known. Isotopic analyses of long-chain alkenones provide a way to constrain the size and shape of the source organism because these compounds are known to be produced in open-oceanic waters by only *Emiliania huxleyi* and the closely related *Gephyrocapsa oceanica* both of which are reasonably similar in size and shape. Moreover, our recent field studies imply that the growth rate of modern alkenone-containing haptophytes is correlated with [PO$_4$]. Therefore, isotopic analyses of alkenones have potential as a CO$_2$ proxy because cell geometry and potentially growth rate of the alkenone-synthesizing algae may be estimated. These relationships have motivated us to recently develop a simple $^{13}$C tracer method to measure *in situ* growth rates of alkenone-synthesizing algae and to use it to field-test the hypotheses that growth rate affects carbon isotopic fractionation in naturally occurring alkenone-synthesizing algae.
The $^{13}$C alkenone-labeling technique we are developing is analogous to the method for determining phytoplankton growth rates using $^{14}$C-labeling of pigments, but uses irmGCMS to determine the rate of incorporation of $^{13}$C into alkenones. The objectives of our laboratory studies are to define the range of growth conditions under which the $^{13}$C alkenone-labeling technique gives reliable estimates of growth rate and to identify under what conditions significant bias may be anticipated. The objective of our field programs are to compare *in situ* growth rates determined using the alkenone $^{13}$C-labeling method with that predicted using laboratory-based fractionation relationship for *E. huxleyi* along a [PO$_4$] gradient in the subarctic Pacific and Bering Sea. These results will allow us for the first time to field-test laboratory-based microalgal fractionation hypotheses.

![Plot of carbon isotopic fractionation versus the specific growth rate divided by the dissolved carbon dioxide concentration for chemostat cultures of marine microalgae. Figure from Popp et al. 1998, Geochimica et Cosmochimica Acta, 62, 69-77.](image)

Although our laboratory results show that we can explain 99% of the variation in carbon isotopic fractionation between marine microalgal species, I feel confident that this research program will continue at least another six years beyond the lifetime of the present grants. First, this research has been largely laboratory-based and more than a single field study is needed to test our laboratory-based hypotheses. Second, although we have made great strides in explaining natural variations in carbon isotopic fractionation in marine microalgae, we are only now beginning to determine how the exact species of inorganic carbon (bicarbonate or aqueous carbon dioxide) used by marine phytoplankton affects its carbon isotopic composition in the
laboratory and in the field. This research has been supported by four separate NSF grants and thus far has supported 3 postdoctoral fellows, 8 graduate students, 5 undergraduate students, a research technician and my stable isotope laboratory manager. I expect that our future grants will support at least another 2-3 graduate students and the technicians.

**Evaluating Unsaturation Patterns in Long-Chain Alkenones (NSF-OCE 0094272; 0324299)**

Unsaturation patterns ($U_{37}^{K'}$) in long-chain alkenones of phytoplankton origin, most notably represented by the haptophyte *Emiliania huxleyi*, have now proven remarkably valuable for paleoceanographic reconstruction of sea surface temperatures. On a nearly global basis, $U_{37}^{K'}$ measured in recent ocean sediments correlates highly with the ‘mean annual’ SST of overlying surface waters. Despite the significance of such endorsement, however, certainty of predictions made using this paleoceanographic technique is not yet assured. For example, we now know that other alkenone-producing haptophytes in the ocean, even other *E. huxleyi* strains, can display quite different quantitative $U_{37}^{K'}$ responses to growth temperature. There is also growing evidence that $U_{37}^{K'}$ is not just set by growth temperature but is affected perhaps significantly by other physiological factors such as nutrient and light availability. Thus, scatter noted in the global dataset for the $U_{37}^{K'}$-mean annual sea surface temperature calibration, which corresponds to a prediction uncertainty of ±3°C, is a concern that cannot be overlooked and clearly warrants further investigation in the modern ocean.

I have been recently working with Fred Prahl (Oregon State University) using our $^{13}$C labeling method to determine the rate of alkenone production and the alkenone unsaturation patterns ($U_{37}^{K'}$) for actively growing cells at discrete depths in the water column at Station ALOHA in the oligotrophic North Pacific gyre. The goal of this work is to define how alkenone productivity and the $U_{37}^{K'}$ value for actively growing cells equate with the concentration of alkenones and $U_{37}^{K'}$-temperature relationship in particulate organic matter. This work is providing a sound biological oceanographic basis to link $U_{37}^{K'}$ and the sea-surface temperature encoded by alkenones. We have just received our second NSF grant to extend this work to the North Pacific Ocean and to the Guaymas Basin in the Sea of Cortez. A new graduate student has just been recruited for this research project.

![Schematic illustration of in situ array used to study alkenone production rate and the growth rate of the alkenone-producing algae. Samples (100 L) are collected from 4 depths, $^{13}$C-labeled bicarbonate added and bottles incubated *in situ* for 24 hours. The $^{13}$C labeled C$_{37:2}$ alkenones are isolated and analyzed isotopically to determine production and growth rate.](image-url)
Origins of $N_2O$ and $CH_4$ in Seawater (NSF-OCE 0240787)

$N_2O$ plays a significant role in the reduction of ozone in the stratosphere and is an important greenhouse gas in the troposphere. The oceans are a source of $N_2O$ to the atmosphere. However, it is not clear which oceanic $N_2O$ production mechanism is most important: nitrification in areally extensive oligotrophic waters where $N_2O$ is only slightly saturated, or denitrification in areally restrictive regions with a strong $O_2$ minimum layer near the surface where $N_2O$ is supersaturated. A major goal of this research program has been to identify, using a combination of laboratory and field experiments, the biogeochemical conditions under which a particular mechanism of $N_2O$ production predominates. We are attaining this goal by 1) determining the $\delta^{15}N$, $\delta^{18}O$ and the isotopomer distribution of $N_2O$ (i.e., determine the position of $^{15}N$ within the linear NNO molecule) throughout the water column in contrasting oceanic regimes (Eastern tropical and subarctic Pacific Ocean, Bering Sea, Black Sea) and use these results to model the sea-to-air flux and the rate of production of $N_2O$, 2) measure the concentration of $N_2O$ in the surface mixed layer and the in air and use well-known air-sea gas transfer coefficients to determine the sea-to-air flux of $N_2O$, 3) measure the rate of incorporation of $^{15}NO_3^-$, $^{15}NO_2^-$, and $^{15}NH_4^+$ into $N_2O$ using in situ or on-deck incubations to independently determine the rate and mechanism of $N_2O$ production, and 4) use these analytical results to refine existing source functions for oceanic $N_2O$ production and to develop and analyze a nitrous oxide cycle component of a general circulation model (i.e., the GFDL Earth System Model).

The results of natural abundance isotopomeric measurements will refine budgets of the global isotopic mass balance of $N_2O$ by allowing prediction of the isotopic compositions of dissolved $N_2O$ exchanging with the atmosphere based on biogeochemical conditions. In addition, the nitrous oxide component of the GFDL model will be used to investigate possible significant increases in global warming potential due to release of $N_2O$. The results of the research program should constrain models of the global $N_2O$ cycle by clarifying the relative importance of nitrification and denitrification in $N_2O$ production under specific oceanic biogeochemical conditions, thus significantly advancing efforts to balance the global $N_2O$ budget. This research has been supported by two NSF grants and has supported one graduate student and numerous undergraduates.

![Plot of the concentration and stable isotopic composition of $N_2O$ from Station ALOHA.](image-url)
The oceans have long been known to be a source of methane (CH$_4$) to the atmosphere, but the processes controlling the origins and distribution of CH$_4$ in surface-ocean waters are poorly understood. In general, the mixed layer typically has CH$_4$ concentrations that are oversaturated with respect to the atmosphere, with a marked maximum associated with the pycnocline. I have been actively studying CH$_4$ dynamics in a variety of marine environments over the past several years. Our study at Station ALOHA indicated that the $\delta^{13}$C of methane in the upper ocean varied between -45 to -47‰ suggesting that the oligotrophic North Pacific Ocean was a source of $^{13}$C-rich CH$_4$ to the atmosphere during 1996-97. Recently, we have expanded our work into the eastern tropical North Pacific (ETNP), a large, well-documented region of high surface-ocean productivity resulting from coastal upwelling of nutrient-rich deep-sea water. Decomposition of the resulting organic matter leads to the formation of a large area of upper-ocean with highly depleted levels of dissolved oxygen. The ETNP has been the site of numerous studies of suboxic and anoxic nitrogen transformations; however, there are few studies of methane cycling in this region. Our preliminary results indicate that the ETNP subsurface CH$_4$ maximum is clearly the largest pool of methane yet discovered in the open ocean, but its exact magnitude is yet to be determined. The deeper half of this pool may be from a coastal source whereas CH$_4$ production in the upper pool appears to be associated with sinking particulate matter. The plume of CH$_4$ in the ETNP can be traced westward and oxidation of the subsurface CH$_4$ pool appears to be the source of the $^{13}$C-enriched methane found at midwater depths in the central North Pacific gyre. We discovered that 1) highly CH$_4$-enriched waters exist throughout the ETNP, 2) CH$_4$ is being released to the atmosphere at rates several times faster than in other parts of the open ocean, and 3) this CH$_4$ is distinctly $^{13}$C-enriched relative to atmospheric methane.

Although we have now documented that the central North Pacific gyre and the ETNP surface waters are sources of $^{13}$C-rich methane to the atmosphere, the mechanisms of methane formation remain enigmatic. Biogenic methane can be produced from a variety of processes, the most common being from the fermentation of acetate or the reduction of carbon dioxide. We are currently using in situ incubations with $^{13}$C-labeled compounds (acetate, trimethylamine, formate and bicarbonate) to determine the pathways of net methane production. Equipment recently delivered to my laboratory will allow us for the first time to measure the D/H ratio of methane in seawater and extend our labeling studies to D-enriched compounds. The hydrogen incorporated into methane produced from CO$_2$ reduction originates from water whereas the source of hydrogen in methane formed by acetate fermentation is thought to be predominantly from the methyl groups of acetate. The differences in the sources of carbon and hydrogen for CH$_4$ formation led to the suggestion that the $\delta^{13}$C and $\delta$D of CH$_4$ could be used to differentiate the mechanisms of CH$_4$ formation. Recently, this classic interpretation of $\delta$D variations in CH$_4$ championed originally by Whiticar, Schoell and colleagues has been challenged for freshwater systems since it is now recognized that enzyme-mediated hydrogen isotope exchange can occur between acetate methyl-hydrogen and water. Although the use of $\delta$D of CH$_4$ as an indicator of the mechanism of CH$_4$ formation in freshwater systems is controversial, it appears that hydrogen isotope exchange between H$_2$O and the methyl group of acetate does not occur in sulfate-rich water. Therefore, the $\delta$D of CH$_4$ produced from acetate in marine environments (i.e., high sulfate) should carry the isotopic signature of organic hydrogen and be distinct from that produced from CO$_2$ reduction. The use of natural abundance carbon and hydrogen isotopic compositions of methane in conjunction with tracer experiments using $^{13}$C- and D-labeled substrates should therefore yield a much better understanding of the mechanisms of methane formation in the ocean. This research has been supported by two NSF grants and an ONR grant and has supported one postdoctoral fellow and two graduate students.
Hydrothermal Vent Biogeochemistry (NSF-OCE 0095297)

Hydrothermal plumes, and the biogeochemical processes active within them, provide dramatic linkages between crustal accretion/subduction processes and the flux of energy and mass to the overlying ocean waters. An important transformation step is the microbial exploitation of reduced inorganic hydrothermal constituents within the plumes. Ammonium is the least studied of metabolically exploitable reduced constituents in hydrothermal plumes. Yet, very high levels of ammonium are discharged from hydrothermal vents at sedimented ridge (e.g., Guaymas Basin) and back-arc basin systems; anomalously high NH$_4^+$ levels are also found in vent fluids from the sediment-starved Endeavour Segment, Juan de Fuca Ridge. Consequently, NH$_4^+$ is a potentially important substrate for chemolithoautotrophic production of organic carbon within the neutrally buoyant plumes associated with these hydrothermal systems.

NH$_4^+$ is readily exploited as a source of energy by ammonium oxidizing Nitrifiers, a group of obligate aerobic chemolithoautotrophic bacteria comprising 5 genera in the family Nitrobacteraceae. The physical and chemical conditions within deep-sea hydrothermal plumes appear to be favorable to NH$_4^+$ oxidation. Recent preliminary NH$_4^+$ loss rate experiments with Endeavour plume waters indicate potential NH$_4^+$ oxidation rates of 5-15 nM d$^{-1}$, and specific scavenging rates of 0.04 to 0.13 d$^{-1}$, nearly identical to that found for CH$_4$ oxidation within Endeavour plumes. Such oxidation rates could produce an amount of organic carbon equivalent to over 100% of the surface-derived organic carbon flux to plume depths. The potential NH$_4^+$ oxidation and organic carbon production rates could be an order of magnitude higher in plumes associated with sedimented hydrothermal systems such as at Guaymas Basin. However, large uncertainties remain, due primarily to the preliminary nature of existing experimental data and the unknown NH$_4^+$ partitioning ratio between oxidation and assimilation processes.
I have been working with James Cowen (UH Oceanography) to address the fate of hydrothermally injected NH$_4^+$. We have measured the rate of NH$_4^+$ removal and its partitioning between oxidation and assimilation processes, using both sensitive fluorometric and stable isotopic tracer techniques. The population dynamics of the relevant nitrifying bacteria in evolving hydrothermal plumes are also being assessed using molecular genetic probe techniques. The biogeochemistry of NH$_4^+$ in the hydrothermal plumes of the Endeavour and Guaymas Basin systems are being compared and contrasted with each other and background (NH$_4^+$-deprived) deep waters. We are particularly concerned with the influence of hydrothermally injected constituents, including NH$_4^+$, on the transfer of (bio) energy and organic-C between hydrothermal systems and the overlying water column. Furthermore, nitrification is a critical component of the global aquatic N cycle. Studies of the marine N cycle have emphasized the upper ocean with relatively few studies extending to the mid-depths. The injection of NH$_4^+$-enriched hydrothermal plumes into the deep sea water column creates a dynamic and spatially-well constrained geomicrobial ecosystem for studying the responsiveness of ammonium oxidizing bacteria to sudden environmental changes (e.g., NH$_4^+$ substrate, particle concentrations, chemical).

Tuna Trophic Ecology and Migration (NOAA Pelagic Fisheries Research Program)

Recent modeling suggests that tuna productivity in the western and central Pacific Ocean is tied to upwelling along the equator in the central and eastern Pacific. We have just begun a three-year project that tests this hypothesis by combining diet analysis, stable isotopic compositions, food-web modeling, and stable isotope markers to trace tuna movements and trophic-level variation in the equatorial Pacific. The hypothesis predicts that tunas that reside near equatorial upwelling fronts feed at relatively low trophic levels. Opposite trends are expected in equatorial regions with little upwelling, such as the warm pool of the western Pacific, where tunas are expected to feed at higher trophic levels and move extensively,
searching for less-abundant prey. Results of this study should help define ecosystem linkages leading to tuna production and the effect of climate variability on the systems. This information is important for both fisheries production and ecosystem modeling of the equatorial Pacific Ocean. This research is supported by a NOAA Pelagic Fisheries Research Program grant and supports a graduate student and technician.

Recent Laboratory Improvements (NSF-MRI 0115958)

We have recently purchased a Finnigan Delta-Plus XP isotope-ratio monitoring Gas Chromatograph/Mass Spectrometer (irm-GC/MS) to expand our research capabilities. This instrument measures carbon, nitrogen and hydrogen isotopic compositions of individual molecules and the isotopomers of N\textsubscript{2}O (i.e., the intramolecular distribution of \textsuperscript{15}N within the linear NNO molecule). We have also purchased a GasBench II for isotopic analysis of carbonates, dissolved inorganic carbon in seawater, oxygen and hydrogen in water, and nitrate in fresh water and seawater. Lastly, a second GC-combustion system was purchased for our Finnigan MAT 252, which gives us separate GC combustion systems for natural abundance and artificially labeled compounds, thereby reducing the risk of cross-contamination of samples and minimize our use of radioisotopes, thus eliminating the safety and disposal issues associated with radioisotope usage. Our Finnigan Delta-Plus remains dedicated to carbon and nitrogen isotopic analyses of organic materials.