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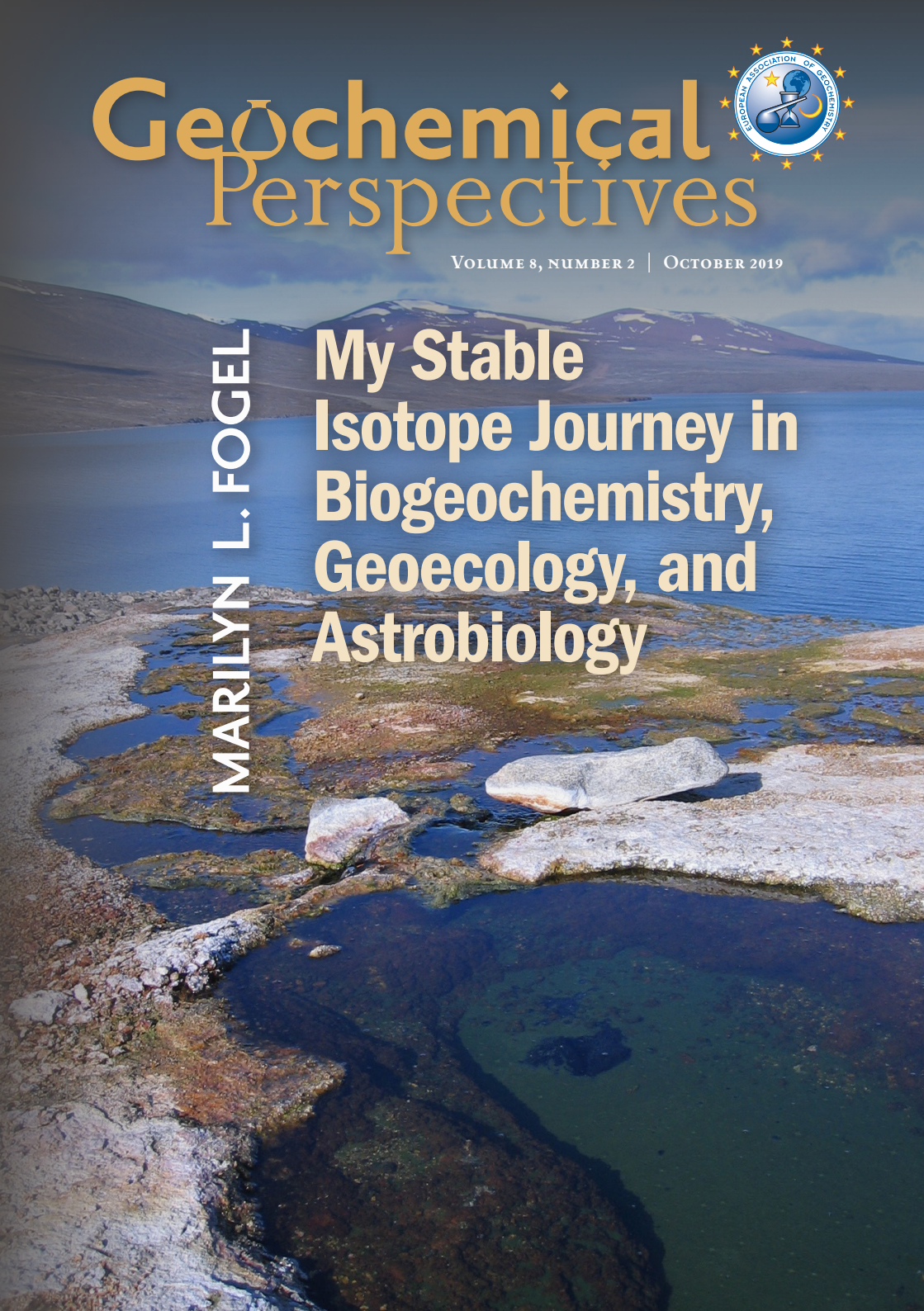

MARILYN L. FOGEL

My Stable Isotope Journey in Biogeochemistry, Geoecology, and Astrobiology

Geochemical Perspectives

Each issue of ***Geochemical Perspectives*** presents a single article with an in-depth view on the past, present and future of a field of geochemistry, seen through the eyes of highly respected members of our community. The articles combine research and history of the field's development and the scientist's opinions about future directions. We welcome personal glimpses into the author's scientific life, how ideas were generated and pitfalls along the way. *Perspectives* articles are intended to appeal to the entire geochemical community, not only to experts. They are not reviews or monographs; they go beyond the current state of the art, providing opinions about future directions and impact in the field.

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About the cover

Jotun Thermal Springs in the Bockfjord Volcanic Complex in Svalbard, one of Fogel's field areas, is one of the most northern thermal springs.

Photo credit: Jennifer L. Eigenbrode

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PREFACE

I am proud to have the honour to handle this issue of *Geochemical Perspectives* (GP) by Marilyn Fogel. She is the first female scientist writing a GP series, and I am happy that I managed to convince her to do so.

I am one of the lucky scientists who was 'adopted' into Marilyn's 'science family' where you are assured support and backing in all scientific and life situations. Marilyn is not just an excellent mentor, but an outstanding role model. She taught me much of what I know about organic geochemistry and astrobiology, as well as what makes a good colleague or mentor to the younger generation. She taught me to always endeavour to do the best science regardless of consequences and to ignore the still strong gender bias in science. She gave me the confidence to stand up for myself and defend my science. That advice in a large part helped me to become the person I am today. Her path as described in this GP issue is a fantastic example of how following your curiosity will keep you content. Best advice ever.

I just want to share a typical Marilyn (Queen Thora) adventure story. In 2010, while in Svalbard on one of our many field excursions linked to the AMASE / ASTEP project, we needed someone to be on 'polar bear watch' while we sampled snow and ice algae on Midtre Lovebreen. Marilyn volunteered with no hesitation and accompanied us the whole day with the rifle on our glacier sampling endeavours; naturally she was more than a polar bear guard, and in her understated fashion helped us better define our scientific questions. With her astute observations, she focussed our sampling and analysis plans.

Three generations of female scientists during a sampling trip to Midtre Lovebreen, Svalbard, Norway in 2010. From left to right: Verena Starke, Liane G. Benning, Marilyn Fogel and Dominique J. Tobler. Photo credit: Liane G. Benning.

Soon after our last trip to Svalbard in 2015, Marilyn was diagnosed with ALS. No words describe the feelings that the ALS news brought up in me adequately so best not to start, but I urge everyone to read this GP issue, to follow Marilyn's "IsotopeQueen" blog (https://isotopequeen.blogspot.com/), and to watch a video that her son Evan Swarth made about Marilyn – the scientist and her ALS – available at https://www.youtube.com/watch?v=yRhpFoUFMyA&feature=youtu.be&fbcli=IwAR11UivstUF8dkB53KdhPhs-f2RUHXXpLotcOqVEd8LVY9sT6vVQbAvSr1u8.

Thank you, Marilyn, for being you.

Liane G. Benning
October 2019
German Research Centre for Geosciences, Potsdam, Germany
Geochemical Perspectives Handling Editor of this issue

FOREWORD

Looking in the rear view mirror at the 50 years of my journey in science, I see a life rich in scientific discovery as well as scientific colleagues, who have without a doubt enriched my life. No one can predict what path their career will follow. Mine began in 1970 at Penn State University as a biology major, where I became intrigued by recent findings of ancient life on Earth. The Viking space probes had landed on Mars, and impacted my curiosity about life on other planets, a theme that remained dormant for many years. I knew at an early age that I wanted to use my interest in chemistry to investigate biological phenomena that happened during the span of Earth's history. My career has encompassed three different fields: biogeochemistry of modern environments and ecosystems; palaeo-biogeochemistry of fossil and historic ecosystems; and astrobiology. What I've learned about how organisms interact with their environment, essentially the study of ecology, and what stable isotope patterns might be associated with those relationships has been critical for interpreting ancient rocks and fossils, as well as signatures of potential life from outer space.

My Ph.D. work in 1974 was funded by a NASA Exobiology grant. Later in my career, it came as no surprise that I would be fascinated by the search for life in the universe. My work came full circle 30 years later with the study of astrobiology in the Arctic, India, and other extreme environments.

The majority of my professional career took place at the Carnegie Institution of Washington's Geophysical Laboratory. I was fortunate to land there at a time when biogeochemistry was in its infancy. Without really being aware of where I was headed, I jumped with both feet into multi-disciplinary work, not

being afraid to collaborate with smart people in many different fields along the way. Carnegie encouraged its staff scientists to think broadly, try new things, and be creative. In the early days from Carnegie Institution's inception until about 1980, we did our research without significant government funding. My early work was supported in part by grants from private foundations, money that I may have never seen explicitly, but nonetheless provided support for my postdocs, lab supplies, fieldwork, and an occasional conference.

As I grew more experienced, I transformed from a shy, quiet, perpetually youthful looking woman into a more outgoing leader. Always serious, it took a while for me to realise that being quiet did not help my career. I attribute the transformation to a supportive husband, motherhood, and great colleagues. My husband, Christopher Swarth, forced me, pleasantly, to stand up for myself and speak out when I had an opinion. As a mother of two young children, I learned more about how to work effectively as a scientist, while taking care of others. Eventually, I developed personal relationships with Geophysical Laboratory scientists, Tom Hoering and Doug Rumble, as well as others who supported my ideas and recognised the value of my hard work.

Mentoring young scientists along the way became one of the most rewarding aspects of my career. Carnegie supported a vigorous postdoctoral programme, and typically I worked with one to three postdocs at any one time. Although the Carnegie Institution did not grant academic degrees, I served on the committees of many graduate students and was an active participant in their research. Bright undergrads and high school students somehow magically appeared each summer and enriched my career with their innate fascination for science. I learned much from these folks.

Below is the story of my career thus far (1970 to 2019). Its path has influenced many of my direct associates as well as those related to them. My academic family tree, thanks to a life time of mentorship, is healthy, active, and strong. These relationships buoy me daily, especially as I transition to the next phase of my career. In 2016, I was diagnosed with amyotrophic lateral sclerosis (ALS; Motor Neuron Disease in Europe), which abruptly changed the way that I had planned to end my career. No longer able to travel at the drop of a hat to far-flung field areas; no longer able to wield the wrenches in the lab; and finding it difficult to travel to conferences, I have had to consider what is most important in this phase of my life and why it might be so. Accordingly, it was finally the right time to write this *Geochemical Perspectives* piece that I hope will serve as inspiration for young and old scientists alike on the joys and challenges of a full, intellectual and personal life.

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Liane Benning, Editor, asked me to write this article in 2015 during my last expedition to Svalbard. We were sitting in a restaurant, drinking a beer, when she put the question to me. Immediately, I wanted to scream "*Yes!*", but coyly thought about it for a minute before replying, "*What an honour. I'd love to.*" Owing to the many obligations I have at the University of California as a faculty member, chair of the department, and Institute Director, I needed a block of quiet time without too many distractions to begin to write. A sabbatical leave from UC Riverside in fall 2018 provided this opportunity. Without the help of my husband, who sees to my care every day, I could not have carried out this task. Thanks also to Seth Newsome (University of New Mexico), former postdoctoral associate and current colleague, for providing a corner of his office desk in Albuquerque for me to write.

Most important in my long career is the love, support, and understanding from my family. A woman does much better with a great partner. Chris Swarth, my husband of over 30 years, has helped my career in innumerable ways – being a dedicated, involved father, a serious ecologist, and a sympathetic ear for rough times. My children, Dana and Evan Swarth, provided realism every day for the past 30+ years. They have challenged me as a mother and supported me as a scientist. I always felt that they were proud of my accomplishments, which made it easier to go to work each day. My father, Arthur Fogel, and mother, Florence Fogel, were always supportive of my "alternative" path, even though they professed to not understand the science that I do.

My sister, Barbara Fogel Lis, was an unflagging cheerleader, who thought I was the smartest women on earth (or at least in the top 5). Having the joy of friends and family contributed mightily to my success as a creative scientist.

Throughout my career I benefited from the wisdom and advice of many at the Geophysical Laboratory: Tom Hoering, Douglas Rumble, Hatten Yoder, Charles Prewitt, Wes Huntress, George Cody, and Andrew Steele. Maxine Singer and Vera Rubin: senior Carnegie women who made it to the very top, were mentors along the way. Maxine's phrase "*Half measures never work*." reminds me daily of how I need to think big and finish things that I start.

There are many special people that shaped my career. Two of my Ph.D. supervisors at the University of Texas, Chase Van Baalen and Patrick Parker, were quiet mentors who guided me without me really realising this. Their subtle encouragement and recognition was crucial for my early successes in science. Next came Thomas C. Hoering, who guided me with his folksy phrases: "*Show it who's boss!!*" and "*Pick 5 well-chosen samples*." I started as his postdoc and ended as his close, and equal colleague. When Tom was near the end of his life in 1985 in a nursing home, he told me how important I was to his career. I was touched. This revelation has remained an inspiration to me throughout my career.

Noreen Tuross and I met in 1978 during my first Carnegie Institution conference on Amino Acids held at the Airlie House conference centre, Virginia, where we shared a room. We have remained colleagues, co-authors, and close friends to this day. Her insights and advice into many of the challenges in my career have helped me along. As "elder stateswomen", we enjoy talking about the past, the present, and the future of our field. Andrew Steele came to the Geophysical Laboratory in the late 1990s. After jokingly referring to me as a middle-aged matron, we struck up a close scientific and personal friendship. I am too young to be his mother, so we consider that we're scientific brother and sister. Always thinking about the bigger picture and empathetic to things going on around us, we made the Geophysical Laboratory a fun place to work for many years.

Many of my colleagues and I worked together for decades – beginning by actively working on projects, and then keeping in touch personally for decades. Giff Miller (University of Colorado) and I made many trips to Australia, visited each other in DC, California, and Boulder. Ken Nealson (USC) and I met in 1977 when I presented my Ph.D. work at an American Society for Microbiology meeting. We've had postdocs in common and continue to think about novel microbial experiments. Ron Benner (University of South Carolina) and I have three decades of research projects in common. He's been instrumental in helping me realise what retirement means.

I continue to be in touch with all of the former and current postdocs and students listed below. Each of them contributed in very different and unique ways, all are gems, and many still close: Stephen Macko (1980-1982); Thomas Stafford (1983-1986); Luis Cifuentes (1987-1990); David Velinsky (1988-1991); Paul Koch (1989-1993); Gray Bebout (1989-1992); Carmen Aguilar (1992-1995);

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Training graduate students has made me realise what I know and don't know. And if I did know a concept, I needed to look back to when I first learned it. Participating in their education has proved instrumental in many ways: Kent Sprague (1984-1986); Kevin Mandernack (1988-1993); Matthew Hoch (1988-1992); James Shores (1993-1995); Matthew Fantle (1997-1998); Felicitas Weidemann (1999-2003); Sean Pack (1999-2000); Kevin Boyce (1999-2000); Albert Colman (1999-2002); Simon Clarke (2000-2003); Katherine Cooney (2003-2005); Maia Schweizer (2004-2006); David Johnston (2004-2007); Paula Zelanko (2005); Stephanie Gudeman (2004-2006); Rachel Schelbe (2005-2006); Isabel Romero (2004-2009); Bianca Mislowak Silver, 2004-2008; Kelton McMahon (2007-2010); Christopher Florian (2009-2011); Ana Laura Liberoff (2009-2010); Charlotte Oskam (2010); Patrick Griffin, (2007-2009); Derek Smith (2010-2013); Nicole DeBond (2010-2012); Joy McDermot (2014-2016); Jonathan Nye (2014 to present); Daniel Toews (2015-present); Bobby Nakamoto (2015-present).

Many individuals helped produce and edit this manuscript: Jeanette Westbrook, administrative assistant of the EDGE Institute: Anat Shahar, Jessica Blois, Susan Ziegler, Peggy Ostrom, Kaycee Morra, Diane O'Brien, Bridget Kelly, Valery Terwilliger, Anne Jakle, and Beverly Johnson provided comments and suggestions on the "Women in Geoscience" epilogue. The full manuscript was given thorough and helpful reviews by Christopher Swarth, Matthew Wooller, Valery Terwilliger, Seth Newsome, Kate Freeman, Peggy Ostrom, and Rob Raiswell.

MY STABLE ISOTOPE JOURNEY IN BIOGEOCHEMISTRY, GEOECOLOGY, AND ASTROBIOLOGY

ABSTRACT

Stable isotope biogeochemistry started in earnest in the 1960s with isotope ratio mass spectrometers hand made in physicists' laboratories. I began my career at the time when people were realising that the biosphere was important in shaping the geosphere. Bringing sophisticated chemical instrumentation to study the relationships between living organisms and their environment, in particular in fossils over geological time, was exploding in the 1970s and 1980s. Follow along on insights gained over a nearly 50 year career.

1. INTRODUCTION

The origin of life on Earth holds many mysteries for scientists of all disciplines. In the 1960s, professors and students at Harvard published short papers describing their findings of trace fossils from early microbes in some of the Earth's oldest Precambrian rocks, 1 to 4 billion years old. At the time, the biological revolution was still a decade away. In earth science, plate tectonics did not yet provide the framework for interpreting how continents were built or how their sedimentary features had been formed. Dating of rocks was just being developed, and many of the Precambrian deposits studied at this time for the presence of fossils had just been dated. A key paper for me was a review in *Scientific American* by Elso S. Barghoorn, Harvard professor and palaeontologist (Barghoorn, 1971) (Fig. 1.1). Along with his student William Schopf, the two published photographs of thin sections and electron micrographs of putative microbial cells (Schopf and Barghoorn, 1967).

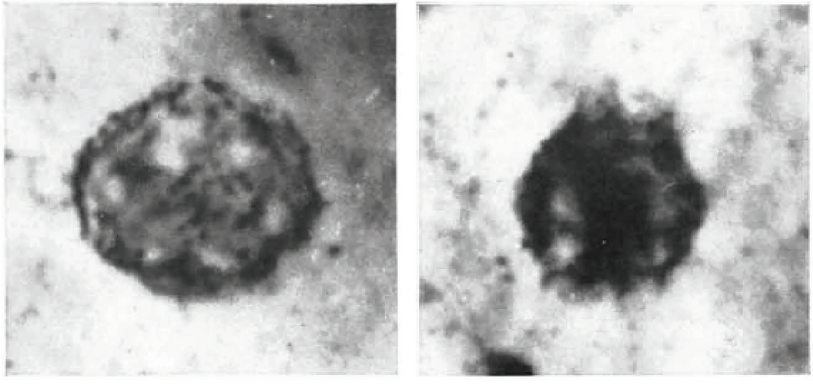

Figure 1.1 These early photomicrographs of putative algal structures in Precambrian rocks were rudimentary compared with those taken today. Nevertheless, they showed for the first time that microbial cells could be fossilised, a huge leap in our understanding of early life on Earth. From Barghoorn (1971) with permission from Scientific American.

Barghoorn noted that life had to conquer three different thresholds to evolve to where it is today. From the chemical soup of the early Earth, organisms needed to develop biosynthetic pathways such as photosynthesis and the tricarboxylic acid cycle. He presumed that the first organisms were heterotrophs, which fed on compounds in the chemical soup. Barghoorn argued that autotrophy was also needed to continue the supply of organic nutrients for heterotrophs. The second threshold was diversification, whereby single celled organisms needed to develop into more complex forms, including chains of cells

or stromatolites, macrostructures seen in sediments over the years. The third threshold that organisms needed to transcend was the formation of cellular microstructures, notably the nucleus, in order to become multicellular organisms. Sedimentary deposits from three different continents, Africa, Australia, and North America confirmed to Barghoorn, Schopf and others that life was widespread on the earth during its first 2 billion years and had originated in similar fashion across the planet (Fig. 1.2).

> *"In the paleontological assessment of minute structures of Precambrian age and of possible biological origin, it is often difficult to differentiate between inorganically produced pseudofossils and partially degraded remnants of primitive microorganisms. This is particularly true in the interpretation of structures from sediments such as those of the approximately 3 billion year old Fig Tree Series. For which there are no known fossils of equivalent age for morphological comparison. Nevertheless, the Fig Tree spheroids are almost certainly of biological origin, probably representing the remnants of single celled algae-like microorganisms. Their organic composition, morphological consistency, limited size range, mode of preservation, and morphological similarity to known spheroidal algae, both modern and fossil, support this interpretation."*

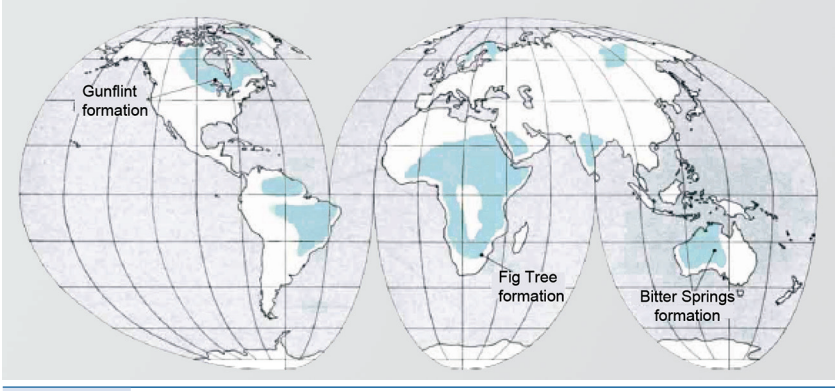

Figure 1.2 Precambrian rocks occupy continental cratons on almost all of the continents. Many of these rocks have been altered by heat and pressure, but some retain biological fossils that were found by Elso Barghoorn and his colleagues. From Barghoon (1971) with permission from the Estate of Bunji Tagawa.

The conclusion that these were fossil microbes (Schopf and Barghoorn, 1967) started a four decade debate about the biogenicity of these structures found in ancient rocks. The authors noted that although it is difficult, I would say impossible, to relate the potentially biological structures to intact cells, they concluded that the spheroids are *"almost certainly of biological origin"* representing cells, most likely algae. Electron microscopy of ancient sediments was a new technique at the time. Microscopic examination coupled with chemical and isotopic analyses seemed to prove the interpretation that these were fossil

organisms. Their paper certainly caught my eye and my interests because it combined new chemical instrumentation to describe once living organisms in a geological context. These three fields of science – biology, chemistry, and earth science – rolled into one sparked my career.

Geochemical evidence came next. Keith Kvenvolden (USGS) isolated what he presumed were amino acids from Precambrian rocks (Schopf *et al.*, 1968); carbon isotopes were measured in several deposits by Dorothy Oehler and others (Oehler *et al.*, 1972). Claims of preservation of molecular fossils multiplied at this time. The earth science community was increasingly excited by the opportunity to study the origin and development of life. Schopf's paper with Kvenvolden showed the level of rigour that was taken in analysing the three specimens. While it is clear that amino acids were found within the rocks, with time it has been shown that indigeneity of the amino acids is impossible to confirm in this and other subsequent studies. More on this subject later.

As a student in Professor Peter H. Given's (Penn State University) organic geochemistry class, this research was all very exciting for me. He revealed this set of fascinating papers to our small group of undergraduate and graduate students. I was immediately hooked! As a biology major, I wanted to know how to identify chert, the rock type where fossils were found. I wanted to use my fascination with biochemistry to find molecular fossils. Last but not least, ironically at the time, I was not at all interested in the carbon isotope papers published by Tom Hoering (1963, and 1967) and others noted above. Little did I realise then that carbon isotopes and the process of isotope fractionation by blue-green algae (cyanobacteria) that would come to occupy the majority of my research career.

Prof. Given's class was an eye opener for me in many ways, because I was able to use my understanding and love of chemistry to begin to understand biological, environmental, and ecological processes. In the 1960s, scientists at the Carnegie Institution led by Philip Abelson, Tom Hoering, and P. Edgar Hare were discovering simple molecules like fatty acids in marine sediments and fossil shells (Abelson, 1956; Hare and Abelson, 1968). Then postdoctoral fellow Patrick Parker published a landmark paper (Parker, 1964) in which he not only described the molecular distributions of fatty acids in marine organisms, but isolated these molecules to measure their carbon isotopic compositions ($\delta^{13}C$). Parker and colleagues at the University of Texas Marine Science Institute (UTMSI) were publishing papers at a rate of one a year in the journal *Science* on new compounds found in sediments, marine ecosystems, and blue-green algae. Parker and Leo's study on branch chained fatty acids in sediments and their conclusion that these were synthesised by bacteria is the first of a very long and fruitful avenue of study using fatty acid compositions to fingerprint bacteria (Leo and Parker, 1966) (Fig. 1.3).

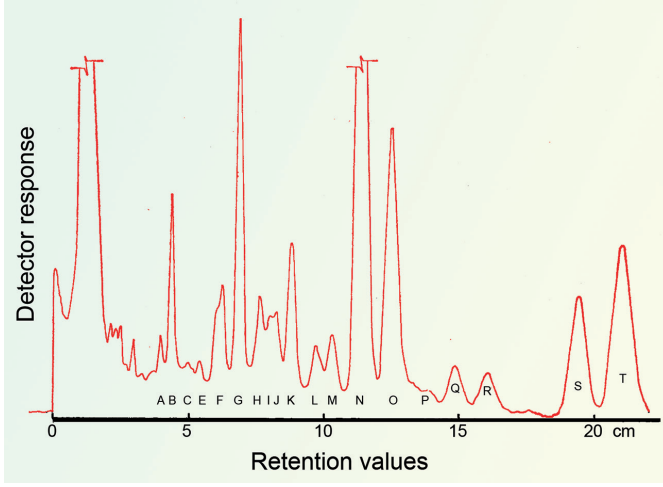

Figure 1.3 This early chromatograph (1966) showed that bacterial lipids were found in sediments, which at the time, was not widely known. Modified from Leo and Parker (1966).

In the early 1970s, organic geochemists like Given and his colleague William Spackman, my professor for Palaeobotany, were studying peat formation in the Florida Everglades. They developed early methods in the field of plant taphonomy, the study of how living organisms change over time after death to become geochemical fossils. With his experience in coal chemistry, Given provided the organic geochemical expertise, while Cohen and Spackman (Cohen *et al.*, 1984) studied the macromolecular changes in plant structures. Peat was a perfect medium to study diagenetic changes in plant morphology. The Everglades boasts 10,000 years of sedimentation allowing researchers to see transformations in morphology from modern living plants to geochemical plant-derived peat.

At the conclusion of my undergraduate years as a biology major, I was convinced that I wanted to combine biology, chemistry, and geology in an interdisciplinary career. Professor Given helped tremendously by suggesting two United States labs and one British group that were engaged in the research I was most interested in. The first was Woods Hole Oceanographic Institution under John M. Hunt. Hunt worked on organic geochemistry of sediments (Hunt, 1970) and their relationship to oil pollution and petroleum formation (Fig. 1.4). He conducted early studies of hydrocarbons and fatty acids in nearshore environments, as well as stable carbon isotope measurements of sediments. Hunt's early work on characterising percent total organic carbon (% TOC) in carbonate rocks and shales was important foundational work on the formation of petroleum

in sediments and rocks. The second was Archie Douglas's lab in Newcastle, England. I visited there in 1973, but his emphasis on coal was not a good fit for me.

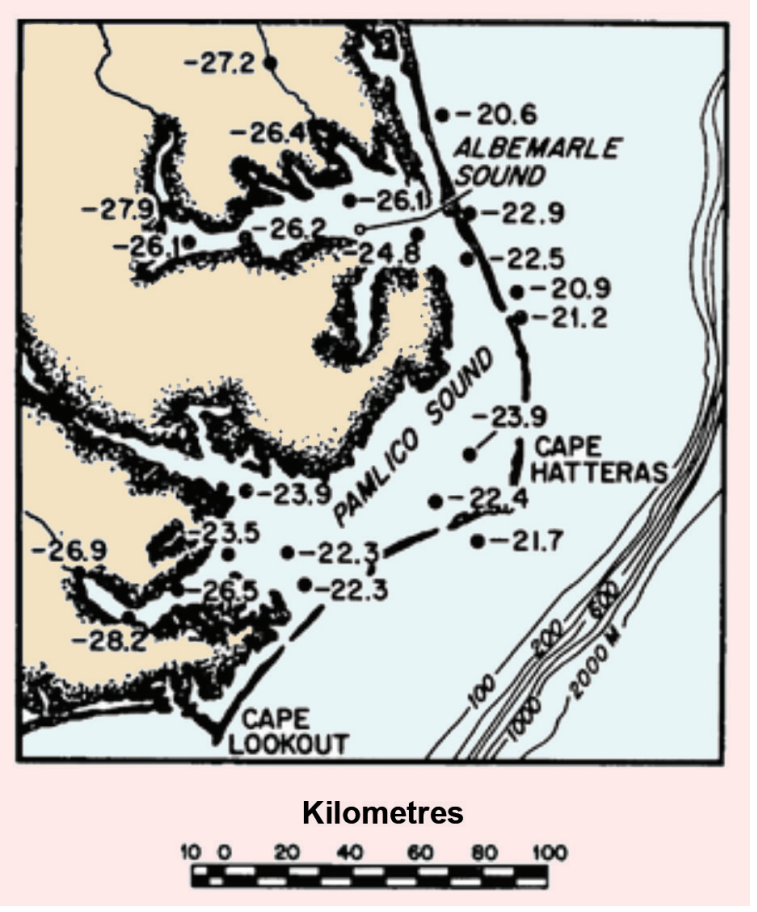

Figure 1.4 Hunt was one of the first to publish carbon isotope measurements of coastal sediments. He was more well known for his studies on petroleum formation. The $\delta^{13}C$ values of -26.9‰ and -28.2‰ in riverine embayments compared with the $\delta^{13}C$ values of -20 to -21‰ offshore showed clearly the terrestrial-marine trends through an estuarine environment. Modified from Hunt (1970).

The third was University of Texas Marine Science Institute (UMTSI) in Port Aransas. Pat Parker provided a positive response to my potential and offered me a position as a graduate student in an interdisciplinary course of

study under the joint supervision of Parker (a marine organic geochemist), Chase Van Baalen (an algal physiologist and specialist in cyanobacteria), and Bill Behrens (a marine geologist). I accepted the position and in January 1974 I headed south to Port Aransas, Texas, on the coast of the Gulf of Mexico. In hindsight, my decision to study at UTMSI with Parker was without question, the right choice. Parker's research on the relationship of modern organisms to the compounds found in Recent sediments, as well as his interest in carbon isotopes, set the path for my career in biogeochemistry.

In early January, I flew down to Texas from my family home in New Jersey. My one suitcase was filled with woolen winter clothes, leather boots, and only a few summer things. I brought my pillow and a typewriter. Parker picked me up at the airport in his old VW bug, rusted out on the sides. He was a modest looking man with a small mustache, a bit of a limp, and shaggy brown hair. I had formed a different mental impression of him based on his *Science* papers. We drove to Mustang Island, where the Marine Science Institute was located, with the final portion of the journey *via* ferry. Coming from urbanised Southern New Jersey, Port Aransas seemed like a town literally at the end of the earth for me. The next day I met Professor Chase Van Baalen. Chase was often mistaken for one of the maintenance staff. A short fellow with a crew cut, Van Baalen smoked a pipe, and was known for shoving his lit pipe deep into the pockets of his jeans. His right hand lab assistant, Rita O'Donnell, took care of the lab glassware and culturing facility for Chase. She was a crusty, Port Aransan, who also owned and managed a popular motel with her husband. Parker's lab manager, Ken Winters, was a former Ph.D. student of Pat's. A real Texan, Ken was polite and funny and fortunately for me, very organised.

I settled in quickly, meeting and getting to know other graduate students and postdocs. My first mini-project was to assay naturally occurring iron-binding compounds made by microorganisms. I gathered algal mats from nearby lagoons and shallow marine sediments, extracted them, and then placed the extracts on a lawn of bacteria that required an iron-binding compound to grow. We discovered that organic, iron-binding compounds were widespread in the natural environment, not just secretions from laboratory microbes. It was a winning small project, and the subsequent publication, standing the test of time, is still relevant (Estep *et al.*, 1975).

2. RUBISCO AND CARBON ISOTOPE FRACTIONATION

Along with Van Baalen and student John Calder, Parker noted that the carbon isotope fractionation between bulk tissue and dissolved CO_2 measured in natural cyanobacterial mats and in algal cultures was highly variable and lower than that measured in Precambrian rocks (Calder and Parker, 1968). The significance of this work, that carbon isotopic compositions of organic matter could be proxies for palaeo-CO_2 concentrations, was only realised much later. When I arrived in Texas, this quandary dominated the Parker-Van Baalen laboratories, since it was assumed that organic matter in Precambrian rocks originated from similar modern environments present in the hypersaline lagoons of south Texas.

After the study on iron-binding compounds, I started my Ph.D. project with funding from a NASA Exobiology grant working with Parker, Van Baalen, and microbiologist/enzymologist F. Robert Tabita. Calder and Parker were the first to determine that the concentration of carbon dioxide in growth media (or seawater) determined carbon isotope fractionation ($\Delta^{13}C$) between CO_2 and fixed organic carbon (Calder and Parker, 1968). Later work by Pardue, Parker, and Van Baalen introduced the concept that substrate limitation, as measured by cell density at harvest, was key to carbon isotope fractionation: slow growth and lower cell density resulted in greater isotope fractionation; faster growth and greater biomass, less fractionation (Pardue *et al*., 1976) (Fig. 2.1). Both of these concepts were exploited by John Hayes, Brian Popp, Bob Bidigare, and Kate Freeman in understanding the role of substrate availability and growth rate on carbon isotope fractionation by marine phytoplankton (*e.g.*, Bidigare *et al*., 1997; Hayes, 2001). My Ph.D. research was to purify the enzyme Rubisco from various organisms and measure the isotopic fractionation *in vitro*.

Harmon Craig wrote the first blockbuster paper on "carbon isotopes in everything" from diamonds to an unwitting discovery of a C_4 grass (Craig, 1953). His later work tracked major carbon isotope systematics in the world's oceans. At the same time, Melvin Calvin and A. A. Benson were using radiocarbon to track the pathways of carbon fixation (Calvin and Benson, 1948). A paper by Park and Epstein (1960) linked the two types of studies and measured, for the first time, the carbon isotope fractionation by the CO_2 fixing enzyme Rubisco. Using a purified enzyme, bicarbonate, and ribulose 1,5-bisphosphate (RuBP), they calculated an isotope fractionation of -24 ‰, indicating the lighter isotope of carbon was favoured over the heavier one during the reaction. Not much was known about the structure and function of Rubisco at this time.

Work in the late 1960s and early 1970s on the structure and function of Rubisco brought new interest to the carbon isotope fractionation studies in plants. Bob Tabita, then a recent Ph.D. and postdoc, brought his skills in purifying Rubisco as an Assistant Professor at the University of Texas. His work centred on isolating and describing the different forms of bacterial Rubisco enzymes – their structures, kinetics, and activations. Rubisco from higher plants

has a molecular weight of 550,000 and is composed of 8 large subunits and 8 small subunits. Park and Epstein's experiments with Rubisco carbon isotope fractionation used enzymes from the tomato plant.

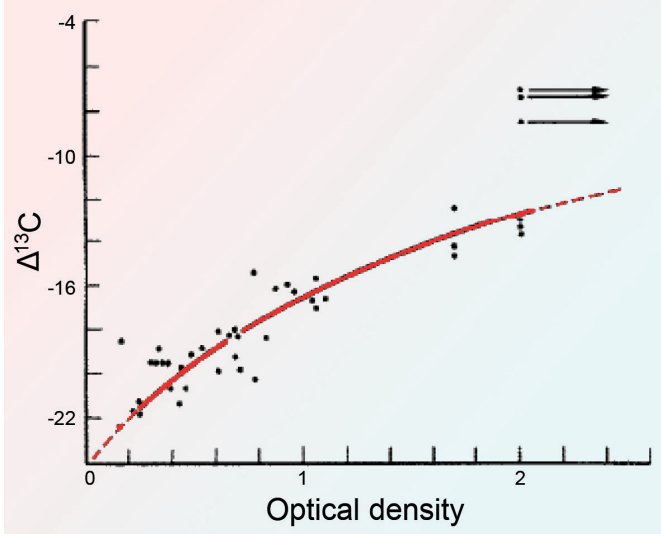

Figure 2.1 This graph was hotly contested when I arrived at UTMSI as a graduate student in 1974. Why the carbon isotope fractionation should decrease with increasing cell density was not known at the time. Now, we know that the cells became CO_2 limited at higher densities (greater O.D.). Modified from Pardue *et al*. (1976).

My Ph.D. research started with purified spinach Rubisco followed by isolating and purifying this enzyme from various microorganisms. The idea behind this work was to figure out why carbon isotope measurements of modern and ancient sediments and rocks were so different from one another. Precambrian stromatolites were thought to be microbially produced structures formed primarily by cyanobacterial primary producers. One question that persisted was whether or not the Rubisco from cyanobacteria had the same degree of carbon isotope fractionation as the enzyme from higher plants. At the time, Tabita and others thought that the cyanobacterial enzyme had a different structural form from the higher plant Rubsico, although subsequently this was shown not to be the case. The photosynthetic bacteria, *Rhodospirillum rubrum,* produced two different forms of the enzyme: form I, the high molecular weight form, and form II, a Rubisco with a smaller molecular weight. We wondered whether the different forms might be associated with different enzymatic mechanisms and consequently different isotope fractionations.

At the time Rubisco isotope research was a very hot field. A group from New Zealand published experiments using soybean enzyme (Christeller *et al.*, 1976), which showed little effect of bicarbonate concentrations or temperature on fractionation. Average values for the soybean enzyme were -28.3 ‰. A team led by Roy Benedict and Bill Sackett at Texas A & M published work using sorghum Rubisco and measured a carbon isotope fractionation ($\Delta^{13}C$) of -33 ‰ at 24 °C and -18 at 37 °C (Wong *et al.*, 1979). We did not find this work compelling given the large differences between the two temperature treatments. Further, the Texas A & M group used a cell-free extract, but not purified protein. Deleens *et al.* (1974) experiments gave carbon isotope fractionation values from -40 to -80 ‰, a range that was completely beyond that of known measurements in natural samples. Neither of these groups used Rubisco from taxonomic groups other than higher plants.

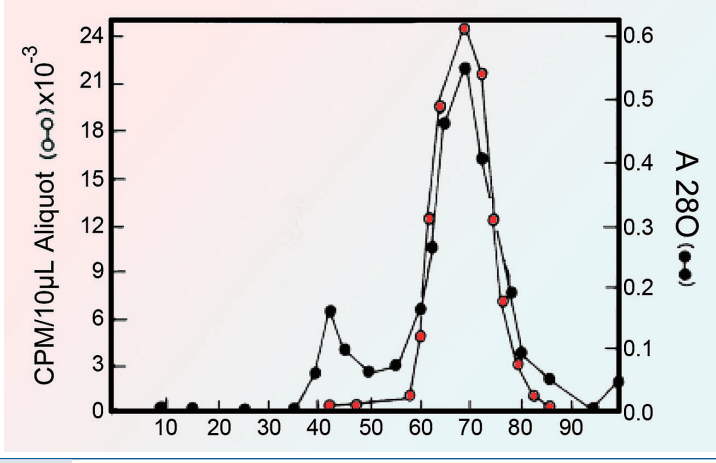

Figure 2.2 I purified Rubisco for most of the experiments I conducted for measuring carbon isotope fractionation. At that time, we were concerned that other enzymes could modify the product, PGA, and change fractionation values. Modified from Estep *et al.* (1978a).

My challenge was to grow microorganisms in large quantities from litres of pure cultures and to purify the Rubisco to homogeneity, *i.e.* isolating it from all other proteins. The work required that I learn sterile techniques and microbial culturing. Once the organisms had been harvested, enzyme purification required column chromatography using size exclusion principles. The location of Rubisco separated by chromatography was assayed by ^{14}C bicarbonate uptake measurements (Fig. 2.2). Once the Rubisco was identified, it was precipitated and washed, re-chromatographed, then analysed on a polyacrylamide gel to confirm homogeneity. I purified large amounts, milligrams of pure protein, for the isotope experiments. Rubisco was incubated with milligram quantities of the substrate RuBP with an excess of bicarbonate in a buffered solution. A

separate enzymatic assay determined when all of the RuBP was used up. At this point, each of the 40 experiments was terminated and the product, phosphoglyceric acid (PGA) was purified by crystallisation.

Once the PGA was purified, I proceeded to Pat Parker's aging "Craig line" where my precious sample was introduced under vacuum. It was then pushed into a furnace in which oxygen was added, the PGA was combusted, and CO_2 was produced. The $\delta^{13}C$ of carbon dioxide was analysed on a 1960s version Nuclide 6" isotope ratio mass spectrometer (IRMS) complete with decade boxes and strip chart recorders for recording isotope ratios. In those days, measurements were completed by hand using rulers and slide rules to calculate $\delta^{13}C$ values. Results from all 40 experiments produced an average $\Delta^{13}C$ of -34 ‰. For spinach (form I higher plants), we measured $\Delta^{13}C$ values of -36.5 ‰, whereas microbial Rubiscos had $\Delta^{13}C$ of -28 to -32 ‰ (Estep *et al.*, 1978a) (Fig. 2.3). Our work showed that different enzyme forms and different metal cofactors resulted in variations in $\Delta^{13}C$. We also showed that the enzymatic isotope fractionation was greater than that measured *in vivo* with whole plants and cells. This finding led to further studies by Farquhar *et al.* (1982), who developed mathematical equations for carbon isotope fractionation by C_3 and C_4 plants.

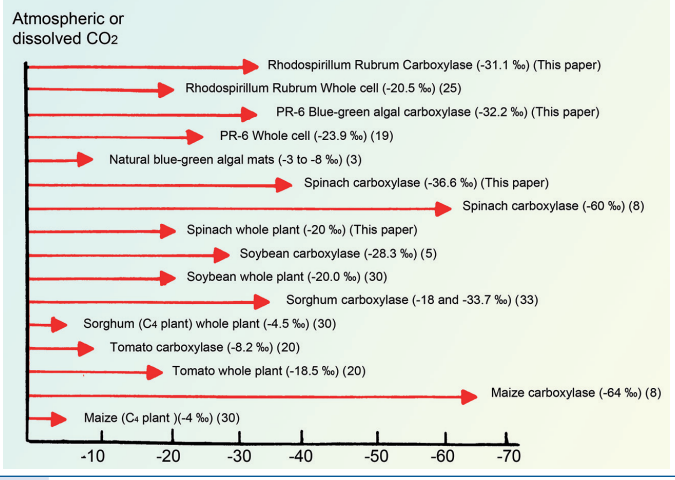

Figure 2.3 Carbon isotope fractionation values($\Delta^{13}C$) for whole plants/microorganisms compared to fractionation values($\Delta^{13}C$ of dissolved CO_2) by their RuBP carboxylases. All fractionations are with respect to dissolved CO_2 or atmospheric CO_2. Length of arrows indicates fractionation value. Modified from Estep *et al.* (1978a).

My final work at UTMSI was the purification of Rubisco from a marine diatom, *Phaeodactylum tricornutum*, with subsequent $\Delta^{13}C$ measurements of -32 ‰ (Estep *et al.*, 1978b). Diatom Rubisco was a form I enzyme, the same form as all of the higher plant and cyanobacterial Rubisco proteins, something that was not recognised at the time (Tabita *et al.*, 2007) (Figs. 2.4 and 2.5).

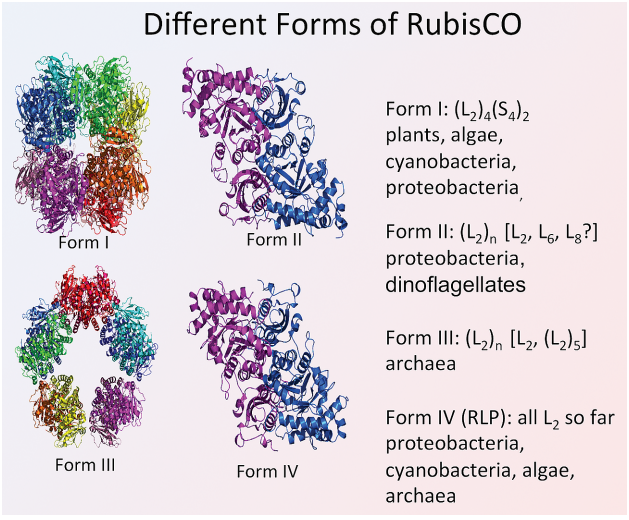

Figure 2.4 Different forms of Rubisco from various organisms. Plants, algae, cyanobacteria, and some proteobacteria have enzymes with both large and small subunits (Form I) (see Tabita *et al.*, 2007). Different Rubisco forms (II, III, and IV) fractionate carbon isotopes slightly different. Figure from F. Robert Tabita.

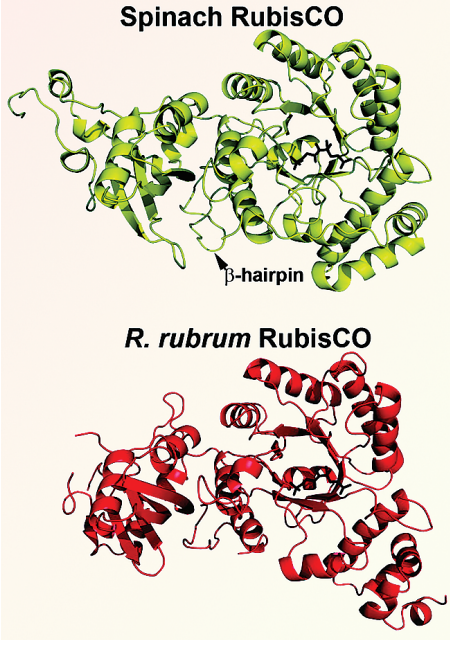

Figure 2.5 The active centre of the spinach and R. rubrum Rubisco enzyme. The active site of the enzyme is highlighted by the amino acids in black. From Tabita *et al.* (2007) with permission from the American Society for Microbiology.

3. EARLY GEOPHYSICAL LAB 1977-1983

Every summer, the esteemed Dr. Tom Hoering would come to the Marine Science Institute in Port Aransas on "vacation" which generally consisted of him hanging around the lab and chatting with the graduate students and postdocs. The summer before I graduated, he stood in the hallway and quizzed me informally about my work and my findings. At the end of the conversation, he remarked that the Geophysical Laboratory might be an excellent place for me to consider for a postdoc. I was thrilled! As the year went by and I wrote up my work, I applied to two places: University of Georgia to work with Clanton Black, a noted plant physiologist who dabbled in stable isotopes, and the Geophysical Laboratory of the Carnegie Institution of Washington. I received offers from both, and it was a fairly easy choice to go to Washington, DC, and work on hydrogen isotopes with Tom Hoering.

The Geophysical Laboratory was the polar opposite of the Port Aransas Marine Lab in almost every way. In Texas, I came to work in shorts, a ragged T-shirt; shoes were optional. At the Geophysical Laboratory, shorts were out of the question. I needed to purchase a week's worth of "work" clothes—khaki pants, blouses, and decent shoes. The staff scientists at that time were all men, who came to work wearing ties and white shirts. The halls of the lab were quiet; the average age of people I saw on a regular basis was about 50. They were all geochemists; I was the only biologist. The desk I was assigned was once occupied by "Mrs. Navrotsky", who I later learned was Dr. Navrotsky, a never married and very distinguished geochemist. I required a sterile environment to do culturing work, so a small plywood "hood" was constructed for me in Tom Hoering's back lab, which I shared with Doug Rumble and a fuming, BrF_5 oxygen isotope extraction line.

I arrived at the Geophysical Lab with cultures of microalgae from Van Baalen's lab. Within a few weeks, I learned how to measure hydrogen isotopes (see Text Box 3.1) in water, oils, and plants. I purchased supplies for growing algal cultures and obtained an old autoclave from our sister institution, the Department of Terrestrial Magnetism. In my office, I set up an aquarium I'd purchased at a yard sale as a water bath, adding gas mixing lines and a temperature controller for growing pure, axenic cultures of microbes. The field of hydrogen isotopes in plants and algae was wide open at this point in time. After six months, I gave Tom Hoering a report of my work:

> *"During the past six months, I have been investigating hydrogen isotope ratios in plants. A survey of δD values of various terrestrial plants, including lichens, liverworts, mosses, a fern, and angiosperm leaves, was attempted. As others have found, δD values of metabolic H in plant tissue were depleted in D by approx. 50 ‰ from associated environmental water. There seemed to be no correlation between δD and the type of terrestrial plant. Second, leaves from certain branches of a certain tree were monitored for three weeks to determine whether sampling introduced large variations in δD. The precision of these leaf δD analyses was generally ±4 ‰. Leaves on different branches of the same tree have basically the same isotope ratio although*

one leaf sample during the three-week period showed a considerable variation, 34 ‰. Similar experiments with three species of mosses showed similar results, although one moss species was consistently enriched in D."

Today viewed from a 40 year perspective, these findings are both simplistic and profound because they highlight biological variations that capture the big trends. In fact, data like these are fundamental to understanding isotope biogeochemistry of hydrogen isotopes in plants. My report also included data on microalgal culture experiments and some phytoplankton samples collected in Chesapeake Bay. After reading my two page report, I met with Tom who criticised my approach and told me "*I wouldn't have done things this way*." I answered that his opinion wasn't my own, and I was proceeding just as I had planned. I am not entirely sure what his approach would have been, but given his strong talent in chemistry, I think he would have moved more quickly to studying plant lipids.

As a biologist seeking replication, I needed to confirm that I wasn't making "one off" measurements with just "5 well chosen" natural samples. Before proceeding further, I needed to know if different taxonomic groups fractionated hydrogen isotopes in the same way or in different ways. These fundamental questions obviously did not appeal to a geochemist, but it was important for me to follow my own ideas to completion.

Text Box 3.1 – Hydrogen Isotope Methodology

In the 1970s, the method for measuring hydrogen isotopes in organic compounds was a three step process. For organic samples, after thorough grinding, about 5 mg was weighed into a ceramic combustion boat and inserted into a vacuum line where oxygen was introduced. The sample was then pushed into a 900 °C furnace. A pump circulated the gases from combustion including carbon dioxide, water, and nitrogen. The water was frozen into a 6 mm Pyrex tube, which was sealed off with a torch. For every single measurement reported in a paper, the sample was analysed about 5 to 6 times. The tubes with water samples were then attached to a vacuum line in which water was converted to H_2 gas by reaction with hot uranium at 750 °C. This vacuum line used a Toepler pump, a frightening, fragile glass contraption that cycled mercury up and down a glass column to move gases around. The combination of 100 kg of mercury and hot uranium made this a particularly dangerous part of the analysis. Hydrogen gas was collected in a sample bulb with a glass stopcock, which was then taken to a Nuclide 3" mass spectrometer especially designed for measuring D/H. The inlet systems of mass spectrometers in the 1960s and 1970s had mercury pistons to compress the gas samples to match the sample and standard pressures. If you were on top of things and paid attention, you could analyse 10-12 individual aliquots in two days, which resulted in two publishable data points.

The reason for the multiple analyses centres are "memory effects", which were difficult to eliminate with these methods. The combustion line was constructed from 8mm Pyrex glass "lines" with ample charged surfaces that had the potential to adsorb water. The uranium conversion line held a "memory" caused by some remaining hydrogen that was adsorbed onto the uranium and other dead volumes in the line. After the organic hydrogen had been converted to H_2 gas, memory effects were diminished, but great care was needed. Within the ion source of the older isotope ratio mass spectrometers, we ran sample *vs.* standards long enough so that mixing within the inlet system or the source did not affect the results.

Other than the research Tom Hoering and I were carrying out at Carnegie during my postdoc, Sam Epstein and his students at Caltech were turning out the majority of work on organic hydrogen isotopes (Epstein and Yapp, 1976). Epstein's work focused on measuring the isotopic composition of non-exchangeable hydrogen in cellulose purified from tree rings. He and his colleagues were using δ^2H values in this fraction for studying palaeoclimate in the Holocene (Epstein *et al.*, 1977) (Fig. 3.1). The basis of their work centred on the assumption that temperature affects the δ^2H of environmental water, and that the hydrogen in environmental water equilibrates with glucose monomers in cellulose during synthesis, often in a matter of hours. The δ^2H of whole or "raw" plants was only loosely correlated with similar values in non-exchangeable hydrogen in cellulose, implying that the δ^2H of whole plant tissue was unreliable as a tracer. The premise of this work was based primarily on temperature-based isotope exchange of water with cellulose. My work showed that the δ^2H of plants was determined by biosynthetic reactions, not simple exchange.

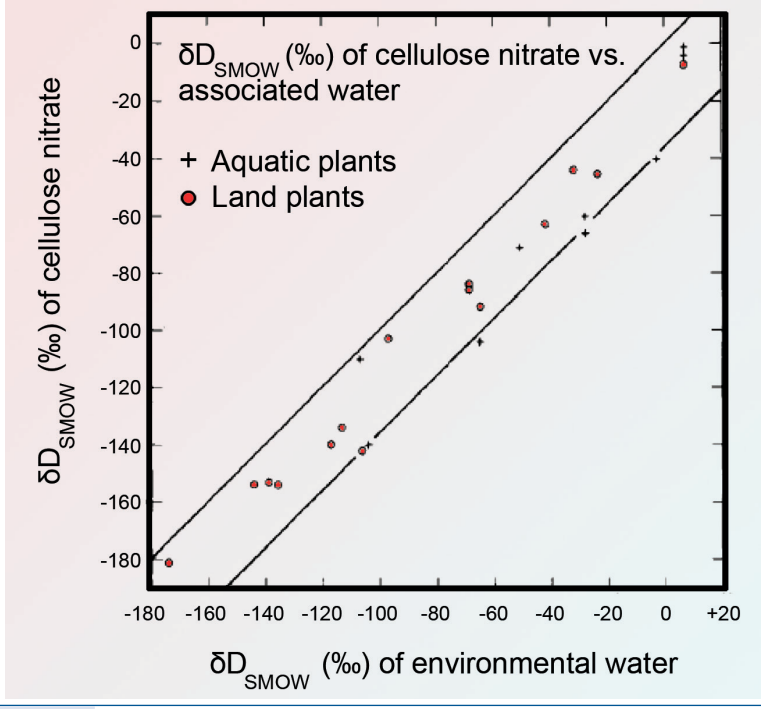

Figure 3.1 This figure summarised the idea in the 1970s by showing that the hydrogen isotope composition of cellulose purified from tree rings could be directly related to the hydrogen isotope composition of environmental water. Subsequent work, however, showed that this relationship was more complicated than originally thought. Modified from Epstein *et al.* (1976).

The experiments that I conducted with microalgae taught me that the water used in actively growing plant cells is not environmental water—there is a considerable lag phase between the time when water enters a cell and when it is used in biosynthetic reactions. Based on changing light conditions and using photosynthetic inhibitors, I learned that NADPH producing reactions in photosynthesis are key to labelling most of the organic hydrogen in plant tissue (Estep and Hoering, 1980, 1981) (Fig. 3.2). It has recently been shown by modelling that NADPH production determines the δ^2H of organic hydrogen, particularly lipid-bound hydrogen (Sachse *et al.*, 2012) (Fig. 3.3).

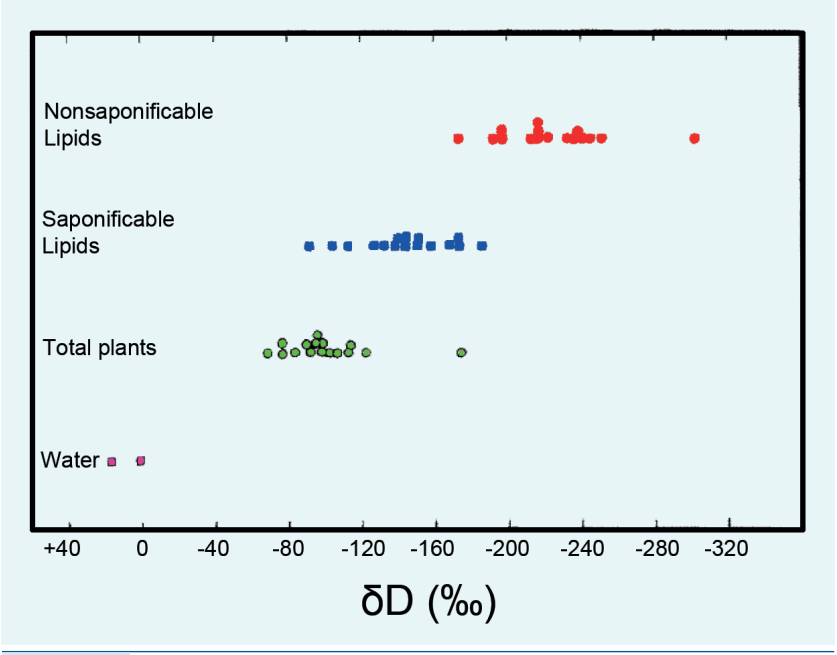

Figure 3.2 The δ^2H (δD as in the graph) of plants and lipids extracted from them are depleted in ^2H relative to environmental water. Nonsaponifiable lipids, *e.g.*, sterols, have much more negative δ^2H than saponifiable lipids, *e.g.*, fatty acids. Modified from Estep and Hoering (1980).

With the advent of compound specific isotope analyses in the early 1990s, the δ^2H of lipids and their relationship with environmental water or water in the growth medium can be accurately measured (Sessions *et al.*, 1999; Sachse *et al.*, 2012). The δ^2H of C-29 hydrocarbons, which originate from plant leaf waxes, show a strong correlation with the δ^2H of rainwater ($r^2 = 0.80$), but the slope of the relationship is only 0.55. This suggests that other sources of hydrogen contribute to the δ^2H value of lipids. In the natural environment salinity, light

intensity, and membrane permeability all affect the production of NADPH in photosynthetic plants, while biosynthetic pathways using the NADPH to form lipids result in additional variations in isotopic fractionation. The δ^2H of C-29 hydrocarbons also varies as a function of the type of plant: C_3 *vs.* C_4 plants, grasses *vs.* forbs, therefore caution is needed when using the δ^2H of plant leaf waxes for reconstructing palaeoenvironmental conditions (*e.g.,* Tierney *et al.*, 2008) (Fig. 3.4). Moreover, when considering marine environments, salinity appears to affect the way in which the δ^2H of lipids are determined, with more positive isotope fractionations in cells grown in more saline water.

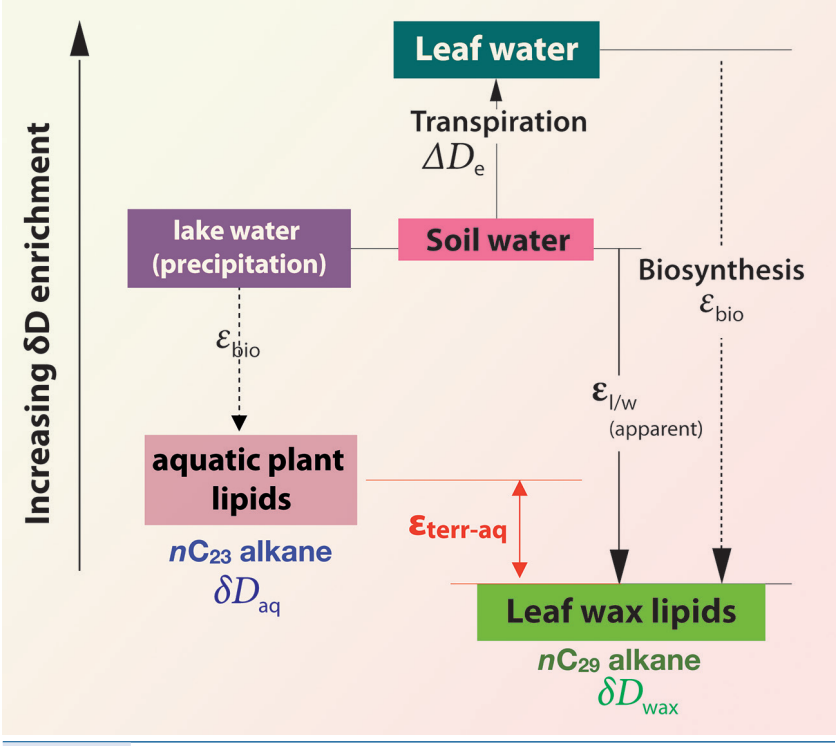

Figure 3.3 Schematic diagram of hydrogen isotope fractionation of lipids and water in plants. Bulk δ^2H of plants fall between the δ^2H precipitation and the δ^2H of plant lipids. After Sachse *et al.* (2012).

Since cell membranes are complex structures, cells are not "open" systems with respect to water. Although some water diffuses through cell membranes, most water passes through specialised proteins called aquaporins that allow neutral water molecules, but not protons, to pass into the interior of the cell, thus enabling the cell to remain at neutral pH (Agre, 2006) (Fig. 3.5). Water in

solution is heavily H-bonded. The pore space of the aquaporin is 2.8 Å, just large enough for one water molecule to pass through at a time. A second step involves the water molecule attaching to an amino acid *via* H-bonding for efficient transport into the cell without resistance. In the 1970s, none of this was known.

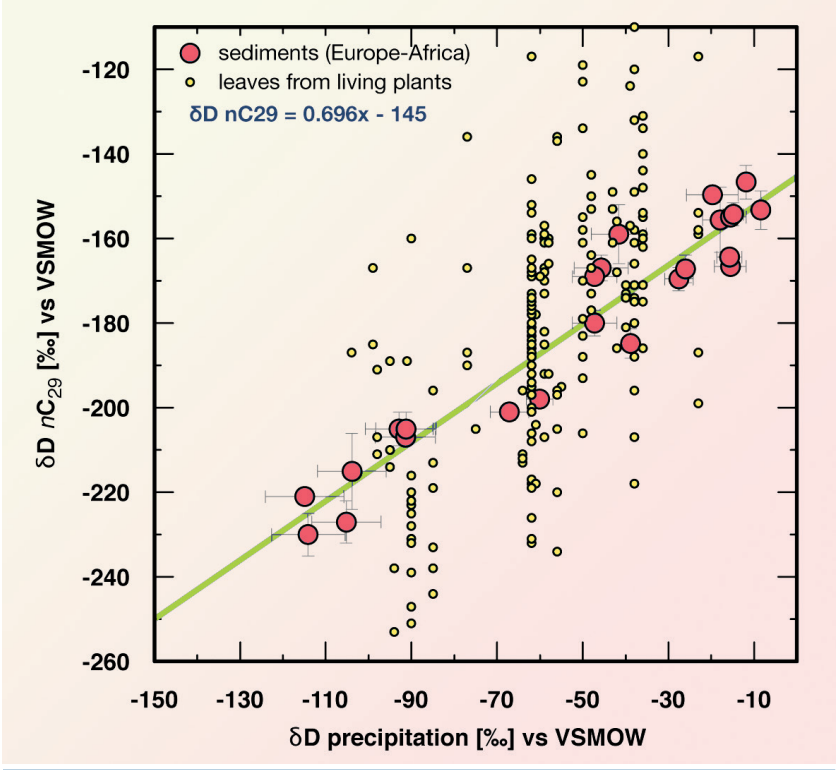

Figure 3.4 Relationship of hydrogen isotope composition in individual alkanes compared with δ^2H in precipitation is not constant. The large variations in plant alkanes needs further study to be useful for paleoclimate reconstructions. After Sachse *et al.* (2012).

Reviews of my first manuscript, submitted in 1979, on hydrogen isotope fractionation by microalgae were harsh. One reviewer remarked with sharpness: "*This paper suffers from a fundamental flaw in experimental design.*" The review primarily criticised the measurements on the bulk or total hydrogen in the algal cultures or plant leaves. The reviewer assumed that in living organisms C-bound hydrogen was not exchangeable with water, but hydrogen bonded to N, O, S, readily exchanged with any contact with water. The exchangeability "controversy" persisted for over 20 years, until finally a number of researchers

repeatedly showed that only about 10-15 % of hydrogen in molecules like keratin (*e.g.*, feathers) is exchangeable, whereas the remainder of the organic H, if bonded by covalent or ionic bonds does not (*e.g.*, Sessions *et al*., 2004; DeBond *et al*., 2013). An important outcome of the above-mentioned review was that I conducted a number of exchange experiments with algal cultures, both alive and dead, which showed that during the processing of samples for analysis, no measurable hydrogen exchange was detected. More drastic measures, like heating or sonication, which ruptured tertiary bonds was needed in order to promote greater hydrogen exchange (Estep and Hoering, 1980, 1981).

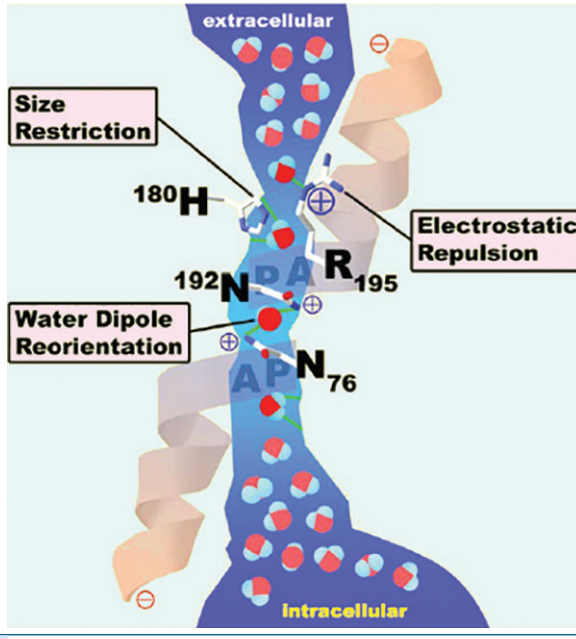

Figure 3.5 Illustration of how aquaporin enzymes operate in cells. Aquaporins are membrane bound proteins that control the movement of water into and out of cells. From Agre (2006).

Over time, ideas about 1) plant cellulose and its relationship to water and temperature and 2) the control of photosynthetic NADPH on isotope fractionation have both been shown to be correct even though substantial gaps still clouded our understanding of the mechanisms. Temperature does not affect the δ^2H of plants *per se*, but does determine, along with many other factors, the isotopic composition of precipitation and the extent of evaporative transpiration. Although a small portion (10-15 %) of organic hydrogen is exchangeable, the majority is not.

During my Ph.D. studies, fellow graduate student Brian Fry (the son of isotope chemist, Arthur Fry) worked with Pat Parker showing that "you are what you eat" in an elegant grasshopper and plant community study (Fry *et al.*, 1978). This study included detailed diet measurements and mass balance equations using the mixtures of C_3 and C_4 plants found in his study area. This paper remains a classic. At the same time, Michael DeNiro and Sam Epstein were growing mice in the laboratory and finding similar results (DeNiro and Epstein, 1978). In 1980, I was intrigued by these studies and set out to determine if hydrogen isotopes could also be used as a tracer for animal diet. My study included a laboratory-based experiment of mice as well as collections of marine snails and potential algal food sources from a natural environment study on the coast of Maine (Estep and Dabrowski, 1980). Essentially, I determined that the primary source of hydrogen in the organic tissues of animals originated from their food, rather than their drinking water (Fig. 3.6). The paper, published in *Science*, attracted the wrath of DeNiro and Epstein, who submitted a technical comment to *Science* arguing that my findings were invalid owing to hydrogen exchange (DeNiro and Epstein, 1981a).

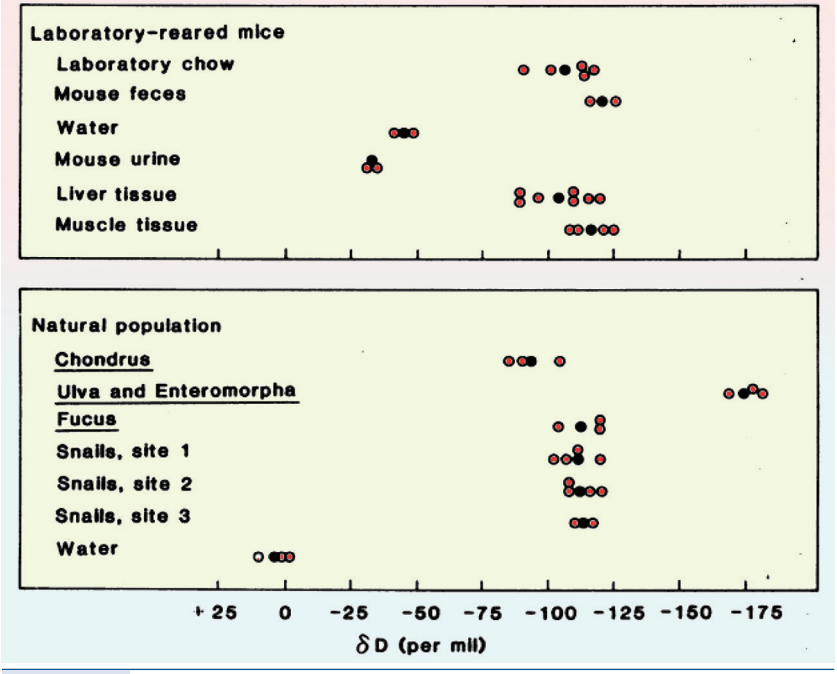

Figure 3.6 In this paper we concluded that the δ^2H of organic matter in animals derived principally from an animal's diet rather than its drinking water. Modified from Estep and Dabrowski (1980).

For a quiet, shy young woman from New Jersey and coastal Texas, being challenged by Caltech folks was unsettling. Tom Hoering supported me in writing my rebuttal: *"At present, there is some uncertainty about whether the isotopic composition of the hydrogen in prey and predators can be used to follow food chains, but similar criticism may also be applied to the use of carbon or nitrogen isotopes in analogous studies"* (Estep, 1981). DeNiro and Epstein's technical comment included data on hydrogen exchange experiments with mouse tissue that had been freeze-dried, ground, steamed at 100 °C, then analysed. It was no surprise that their experiment showed considerable isotopic exchange with the hydrogen in steam. I argued that because tissues were treated carefully and at room temperature in my studies, exchange was not the major controlling factor in the hydrogen isotopic composition of animal tissues. With time, it has been shown that about 20-30 % of the hydrogen in animal tissues comes from drinking water – not by random exchange, but by direct incorporation. The remaining 70-80 % originates from the hydrogen in the diet (Hobson *et al.*, 1999 and others).

Interestingly, although my original paper was the first to show the utility of hydrogen for tracing diets, it has only been cited about 120 times since it was published in 1981. This work had little impact until almost twenty years later when the methods for measuring δ^2H became easier using a thermo-chemolysis procedure that is fully automated. Although methodology was an important factor in delaying the impact of my work, another reason why this paper was largely ignored by the ecological community studying animal migration is that they did not, and still don't, care about hydrogen in food. All of the figures in ecological publications regress precipitation δ^2H *versus* tissue δ^2H. Those isotope biogeochemists in the know realise that food reflects local precipitation as water is the only source of hydrogen available to primary producers to build organic tissues. Broadening the ecological community's perspective on the influence of hydrogen from all of the major biochemical sources available to an animal has been a challenge.

During my two year postdoctoral fellowship funded by the Carnegie Corporation of New York, I started to apply for permanent positions in academia. I had produced solid work on the enzymology and isotope fractionation by Rubisco. I had transitioned to a new isotope system at the Geophysical Lab and showed I had become independent from my three Texas advisors and from Tom Hoering. My undergraduate degree was in Biology and my Ph.D. was in Botany (Marine Science), and now I was completing a postdoc at a prestigious earth science laboratory. With several papers and presentations under my belt, I began sending out applications for faculty positions. At that time (1978-1979), it was not an advantage to be a woman in almost any scientific discipline. Today, women scientists with talent often compete well for positions. Not so in the 1970s. Further, I didn't fit easily into either a regular biology department or an earth science department. Marine science positions were relatively rare, and

if they did exist, many did not have the type of analytical support (*i.e.* mass spectrometers) that I needed to do my work. At that time, it wasn't clear to me that being an interdisciplinary scientist could have its drawbacks.

After receiving over 20 letters of rejection, I finally had one offer for a second postdoc with a promise of a faculty position in two or three years at a marine science laboratory in South Carolina. At the same time, the Geophysical Lab was searching for a biogeochemist to fill a vacant staff member position. There was no position description and applications were by invitation only. Initially, I was not asked to apply. A couple of men, at my level of experience, interviewed. Things changed for the better when I gave a talk on my hydrogen isotope work at the Geological Society of America meeting in 1979. I was scheduled to speak just prior to an awards session honouring Harmon Craig. The lecture hall was packed with over 400 people in anticipation of hearing the great man speak. With good fortune, as I gave my talk, I noticed the President of the Carnegie Institution, Dr. Philip Abelson, directly in front of me in the audience. As I made my points, he nodded his head in agreement. I felt as though I was talking directly to him, and perhaps I was. The following week, I was called up to the Geophysical Laboratory Director's office to meet with Dr. Hatten S. Yoder to "*discuss my future plans*."

Apparently President Abelson returned from the GSA conference and phoned Yoder directly asking him, "*Why haven't I heard about this postdoc, Marilyn*?" I was invited up to talk with Hatten Yoder, who asked me to write a summary of what I might work on if I were to remain at the lab as a new staff scientist. After this conversation I floated out of Dr. Yoder's office elated and headed immediately to Tom Hoering's office. He knew all about this, of course, and encouraged me to write up my ideas. My proposal had three major projects that I would work on for the next three years: 1) the biogeochemistry of oxygen isotopes, 2) the role of bacteria in determining the isotopic compositions of sedimentary organic matter, and 3) biochemical processes of biomineralisation. I did not carry out these projects exactly as I'd written, but carved out studies with oxygen isotope fractionation of molecular O_2 during biological reactions, work on thermophilic microbes, as well as investigations into bacterial manganese mineral formation (see below).

Director Hatten Yoder wrote to then Carnegie President James Ebert the following:

> "*The present staff has the expertise to measure the principal stable isotopes and characterize the complex amino acids and mineral structures, but they do not have the knowledge to maintain the primitive living organisms needed in the proposed studies. After a thorough search, the staff organic geochemists and I have concluded that we already have a most talented, highly motivated, and successful potential staff member among our Postdoctoral Fellows*."

Within a week or so, I had an offer for a Temporary Staff Member position starting July 1, 1979. I was off to a solid career in Biogeochemistry.

4. INTERSECTION OF GEOCHEMISTRY AND ECOLOGY

As a new staff member, it was exciting to move into my own dedicated laboratory space. The original Geophysical Laboratory on Upton Street in Washington, DC, was built in 1908, had 18-inch walls and big hallways. Director Yoder assigned me a laboratory to share with retiring staff scientist, Gordon Davis. Davis' career focused on radiogenic dating of rocks. His laboratory was primarily a preparatory, "semi-clean" lab where he purified lead and other radiogenic isotopes for dating. For about a year, we "shared" the space with my culturing equipment on benches in the clean lab. It was an arrangement that would never really work, because I did not have full control over my lab space. Only many years later did I realise that this arrangement put the start of my career at a moderate disadvantage. Fortunately for me, after one year Gordon fully retired and I was able to renovate the laboratory, move in two isotope mass spectrometers, and build my first original vacuum line.

After investigating hydrogen isotopes for several years, my interests turned to oxygen in organic matter with an experimental plan similar to that I had carried out for hydrogen isotopes. The analysis of organic oxygen was, at this time, limited to materials with only C, O, and H, namely carbohydrates. The Caltech group was analysing cellulose in plants and tree rings. John Hayes and his student Kim Wedeking at Indiana University (Wedeking and Hayes, 1983) were revising a method, originally described by Rittenberg and Ponticorvo (1956), for analysing proteins and kerogens, compounds with N, S, and other elements. Tom Hoering and I followed their work closely. Their method, published only in Kim Wedeking's dissertation (Wedeking, 1983), was based on a reaction with mercuric chloride ($HgCl_2$) to form a mixture of CO and CO_2. Tom Hoering and I were working on a similar method. Organic matter was heated in an evacuated sealed tube with $HgCl_2$ at 500 °C. Products included both CO and CO_2, with HCl and other impurities that were separated by gas chromatography (Hoering and Estep, 1981) (Fig. 4.1). CO was converted to CO_2 by disproportionation in a high voltage discharge apparatus where excess carbon is plated out on platinum electrodes. The method was never robust, and as a byproduct of the high voltage discharge in the reaction chamber, NO_2 was formed from N_2 in organic matter. Multiple analyses of biological samples introduced NO_2 unwittingly into the flight tube of our homemade IRMS. NO_2 is notoriously sticky on metal surfaces. Eventually the IRMS would not pump down. A real disaster resulted in which Tom Hoering removed the flight tube, took it out on the lawn of the Geophysical Laboratory, and sandblasted it. In the environment of the Geophysical Lab, where everyone seemed to have research successes all the time, I felt deflated, maybe an "imposter", but I immediately moved on to other projects.

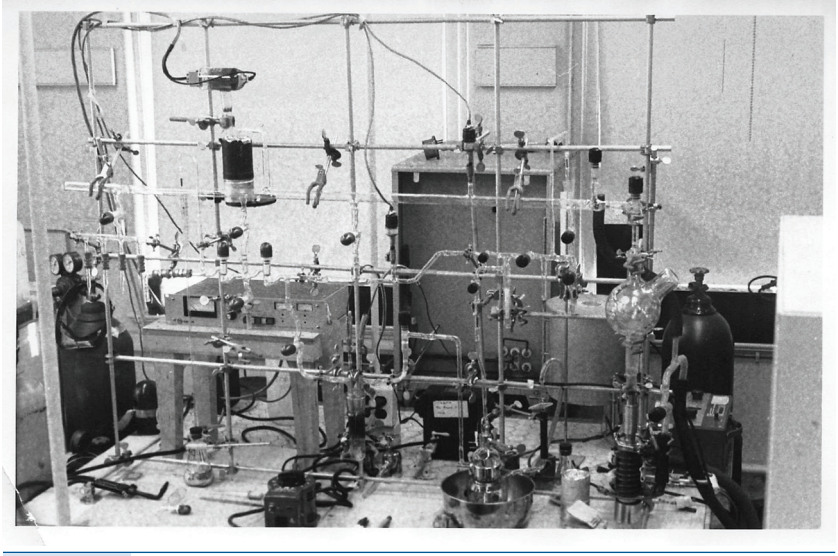

Figure 4.1 Oxygen isotope line in my laboratory at the Geophysical Laboratory on Upton St., Washington, DC. Photo credit: M. Fogel.

The analysis of oxygen isotopes in organic matter was my first failed project, but I learned a lot during the process. The most important lesson was learning to deal with failure. A scientist's life is filled with a certain amount of rejection: manuscripts, proposals, ideas. Only rarely does one get affirmation and accolades. Learning to not give up, but to keep searching for the next promising idea, is key to a successful long term career. For example, the vacuum line I designed was my first. Parts of it were constructed using Swagelock fittings for water-cooling lines. When the water was first turned on, the line dripped at every connection because I did not know how to assemble a Swagelock fitting properly. As Tom noted: "*You have these in ass-backward.*" In this system, I learned preparative gas chromatography, a skill needed for later work on oxygen isotopes in molecular O_2 and even later for continuous flow gas chromatography-combustion-IRMS (GC-C-IRMS).

Fortunately, I had the ability to shift my emphasis to two other major projects: the biogeochemistry of extremophiles living in hot springs and stable isotope biogeochemistry of nitrogen. On the way to attending a Plant Physiology conference in eastern Washington state in 1980, I made a trip through Yellowstone National Park armed with a newly purchased book authored by Thomas Brock entitled *Thermophilic Microorganisms and Life at High Temperatures* (1978). The juxtaposition of my earlier work with microalgal cultures and isotope fractionation in comparison to naturally growing algal and bacterial

mats struck me as the perfect analogous system with which to study Precambrian stromatolites. I devoured Brock's book and was determined to set my sights on carrying out an ambitious field-based study in summer of 1981 in Yellowstone.

I wrote and submitted a memo to Director Hat Yoder requesting permission to spend four months studying and collecting thermophiles in Yellowstone National Park:

> *"Carbon isotope ratios of naturally occurring blue-green algae have yet to be measured that show a similar fractionation to the Precambrian carbon. Some of these modern stromatolites consist primarily of photosynthetic, mat-forming bacteria, Chloroflexus. Perhaps the difference between modern blue-green algal* $\delta^{13}C$ *and Precambrian* $\delta^{13}C$ *is that the photosynthetic bacteria, not the blue-green algae, were responsible for the stromatolites found in the Precambrian."*

No one had measured the isotopic compositions of any of these organisms before. The idea that different carbon assimilation and metabolic pathways might impart different isotope fractionations was an exciting new lead. Yoder liked to keep a "tight ship" and to know what his staff were doing at all times. Every year in April, Director Yoder sent out a memo to staff members detailing the timeline for writing and producing the Carnegie Institution's Annual Yearbook Report. We termed this the "rigid adherence" memo because in the memo's first paragraph Yoder stated "*Rigid adherence to the schedule*" was required. I had to promise to handle all of my Yearbook submissions prior to departing for the west. Somewhat reluctantly, Hat Yoder approved my request. Armed with a permit from Yellowstone National Park (YNP), my first big field season began.

Carnegie scientists had previously worked in Yellowstone National Park. In 1935, E.T. Allen and Arthur Day published a megavolume on the chemistry and location of all of the thermal springs. Allen and Day consulted with a University of California botanist W. A. Setchell to learn about the organisms in the hot springs they were studying:

> *"Accepting the facts of biological research, the writers concede that algae are a factor favoring the precipitation of both travertine and silica from these thermal waters. Professor W. A. Setchell concludes from his observation in the Yellowstone Park and elsewhere, that a few species of blue-green algae are probably instrumental in the formation of limited amounts of both travertine and silica deposits, though they are not responsible for anywhere near all of either. Dr. Setchell lays special emphasis on several species of algae, within whose woolly or gelatinous layers masses of travertine, shaped by the growing plant, accumulate. These particular organisms may indeed exercise a more potent influence than the rest; still, from the chemical viewpoint, all photo-synthetic plants, so far as they derive their carbon dioxide from the water, should be regarded as a factor in the formation of carbonate."*

My field plans included sampling a series of hot springs with very different water chemistries. I highlighted carbonate springs *vs.* silica depositing springs *vs.* highly acidic springs. Brock and others had shown that even in nearly boiling

water at pH 2.0, organisms could survive and even thrive (*e.g., Sulfolobus*). For my first year of fieldwork, I had to choose hot springs that were within 1 to 5 km of a road as well as relatively hidden from Park visitors (Figs. 4.2 and 4.3). At this time, park regulations stipulated that the public should not view researchers in action, a policy that has changed since then. The silica depositing springs I chose were in the Lower Geyser Basin: Fairy Creek, Queen's Laundry, Wiegert's Channel, and Octopus Spring. As part of this work, I incubated glass slides in the spring to sample freshly grown material that matched with the environmental conditions that I was measuring at the same time (*e.g.*, temperature, pH, CO_2 concentration). Once sufficient growth was noted on the glass slides, biomass was scraped off and dried for isotopic analysis.

Figure 4.2 I am inspecting the thermophilic microorganisms in Mammoth Hot Spring, Yellowstone National Park, 1982. Photo credit: M. Fogel.

There were three major photosynthetic organisms in the neutral pH, silica depositing springs: *Synochocccus lividus* (coccoid, yellow cyanobacteria); *Chloroflexus* sp. (a phototrophic eubacteria); and *Phormidium* sp. (orange, filamentous conophyton-forming cyanobacteria). Rarely were these organisms found in isolated forms, however, temperature segregated the populations. *S. lividus* grew at the highest temperatures (60-75 °C); *Chloroflexus* slightly lower (48-65 °C); *Phormidium* at the lowest temperatures (40-48 °C). Carbon isotope compositions of each group varied by several per mille. *S. lividus* averaged $\delta^{13}C$ =-22.0 to -23.5 ‰ depending on the hot spring; $\Delta^{13}C$ = 14.5 ‰; ($\Delta^{13}C = \delta^{13}C$ inorganic

carbon source – $\delta^{13}C$ organic carbon). *Chloroflexus/S. lividus* mixtures averaged $\delta^{13}C$ =-22 ‰; $\Delta^{13}C$= 12.2 ‰, while *Chloroflexus* averaged only $\delta^{13}C$ =-16.2 ‰; $\Delta^{13}C$ = 7.2 ‰. *Phormidium/Chloroflexus/S. lividus* stromatolitic features called conophytons had $\delta^{13}C$ ranging from -18 to -20 ‰; $\Delta^{13}C$ = 9.4 ‰. The pink filaments from Octopus Spring, identified in the 1990s as *Aquifex* species (Flores *et al.,* 2012) had an average $\delta^{13}C$ of -16 ‰; $\Delta^{13}C$ = 7.3 ‰ (Fig. 4.4).

Figure 4.3 A neutral pH, silica depositing hot spring in the Upper Geyser Basin, Yellowstone National Park, 2016. Photo credit: M. Fogel.

In the early 1980s, the carbon fixation pathways in many of these organisms were not known. In the ensuing decades, detailed pathways for the diversity of microbial carbon fixation were discovered (Fuchs, 2011) (Fig. 4.5). *Chloroflexus*, for example, uses the 3-hydroxyproprionate cycle, whereas *Aquifex* sp. uses the reductive TCA cycle. Cyanobacteria all use Rubisco and the pentose phosphate pathway for CO_2 fixation. Unwittingly, I had been measuring microbes with three different CO_2 fixation pathways with attendant $\delta^{13}C$ signatures. House *et al.* (2003) published a paper summarising literature data from cultures, along with original data from culture experiments. Organisms using the 3-hydroxyproprionate pathway had the smallest $\Delta^{13}C$ averaging around 5 ‰; $\Delta^{13}C$ associated with the reverse TCA cycle was 5-10 ‰; and the $\Delta^{13}C$ of pentose phosphate pathway, 15-20 ‰ (Fig. 4.6).

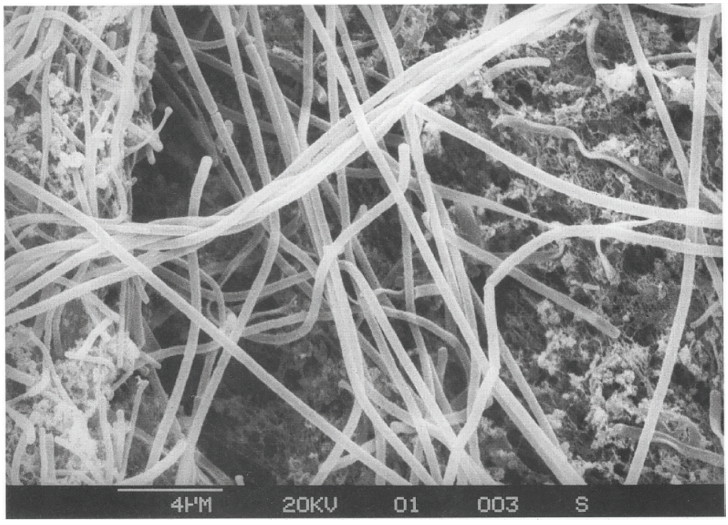

Figure 4.4 Pink filamentous bacteria, Aquifex sp. similar to those I sampled at Octopus Spring, Yellowstone National Park. From Reysenbach *et al.* (1994) with permission from the American Society for Microbiology.

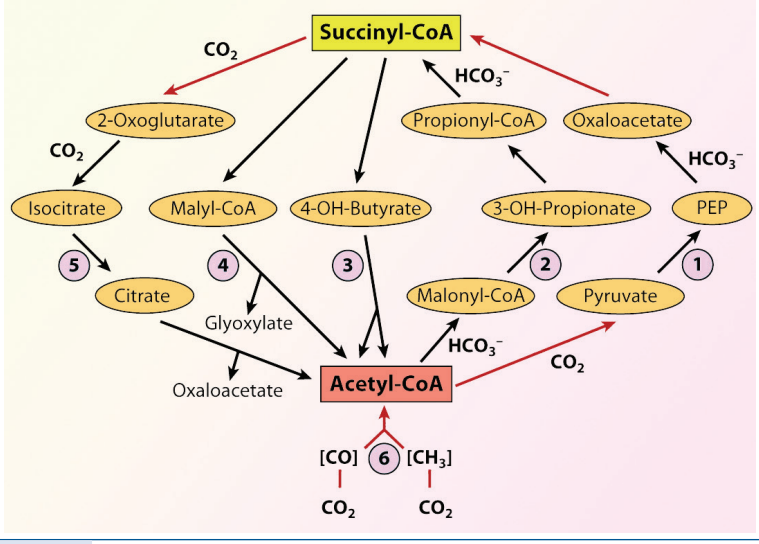

Figure 4.5 Six different carbon fixation pathways showing primordial central carbon metabolism. These different pathways (or routes) are combined in various ways for microbial carbon metabolism. Modified from Fuchs (2011).

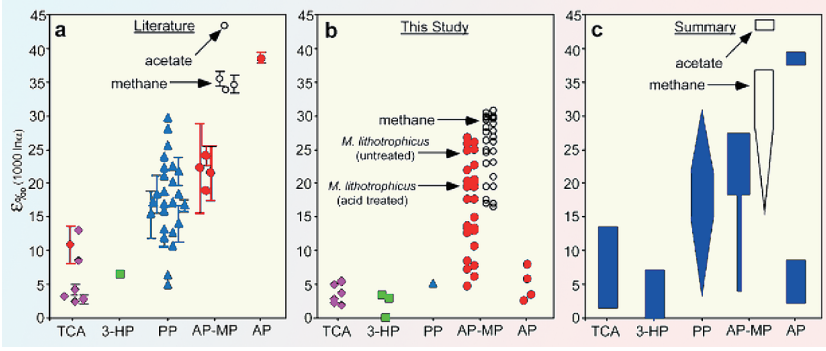

Figure 4.6 Illustration of what we now know about carbon isotope fractionation by microbes that use different carbon fixation pathways. Modified from House *et al*. (2003).

From this project, I learned how to carry out field work in a remote, distant location where one had to be resourceful and calculating. There were grizzly bears in the Park, bison and elk that commonly charged tourists, and of course, boiling hot springs with fragile crusts. The work required permitting, regulations, and reporting. The park supervisor required that researchers do their work out of the public view, something very difficult in crowded and popular areas. I chose sample locations off the beaten track. Taking samples and setting up biogeochemical measurements in the field, preserving the samples properly for subsequent isotopic analysis, and creating experimental protocols were all skills learned during the three summers in Yellowstone and carried through my years as a field savvy biogeochemist.

One of the major findings of my studies at Yellowstone was discovering the relationship between the concentration of inorganic CO_2 with the $\delta^{13}C$ of organic carbon fixed by cyanobacteria and other photosynthetic microorganisms (Estep, 1984) (Fig. 4.7). The relationship between $\delta^{13}C$ and carbon dioxide concentration was not well known at the time. I hypothesised that high concentrations of CO_2 in the Precambrian would have resulted in more negative $\delta^{13}C$ because Rubsico would have an unlimited supply of carbon for fixation. Organisms collected in acidic springs (pH 4-5) plotted on a different line, probably because the inorganic carbon source was CO_2 rather than bicarbonate at this pH. Around the same time, Farquhar *et al*. (1982) published their equations describing how intra- and extracellular partial pressures of CO_2 influence carbon isotope fractionation in higher plants. Subsequently, such relationships have proven to be important for interpreting palaeo-CO_2 conditions by measuring biomarkers in marine sediments (see Freeman, 2001, for a review). I also found that glucose and other organic compounds were readily taken up by these photosynthetic microbes (unpublished data). Today, the question of

photoheterotrophy by cyanobacteria remains an important consideration and may explain how the biological soil crusts in arid ecosystems are able to function at high temperatures and low water availability (*e.g.,* Stuart *et al.,* 2016).

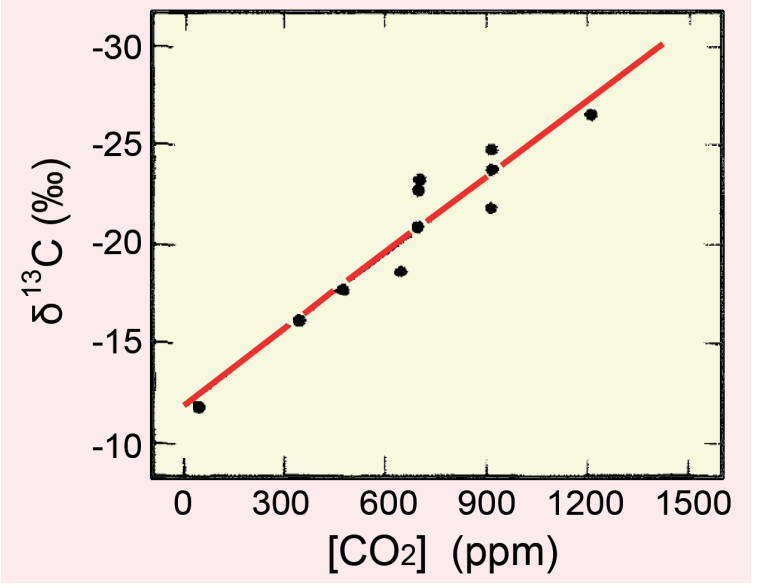

Figure 4.7 Relationship between $\delta^{13}C$ and CO_2 concentration at neutral pH springs in Yellowstone National Park. Modified from Estep (1984).

5. NITROGEN ISOTOPE BIOGEOCHEMISTRY

In September 1982, my first postdoctoral fellow, Dr. Steve Macko, arrived from UTMSI. He brought with him not only boundless enthusiasm and a plethora of ideas, but the technical wherewithal to measure nitrogen isotopes in organic matter. Macko quickly set up sealed tube methods for analysing organic matter at the Geophysical Lab. Steve came with a host of marine science projects, collaborators from around the globe, as well as a zeal to learn as much as he could. His main project was to culture microorganisms, then separate and purify amino acids for isotopic analysis, similar to the original work of Abelson and Hoering (1961).

This work involved all three staff members in biogeochemistry: me for culturing and biological aspects, Ed Hare for separation of the amino acids; and Tom Hoering for IRMS and other isotope support (Fig. 5.1). To carry out these experiments, I needed to ramp up my culturing ability in order to grow the substantial amounts of material needed for amino acid isotope analyses. Ed Hare needed to produce grams of the special ion exchange resin required to separate milligram quantities of amino acids. Hare and Macko worked hard in order to have the amino acid analysers going full time to assay compounds as they eluted from the separation column. We used two IRMS instruments for the isotope measurements: a newer Nuclide 6″ IRMS for carbon and for nitrogen a much older IRMS constructed at the Geophysical Laboratory by Hoering and others.

Figure 5.1 P. Edgar Hare (left), Tom Hoering (right), and me at the Airlie House, 1982. Photo credit: M. Fogel.

It took several weeks to grow the bacterial and algal cultures. We analysed the bulk isotope values, then hydrolysed the sample to produce hydrochloride salts of amino acids. Individual amino acids in the hydrolysate were separated using column chromatography which took 24 to 48 hours to complete. The fact that amino acids do not separate at regular or specific time intervals was a problem. The trick was to use a separate liquid chromatography system to determine when each amino acid started to elute and when it finished eluting. In this way we could to isolate and capture each amino acid in its entirety but separate it from other amino acids in the sample. Fractions were collected from the eluant every few minutes. After analysis *via* liquid chromatography, fractions were pooled to capture the entire amino acid eluting from the column. This result resulted in about 13-15 samples, each containing the hydrochloride salts of a single amino acid. The samples were then dried by evaporation, and the amino acids were weighed into quartz tubes, sealed, and combusted. The final steps were purification of gases and mass spectrometric analysis. This was an elaborate and slow process where each sample took a total of about 4 to 6 weeks from start to finish, depending on whether everything was working in tip-top order (Macko *et al.,* 1987). Today, experiments of this sort can be accomplished with much less material (10 mg), and by automated analysis, where five to ten samples can be measured for both $\delta^{15}N$ and $\delta^{13}C$ in a week, including isotope standards.

This laborious work established that the framework we established in the 1980s was important for subsequent measurements of $\delta^{15}N$ in amino acids from animals, a critical method that is required to study ecological trophic levels. With the exception of aspartic acid in one of the cultures, the $\delta^{15}N$ of glutamic acid had the most positive values. In experiments where *Anabaena*, a filamentous N_2-fixing cyanobacterium, was grown on either NO_3, NH_4, or N_2 as the nitrogen source, there was considerable difference in the bulk $\delta^{15}N$ between the three different sources. Amino acids from cells grown on NH_4 had the most negative values, whereas amino acids from cells grown on NO_3, had the most positive $\delta^{15}N$ values.

The majority of amine groups on amino acids are transferred *via* transamination from one amino acid to the keto-group of an organic acid. Evidently, there is significant isotope fractionation in this step, otherwise all of the amino acids would have very similar values. For *Vibrio harveyii* cultures, we measured patterns similar to the cyanobacterial data which meant that the two diverse organisms used many of the same enzymes and biosynthetic pathways. Two small differences were measured in the difference between the $\delta^{15}N$ of glutamate and the $\delta^{15}N$ of phenylalanine ($\delta^{15}N_{glu} - \delta^{15}N_{phe}$). In the cyanobacterial samples, the $\delta^{15}N_{glu} - \delta^{15}N_{phe}$ was +1 to -2 ‰, whereas in the *Vibrio* cultures, it was +2.5 and +4.5 ‰. Glutamate is synthesised from an intermediate of the TCA cycle and is involved in many reactions in which nitrogen is transferred to other amino acids. Phenylalanine, on the other hand, is formed at the end of a series of biochemical transformations and is not routinely involved in other pathways, with the exception of tyrosine formation in high plants. $\delta^{15}N_{glu} - \delta^{15}N_{phe}$ of

primary producers (β) is now an essential parameter in amino acid $\delta^{15}N$ based equations that estimate animal trophic position (McMahon and McCarthy, 2016). Ecologists are using a canonical value of +3.4 ‰ for β to interpret marine food webs, even though there is no clear understanding of why the isotopic relationship between glutamate and phenylalanine should not vary among organisms that can synthesise these two amino acids *de novo*.

The $\delta^{13}C$ of the amino acids in both the cyanobacteria and the *Vibrio* cultures were remarkably similar (Macko and Estep, 1984; Macko *et al.*, 1987). More recently, scientists have used linear discriminant analyses to create isotopic "fingerprints" of primary producers (Larsen *et al.*, 2009, 2012). These are helpful for determining the relative importance of different primary producers in fueling food webs, and for identifying dietary resources for animals (*e.g.,* detritivores) for which traditional proxies are not useful. The isotope fractionation patterns we measured back in 1982 (and published formally five years later) were related to the biosynthetic pathways that created them. In subsequent reviews (*e.g.*, Hayes, 2001), $\delta^{13}C$ patterns were related to specific biosynthetic pathways: glycolysis, the TCA cycle, or branched chain amino acid pathways. We also determined that the range in $\delta^{13}C$ of amino acids is almost twice as large in primary producers as it is in secondary consumers (*i.e.* bacteria). It was not until we extended these measurements to animal tissues that their power became evident (Hare and Estep, 1983). The $\delta^{13}C$ can be used to distinguish the input of essential amino acids originating from the base of the food chain.

Another pivotal postdoctoral fellow from this time was Michael Engel. Engel and Macko became fast friends and colleagues, a relationship that has lasted over 30 years. Michael Engel and his major professor Bart Nagy at the University of Arizona measured an enantiomeric excess of some acids found in the Murchison meteorite (Engel and Nagy, 1982). Engel came to work with Ed Hare to learn as much as he could about amino acid analytical techniques. Macko and Engel published their first continuous flow paper in 1991 (Silfer *et al.*, 1991), with multiple papers published in the coming decade on isotopic compositions of amino acids from meteorites, which confirmed Engel's earlier assertion that the enantiomeric excess he measured and published with Nagy in 1982 was in fact correct.

While Engel and Macko were at the Geophysical Laboratory, we conducted experiments measuring nitrogen isotopic fractionation by the glutamate-aspartate transaminase (Macko *et al.*, 1986) (Figs. 5.2 and 5.3). This was my second enzyme isotope fractionation experiment and for several reasons was much easier than working with Rubisco. First, we could purchase the enzyme directly from Sigma Chemical Company. The reactants (aspartate, glutamate, α-ketoglutarate and oxaloacetate) were all inexpensive and readily obtainable. We determined an isotope fractionation of 8.3 ‰ for the reaction from glutamate to aspartate, and a fractionation of 1.7 ‰ for the aspartate to glutamate direction. This study is widely used to interpret the differences measured in the $\delta^{15}N$ in separated amino acids. Isotope branching points largely determine the differences between amino acid pairs.

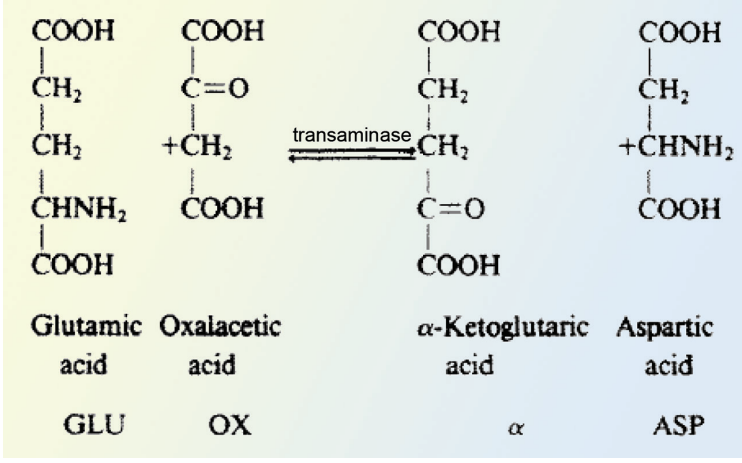

Figure 5.2 Glutamate transaminase reaction. Transaminase enzymes shuttle amino groups from an amino acid to an alpha-keto acid. From Macko *et al.* (1986) with permission from Elsevier.

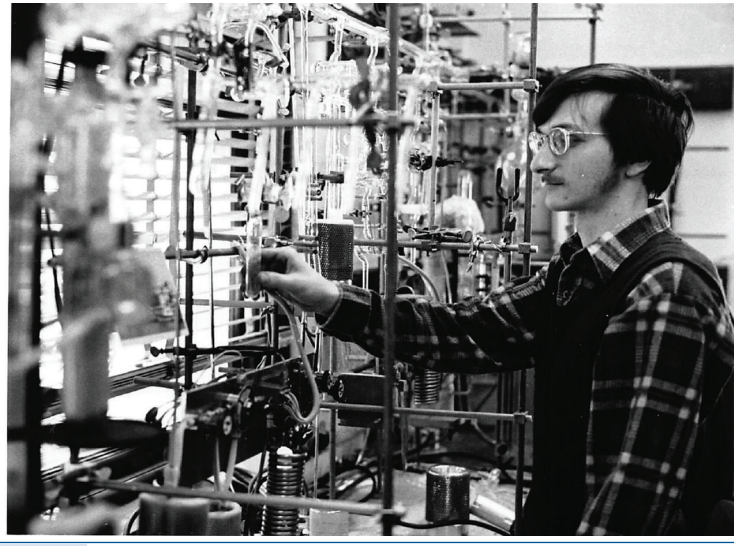

Figure 5.3 Stephen Macko at the nitrogen isotope vaccum line that he designed at the Geophysical Laboratory, Washington, DC, in 1982. Photo credit: M. Fogel.

In addition to my earlier work on compound specific amino acid studies, having the capability for measuring N, C, O, and hydrogen isotopes in my laboratory opened up the entire world of isotope biogeochemistry for me (Fig. 5.4). Every experiment that we could think of was new, and the outcomes unknown. At that time, the literature on stable isotopes was so small that you knew each publication that came out, memorised its contents, and thought about it long and hard. The work on amino acids quickly progressed to investigating fossil collagen samples and lab grown animals, a field that was dormant for 20 years, before resurging as continuous flow methods became more robust.

Figure 5.4 Geophysical Lab staff (1984). I am seated in the front row on the right between T. Neil Irvine (left) and Robert M. Hazen (right). Photo credit: M. Fogel.

6. ESTUARINE AND OCEAN BIOGEOCHEMISTRY

By the mid-1980s, nitrogen isotope biogeochemical methods were firmly established at the Geophysical Lab; other projects followed. In 1984, Hoering and I connected with Professor Jon Sharp and his student Luis Cifuentes at the College of Marine Studies, University of Delaware. Luis was an up and coming graduate student and interested in using stable isotopes to track estuarine processes in the Delaware Bay. Wisdom on how organic matter transferred from terrestrial to marine environments was based primarily on simple mixing models using terrestrial and marine organic matter as end members. For example, using carbon isotopes from suspended particulate organic matter (POM) collected along the salinity gradient of estuaries, the $\delta^{13}C$ of terrestrial plants, nominally -27 ‰, was mixed with marine POM, -20 ‰, in the estuary, without much consideration for primary production in the estuary itself. Hedges and Parker's elegant work (1976) took a different approach that correlated lignin phenol parameters with the $\delta^{13}C$ of POM in the Mississippi River delta and Gulf of Mexico. Pairing the two measurements, they clearly showed the influence of terrestrial organic matter on POM in this environment. Luis, Jon, and I planned a similar type of approach by combining chemical, biological, and isotopic measurements to study the Delaware Bay. In particular, we were interested in linking the estuarine processes of nitrification, primary productivity, and ammonium uptake to changes in isotopic compositions of POM.

Nitrogen isotope studies were less common in the 1980s than they are today. Nick Owens of Plymouth University (1987) published one of the earlier papers showing that $\delta^{15}N$ of POM was not a simple mixture of terrestrial and marine organic matter (Fig. 6.1). He proposed that *in situ* reactions were more important: the mixing of freshly produced planktonic organic matter with detrital organic matter having an altered isotopic composition owing to degradation. Following up on his work, we began a series of research cruises on the R/V Cape Henlopen from Trenton, NJ, to the mouth of the Delaware Bay, during which we collected POM and measured many parameters (*e.g.*, NH_3/NH_4, NO_3, dissolved oxygen, chlorophyll a concentrations) that might influence the $\delta^{15}N$ of POM. For example, high concentrations of chlorophyll a indicate fresh POM, perhaps from bloom conditions. The cruises not only spanned the entire salinity gradient from freshwater to open ocean salinities (35 ppt), but were undertaken monthly so as to document whether seasonal trends in productivity and biological processes within the estuary were important. Our station #14 was under the Delaware Memorial Bridge on Interstate 95. Every time I crossed this bridge on trips to see family in New Jersey, I pointed this out.

The $\delta^{15}N$ of POM was highly dynamic and varied both spatially and temporally. In winter, $\delta^{15}N$ was similar to classical mixing of terrestrial and marine OM end members. In early spring, in concert with thawing and a spring bloom of diatoms, the $\delta^{15}N$ of POM increased in the mid-estuary by almost 8 ‰

and could be related to increased rates of primary production. By early summer, as secondary production matched primary production, $\delta^{15}N$ values returned to "conservative", nearly uniform values throughout the entire salinity gradient. These $\delta^{15}N$ values were related to the abundance of detrital OM in the estuary and the concentrations of ammonium, nitrate, and chlorophyll (Cifuentes *et al.*, 1988a). This work led to a second paper on the $\delta^{15}N$ of estuarine ammonium which, when coupled to $\delta^{15}N$ in POM, introduced the concept of variability in isotope fractionation (Cifuentes *et al.*, 1988b, 1989) (Fig. 6.2).

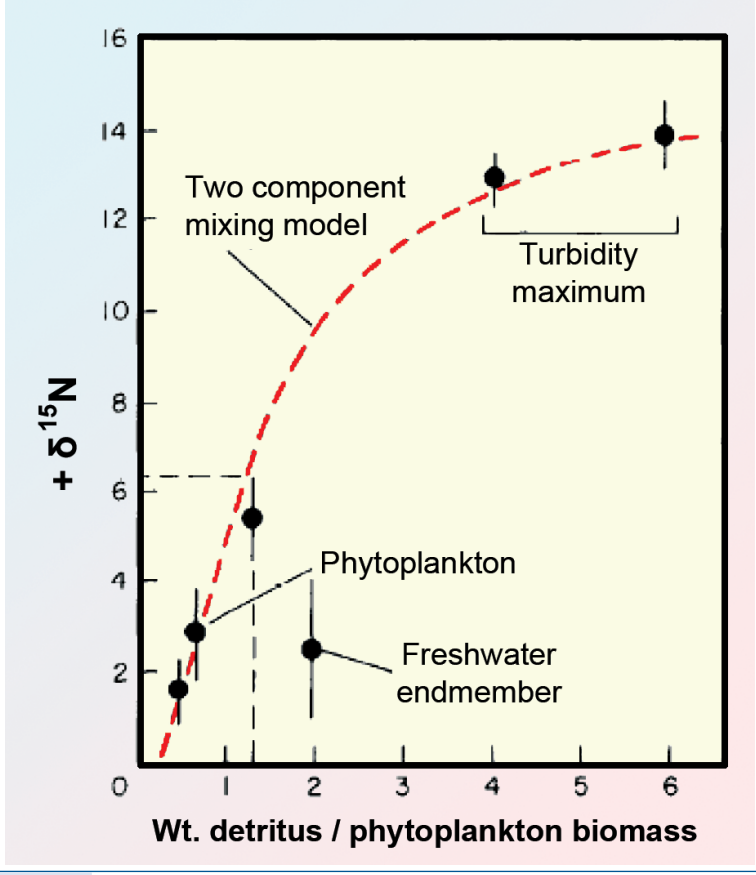

Figure 6.1 Early interpretations of estuarine POM isotope values were modeled as simple mixing of POM from freshwater and seawater. In this graph, Owens has plotted the relationship between the $\delta^{15}N$ of fresh POM to the ratio of detrital to fresh material in the sample, implying that POM was produced within the estuary, not simple mixtures. Modified from Owens (1987).

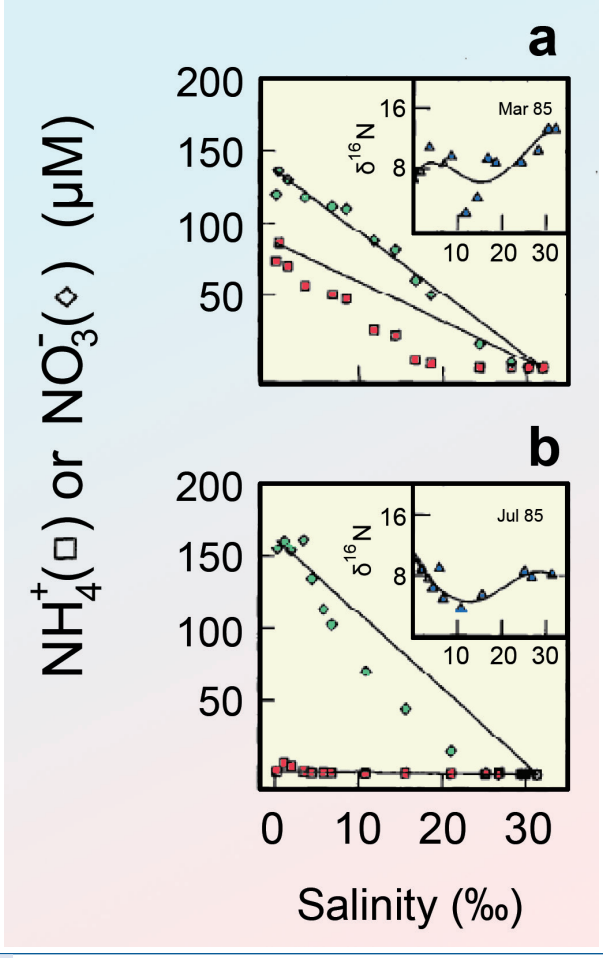

Figure 6.2 The relationship between salinity and nutrient concentrations in the Delaware estuary. The straight lines are conservative mixing lines between freshwater and marine concentrations. Data points plotting above the line denote a source within the estuary, whereas those below the line, a sink. The insert panels show the $\delta^{15}N$ composition of POM. The March data **(a)** shows the spring bloom; the July data **(b)** shows heterotrophic uptake. Modified from Cifuentes *et al.* (1988a).

After completing the studies of the $\delta^{15}N$ in POM, we worked on nitrogen isotope fractionation by a pure diatom culture (Pennock *et al.*, 1996) and a pure bacterial culture (Hoch *et al.*, 1992) in which $\Delta^{15}N$ (nitrogen isotope fractionation; $\delta^{15}N$ of organic nitrogen – $\delta^{15}N$ dissolved inorganic nitrogen) was related

to the concentration of ammonium/ammonia. Matthew Hoch, graduate student working with David Kirchman at the University of Delaware, worked with me on my third and fourth enzyme experiments using glutamine synthetase and glutamate dehydrogenase. In experiments with both diatoms and bacteria, we found that $\Delta^{15}N$ of NH_3/NH_4^+ or NO_3^- was dependent on the concentration of the inorganic nitrogen substrates. Cells use active transport to bring NH_4 across membranes in addition to a small amount of gaseous NH_3 which enters by diffusion. Higher concentrations of NH_3/NH_4 also trigger enhanced glutamate dehydrogenase activity while suppressing glutamine synthetase activity, which further complicates $\Delta^{15}N$ studies. Recent work with a strain of *E. coli* confirmed that $\Delta^{15}N$ varied as a function of ammonium concentration and elegantly documented the importance of the cellular functions of assimilation and membrane transport (Vo *et al.*, 2013) (Fig. 6.3).

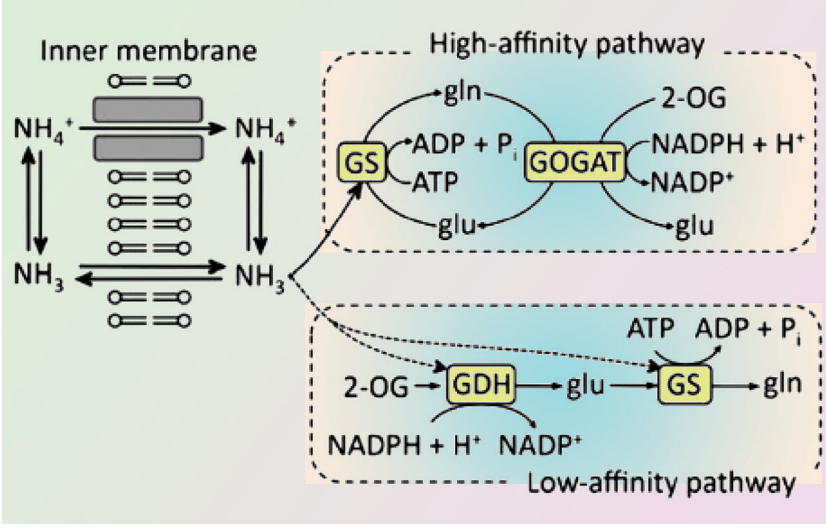

Figure 6.3 Microbes obtain ammonia/ammonium by diffusion or active enzymatic uptake. In this schematic diagram of nitrogen assimilation from ammonium by *E. coli* K12, two assimilation pathways are highlighted, each of which is associated with different nitrogen isotope fractionations. Modified from Vo *et al.* (2013).

My involvement in the marine and estuarine science community encouraged me to jump into the field of diagenesis of organic matter, a research area led by John Hedges of the University of Washington and Ron Benner, then a postdoc at the University of Georgia. In the 1980s, one of the most contentious estuarine theories involved the role of C_4 grass *Spartina alterniflora*, which dominates salt marshes in North America, for sustaining secondary production in this highly productive ecosystem. Carbon isotopes of POM, sediments, primary

producers, and small invertebrates (*e.g.*, mussels and crabs) were measured from Woods Hole, Massachusetts, to Georgia. Every study found that the $\delta^{13}C$ in these organic matter pools had values closer those of phytoplankton that ranged from -18 to -20 ‰ rather than the range of -12 to -13 ‰ of *Spartina*. The idea that salt marshes were not important contributors to food web dynamics did not make sense to me. The interpretation of the $\delta^{13}C$ data had important implications because salt marshes were threatened habitats. If estuarine scientists could prove that these wetlands were nurseries and energy sources for commercial seafood, they were more likely to be protected from development (Fig. 6.4).

Figure 6.4 *Spartina* marsh on the shores of the Chesapeake Bay. Mat Wooller and I are seining for fish in the shallow tidal water. On the banks are Casey Gustawarra, Maia Schweizer, Quinn Roberts, and Andrew Steele (left to right). Photo credit: M. Fogel.

My involvement with *Spartina* ecosystems began in 1985, when I was contacted by Kent Sprague, a student from the University of Georgia who was studying estuarine sediment deposits from palaeo-salt marshes. He came to the Geophysical Laboratory with peat cores, in which he could pick out fragments of nearly intact *Spartina* that were hundreds to thousands of years old. The $\delta^{13}C$ of these fragments was -18 ‰, similar to modern bulk sediment $\delta^{13}C$. We used pyrolysis-gas chromatography-mass spectrometry coupled to stable isotope analysis to provide further information on the structural and chemical composition of organic matter sources from these marshes (Fogel *et al.*, 1989a). Then, using lignocellulose fragments as a proxy for *Spartina*, saturated C_{15}-C_{19} hydrocarbons as a proxy for diatoms, and saturated C_{22}-C_{27} hydrocarbons as

a second proxy for *Spartina* and other higher plants, I plotted the results for sediments and plant fragments on a ternary diagram to estimate the sources of organic matter in these pools (Fig. 6.5). Sedimentary material and plant fragments plotted firmly in the space showing that *Spartina* contributed roughly 50 % of the organic matter in sediments. Discovering why the sediments and plant fragments from *Spartina* had more negative $\delta^{13}C$ values involved more work.

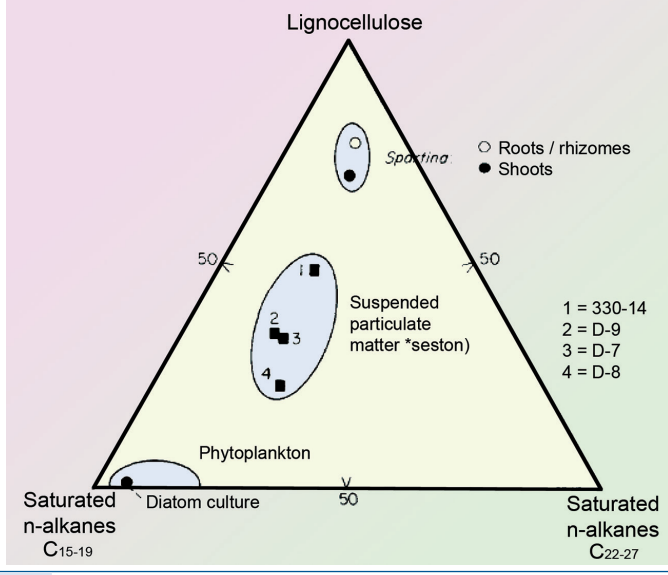

Figure 6.5 We developed a ternary diagram of relative proportions of marker compounds in *Spartina*, phytoplankton, and suspended particulate matter to reflect three component mixing which was not previously considered. Modified from Fogel *et al.* (1989a).

Ron Benner was conducting litter bag experiments with *Spartina*. Dried plants are weighed, sewn into nylon or polypropylene bags with defined mesh sizes, and then incubated in the environment either under aerobic or anaerobic conditions. Bags are periodically (*e.g.*, weekly or monthly) removed from the environment, and the remaining plants in the litter are dried and subjected to various types of analyses. Kent encouraged Ron to write to me about analysing the *Spartina* in the litter bags for carbon and nitrogen isotopic compositions. Using the bulk *Spartina* material, Benner used chemical methods to separate the major plant structural biochemicals: lignin, cellulose, and hemicellulose. In 1985, Ron wrote to me:

> *"I welcome your suggestions and I believe that the carbon isotope measurement on the chemically fractionated material is a good one. Am I correct to assume that the carbon isotope ratios among these particular fractions (cellulose, hemicellulose, lignin) will probably be indistinguishable?"*

Samples from an 18 month experiment were brought up to the lab by Kent Sprague and analysed using the sealed-tube combustion methods. The results were striking (Benner *et al*., 1987). Although bulk $\delta^{13}C$ of *Spartina* was -13 ‰, the biochemical fractions were very different. Cellulose and hemicellulose had $\delta^{13}C$ of -11.7 ‰, a 1.3 ‰ enrichment in ^{13}C from bulk *Spartina*, while the lignin $\delta^{13}C$ was more negative with a value of -17.4 ‰. Uncharacterised material, suberins and other insoluble material, had a $\delta^{13}C$ of -16.4 ‰. As the relative proportion of lignin relative to other compounds increased in the litter bags from 10 to 15 %, the $\delta^{13}C$ of the remaining *Spartina* decreased from -13 to -14.2 ‰. The decrease in the $\delta^{13}C$ value demonstrated that as decomposition proceeded, the celluloses were preferentially decomposed leaving more isotopically negative lignin. The $\delta^{13}C$ of the remaining plant material approached the $\delta^{13}C$ values measured in natural salt marsh sediments and POM.

Ron, Kent, and I widened the study to measure biochemical fractions from eight other species of plants (Benner *et al*., 1987). In all cases, the $\delta^{13}C$ of lignin was 2.5 to 5.0 ‰ more negative than the $\delta^{13}C$ of bulk plant material. The $\delta^{13}C$ of the cellulose and hemicellulose fractions was in general 1 to 2 ‰ more positive than the bulk plant, but there was considerable variation. Subsequent studies with compound specific isotope analysis of individual carbohydrates (Teece and Fogel, 2007) showed that the major 6-carbon sugars in celluloses, glucose, galactose, and mannose, could have quite variable $\delta^{13}C$ values. In higher plants, the synthesis of these monomers and their translocation to wood, stems or rhizome is probably associated with additional isotopic fractionation. Carbohydrate monomers are the most biosynthetically-active and labile molecules in an organism and in the environment, and the $\delta^{13}C$ of sugar monomers at the compound specific level reflects this dynamic nature.

6.1 Atmospheric Nitrogen Deposition to Coastal Areas

In 1989, out of the blue I received a phone call from Hans Paerl of the University of North Carolina's Institute of Marine Science. Hans has devoted his career to studying the harmful effects that excess levels of inorganic nitrogen in estuarine and coastal waters can have on cyanobacteria and other algae. He had read the papers I'd published on the Delaware estuary and wondered if I would join him in a study on the effects of acid rain on the coastal zone. At that time, the U.S. Environmental Protection Agency was monitoring acid rain and its nitrate and ammonium concentrations across the entire continental United States. Their monitoring programme did not extend past land, leaving a gap in our understanding of how important acid rain might be for altering coastal ecosystems. We brainstormed and concluded that stable isotopes might be able to distinguish sources of nitrogen from rainfall *vs*. fertilisation or natural nitrogen fixation.

What followed was nearly a decade of research, the exchange of graduate students and postdocs, and valuable shipboard and leadership experience for me. Our work started without government funding. Hans shipped litres

of frozen rainwater to Washington, DC, where I isolated the ammonium and nitrate using methods worked out for the Delaware project. Hans was, and probably still is, a relentless collaborator. He thinks of ideas on the fly, likes to do his writing in a group, and always plots his next scientific move. We submitted three NSF proposals before one was finally funded in 1993 to carry out this work properly. In 1994, we published our first paper with measurements of the $\delta^{15}N$ in ammonia and nitrate in rains collected in North Carolina. We discovered that the $\delta^{15}N$ of ammonium was more negative (-12 to +3 ‰) than both the $\delta^{15}N$ of nitrate and dissolved organic nitrogen, which have average values of +1 ‰. We carried out mesocosm experiments in which four litres of estuarine waters were incubated with an aliquot of rainwater, then POM was filtered and analysed. Our results showed that phytoplankton used the nitrogen from rainwater resulting in increased primary productivity (Paerl and Fogel, 1994).

Nitrogen in rainfall in coastal areas could be considered as "new" nitrogen in areas of the ocean where phytoplankton production was nitrogen limited. Our work extended to the Sargasso Sea, a region in the central gyre of the North Atlantic Ocean. Over a period of three years, we conducted six cruises on the R/V Cape Hatteras from Beaufort, North Carolina, through the Gulf Stream, into the calm, warm waters of the Sargasso Sea. On our first cruise, Hans served as Chief Scientist, a position that requires 24 hour interaction with scientists, the crew, and most importantly the Captain. We sampled POM, nutrients, zooplankton,and floating *Sargassum* and *Trichodesmium*, while conducting onboard measurements of primary and bacterial productivity and dissolved organic matter concentrations. Postdoctoral researcher Carmen Aguilar and I filtered hundreds, if not thousands, of litres of seawater in a pressurised system using nitrogen gas. In addition, Carmen and Hans' students conducted mesocosm experiments on deck, as Hans and I had done previously.

On our third cruise, I served as Chief Scientist. This particular cruise was filled with high adventure. In my first meeting with the Captain, I laid out my cruise plans to stop on station every 100 km to sample the water column. The captain looked at me with slight derision, then remarked that he used nautical miles, so what did 100 km mean in nautical miles? I was put in my place. With my tail between my legs, I retreated, converted units, then proposed we stop every 54 nautical miles until we reached the centre of the Sargasso Sea. After only one day at sea, seriously rough weather began to affect our sampling. Some of the scientific party got sea sick. By late afternoon on the second day, our ship was required to "standby" to assist a sailboat with a snapped main mast, as the Coast Guard came to their rescue. By the end of that day, the Captain informed me that Hurricane Gordon, previously thought to have gone into the North Atlantic, had changed direction and was projected to intersect with our cruise track within 24 hours. We decided to head back to Beaufort rather than risk collision with the storm. I was disappointed.

When we reached port, those who had been sea sick were relieved to be off the rolling ship. By next morning, the weather report showed that the storm had gone "safely out to sea" again, so we were cleared again for departure. Only half the scientific crew returned for the second leg of the voyage, but we left the dock in a hearty mood. Our strategy now was to compare the measurements we'd made before Hurricane Gordon to those taken after the storm had churned up surface waters. It wasn't long before Gordon reversed course again, and turned back towards our ship. We made it out just to the edge of the Gulf Stream around 9 o'clock at night. Waves were crashing over the bridge – three stories up. The Captain, himself, was looking grim and said to me in his thick Southern accent, "*Marilyn, we've got to turn around*." I said, "*One more sample*."

Lashed with a rope to the deck of the ship, I staggered outside in the wind and rain to collect that last sample, Station 17. As I came inside, I radioed to the bridge, "*Ok, let's go in*." Refrigerators, the scintillation counter, and freezers were flung back and forth like billiard balls. All of us were ordered to our staterooms, while the Captain was lashed to the ship's controls. Years later, I can still feel the drama that made us a captive ship in a huge storm. Was it worth it?

Not many people have the chance to sample before and after a hurricane. Once we were safely back to port, we had the sense that Hurricane Gordon provided us with a unique scientific opportunity. Now, owing to global warming, hurricanes are more intense than they were 20 years ago. We documented the following (Fogel *et al*., 1999):

> *"Meteorological forcing resulting from wind generated by this storm resulted in significant changes in primary production in the continental shelf photic zone. Resuspended sediments laden with microorganisms and dissolved, growth-limiting nutrients were mixed into the water column. Significant increases in Chl a, CO_2 fixation, and bacterial production were observed over relatively large areas. Stable isotopic compositions of suspended particulate material shifted quickly and recorded the biological perturbations to the water column. Increases in the rate of primary production in association with major storm events could therefore be important in the calculation of coastal and global ocean production and may influence the sedimentary organic C record in coastal areas".*

Subsequent cruises revealed that phytoplankton, which were primarily *Prochlorococcus* sp., were stimulated by both nitrogen and phosphorus, substantial constituents of continental rainwater source. On land, acid rain might be problematic, but in the open ocean, "fertilisation" by atmospheric deposition could be a good thing (Paerl *et al*., 1999).

Others followed us to measure the $\delta^{15}N$ of rainwater and found similar results (Altieri *et al*., 2014; Felix *et al*., 2017). The widespread occurrence of *Prochlorococcus* sp. was just being discovered (Partensky *et al.,* 1999), and at that time, its importance in ocean productivity, particularly in oligotrophic areas, was unknown. Today our work is even more relevant for understanding the effects that major ocean storms may have on marine primary productivity as our climate changes.

7. OXYGEN ISOTOPES IN ATMOSPHERIC OXYGEN AND THE DOLE EFFECT

One of the more intriguing intersections, to me, between botany and biogeochemistry was the "Dole Effect" (see Text Box 7.1).

Text Box 7.1 – The Dole Effect

Malcolm Dole, a chemist at Northwestern University, had been measuring oxygen isotope fractionations in inorganic experimental systems for over 15 years starting in the 1940s. He and others, including Harold Urey, noted that the oxygen in the atmosphere had an excess of ^{18}O relative to what it should be if atmospheric O_2 was in equilibrium with water (Dole, 1935; Morita, 1935). In 1956, Lane and Dole (1956) published experiments that measured oxygen isotope fractionation during respiration by various organisms (Fig. 7.1). Earlier experiments had shown that oxygen evolved from photosynthesis had the same isotopic composition as that of the water in the plant or the medium (Dole and Jenks, 1944). In the 1956 study, respiration was associated with an oxygen isotope fractionation between 10-25 ‰. The authors concluded that, "*the O^{16}/O^{18} ratio of atmospheric oxygen has risen to a point such that the ratios for photosynthetic oxygen delivered to the atmosphere and the oxygen extracted from the atmosphere by respiration are equal*." The problem of the Dole Effect persisted. Studies by Michael Bender, then at the University of Rhode Island, showed that oxygen isotope fractionation during respiration by marine phytoplankton and microbes in oceans was considerably less (17 ‰) than the 23.5 ‰ needed to explain the Dole Effect (Bender and Grande, 1987).

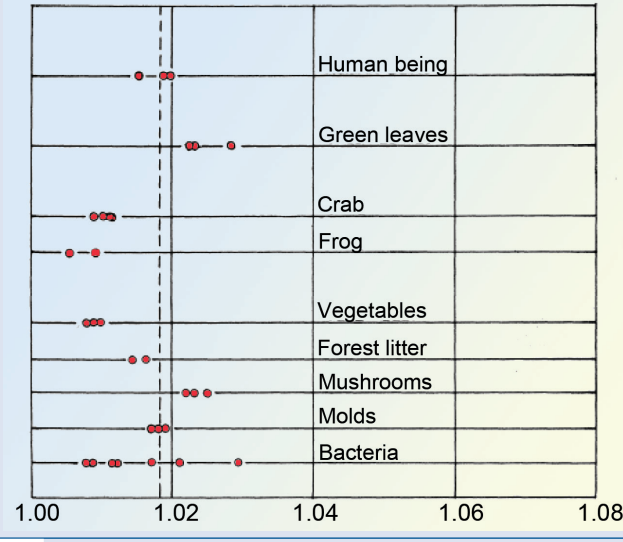

Figure 7.1 Oxygen isotope fractionation factors for various biological systems. Modified from Lane and Dole (1956).

In 1985, I was looking for additional challenges to my work as an isotope biogeochemist. I had spent eight years at the Geophysical Laboratory in the company of geochemists and earth scientists, far removed from biologists and botanists at the start of my career. I met Joe Berry at Carnegie's Plant Biology Department at a Carnegie Institution of Washington meeting, and we immediately struck up a conversation about how we could weave together experiments on modern plant biology to tackle the potential factors that cause the Dole Effect. Berry, Chris Field, and Olle Bjorkman, all staff members at Carnegie's Department of Plant Biology, were studying photorespiration in leaves, as well as conducting *in situ* work with Rubisco. If conditions are right, Rubisco has the potential to fix O_2, rather than CO_2. This can occur when temperatures are high, and the plant has made an excess of oxygen that might damage the plant *via* free radical production.

At that time, we could not account for the factors that caused the Dole effect, which had implications for how biological processes were balanced globally. Clearly, the measurements of isotope fractionation from respiration of O_2 or from net oceanic or terrestrial processes could not resolve the problem Thus, I started on a major discovery endeavour to describe the oxygen isotope systematics of photosynthesis, photorespiration, and the other plant processes that could explain why the atmosphere has a $\delta^{18}O$ of +23.5 ‰.

Berry and I submitted a proposal to the Department of Energy and received the following reviews:

> *"The investigators underestimate the pitfalls of their experimental technique. The trapping of oxygen "on a molecular sieve column" (page 8) and the application of an oxygen electrode (pages 8,9) shift the ratio of the two oxygen isotopes. The hypothesis that photorespiration can account for part of the photosynthetic fractionation is interesting. Is there any reason (literature, preliminary data) to expect it to be so?"*

Amazingly, the proposal was funded and on we went (Fig. 7.2).

To purify atmospheric oxygen from argon in air samples, Tom Hoering and I constructed our first vacuum line at the Geophysical Laboratory in Washington, DC. After oxygen was separated from argon, it was converted quantitatively to CO_2. In late February 1985, we deconstructed the line piece by piece and shipped it to the Department of Plant Biology in Palo Alto, California. Working closely with postdoctoral fellow Robert Guy, we designed and built the "biological" part of the extraction line where enzyme reactions and cells would be incubated for experiments. Experiments began in earnest in 1986, when I spent a year's sabbatical leave at Plant Biology.

Our first experiments were with *Anacystis nidulans*, an easy-to-culture cyanobacterium. Cells were grown in the lab, then transferred to a collapsible bag that was sparged with helium to remove all traces of atmospheric oxygen. We had an oxygen electrode mounted in the bottom of the apparatus, which was constantly stirred. Light was turned on, and oxygen evolved. To kill the reaction, we added phosphoric and salicylic acids, then extracted the dissolved oxygen in a vacuum system. We confirmed earlier studies that showed there

was little to no oxygen isotope fractionation during photosynthesis (*e.g.*, Stevens *et al.*, 1975). These first photosynthetic O_2 measurements allowed us to refine our techniques and understand the various pitfalls. Typically, we would start on Monday to grow cells, get the chamber and line in good shape on Tuesday, run the first set of experiments on Wednesday, then repeat Thursday and Friday. Samples of CO_2 were sealed into Pyrex ampules. Every two weeks or so, I travelled down to NASA's Ames Center at Mountain View to work in David DesMarais's lab, who kindly allowed me to use his isotope ratio mass spectrometer.

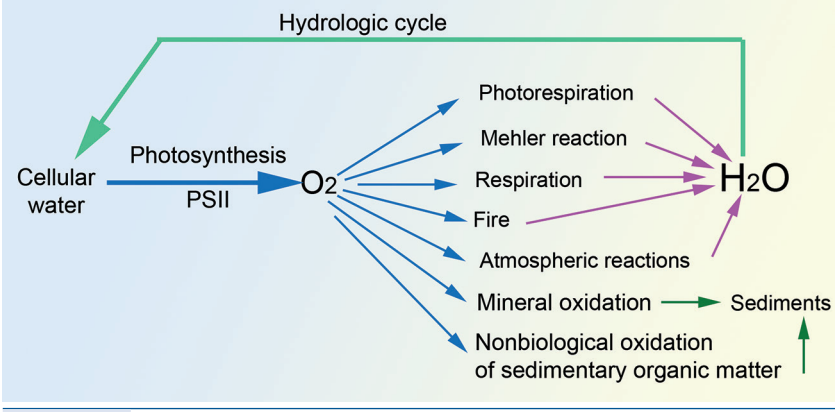

Figure 7.2 Processes involved in the geochemical cycling of oxygen taken from a proposal submitted (and funded) by the Dept. of Energy. Prior to my work with Joe Berry on this project, photorespiration and the Mehler reaction, two important plant-based oxygen uptake mechanisms, had not been considered by other researchers. Unpublished graph of M. Fogel.

Rubisco, which stands for ribulose 1,5 bisphosphate (*Rubis*) carboxylase (*c*) oxygenase (*o*), is an unusual enzyme in that it catalyses the uptake of both CO_2 and O_2. Our second set of experiments was a "revisit" to my Ph.D. dissertation and the re-measurement of both carbon and oxygen isotope fractionation by Rubisco. Times had changed. Rubisco from spinach, cyanobacteria, and *R. rubrum* could now be expressed in *E. coli*; no need for growing massive amounts of cells or purifying enzymes. For these experiments, rather than the time consuming purification and crystallisation of PGA, we simply measured the isotopic composition of the remaining CO_2. It turns out that the values I measured for my Ph.D. were several ‰ more negative than those I measured in California, because the $\delta^{13}C$ of RuBP in my earlier experiments was influenced by unknown contamination. By measuring only CO_2, we avoided that problem. We measured an isotope fractionation for carbon in CO_2 of 29.4 ‰, similar to recent experiments by Roeske and O'Leary (1984).

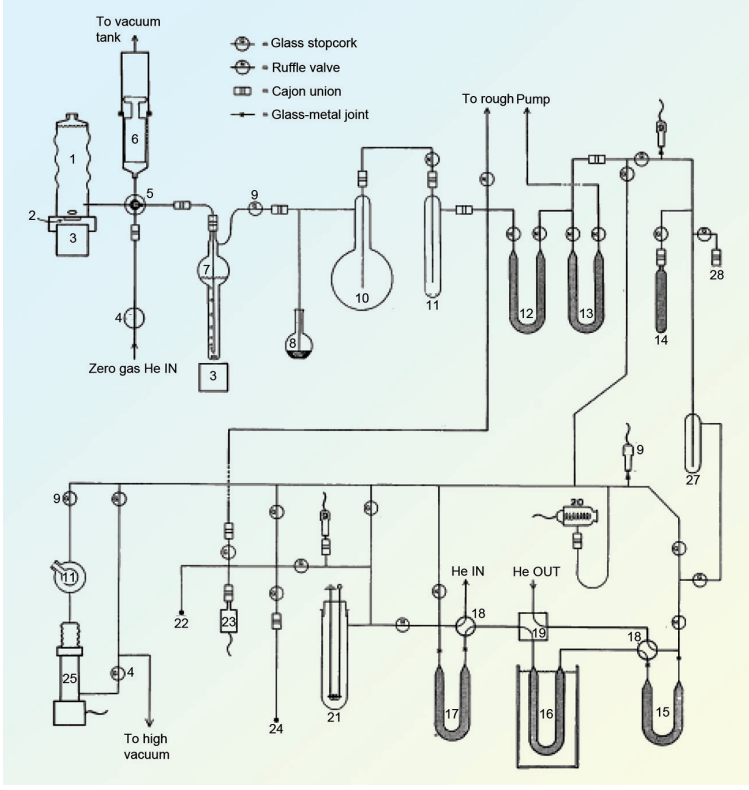

Figure 7.3 Schematic diagram of a vacuum line used for purifying oxygen for isotope experiments. Cells were incubated in the apparatus (1, 2, 3) then transferred through various traps (10, 11, 12, 13) before being purified by a homemade gas chromatograph (15, 16, 17). Tom Hoering and I designed the original line in Washington, DC, and shipped it to Stanford, CA. Rob Guy and I designed the remainder of the line while I was on sabbatical at Carnegie's Plant Biology lab, Stanford, CA. After Guy *et al.* (1989).

Oxygen isotope fractionations ($\Delta^{18}O$) were calculated using Rayleigh equations that compared the $\delta^{18}O$ of the O_2 in our reaction chamber at the start of the experiment to the $\delta^{18}O$ of O_2 when we took a sample. Using our oxygen electrode, we could calculate the fraction of O_2 that was consumed (Guy *et al.*, 1993) (Fig. 7.3). We determined the isotope fractionation from the oxygenation part of the Rubisco reaction: 20.7 ‰, very similar to what was required to explain the Dole Effect. Approximately 30 % of all O_2 produced by photosynthesis is "fixed" by Rubisco prior to its being released. Another 20 % is taken up by the Mehler reaction in which oxygen radicals are converted to peroxide, then converted *via* catalase to H_2O. The isotope fractionation during this reaction

yielded a $\Delta^{18}O$ value of 15.3 ‰. We followed with measurements of oxygen isotope fractionations by glycolate oxidase *in vitro* and cytochrome oxidase. The experiments with glycolate oxidase were relatively simple with $\Delta^{18}O$ values of 22.7 ‰. Cytochrome oxidase experiments were another story. To perform these experiments, we needed large quantities of the enzyme cytochrome oxidase, which we could obtain from Sigma Chemical Company. Cytochrome c, on the other hand, was expensive – about $500 per sample – and it was destroyed at the end of each experiment. We managed to only have one or two successful runs, measuring an isotope fractionation of 6.6 ‰, hardly enough to explain the Dole Effect.

Rob Guy took the lead on respiration experiments (Guy *et al.*, 1989) in which we investigated an alternative respiration pathway in plants that was cyanide-resistant. We used cells, isolated mitochondria, sub-mitochondrial particles, whole seedlings, as well as purified enzymes to show that whole plant respiration had a $\Delta^{18}O$ fractionation of about +20 ‰, whereas the cyanide resistant respiration $\Delta^{18}O$ was +25 ‰. Experiments with yeast gave $\Delta^{18}O$ fractionations of about 16-18 ‰, much less than plants, and similar to previous measurements by Dole and others. Our final enzymatic experiment was with soybean lipoxygenase, an enzyme that used linolenic acid as a substrate ($\Delta^{18}O$ = 9 ‰, but was O_2 concentration dependent) (Fig. 7.4).

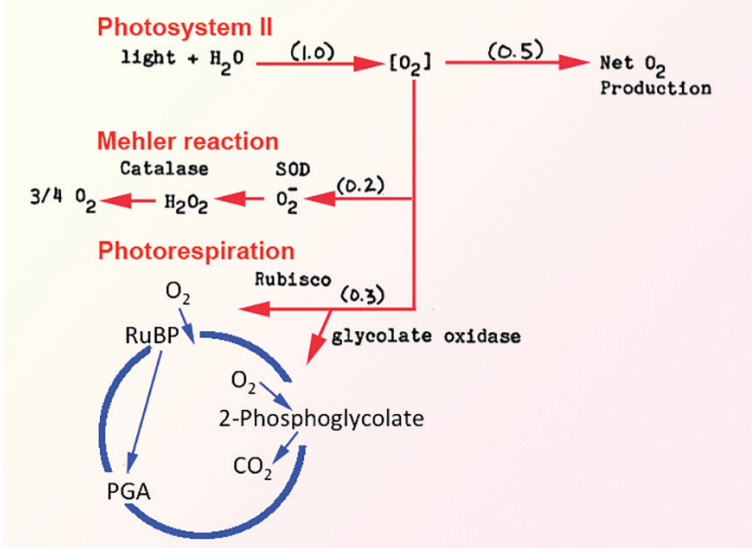

Figure 7.4 Diagram in Dept. of Energy proposal by Fogel and Berry outlining where we hypothesised that fractionations might take place. Unpublished graph of M. Fogel.

Years later, Luz and others added measurements of ^{17}O to further understand how atmospheric O_2 is cycled, going several steps beyond our experiments with ^{18}O and ^{16}O (Luz *et al.*, 1999; Luz and Barkan, 2005). Today, it is recognised that the oxygen in air has a depletion of ^{17}O that derives from mass independent isotope effects in the upper atmosphere. This small but measurable $\Delta^{17}O$ anomaly in atmospheric oxygen allows geochemists and ecologists to use ^{17}O as a tracer for measuring gross and net primary production, as well as for studies of respiration (Whiteman *et al.*, 2019).

8. ISOTOPE BIOGEOCHEMISTRY OF FOSSIL ORGANIC MATTER, ARCHAEOLOGY, AND PALAEOCLIMATE

Since the early 1960s, Philip Abelson, Ed Hare, and Tom Hoering had been studying various types of ancient organic matter. Abelson was one of the first to find amino acids in fossil shells (Abelson, 1956) and as Geophysical Laboratory Director, he hired Ed Hare as a geological chemist in the late 1960s. Ed's specialty was dating fossils by amino acid racemisation methods. Basically, all amino acids in living organisms are in the L-form, but after an organism dies, the remaining amino acids in fossil proteins can convert to the D-form. The Arrenhius equation, which describes the rate of conversion from the L- to D-form as a function of temperature, can be used to estimate time, an approach that is particularly useful in the lab where temperature can be constrained. Hare and his postdoctoral fellows were key in developing this as a robust, reliable method for dating mollusc shells, eggshell, and bone (Brooks *et al*., 1990). A pinnacle of Ed's career was his measurement of D/L ratios in a piece of the Shroud of Turin, a scrap of linen cloth that supposedly bears the negative image of a man thought to be Jesus. Ed determined that the Shroud was only a few hundred years old, and not likely the original burial cloth from Jesus's tomb.

Hare's lab was state-of-the-art in terms of dating fossils. It was filled with boxes, some opened and analysed and some not, of interesting very old specimens from around the world. He had samples of dinosaur teeth, bones from the oldest humans from North America, and many mammoth and mastodon fossils. When postdoctoral fellow Noreen Tuross arrived in 1985, we began to work as a team to investigate stable isotopes in fossil materials. At the time, I had a "new" IRMS system, a modified double-focusing 491 Dupont mass spectrometer that the Nuclide Corporation had retrofitted for nitrogen analyses. The instrument had an inlet system built with a pair of mercury pistons for compressing small amounts of N_2 gas right up to the capillary leading to the ion source. The machine was placed within easy reach of my desk in my office that I shared with various postdocs including David Velinsky and a host of visitors.

South African scientists, Nick van der Merwe and John Vogel, who were actively dating fossil bones using radiocarbon techniques, had started publishing carbon isotope papers in the late 1970s and early 1980s. At this time, it was well known that C_3 ($\delta^{13}C$ =-27 ± 2 ‰) and C_4 ($\delta^{13}C$ = -12 ± 1 ‰) plants had distinct carbon isotope compositions. This separation results from differences in the degree to which the first committed CO_2 fixing enzymes in photosynthesis, Rubisco and PEP carboxylase, discriminate against ^{13}C. As a byproduct of radiocarbon dating, the $\delta^{13}C$ of bones from archaeological sites was also measured. Early work by van der Merwe, Vogel, Henry Schwartz and others concentrated on differences in the $\delta^{13}C$ of fossil human skeletons as a function of whether they had consumed C_4-based corn (van der Merwe, 1982) or marine foods in their diet (Chisholm *et al*., 1982) (Table 8.1). Very few labs at that time had the capability to analyse nitrogen isotopes in fossil materials:

Caltech, UCLA, Carnegie, and Macko's new lab in Newfoundland, Canada. At the Geophysical Laboratory, we had the added advantage of having Ed Hare's amino acid dating capabilities as well.

Table 8.1 Average $\delta^{13}C$ values relative to PDB for diet and consumer samples obtained from terrestrial and marine ecosystems in Canada. The positive values for Southern Ontario humans after 1000 A.D. reflect the influence of maize on the diet. Data from Chisholm *et al.* (1982)* and Katzenburg *et al.* (1995).

Description of samples	N	$\delta^{13}C$ (per mil)	Description of samples	N	$\delta^{13}C$ (per mil)
Dietary materials					
Terrestrial mammals	27	–25.5 ± 1.5	Marine mammals	4	–17.5 ± 0.9
Terrestrial birds	15	–25.2 ± 1.5	Marine fish and shrimp	20	–17.5 ± 1.5
Freshwater fish	4	–28.8 ± 2.2	Littoral species	7	–18.7 ± 1.2
Population mean and error		–25.7 ± 0.3			–17.8 ± 0.3
Human bone collagen					
Northern European C3 consumers	81	–19.6 ± 1.6	Greenland Eskimo	2	–12.8
Ottawa Valley consumers	17	–19.6 ± 0.9	British Columbia consumers, coastal area	40	–13.4 ± 0.9
British Columbia consumers, interior area (N = 5) –15.4 ± 0.3					
Southern Ontario consumers prior to 1000 A.D.	16	-19.0 ± 1.2			
Southern Ontario consumers post 1000 A.D.	11	-12.8 ± 1.4			

* Literature was collected from the following sources: data on the $\delta^{13}C$ values of Greenland Eskimo are from H. Tauber, in *Radiocarbon Dating*, R. Berger and H. E. Suess, Eds. (Univ. of California Press, Berkeley, 1979), p. 447; data on $\delta^{13}C$ values for northern Europeans were taken from various issues of *Radiocarbon*.

My first paper on stable isotopes in fossil bones, with Tuross and Hare, included not only bulk data for a set of fossil samples, but also compound specific carbon and nitrogen isotope analyses of amino acids purified from fossil collagen (Tuross *et al.*, 1988). One of our principal findings was that the $\delta^{13}C$ of glycine had values 10-14 ‰ more positive than bulk collagen; serine $\delta^{13}C$ values were also quite positive. It had been shown previously (*e.g.*, DeNiro and Epstein, 1978) that the $\delta^{13}C$ of collagen is about 5 ‰ more positive than bulk tissue $\delta^{13}C$ (Fig. 8.1).

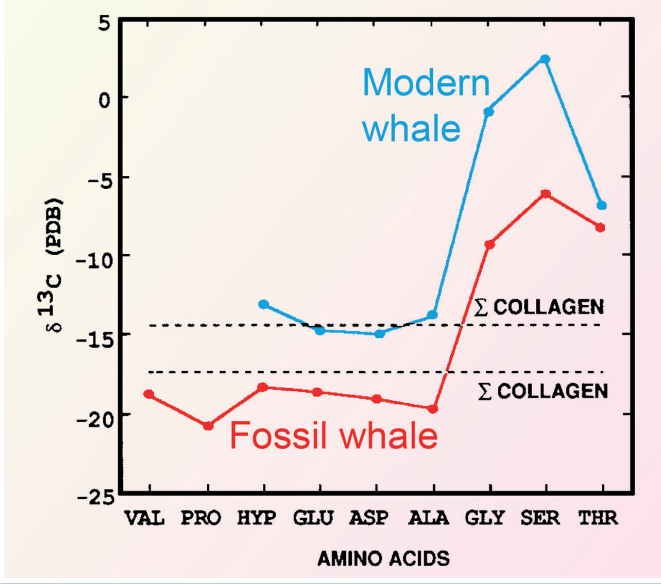

Figure 8.1 Individual amino acid $\delta^{13}C$ from hydrolysates of insoluble fossil and modern whale collagen. Dashed lines indicate the $\delta^{13}C$ of the total collagen. Modified from Tuross *et al.* (1988).

This isotopic enrichment could now be explained by collagen's unique amino acid composition where one third of its amino acid residues are glycine. Another interesting observation was the very negative $\delta^{15}N$ values of threonine. At the time, it was not known if these unusual values were "real" or an artefact of chromatography or preparation. Now, the $\delta^{15}N$ of threonine can be used as a tracer of trophic level because with each trophic step there is a pronounced, almost uniform depletion of $\delta^{15}N$ in threonine.

Beyond the compound specific data, early work during this period investigated whether fossil collagen had enough of an intact structure, free of humic acid contamination, to produce a valid isotopic composition. Methods for purifying collagen from fossil bones and teeth include extraction by 1 or 0.1 N HCl, extraction by EDTA, and gelatinisation (weak acid solubilisation). We demonstrated that EDTA extraction resulted in the highest yield and the purest collagen. Commonly now, the C:N ratio of fossil collagen is measured during elemental analysis. Values of C:N > 4 are considered to reflect collagen that has been compromised by contamination or diagenetic alteration (Ambrose, 1990) (Fig. 8.2). Collagen concentrations in bone (about 20 %) and teeth (about 10 %) decrease in fossil materials. Once collagen concentrations reach levels of 2 % or less in bones or teeth, the C:N ratios can be variable (2-10), and the isotopic compositions for both C and N do not reflect original material.

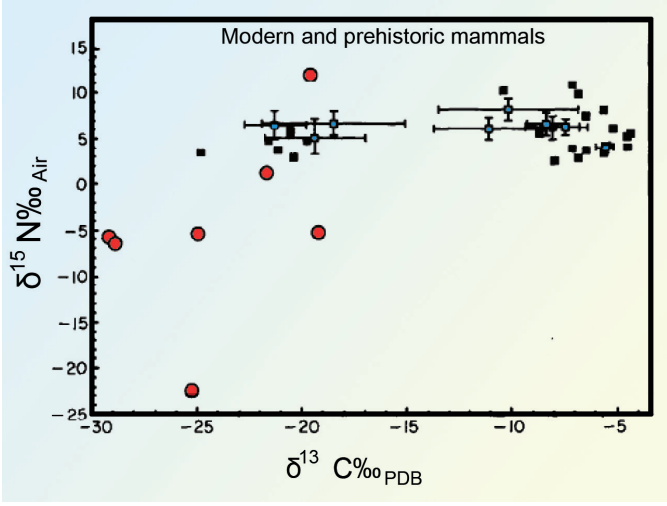

Figure 8.2 Stable carbon and nitrogen isotope compositions of collagen from modern herbivores and archeological specimens of the same species from the Kenya Rift Valley. Black symbols are well-preserved collagens, whereas red circles were poorly preserved collagen. Modified from Ambrose (1991).

The second important substrate from fossil bones and teeth is the apatite phase. The mineral apatite contains a carbonate ion that is incorporated into biologically formed apatite when teeth and bones are grown. Julia Lee-Thorp and van der Merwe (1987, 1991) found that the $\delta^{13}C$ of the apatite is stable for at least 1 million years or more, but the $\delta^{18}O$ of the carbonate in apatite can be influenced by exchange with environmental water, precipitation of secondary carbonates, or re-precipitation of fluroapatite. Postdoctoral fellow Paul Koch was my next colleague to work on fossil materials. He worked out methods to obtain faithful isotopic signals from carbonate by treating apatite with dilute, buffered acetic acid which removes contaminating carbonates from indigenous apatites (Koch *et al.*, 1997).

Archaeologists trained in Michael DeNiro's laboratory at UCLA continued studies of zoo-archaeological and human samples. Stanley Ambrose's early work on African specimens introduced the community to several of the environmental effects on isotopic compositions of collagen (Ambrose, 1991). Based on early work by Minagawa and Wada (1984) and DeNiro and Epstein (1981b), we know that the $\delta^{15}N$ of animal tissue is about 3-4 ‰ more positive than the diet. Measurements of $\delta^{15}N$ in food, tissues, faeces, and urine in several animals showed that while the $\delta^{15}N$ of faeces is about 1 ‰ more positive than diet, the $\delta^{15}N$ of urine, is usually 3-4 ‰ more negative than diet (*e.g.*, Sponheimer *et al.*, 2003). The loss of isotopically "light" nitrogen in urine is the principal reason for the isotopic enrichment in animal tissue.

The popular phrase *"you are what you eat"* with the canonical value of *"plus 3.4 ‰"* has been measured and reproduced repeatedly over the past 30-40 years in a large variety of animals. In general, the +3.4 ‰ value explains the nitrogen isotope fractionation between an animal's tissues and its diet for the majority of organisms studied. However, there are important variations related to the quality and quantity of the diet (*e.g.,* Caut *et al.,* 2009) (Fig. 8.3). For example, a high protein diet can be routed directly to tissue with a much smaller isotope fractionation. In contrast, a heterogeneous diet composed of detrital material and non-digestible material can yield variable N isotope fractionations between diet and tissue depending on which fraction the animal is actually digesting. The variability in isotopic fractionation due to metabolism will be discussed later.

My colleague Noreen Tuross, who began her first postdoc at the National Institute of Health working on the chemistry of bone formation, came to the Geophysical Lab for a second postdoc (Fig. 8.4). Noreen specialised in both the modern and fossil protein fields, having training in immunology and ancient DNA methodology, as well as protein biochemistry. She wanted to investigate the nitrogen isotope fractionation between

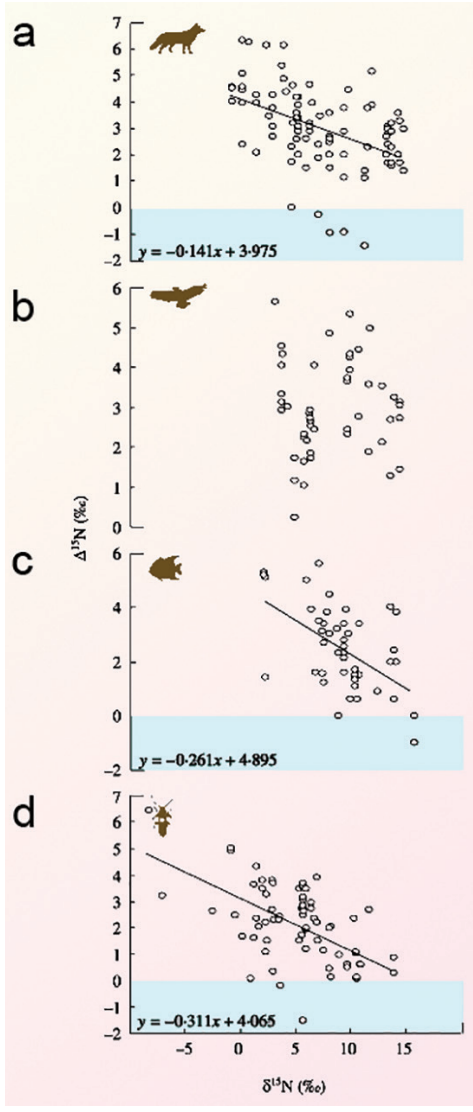

Figure 8.3 Relationship for an animal's taxonomic class and estimates between estimates of $\Delta^{15}N$ and nitrogen diet isotopic ratio $\delta^{15}N$. Each taxonomic class is represented by a pictogram. Regressions are only shown when significant. Modified from Caut *et al.* (2009).

a mother and her nursing infant. This idea was important for understanding why the human population rose dramatically after the origin of agriculture. Theoretically, infants should have a $\delta^{15}N$ that is 3 to 4 ‰ more positive than their mothers, similar to that measured in other animals. Our hypothesis was the following: prior to a secure source of food for raising children, mothers (*i.e.* hunter gatherers) needed to nurse their children for longer periods of time. Once food could be grown and cached, mothers (*e.g.*, agriculturalists) could wean their children earlier, get pregnant and have more children, thus leading to a rise in population. At the time of this study, I was pregnant with my daughter Dana. We eagerly anticipated her birth and began sampling her fingernails and mine right after she was born. In addition, samples were obtained from other mothers and their infants who were exclusively being nursed. As we predicted, we found a 3 ‰ nitrogen isotope fractionation between mothers and infant pairs. As the babies were weaned onto solid diets comparable to that their mothers were eating, the nitrogen isotope fractionation between mothers and babies decreased over time (Fogel *et al.*, 1989b) (Fig. 8.5). After babies were fully weaned, their $\delta^{15}N$ values were the same as their mother's.

Figure 8.4 Noreen Tuross and I (centre), Airlie House, Virginia, 1984. Photo credit: M. Fogel.

We then recruited Douglas Owsley, a forensic anthropologist at the Smithsonian Institution, to analyse human bones from two populations: hunter gatherers from Tennessee and corn-growing Indians from South Dakota. We found that even though these two populations depended on very different sources of food, children were weaned about at the same age, roughly a year old, and their $\delta^{15}N$ values in bones matched the adults in the population by the time they were two to three years old (Fig. 8.6). The nursing effect has been

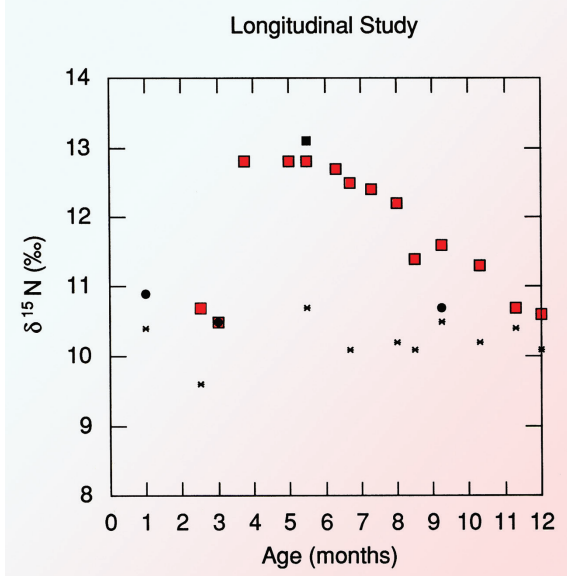

Figure 8.5 Fingernails sampled from my infant daughter, husband, and me when I was breast-feeding and weaning. Dana's fingernails are red squares, mine are small "x"; my husband's, circles. Black squares are samples of Dana's hair. Modified from Fogel *et al.* (1989b).

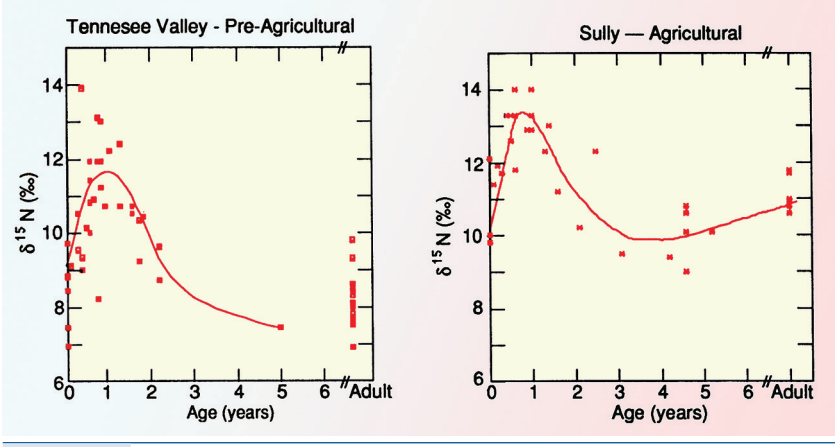

Figure 8.6 Nitrogen isotope composition of human bone collagen from Tennessee Valley (Pre-agriculture) and Sully site, South Dakota (Maize agriculture). Age is at the time of death. Modified from Fogel *et al.* (1989b).

measured in populations of other mammals – seals (Newsome *et al*., 2010a), cave bears, killer whales (Newsome *et al*., 2009a), as well as being a keystone work for anthropological investigations of ancient civilisations (Xia *et al*., 2017; Katzenberg and Waters-Rist, 2019).

8.1 Australia Palaeoclimate

Stable isotopes of oxygen in marine foraminifera and their incorporation into carbonaceous sediments are the basis for dating marine sediments (Shackleton and Opdyke, 1973). Marine isotope measurements of oxygen record palaeo-sea levels and palaeotemperatures. The $\delta^{13}C$ of marine carbonates reveal insights about primary productivity and decomposition, Ice Ages, warm periods, and the activity of the biological pump. Terrestrial records of carbon cycling primarily are studied in laminated lake sediments by analysing various parameters going down core and associating them with sediment age. In the early 1990s, Ed Hare and his colleague Allison Brooks, an archaeologist at George Washington University, developed techniques for dating fossil ostrich eggshells, which are ubiquitous in many African archaeological deposits (Brooks *et al*., 1990). As opposed to bone or tooth, eggshell holds onto its protein matrix and is not affected by groundwater leaching even over 100,000s of years. Von Schirnding *et al*. (1982) measured carbon isotopes in ostrich eggshell to demonstrate that the $\delta^{13}C$ holds a signal of the bird's diet at the time it laid its egg.

In 1991, Beverly Johnson, then a Ph.D. student with Gifford Miller at the University of Colorado, came to the Geophysical Laboratory for several months to pilot a study on stable carbon and nitrogen isotopes in fossil and modern flightless bird eggshells (*e.g*., ostriches and emus). The work turned into her Ph.D. dissertation and began a 28 year project that I have continued with Miller. Using the old-fashioned sealed tube methods, Beverly had to dissolve the eggshell, and then precipitate the calcium using hydrofluoric acid, otherwise the salts reacted with the quartz tube and cracked it. This was a very tedious and time consuming process. Beverly managed to locate eggshells from farm-raised ostriches in the United States, along with their ostrich chow diet (Johnson *et al*. 1998). She also field collected ostrich eggshell from South Africa to calibrate the $\delta^{13}C$ differences between diet, carbonate $\delta^{13}C$, and organic eggshell $\delta^{13}C$. Her work on Equus Cave, South Africa, determined changes in vegetation, rainfall, and temperature over the last 17,000 years (Johnson *et al*., 1997) (Fig. 8.7).

As this work was being completed, Miller was starting a collaboration with John Magee and others at the Australian National University in Canberra. Emu eggshell and eggshell fragments from the extinct fossil bird *Genyornis* were plentiful in sand dune deposits throughout much of the arid interior of the Australian continent (Fig. 8.8). *Genyornis* was a two metre high flightless bird endemic to Australia. Beverly, now a postdoc, Giff, and Magee joined my family for my first trip to Australia in 1994. My son was 3 years old and just recovering from a nasty bout of chickenpox. My daughter, 7 years old, was

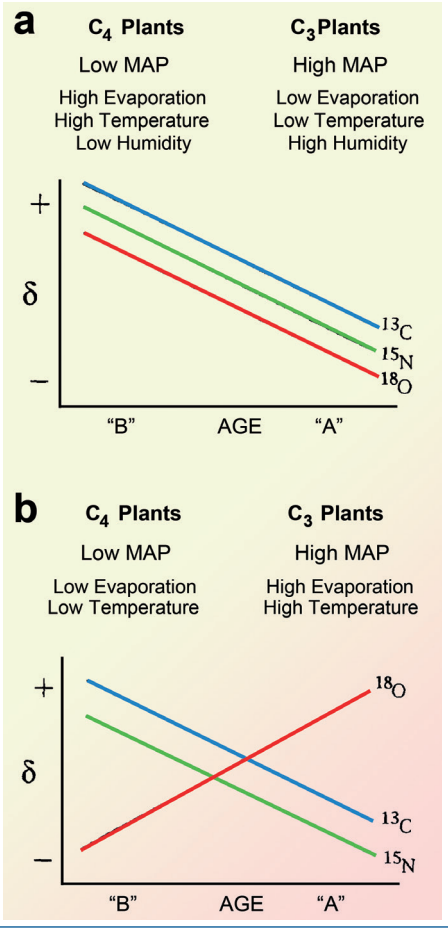

Figure 8.7 **(a)** Modelled paleoenvironmental interpretations from the stable carbon, nitrogen, and oxygen isotope compositions over two arbitrary times (B and A), where all three isotopes co-vary. **(b)** Theoretical paleoenvironmental interpretations from the stable carbon, nitrogen, and oxygen isotope compositions over two arbitrary times (B and A), where the oxygen trend is decoupled from the carbon and nitrogen trend, and primarily reflects a temperature signal. Modified from Johnson *et al.* (1997).

very interested in animals and plants. My husband served as driver, campsite manager, babysitter, and principal ornithologist. We travelled from Adelaide, in Southern Australia, all the way north to Kakadu National Park, almost in Darwin, NWT, and then back again, a ~6,000 km roundtrip. All of the fieldwork was in remote regions of the Outback where we pitched a tent and prepared meals over a campfire. I collected plants and soils along the transect, learning to identify new species in the different regions of the continent (Fig. 8.9).

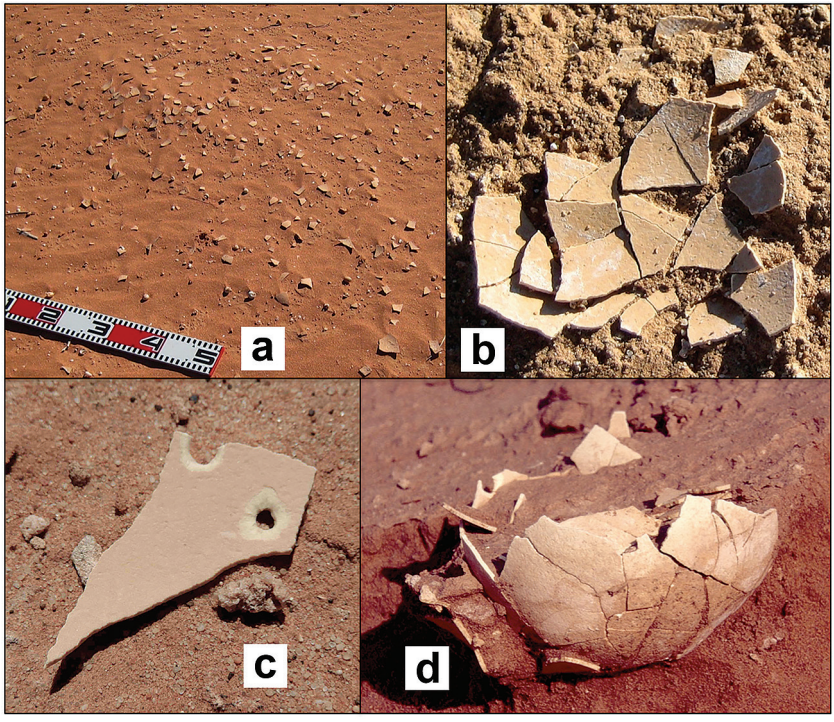

Figure 8.8 *Genyornis* eggshell in settings where they were found. **(a)** Surface clusters of eggshell fragments from the WA region were common. **(b)** Broken *Genyornis* egg cemented in a soil profile from the Lake Frome region. **(c)** *Genyornis* eggshell fragment, NW of Port Augusta with a characteristic hole produced by a predator. **(d)** An intact *Genyornis* before collection, Port Augusta region. All photos by G. Miller. From Miller *et al*. (2016a) with permission from Elsevier.

This trip was my first international field expedition. I did not have proper plant import permits from the United States Department of Agriculture, so when we returned to the United States, my plants were confiscated at the border. I learned my lesson. Through contacts at the Smithsonian, I obtained a permit and after a nervous month, all of my samples entered the United States and were delivered safely to my lab. I brought back 250 plant specimens and over the next year we analysed their carbon and nitrogen isotopic compositions. Grasses in Australia shift from C_4 metabolism in the North to mixtures of C_4 and C_3 grasses in the very south. *Acacia* species, both shrubs and trees, and eucalyptus trees are C_3 plants. In general, the vegetation in Australia has mixtures of grasses, *Acacia*, and Eucalypts with variable proportions of herbaceous C_3 plants and chenopods. Our goal was to determine the extent of C_4 grasses in an Australian animal's diet. Chenopods can be the predominant

plants in many locations. Individual species of chenopods can use C_3, C_4, or a combination of C_3-C_4 types of photosynthesis, therefore it was important to keep plant composition in mind when interpreting the $\delta^{13}C$ in eggshells.

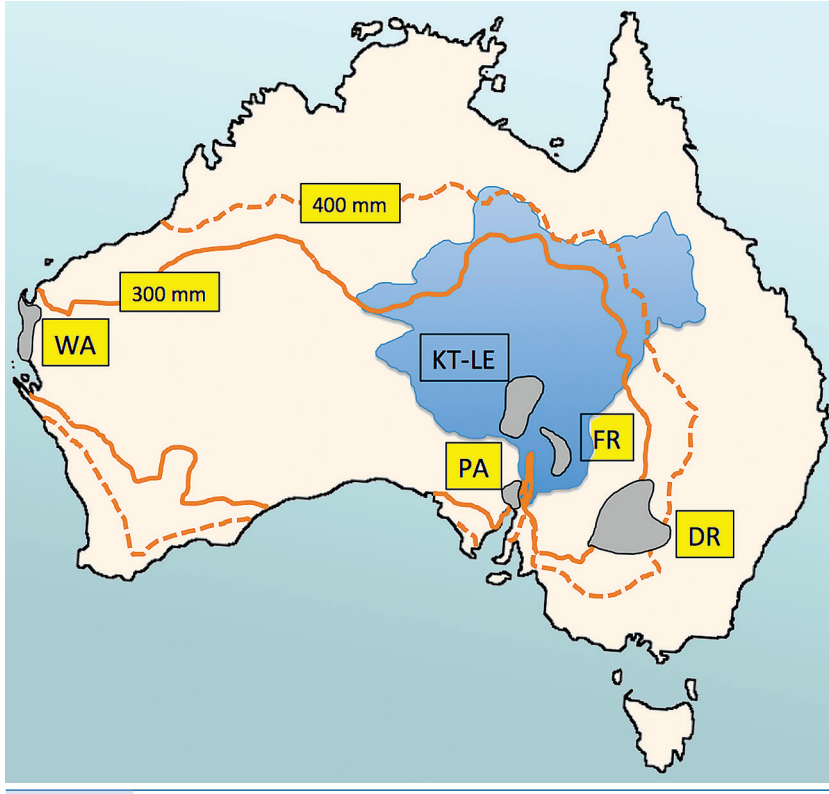

Figure 8.9 Map of Australia, showing the five regions from which avian eggshell were collected (gray). The 300 and 400 mm isohyets show modern mean annual precipitation. The Lake Eyre Basin catchment is highlighted in blue. WA: sites in the North West Cape region of Western Australia, KT/LE: sites around Kati Thanda – Lake Eyre, South Australia, FR: sites around lakes Frome, Callabonna, Blanche, and Gregory, South Australia, PA: sites between Port Augusta and Lake Torrens, South Australia, DR: sites in lake-shore lunettes along the lower Darling River, the Willandra Lakes, and Lake Victoria, western New South Wales. From Miller *et al.* (2016a) with permission from Elsevier.

Because we were ultimately studying the diets of emu and *Genyornis*, my field collections centred around two things: the general vegetative landscape and potential emu diet. Emus consume the seeds and flowers of all plants except eucalypts; they sometimes eat whole leaves and consume fruits when available.

Without question, emus will also eat any insect or lizard that they can catch, which augments their protein intake substantially during the nesting season, particular in arid areas. With subsequent trips in 1998, 1999, 2000, 2001, and 2008, I amassed nearly 1000 plant specimens from all over the continent. Based on this work, we were able to assemble a graph of the $\delta^{15}N$ of plant tissue as a function of precipitation. Also, we determined a $\delta^{13}C$ gradient for C_3 plants, as a function of precipitation with $\delta^{13}C$ values ranging from as positive as -22 ‰ in the most arid regions to -32 ‰ in the wet tropical north.

Fortunately by this time, we were using a new elemental analyser combustion system to measure the carbon and nitrogen isotope values of eggshell organic matter. We developed a streamlined technique acidifying the carbonate directly in a muffled 5 x 9 mm silver boat, reacting the sample overnight, then drying under a stream of N_2. With a one cm^2 fragment of fossil eggshell, we could obtain a radiocarbon date, an amino acid racemisation date, the $\delta^{13}C$ of both organic and inorganic fractions, and the $\delta^{15}N$ of the organic fraction. From these measurements we learned the age of the sample, the diet of the bird, the proportion of C_3 and C_4 plants on the landscape, and the palaeo-precipitation and transpiration climate parameters (Johnson *et al*., 1999; Miller *et al*., 1999). With ever-increasing geographical coverage of Australia's arid interior based on many months in the field especially by Giff Miller and John Magee, we assembled ~150,000 year climate records from several places, enabling us to date and determine major ecosystem shifts.

Our work in Australia intersected with one of the major controversies in palaeontology today: whether the extinctions of megafauna occurred because of human interactions or because of climate change. On every major continent, large mammals, reptiles, and birds have become extinct in the past 10,000 to 50,000 years (Barnosky *et al*., 2004). In North America, mammoths and mastodons were extant when the earliest humans arrived a few thousand years after the Last Glacial Maximum (LGM). These huge animals went extinct within 4,000 years of human arrival. In Australia, the timing of the extinction event as well as the arrival of humans was unknown when we began our work. Because each eggshell sample was dated by amino acid racemisation, we could determine that *Genyornis* went extinct at 45,000 ± 2,000 years ago throughout Australia. An early criticism of our extinction dates was that our samples were collected only in the Lake Eyre basin. Subsequent field work across western Australia, as well as in the Lake Frome and the Murray-Darling River Basin in the south, proved that we were recording a continent-wide extinction event.

Carbon isotopes in the eggshells opened a new window and revealed much about how the Australia Outback ecosystem changed over time. Prior to the arrival of humans, the $\delta^{13}C$ of emu eggshell reflected the full range in potential diet from 100 % C_3 to 100 % C_4 vegetation (Fig. 8.10). *Genyornis'* diet was less varied than that of co-exisiting emu and always included a significant component of C_4 vegetation (presumably grasses) (Fig. 8.11). Earlier estimates by palaeontologists had presumed that *Genyornis* was a browser, eating only

leaves from trees. We found this not to be the case. The carbon isotope data throughout the continent portrayed a rich mosaic of vegetation composed of both C_3 and C_4 plants prior to the arrival of humans and the megafaunal extinction.

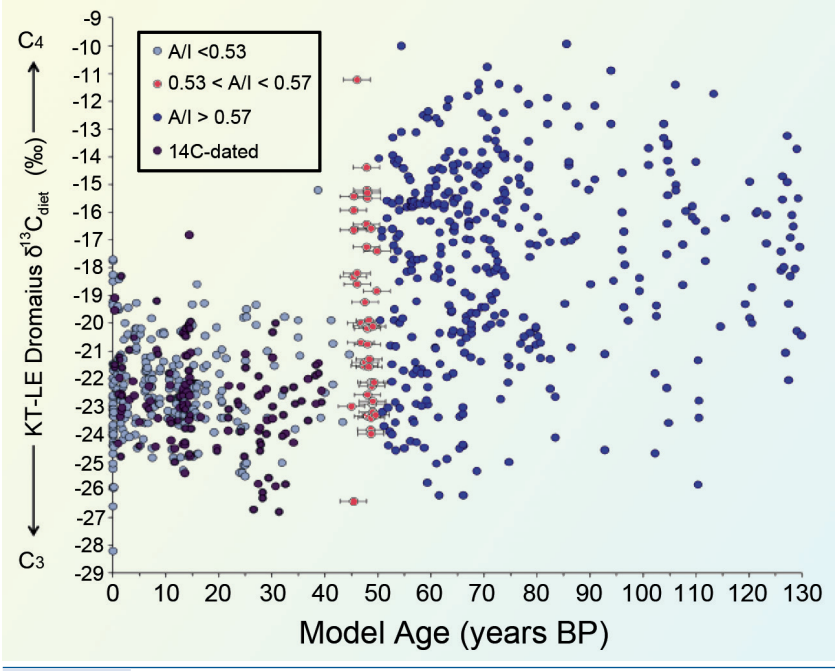

Figure 8.10 *Dromaius* (emu) $\delta^{13}C$ from the Lake Eyre region show a dietary shift after 50 years BP. Ages of these eggshells were determined by amino acid racemization and confirmed with 89 ^{14}C-dated samples (dark blue) are plotted on their calibrated ^{14}C age. Samples with model ages between 50 and 45 ka, corresponding to the extinction window, are plotted as red circles indicating uncertainty in these dates. From Miller *et al.* (2016a) with permission from Elsevier.

After *Genyornis* disappeared from the fossil record, the $\delta^{13}C$ of emu eggshell shifted dramatically to more C_3 vegetation indicating that there was considerably less plant diversity in their habitat. We termed this an "ecosystem collapse", where large animals like *Genyornis* and *Diprotodon* that relied on grass could no longer be sustained by diminished grasslands. *Diprotodon,* the first fossil mammal described from Australia (Owen, 1838) was a large wombat-like marsupial that was widespread across the continent when humans arrived. The widespread ecosystem collapse – meaning vegetational change – requires large scale phenomena to drive the continental shift in vegetation composition. We proposed that human use of fire might well have caused the collapse.

Figure 8.11 The climate evolved along with the diet of *Genyornis*. The 20-point dietary running mean is shown by the heavy top black line and highlights a slow increase in C_4 dietary for *Genyornis* beginning about 100 BP. *Genyornis* went extinct at 50 year BP. From Miller *et al.* (2016a) with permission from Elsevier.

Vegetation in Australia today is principally fire-adapted, as it is in Africa and arid regions of North America. Fire-adapted plants re-grow rapidly after fire, and some even require fire for seed germination. Early humans practised landscape burning to clear the ground to enable easier movement, for hunting, and for communicating between distant groups of people. Many of Australia's plants, such as *Eucalyptus* and *Acacia* species, can tolerate being burnt once every 25-30 years. They re-grow from the burnt stumps and within a few years are fully re-established again (Latz, 1995). The grasses also are fire-adapted, but if fires occur more frequently, for example every 5-10 years, more palatable grasses disappear and are replaced by the spinifex grasses (*Triodia* and *Pletrachne* sp.). Spinifex species are much less palatable for native animal species, such as emus and kangaroos, even though these plants are widespread. In addition to changing the type of vegetation, frequent burning can result in lower soil organic matter contents, which in turn decreases the soil's ability to hold moisture. Soil moisture affects how far the Asian monsoon can penetrate into the Australian outback. Lower soil moisture results in lower rainfall in the continental interior. A decrease in annual rainfall affects the type of vegetation, including both fire-adapted and drought-adapted plant species.

The oxygen isotope data from emu eggshell holds a moisture and rainfall signal: Point Potential Evaporative Transpiration (PPET). PPET uses both temperature and rainfall data to predict the amount of evaporative transpiration that can occur in a landscape. Higher PPET values indicate drier climate and *vice versa*. Miller and I completed a study of modern emu eggshell that could be correlated to measured PPET (Miller and Fogel, 2016). We determined that the PPET during March and April correlated most strongly with our eggshell data from modern specimens. Once we determined this calibration, we interpreted our data from the past 150,000 years (Fig. 8.12). At the time of the megafaunal extinction 45,000 years ago, our data shows that PPET remained fairly constant until the LGM, when dry conditions were present throughout the continent. At Lake Eyre, there was a slight increase in aridity, which was not measured in any of the other locations. While others (Cohen *et al*., 2015) argued that increased aridity caused the extinction, our data does not fully support this conclusion.

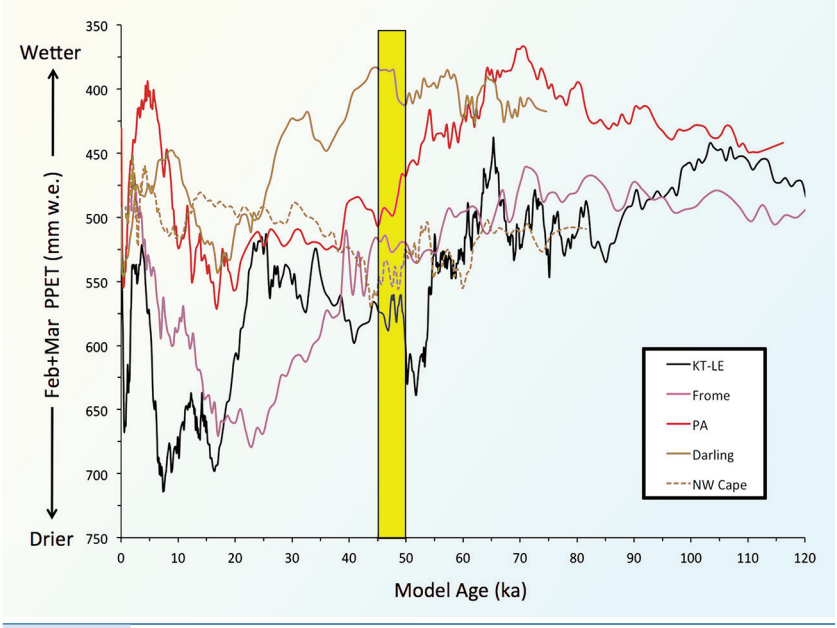

Figure 8.12 Based on a set of modern emu eggshells collected throughout Australia, we developed a relationship between Point potential evaporative transpiration and $\delta^{18}O$ of eggshells. This graph shows reconstructed PPET from *Dromaius* eggshell $\delta^{18}O$ from five regions. All isotope records show relatively moist climates prior to 60 ka, although varied. After the arrival and settlement of humans (marked by the shaded box), arid conditions were found, but occurred after the shift in carbon isotopes. From Miller *et al*. (2016a) with permission from Elsevier.

Nitrogen isotopes in eggshell provide information on diet as well as mean annual precipitation (MAP). Based on a "training set" of modern emu and plants sampled from similar environments, we established a relationship between $\delta^{15}N$ and MAP for plants and emus (Newsome *et al.*, 2011a). At very low MAP amounts (<200 mm per year), the $\delta^{15}N$ of emu eggshell was considerably more positive than the expected 3-4 ‰ enrichment for most other trophic pairs (*i.e.* an animal and its diet). We argued that the more positive $\delta^{15}N$ in emu eggshell reflected a change in diet towards a more carnivorous diet containing insects and small reptiles (Fig. 8.13). These data also indicated increased consumption of chenopods, arid plants with more positive $\delta^{15}N$ values that are widespread in the Australian deserts. *Genyornis* and emu eggshell prior to the extinction had $\delta^{15}N$ values 2.5 ‰ lower than modern emu, indicating that MAP precipitation was wetter 75-130 kyr ago with rainfall amounts exceeding 300 mm per year.

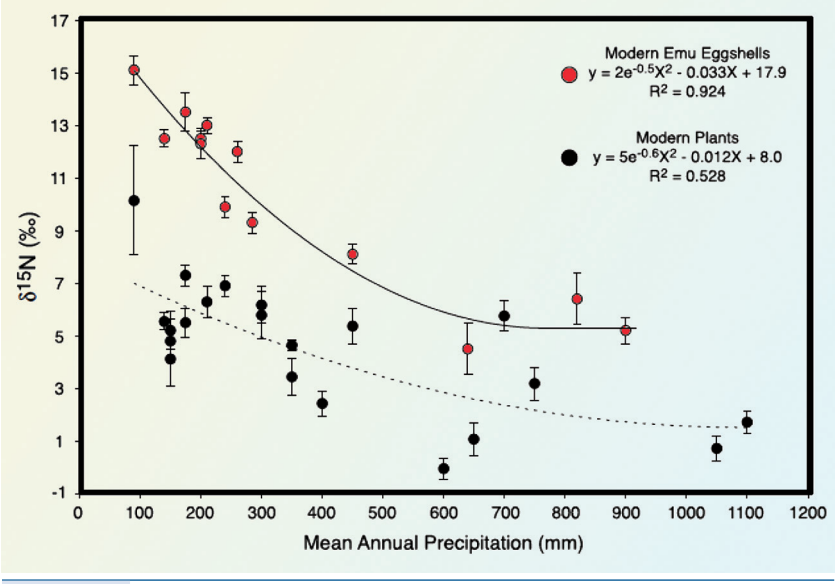

Figure 8.13 Plants were collected after every field trip I made to Australia. We measured the isotopic composition of all of them, a database of over 1,000 specimens. The plant data was compared modern emu eggshells collected by Giff Miller and John Magee. Similar to other continental scale measurements, $\delta^{15}N$ varied as a function of mean annual precipitation. Modified from Newsome *et al.* (2011a).

With two decades of work on Australia's palaeoclimate, Miller, Magee, and I are experts on ages and isotopic compositions of fossil eggshells. The larger scientific community in Australia remains skeptical, including challenging our identification of the *Genyornis* eggshell (Grellet-Tinner *et al.*, 2016;

Miller *et al.*, 2017). Interestingly, no other group in Australia or elsewhere has produced evidence to refute our findings with eggshells. What exactly caused megafaunal extinction, to me, is clearly linked to human expansion on the continent. In Western Australia's coastal region, we found that human use of fire for cooking *Genyornis* eggs could explain at least some of the decline of this species (Miller *et al.*, 2016b). General Circulation and other climate models that take into account vegetation, soil moisture, as well as temperature, can now be used with our data to explain on larger and more complex continental scales how Australia's ecosystems evolved over time (*e.g.*, Wyrwoll and Valdes, 2003; Wyrwoll *et al.*, 2007). Only a combination of factors, including landscape burning, vegetation changes, hunting, and climate forcings, can explain megafaunal extinctions, in Australia and other continents.

9. MICROBIAL BIOGEOCHEMISTRY

In my 1979 application to be a staff member of the Geophysical Laboratory, I wrote "*Geochemists base much of their interpretations of sedimentary carbon on the premise that bacteria have little to do with changing or fractionating isotopes in sediments*." I proposed to examine the isotope fractionations in lipids from cultures of microbes, to isolate microbes from various environments, and to incubate samples *in situ* and follow changes in their chemistry and isotopic compositions. My early work in this area started with the microbes at Yellowstone National Park (see above; Estep, 1984 and Estep and Macko, 1984). Investigations with microbes followed throughout my career.

9.1 Manganese Minerals

The role of microorganisms in the formation of ore deposits was a theme that I was encouraged to study by Carnegie Director Hatten Yoder. In 1983, I organised my first Airlie House conference on Organic Matter in Ore Deposits, inviting an interdisciplinary group of scientists from around the world. Ken Nealson, then at Scripps Institution of Oceanography, was working on manganese oxidising bacteria, which related presumably to the formation of deep sea manganese nodules. The biogeochemistry field at this time was small enough that you bumped into people at conferences as disparate as the American Society of Microbiology (ASM) and the Geological Society of America (GSA). I was fascinated by the possibility that stable isotopes might prove useful in studying the mechanism by which microbes turned soluble manganese into oxide precipitates. It was unknown if manganese oxides were formed with molecular oxygen or with oxygen from water, or if microbial precipitations were formed by different mechanisms from abiotic manganese oxides.

I ended up working on this problem with Ken's Ph.D. student Brad Tebo, who later replaced Ken at Scripps when he moved to the University of Wisconsin. We also worked with Brad's first graduate student, Kevin Mandernack, who grew the microbial cells, isolated the manganese oxides, then came to Washington, DC, to analyse the $\delta^{18}O$ of the oxides *via* fluorination. This was my first and last project to use BrF_5 at high temperatures and pressures to measure a sample. I learned the technique from Carnegie postdoc Page Chamberlain, who was working on metamorphic rocks in Doug Rumble's lab. The work was physically demanding in several ways. You weighed out a few milligrams of sample into a nickel "bomb" – a cylinder of nickel metal about 40 cm long – and attached it to a vacuum line where BrF_5 was frozen into the bomb, then heated to 600 °C for 20 hours. Often, the teflon seals on the bombs leaked. Manganese minerals had never been analysed before using this technique, so we had to develop methods to remove adhering water before reacting the manganese oxides.

We collaborated also with Alan Stone at Johns Hopkins University, who prepared abiotic manganese minerals of various oxidation states: Mn(III) (hausmanite) and Mn(IV) (buserite). We compared these minerals with ones that were biologically precipitated by active live, growing microbes or passive spores (Mandernack *et al.*, 1995). Oxygen isotope analysis of the Mn(IV) minerals revealed significant incorporation of molecular O_2 with both abiotic and biological mechanisms (32-50 %). Kinetic isotope fractionation values were calculated for molecular O_2 incorporation and were quite large: -13 ‰ for chemical manganates and -22 ‰ for spore-precipitated oxides. Oxygen from water was incorporated with minimal isotopic fractionation: -5 ‰ for abiotic precipitation and +1 ‰ by inactive spores. In contrast, the active microbial culture produced minerals showing only a small negative isotope fractionation for either oxygen source.

Brad Tebo, now at Oregon Health and Science University, continues to investigate bacterially-mediated manganese cycling and is a leader in that field. He has isolated many strains of bacteria, as well as studied their genomes to determine which enzymes are involved in the catalysis. Tebo's recent work challenges the paradigm that the manganese cycle operates between soluble Mn(II) and solid Mn(IV) phases. Mn(III) is now seen as an important intermediate formed by microbes in many environments (Oldham *et al.*, 2017) (Fig. 9.1). Tebo and his colleague George Luther (University of Delaware) have made significant strides in unraveling the complexity of manganese cycling to a much greater degree than our earlier experiments in the 1990s. Recently, Sutherland (Sutherland *et al.*, 2018) in Scott Wankel's lab at Woods Hole measured the $\delta^{18}O$ of manganese minerals produced by microbes and obtained results similar to our initial experiments, but by using very different

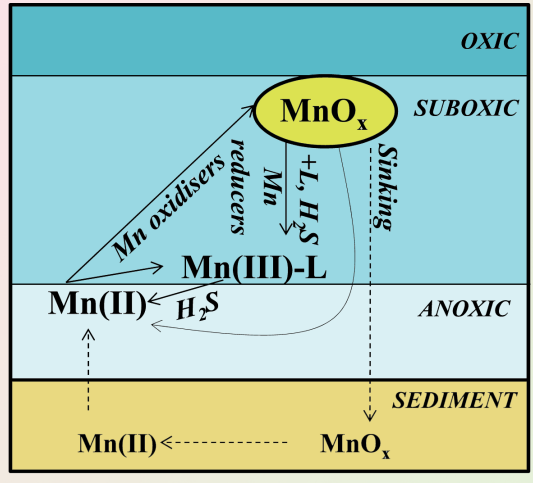

Figure 9.1 Earlier work highlighted only Mn (II) and Mn (IV) without knowing the Mn (III) was an important intermediate. Manganese cycling in and below the suboxic zone in the water column of Chesapeake Bay is more complicated than originally thought. Dashed lines indicate processes that are predominantly occurring before summer stratification and anoxia develops. Modified from Oldham *et al.* (2017).

methods. They measured O_2 isotope fractionations directly by measuring the isotopic composition of O_2 in solution as a function of Rayleigh distillation, similar to the methods we used to study the Dole Effect (Guy *et al.*, 1993). The manganese minerals were analysed directly *via* thermal pyrolysis, automatically and without needing to use dangerous fluorine. Perhaps these new techniques will open up more isotope research now that the molecular mechanisms for manganese precipitation are becoming well known.

9.2 Microbes in Extreme Environments

One of the major themes of research at the Geophysical Laboratory is the effect of high pressure on the formation and properties of minerals. Colleagues subjected mineral samples to Gigapascals of pressure in microscopic diamond anvil cells, where they could interrogate the chemistry of the minerals through phase changes. Diamond anvil cells could also be heated internally, so that the effects of both pressure and temperature could be modified to simulate the Earth's geophysical gradient. Postdocs Anurag Sharma, a mineral physicist, and James Scott, a microbiologist working in my lab, developed a productive and creative bond that led to experiments subjecting microbes to extremes in pressure and observing how they behaved. The two fed off each other's strengths: James, a big thinker, and Anurag, an accomplished experimentalist. James cultured cells of the super bug *Shewanella oneidensis* and the ubiquitous *E. coli*; Anurag loaded them carefully into diamond anvil cells. We were able to measure formate oxidation spectroscopically and determined the cells were metabolically active up to pressures of 1000 Megapascals (Sharma *et al.*, 2002). Our work, however, was controversial. Negative comments by experts in the field of high pressure microbiology followed the publication of our high profile paper (Yayanos, 2002). We prepared a response hoping to carry additional experiments (see article by Yayanos, 2002). Unfortunately, Scott died tragically from a heart attack while he and Anurag were in the midst of continuing their work using other organisms and new methods for confirming that the microbial cells remained intact and metabolically active.

The work continued with postdoc Adrienne Kish and graduate student Patrick Griffin, who developed different techniques using the cold seal apparatus that took pressures only up to 400 Megapascals. The beauty of these experiments is that the cells could be readily recovered and cultured to determine how many of them could survive pressure treatment. Halotolerant species survived completely after pressurisation, far surpassing the survival rate of *E. coli*, which is not generally known for being a piezophilic microbe (Kish *et al.*, 2012). Pressure experiments with microbes are difficult and few have followed up on these experiments, however the concepts developed from our studies of microbial pressure tolerance have prompted others to extend the habitable zone in planets to greater depths.

9.3 Molecular Biogeochemistry

Understanding the role of microorganisms in influencing sedimentary organic matter evolved rapidly after 1979. Today, there are more studies of the structure and isotopic compositions of microbial biomarkers and molecules than there are of compounds from higher plants (*e.g.*, Schouten *et al*., 2002; Hopmans *et al*., 2004; Schouten *et al*., 2013). Following on the earlier work with Ron Benner on *Spartina* decomposition, I started a study in 1991 using modern molecular techniques for determining how microbes changed the original isotopic compositions of plants into sedimentary organic matter. My husband Chris Swarth was the Director of the Jug Bay Wetlands Sanctuary in Maryland, and our family spent every weekend there for almost 8 years. Every year, I watched the growth of lush marsh vegetation in late spring, followed by rapid decomposition in early fall. It seemed like a no-brainer to start a litter bag decomposition project here.

I followed the progression of decomposition in my samples with compound specific isotope analysis (CSIA) using gas chromatography-combustion-continuous flow methods, which had been used in my lab for about 5 years. Noreen Tuross, now a scientist at the Smithsonian Institution, taught me how to conduct ELISA (enzyme linked immunosorbant assay) using two monoclonal antibodies developed for the enzyme Rubisco and a lab synthesised humic acid. I also learned modern techniques in protein purification and Western blotting, which visualised molecular fragments by reaction with specific antibodies.

I was still fascinated with Rubisco and was curious how this major plant protein degraded along with the other major plant biochemicals. In non-woody herbaceous plants, Rubisco declined by 40-80 % in three months time, but we could still detect intact high molecular weight, 55,000 dalton Rubisco subunits (Fogel and Tuross, 1999). In upland plants with thicker, waxy leaves (*e.g.*, mountain laurel), 80 % of the original Rubisco remained intact after one year of incubation in anaerobic sediments. One of the plants I included in the experiment was leaves from locally grown corn plants. Corn, a C_4 plant, was incubated in the C_3 plant environment of the wetland (Figs. 9.2 and 9.3). My hypothesis was that over time, I would be able to detect carbon isotope shifts from corn's C_4 values ($\delta^{13}C$ = -12 ‰) to C_3 values originating from organic matter in the wetland ($\delta^{13}C$ = -27 ‰).

I was able to purify the different subunits of Rubisco from fresh corn, then measure the $\delta^{13}C$ of amino acids from the two subunits, as well as the amino acids in the bulk corn tissue. We found that the $\delta^{13}C$ in amino acids in purified Rubisco subunits had different compositions with respect to bulk protein. In addition, the amino acids within large and the small units in fresh Rubisco had different $\delta^{13}C$ values from each other. The small subunit is coded by a gene in the nucleus but is synthesised in the cytoplasm primarily in leaf tissue, as well as in stems and petals. The large subunit, however, is coded by a gene in the chloroplast and is synthesised within the chloroplast. The

complete Rubisco molecule is assembled after the small subunit is imported into the chloroplast (Gutteridge and Gantenby, 1995). Thus, it is not surprising that the $\delta^{13}C$ of individual amino acids from each subunit should have different isotopic compositions.

Figure 9.2 Fresh corn (Zea mays) leaves that formed the basis of plant taphonomy studies at Jug Bay Wetlands Sanctuary. Photo credit: M. Fogel.

We also compared the $\delta^{13}C$ pattern of amino acids in fresh plant material with those in decayed plant material. In most modern plants, the range in $\delta^{13}C$ is typically about 20 ‰. The simpler amino acids (*e.g.*, serine, glycine, alanine) have more positive $\delta^{13}C$ values, whereas the more complex ones (*e.g.*, valine, leucine, phenylalanine) have more negative values (Fogel and Tuross, 2002). For geochemical studies, a sample's amino acid fingerprint – the relative differences in $\delta^{13}C$ among amino acids – could be altered during microbial decomposition either by the preferential breakdown of amino acids or addition of microbial proteins leading to a heterogeneous mixture. With postdoc Susan Ziegler (Ziegler and Fogel, 2003), we compared the $\delta^{13}C$ of a diatom fraction with general POM, bacterial, and dissolved organic matter in the Jug Bay estuary where the earlier study (Fogel and Tuross, 1999) had taken place. The fingerprint for the POM fraction was very similar to the bacterial fraction on the basis of amino acid fingerprints. Interestingly, although bulk $\delta^{13}C$ of POM was almost constant throughout the year, the $\delta^{13}C$ of some amino acids, specifically alanine varied by 12 ‰. This particular study is important because it demonstrated the power of compound specific analyses for studying dynamic ecosystems with significant microbial inputs.

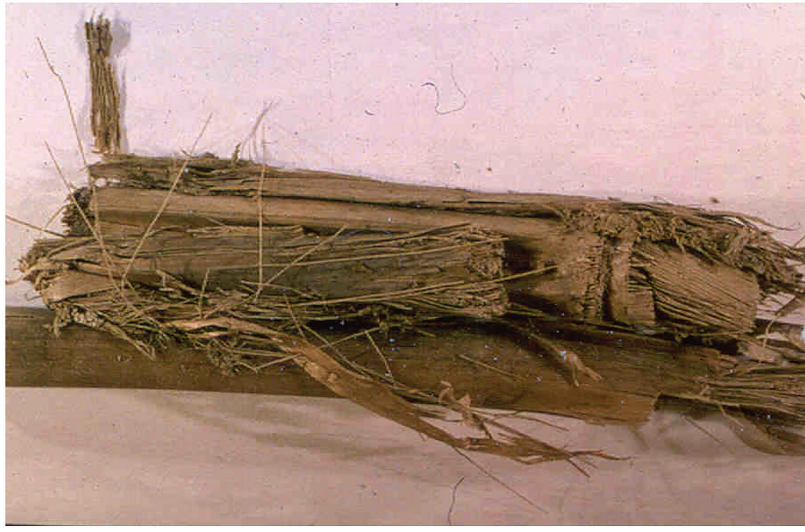

Figure 9.3 Decomposed corn leaves after one year from sediments at Jug Bay Wetlands Sanctuary. Photo credit: M. Fogel.

The isotope fingerprint approach was first developed by James Scott and me with cultures of microbes from the three domains of life (Archaea, Eukaryotes, and Eubacteria) including different carbon assimilation pathways, anaerobic *vs.* aerobic growth, and energy sources (Scott *et al.*, 2006). Using discriminant function analysis (DFA), we separated heterotrophs from autotrophs and from those organisms that used a tricarboxylic acid bypass shunt for carbon metabolism. Even though the enzymes associated with amino acid biosynthesis were fairly well known in general, it was not specifically known at that time which enzymes were active in many species of microbes. The DFA results showed that Archaea clustered with Eubacteria based on their carbon metabolisms, rather than their genetic lineages. The relationships among amino acids were also highlighted by linear regression analysis of amino acid pairs (*e.g.*, alanine and glutamate) that had slopes ranging from 0.80 to 1.17. Alanine and aspartate, for example, are highly correlated even though alanine is not a direct precursor to aspartate. For each of these pairs (*e.g.*, glutamate and proline), we calculated the intercept, which essentially is the isotope fractionation between the two amino acids. Intercepts, hence isotope fractionations, ranged from -15 to +5 ‰. The only pair with a positive isotope fractionation was alanine to serine (5 ‰); the most negative isotope fractionation was for alanine to leucine, which in fact requires several enzymatic steps.

Thomas Larsen and Diane O'Brien at the University of Alaska (Larsen *et al.*, 2009) picked up on this idea of using plant, bacterial, and fungal carbon isotope fingerprints, particularly using the $\delta^{13}C$ of essential amino acids: *i.e.* threonine, valine, leucine, isoleucine, methionine, phenylalanine, and lysine. In their first paper, they used linear discriminant analysis to test their hypothesis that essential amino acids could be segregated by statistical methods and these could be used as mathematical isotopic fingerprints (Fig. 9.4). Non-essential amino acids did not separate as well. Larsen *et al.* (2012, 2013) developed the amino acid fingerprinting approach further to include different types of algae as well as to analyse animal tissues to determine whether the approach was viable for diet source analysis. Nielsen *et al.* (2018) summarised various techniques for assessing the diets of animals (Fig. 9.5). The drawback with using only bulk isotope data to determine diet is that there are many potential sources of food for most organisms, but only a few may be consumed and assimilated. When coupled with models, isotope fingerprinting could be an even better method for determining diets, particularly with organisms that can't be observed feeding, including all those found in the fossil record. More on this later.

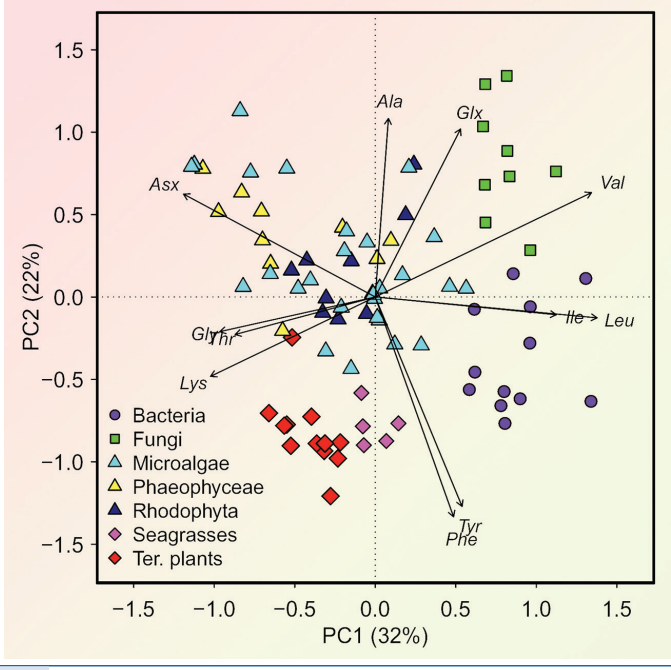

Figure 9.4 A principal component analysis of $\delta^{13}C$ of amino acid values of different producers show a range of different isotope patterns between bacteria, fungi, and terrestrial vascular plant and algae. From Larsen *et al.* (2013).

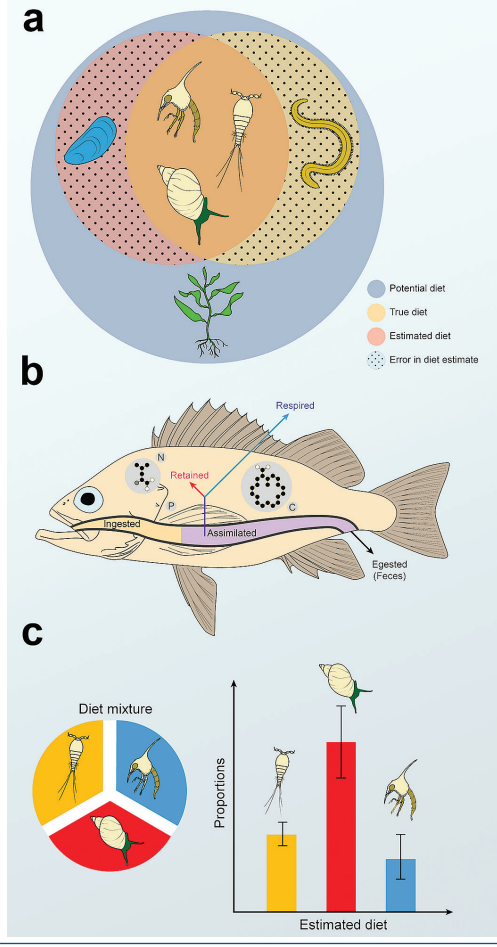

Figure 9.5 Conceptualisation of diet tracing. **(a)** Diet in the environment: A consumer's actual diet (yellow shaded area) represents some fraction of all potential diet items (blue shaded area) available in the ecosystem, while the estimated diet (red shaded area) represents diet identified by the specific diet analysis method. Mismatch between yellow and red shaded areas represents error in the diet estimation (black dotted area). The aim of diet estimation is to maximise the overlap of the true and estimated diet (orange shaded color). **(b)** Diet in the consumer: Ingested and digested diet is detected in stomach, gut or feces content (*e.g.*, visual or molecular diet analyses). Some fraction of ingested material is then assimilated, and a subset of the assimilated material is retained in the consumer's tissue, whereas non-assimilated material is respired or excreted. It is important to note that different methods measure the diet at various stages during ingestion and assimilation. **(c)** Dietary analysis: The selected method should be able to separate the different consumed items and to quantify the proportions of the individual diet items. From Nielsen *et al.* (2018) with permission from the British Ecological Society.

10. GEOCHEMICAL APPROACHES TO ANIMAL ECOLOGY

Geochemists traditionally pursue discovery based science, whereas ecologists often follow a more traditional and rigid hypothesis-based approach to their work. As a biogeochemist, I have followed both approaches and let my curiosity lead me to the most interesting science. Stable isotope methods and instrumentation "grew up" in earth science departments, but eventually ecologists and biologists have realised how powerful these methods are for figuring out major questions such as who is eating whom in a food web. Not only is the source of an animal's diet important, but dietary resources and thus energy flow in an ecosystem is something that can be influenced by environmental parameters, specifically climate change over time. Answering questions about why organisms go extinct transcends the distinct disciplines of evolutionary biology and palaeontology. Stable isotope biogeochemistry has proven to be a strong factor in uniting and integrating these two fields of study.

In 1999, the first IsoEcol conference was held in Saskatoon, Saskatchewan, Canada, hosted by Len Wassenar and Keith Hobson. I was the invited keynote speaker. Everyone at the conference was measuring or was working with someone who could measure bulk isotopic compositions in plants and animals. For the first time while presenting isotope data like this to ecologists and biologists, I didn't get the usual question from the audience "*Do you really believe these magic numbers can tell us anything important?*" However, only one or two other attendees were using compound specific isotope analysis (CSIA) methods, so our Geophysical Laboratory contingent, Mark Teece and Matt Fantle, really stood out from the crowd. The field of isotope ecology has grown so much that almost each issue of every ecological journal includes at least one stable isotope paper. While the eco-bio people have embraced the approach, it is the geochemists who have pushed the envelope by developing new techniques. Such innovations in techniques have all come from geochemists – in my field biogeochemists or organic geochemists. The compound specific isotope analysis field is an excellent example.

While our group at the Geophysical Laboratory forged ahead with CSIA using liquid chromatography methods, these were tedious, time-consuming techniques, and really forced us to measure what Tom Hoering termed "*five well-chosen samples*." John Hayes and his students were pioneering continuous flow methods that worked directly with gas chromatography-IRMS (Matthews and Hayes, 1978), see Text Box 10.1.

Text Box 10.1 – Development of GC-C-IRMS

At this time, both of the major IRMS companies, Finnigan MAT and VG Instruments, were perfecting versions of an interface that allowed compounds in a stream of He to enter the IRMS source at pressures that could be handled by the source turbo pump. Computer software was also being drafted to handle ion counts from

distinct peaks rather than static measurements from dual inlet systems. Finnigan's team included John Hayes (Indiana University), Martin Schoell (Chevron), Kate Freeman (Indiana University graduate student), Willi Brand (Finnigan), and Bob Dias (Chevron) (Freeman *et al*., 1989). They worked with software engineer Margaret Ricci (Penn State) to produce Finnigan's first commercial model of the gas chromatograph-combustion system (GC-C-IRMS). At VG, Jeanette Jumeau, Steve Macko (University of Virginia), and Michael Engel (University of Oklahoma) developed a parallel instrument. Competition for customers was fierce!

I received NSF funding for one of these instruments in 1990, and Tom Hoering and I travelled to see both companies' products. In the VG factory in Manchester, England, the PRISM IRMS system in their demo lab was not operational. We spent two days looking at the GC-C set up, but were unable to determine how it worked. We took a second trip to John Hayes' lab where the Finnigan instrument was demonstrated by Kate Freeman, who has now been elected to the National Academy of Science. Kate started her earth science career as an undergraduate intern at the Geophysical Laboratory, washing glassware for me and working on some organic extractions for Tom, so we knew her well. The 252 IRMS was a real winner, and had the backing of people that we knew well, Freeman and Hayes, sealed the deal for us. Kate recalls, "*I remember showing the instrument to Tom. It was an exciting moment for me (and a bit terrifying). Tom walked into the room and immediately disappeared. We were startled but quickly found him crouching behind the instrument to see the pumps and guts of the thing.*"

The first system I received was relatively primitive compared to the GC- Isolink systems used today. There were four needle valves for controlling GC flow that needed to be fiddled with daily, if not before every sample. Combustion reactors were expensive, $1000 in 1992, and broke easily when they were installed. Jeff Silfer (Michael Engel's Ph.D. student) joined the Geophysical Laboratory for a short time as a postdoc and helped get our system running. It worked slightly better than the old liquid chromatography methods, and we could measure hydrocarbons, fatty acids, and other molecules that liquid chromatography could not easily separate. Much of our work at that time was discovery-based and unpublished. Our questions were very simple: what is the variation in the $\delta^{13}C$ of certain compounds in a plant? How do the $\delta^{13}C$ values compare between species? Silfer brought his amino acid methods (Silfer *et al*., 1991) to the Geophysical Laboratory and we were soon off and running on CSIA of amino acids, methods I still use today. Upwards of 40 % of presentations at the 2018 IsoEcol meeting included amino acid isotope measurements.

The impact and promise of CSIA in amino acids has been fully realised in marine science and animal ecology. A collaboration with colleagues at University of Delaware on the growth of commercially important and tasty blue crabs (*Callinectes sapidus*) resulted in one of the first studies on CSIA-AA that tested different diet sources for early growth stages in a lab setting (Fantle *et al*., 1999). Our work using both bulk tissue and amino acid $\delta^{13}C$ data showed that diet quality was important for determining the isotope fractionation between diet and animal tissue, not simply the diet quantity or source. Using nitrogen as an additional tracer, it became clear that juvenile blue crabs not only lived in and

depended on *Spartina* marshes as a sanctuary while young, they also depended on marsh carbon and nitrogen for survival. CSIA-AA showed that detrital material is isotopically heterogeneous, cautioning those using bulk tissue isotope values for diet sourcing to think about diet quality. Kelton McMahon, a graduate student from Woods Hole Oceanographic Institution, started his CSIA "career" with me at the Geophysical Laboratory and investigated the idea of determining diet quantity, quality, and source even further with fish experiments. Kelton's goal was to measure CSIA-AA in fish otoliths, the tiny ear bones that record a fish's natal habitat in rings laid down annually over a fish's lifetime. After working out the procedures (McMahon *et al.*, 2010), he went on to use this approach coupled with $\delta^{13}C$ fingerprints to study the importance of mangrove habitat for reef fishes (McMahon *et al.*, 2011).

Diane O'Brien, a postdoc from Stanford who came to the Geophysical Laboratory for long stretches of time, furthered CSIA-AA with a series of papers on dietary resources for butterflies (O'Brien *et al.*, 2002, 2003a,b, 2005). During the caterpillar life stage of butterflies and moths, plants are their only source of nutrition for these insects. Once they hatch into butterflies and moths, the adults only consume sugary nectar that contains carbon, but no nitrogen. While the adults can make all of their non-essential amino acids from nectar, they must rely on their essential amino acids from the plants consumed during the caterpillar life stage. Based on controlled feeding experiments, O'Brien confirmed that this was in fact the case, demonstrating that sugars were turned into non-essential amino acids, while essential amino acids were routed through the butterflies' various life stages (Fig. 10.1).

One of my more unusual studies was on the periodical cicada which erupts in 13 or 17 year cycles. The larval stage of the cicada remains underground for 13-17 years, feeding primarily on the fluid from root xylem tissues of deciduous trees in the area where they are developing. When cicadas emerge from the ground, they then metamophose into adult flying insects, mate briefly, lay eggs, then perish. The hatched larvae fall to the ground after 6-8 weeks, then burrow into the soil where they exist for 13 or 17 years, arguably one of the most amazing life cycles of any organism. The amino acid $\delta^{13}C$ values and the concentration data showed that xylem tissue did not have all of the essential amino acids to support the larval stages. It is well known that bacteria inhabit the guts of these organisms, and we were able to show that a significant portion of the essential amino acids in the cicadas was supplied by this beneficial microbial symbiosis (Christensen and Fogel, 2011).

In the marine realm, Matt McCarthy, who was first a student from University of Washington, then a postdoc at the Geophysical Laboratory, and now Professor at UC Santa Cruz, worked with me to assess whether CSIA of amino acid isotope analyses might be useful in understanding the production and cycling of dissolved organic matter (DOM) in the ocean. DOM is composed of 40 % defined, characterised organic compounds, like carbohydrates, amino acids, cell membrane components, while the remaining 60 % is uncharacterised

organic matter. The relationship among bacteria, phytoplankton, zooplankton, and DOM has intrigued scientists working in this field for many years (*e.g.*, Hedges *et al.*, 1997).

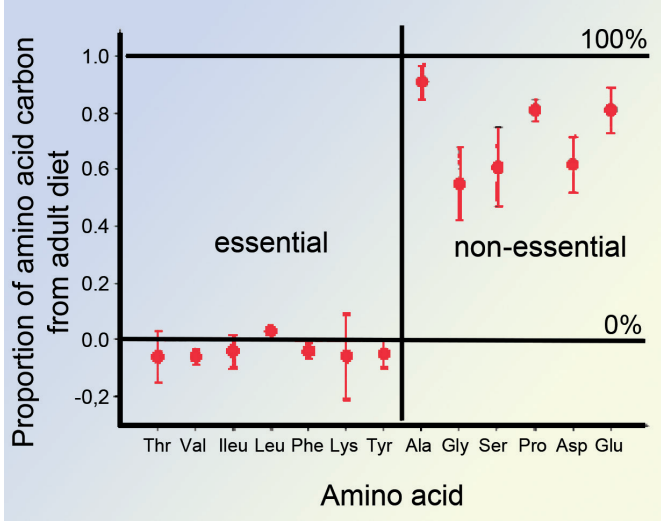

Figure 10.1 Carbon isotope compositions of essential amino acids in butterfly eggs come directly from plants that caterpillars consumed. Non-essential amino acid carbon is derived from a combination of original plant material and nectar. Modified from O'Brien *et al.* (2002).

In the 1990s, marine chemists were working out the methods for measuring DOM accurately as well as methods for concentrating this fraction from seawater. Ron Benner, at that time a Professor at UTMSI, my old alma mater, and John Hedges, University of Washington, were using ultra-filtration to separate DOM so that its complex chemistry could be studied with advanced instrumentation (Benner and Hedges, 1993; Hedges and Keil, 1995). One of our first challenges was to measure the $\delta^{13}C$ of D- and L-alanine. D-alanine is one of the principal components of bacterial cell walls and is found in DOM. Our goal was to measure the $\delta^{13}C$ of both isomers of alanine to compare a pure bacterial signal with a mixed isotope signal. Using material from sediment traps, we measured the $\delta^{13}C$ fingerprint of autotrophs (primarily cyanobacteria and diatoms), heterotrophic bacteria and microzooplankton, sinking particles, and DOM from various depths. Patterns of $\delta^{13}C$ in DOM more closely resembled autotrophs, whereas sinking particles were decomposed and resembled bacterial fingerprints. D- and L-alanine $\delta^{13}C$ from DOM were indistinguishable suggesting that much of the DOM could be attributed to cyanobacteria. We concluded that the rapid cycling of POM in the surface ocean is decoupled from the fast sinking of unaltered DOM from autotrophs (McCarthy *et al.*,

2004). Isotope fingerprinting of essential amino acids (Close, 2019) is currently the most popular use for carbon isotope data, but still remains an uncommon practice for most marine scientists.

CSIA of nitrogen in amino acids is another story and is primarily based on the work of McClelland and Montoya (2002) with marine zooplankton. They proposed that in animals some amino acids have more positive $\delta^{15}N$ values as they are metabolised, while other amino acids undergo minimal isotopic fractionation as they move up the food chain and largely retain the $\delta^{15}N$ values from primary producers at the base of the food chain. Those amino acids with more positive values are termed "trophic" amino acids, whereas those amino acids with unchanging $\delta^{15}N$ values are "source" amino acids. The concept has spawned an entire new field within isotope ecology.

Our early work on human CSIA-AA $\delta^{15}N$ in human bones compared just two specimens (Fogel *et al.*, 1997) and never really took off in the archaeological community, probably because the methods are not routine. Ecologists, though, have embraced the trophic and source amino acid idea and heavily exploited it (*e.g.*, McMahon and McCarthy, 2016; Ohkouchi *et al.*, 2017). That said, the fundamentals behind the use of $\delta^{15}N$ in amino acids for calculating trophic position are on uncertain ground. Inherent in all of the calculations, is the beta (β) value, which is the difference between the $\delta^{15}N$ of a trophic amino acid like glutamate (or proline) and that of a source amino acid like phenylalanine. Based on a few original papers (*e.g.*, McClelland and Montoya, 2002; McCarthy *et al.*, 2007) this value (3.4 ‰ in marine systems) has been assumed to be invariant. As more data are collected, we now know that there can be considerable variation in this measurement depending on the ecosystem. Similarly, for terrestrial ecosystems, the β value has recently been shown to be dependent on whether the tissue originates from a plant with a low amount of lignin (*e.g.*, aquatic plants) or a higher amount of lignin (*e.g.*, tree leaves) (Kendall *et al.*, 2019) (Fig. 10.2).

An additional confounding factor is that with each step up the food chain, the nitrogen isotope fractionation between glutamate and phenylalanine decreases because of direct routing of amino acids into animal tissue (McMahon *et al.*, 2015). Last, of the original proposed "source" amino acids, only phenylalanine, tyrosine, and lysine remain as true source amino acids; the relatively simple (non-essential) glycine and serine are no longer considered to be reliable source amino acids. If recent work on the influence of gut microflora pertains to most animal species, this amino acid $\delta^{15}N$-based method to estimate trophic level will need a critical reassessment.

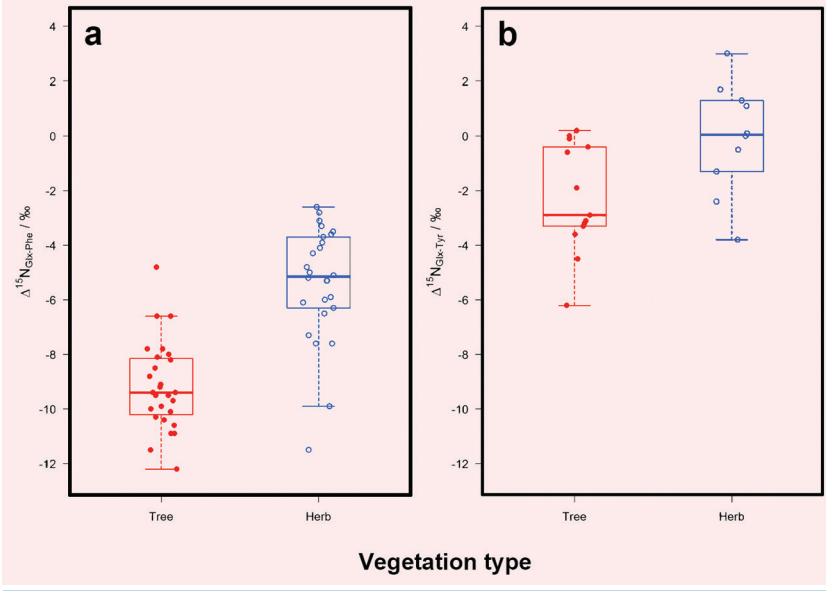

Figure 10.2 The difference between the $\delta^{15}N$ of glutamate and phenylalanine or tyrosine in plants is known as beta, a parameter used in determining trophic position using nitrogen isotopes in amino acids. Beta can be extremely variable in terrestrial systems, based on the amount of lignin in a plant's tissues. Modified from Kendall *et al.* (2019).

11. ASTROBIOLOGY

> *"The probability for the chance of formation of the smallest, simplest form of living organism known is 1 to 10,340,000,000.... The size of this figure is truly staggering, since there are only supposed to be approximately 10^{80} electrons in the whole universe!"* Harold Morowitz, in *Energy Flow in Biology* (1968).

In 1996, NASA announced that it had found evidence of life contained in the Martian meteorite Alan Hills 84001 (McKay *et al.*, 1996). They held press conferences and presented evidence for what they termed bacterial cell structures in the meteorites, along with some amino acid profiles. News spread like wildfire among Mars aficionados and sceptics alike. The search for life on Mars stimulated several lander and orbital missions to Mars. At the same time, NASA under the advice of Wesley T. Huntress, Associate Administrator for Space Science, had the idea to form the NASA Astrobiology Institute, a virtual institute in which scientists in disciplines as far-ranging as astronomy and astrophysics would regularly engage with molecular biologists and geochemists. Objectives central to this effort were to understand how life arose on planet Earth, to determine when it arose, and to devise a set of criteria everyone could agree on that constituted evidence of life.

This task has been more difficult than it sounds. To this day, NASA and NAI scientists still are discussing how they will determine which chemical or physical line(s) of evidence constitute proof that there is life on another planetary body. I was part of two original NAI teams that took very different approaches to search for life. The Jet Propulsion Lab (JPL) team focused on biosignatures. Ken Nealson, who specifically moved from University of Wisconsin to JPL, led the effort. Biosignatures are fingerprints of life such as isotope fractionation patterns or elemental ratios. Our work was carried out in both modern extreme environments as well as in ancient rocks. Being on the JPL campus, this NAI team used several new techniques that were being specifically designed for space flight. The goal of this group was to develop biosignatures in tandem with new analytical methods.

From the first report in 2000 (Fig. 11.1):

> *"Fogel and Ziegler established the isotopic biosignatures of bacteria and how these organisms transform primary, in this case photosynthetic, isotopic and protein signals, into altered microbial biosignatures. We found that bacteria totally resynthesized and scrambled the isotopic patterns in amino acids. The process was rapid for soluble proteins, e.g. Rubisco. Bacterial proteins were mineralized and recycled as well. Our data shows that on very short time scales, hours to days to months, microbial products are formed and degraded. The biologically-resistant material gradually obtains an isotopic and chemical composition that has little resemblance to original biochemical compounds."* (National AstrobioIogy Institute, 2000).

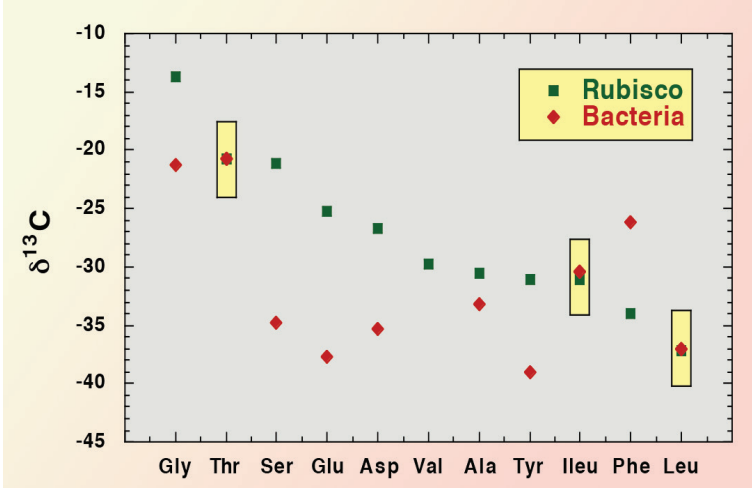

Figure 11.1 Compound specific amino acid $\delta^{13}C$ from bacteria using Rubisco as a carbon source. Microbes changed the isotopic composition of every amino acid except for three amino acids with more complex branched structures: threonine, leucine, and isoleucine. Unpublished graph of M. Fogel.

The approach by the Carnegie team, which I was also working with, was completely different. Geophysical Laboratory staff member Bob Hazen and Harold Morowitz (George Mason University) had the idea that we might be able to study some of the most basic reactions of the TCA cycle using hydrothermal techniques rather than biological enzymes. Geophysical Laboratory staff member George Cody, former Geophysical Laboratory Director Hat Yoder, and myself as well as Bob and Harold, postdocs Mark Teece, Jay Brandes, Tim Filley, Jennifer Blank, and Nabil Boctor discussed how this could be carried out. Bob and postdocs would seal organic reactants into gold tubes; Hat Yoder would subject them to high temperatures and pressures in his internally heated, gas media pressure bombs; George and postdocs would analyse the products of the reaction; Mark Teece and I would measure the CSIA on products (*e.g.*, Teece *et al.*, 1999); Harold and the rest of us would interpret the results. Experiments were more complicated than we ever anticipated.

The team learned that the anticipated products were unstable at high temperatures (*e.g.*, 170 °C). Rather than studying synthesis, the work slowly evolved to studying decomposition (Fig. 11. 2). Further, although the starting reactants were often a single molecule (*e.g.*, citrate), the products were very complex. We never made it to the point of measuring stable isotopes. Bob and George ran hundreds of reactions, then shifted to investigating metal-sulphide catalysts and Fe-nickel catalysts, searching for the Holy Grail of recreating the TCA cycle *via* hydrothermal reactions. From the NAI 2000 report,

"Cody et al. have established a potential entry point into the reductive TCA cycle utilizing transition metal sulfides and reduced carbon bearing fluids. This pathway, named the hydrothermal redox pathway, is not used in any extant organisms but may have been the ignition point for primary metabolism. Cody et al. have begun to explore the potential role of organometallic phases that may be intrinsic to hydrothermal systems as sources of primitive biological energy conversion functionality. The potentially critical catalytic role of such species may be a crucial link between geochemistry and biochemistry at the point of life's emergence... Integrating all of this, we find ourselves within one reaction of demonstrating a purely geochemical carbon fixation pathway that closely mimics the combined acetyl-CoA and reductive TCA pathways. Whether this pathway was the focal point for emergence of primary metabolism remains to be established, but the facility of the reactions makes a strong case for such a pathway lying at pre-enzymatic roots of biochemistry." (National Astrobiology Institute, 2000).

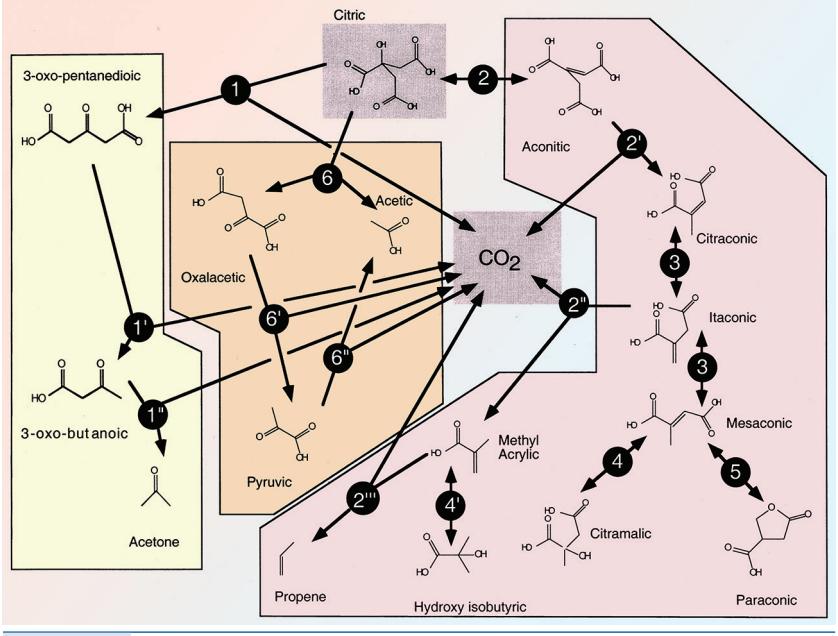

Figure 11.2 Cody *et al.* (2001) highlighted the complex pathways that the simple molecule citric acid could take during decomposition. This was painstaking work that George Cody worked on for years analysing many dozens of hydrothermal experiments using gas chromatography-mass spectrometry. Modified from Cody *et al.* (2001).

Eventually, the stable isotope biogeochemistry studies of the Carnegie team shifted in emphasis. In phase two of the NAI project at the Geophysical Laboratory, we began to study the isotopic compositions of ancient sedimentary rocks and stromatolites and began an ambitious field campaign in Svalbard, an

archipelago high above the Arctic Circle in a project termed AMASE: Arctic Mars Analogue Svalbard Expedition. Both the NAI and AMASE endeavours required field trips to far flung places to collect samples from ecosystems of interest to astrobiologists.

11.1 Arctic Mars Analogue Svalbard Expeditions (AMASE)

Since the emergence of the field of astrobiology in the late 1990s, fundamental questions still persist. What are the basic characteristics of life? How do we recognise life if it does not resemble any known life forms on Earth? These questions have been long debated by National Research Council (NRC) committees, NAI teams, and numerous panels. For example, in a 2007 report (National Research Council, 2007), the following requirements for life were listed:

"A thermodynamic disequilibrium;

An environment capable of maintaining covalent bonds, especially between carbon, hydrogen, and other atoms;

A liquid environment; and

A molecular system that can support Darwinian evolution."

In 2000, I helped organise a workshop held at the Geophysical Laboratory, and sponsored by the National Research Council. In our report titled "*Signs of Life: A report based on the April 2000 Workshop on Life Detection Strategies*" (National Research Council, 2002) we wrote the following:

> *"We make the assumption that if life exists on other planets or moons, it will be carbon based and dependent on liquid water. It will also be self-replicating and capable of evolving. Carbon is the best element for creating macromolecules; it can form chemical bonds with many other atoms to produce biochemical complexity. This complexity consists of thousands of catalytic and structural proteins and nucleic acids, the informational macromolecules involved in protein synthesis. All life on Earth evolved from a single type of cell, referred to as the last common ancestor, and thus shares the same genetic code and central biochemistry. Consequently, all terrestrial life can be compared via phylogenetic trees based on small-subunit ribosomal RNA sequences....A converse difficulty, of course, is that extraterrestrial life could be so different from life on Earth that modern methods would fail to detect it."*

AMASE expeditions began in 2003, with Hans Amundsen and Bjørn Jamtveit of the University of Oslo as leaders. They had assembled an international team of scientists and expedition artists for a voyage to the northern islands of the Svalbard Archipelago. Svalbard is located at about 80° north latitude, the same latitude as northern Greenland. Northern Svalbard is an Arctic desert, which was one of the principal Mars analogue traits important to our ecosystem studies. It is serviced by flights into the major town of Longyearbyen, a combination frontier and tourist destination visited in summer by people from around the world. Like Mars, Svalbard is cold, dry, and virtually devoid of biomass – with exposed rock formations, as well as thermal springs and dormant volcanoes, all important characteristics for our study. My Geophysical

Laboratory colleague Andrew Steele and his student Maia Schweizer were invited on the 2003 trip. Steele was a novice field scientist, having worked primarily in the lab on experimental studies. They were accompanied on the trip by Liane Benning of Leeds University, who became a close scientific partner of mine during future AMASE trips. Steele and Schweizer brought back interesting microbial samples and rocks from Svalbard volcanoes to examine traces of microbial life and organic carbon concentrations.

In the laboratory, I began to engage in the analyses of the samples finding small amounts of carbon and nitrogen in mantle xenoliths, as well as measuring carbon and oxygen isotopes in a variety of carbonates, some of which were cryogenically precipitated. Amundsen visited the Geophysical Laboratory in December that year and learned that I had a perspective that had not yet been considered. Not only were stable isotopes key for all the samples we collected, but as a biogeochemist and geo-ecologist, I could bring a different perspective to sampling a Mars analogue site. I was therefore invited to participate in the AMASE 2004 expedition the following summer.

The Geophysical Laboratory group in 2004 consisted of Andrew Steele, Maia Schweizer, Jan Toporski and Jake Maule (Steele's postdocs), Verena Starke (Steele's graduate student), and I. The Director of the Geophysical Laboratory at that time was Wes Huntress, former NASA Associate Director and champion of the Astrobiology programme. He provided special support for several of us to participate in the expedition. We took with us numerous small items of equipment designed to make measurements of nutrients and bacterial loads in the field. There were boxes of 50 ml Falcon tubes, rock bags, reagents, and rock hammers. We all packed duffle bags full of winter clothes, hiking boots, liquid nitrogen dewars, and other field gear. Our departure from Dulles International Airport was complex because of extra bags, travel from one airline to another, and the remote destination in Svalbard. Miraculously, we all arrived in Longyearbyen with our scientific and personal gear ready to meet other AMASE participants and train for the voyage to our field sites.

After arriving in Longyearbyen, we settled into the local hostel, tested our equipment, purchased more Arctic-worthy gear, and learned about rifles and polar bears. The polar bear is the top carnivore in the Arctic. Typically, these bears spend much of their time on the ice pack hunting seals, but in the summer, when the ice pack retreats, the bears move onto land, give birth to their cubs, and do most of their hunting near shore. Polar bears are a protected and endangered species for a number of reasons, but polar bears and humans should not mix. The AMASE team was taken to the University Centre in Svalbard (UNIS) rifle range to learn how to protect ourselves and our fellow scientists if we did have a close encounter with a bear. Fortunately, I grew up with a father who was a hunter and taught me how to shoot a rifle, albeit a rather small one, at targets. The rifles we had in Svalbard were German Mausers, comparable to 30-06 rifles in the United States. They were heavy and manually operated; automatic weapons are banned in Norway. We learned loading and unloading of ammunition first, then the three positions for firing.

Our group of about 15 was splayed out on our stomachs, the first shooting position we learned. The rifles had a substantial kick to them, and it took a steady hand to control the rifle as the shot was fired. We each fired off a round of 4 bullets at the target, learned to carefully check our weapon to see if it was emptied of bullets, and laid down the guns. Our trainers checked the targets. I hit mine every time – not in the centre, but in a respectable area that may have been lethal. Our second position was kneeling, which required greater control of the heavy rifle, but improved our ability to aim it properly. Finally, we learned to fire the rifle standing up, the most comfortable pose, but also requiring attention to detail and a strong stance. My aim was decent and I passed the test to be able to defend myself and others from polar bears. Of course, we all hoped we would never have to actually fire the gun at a bear.

We departed from Longyearbyen about a week after arriving in Svalbard (Fig. 11.3). Our vessel was the M/V Polarsyssel, an ice breaker once owned by the Governor of Svalbard, and now available for hire. It was an older ship, not fitted out for scientific study. After loading our gear, we underwent our next training on how to don survival suits and learn "man overboard" drills. The bulky orange suits made us feel like monsters, and we laughed as we put the giant Norwegian-sized suits on and hopped around the deck of the ship. When at our field sites, we wore these suits as we travelled to shore in zodiac boats. In ten years of AMASE expeditions, we never had a serious safety issue in the field.

The 2004 field season determined largely how the team would expand and contract into the future (Fig. 11.4). Hans Amundsen, a descendent of the famous Norwegian explorer Roald Amundsen, is a tall, blonde, commanding figure with a penchant for charging up mountains, whacking rocks with a huge hammer called "Thor", and uproarious laughter. It was his vision and persistence that got AMASE started and rolling for ten years. AMASE expeditions always included strong European and United States components, a mix of scientists and engineers, a mix of field and lab enthusiasts, and a blend of senior and more junior scientists. In addition, Hans always included safety personnel and often invited media (*e.g.*, television, radio, newspaper) folks along to document the trips. We were joined by Kjell Ove Storvik, who was the expedition photographer with many years of experience working in the Arctic with novices. Ivar Mitkandal, a graduate student at the University of Oslo, was raised in the Norwegian mountains, was a hunter, and provided not only sage advice on safety, but also great insight into the geology.

Our primary goal was to characterise the environment in terms of what a Mars analogue site should look like. From the geologists' perspective, easy access to rocks from all ages and types – Precambrian to modern sediments and volcanic, mantle rocks to sedimentary sequences – was a strong positive reason for choosing Svalbard. Specific field areas were primarily selected in terms of geological outcrops. From the ecologist's perspective, fewer than 200 plant species occur on these islands. All of them need to reproduce and grow in a very short summer. Animal life was limited to abundant seabird breeding colonies and migratory shorebirds. A few land birds (*e.g.*, ptarmigan and Snow Bunting) occupy Svalbard year round. Soils were mostly thin to non-existent.

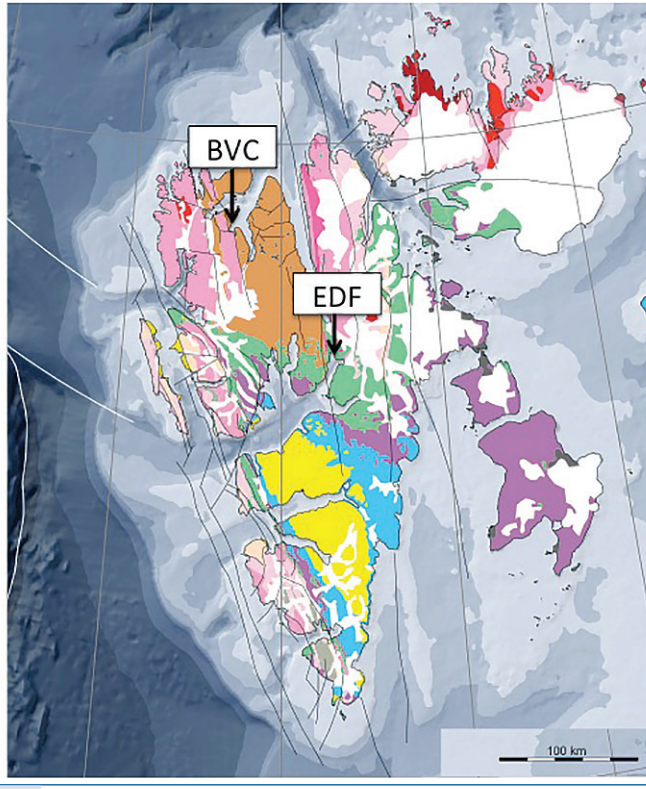

Figure 11.3 This map of Svalbard shows our proposed field areas for AMASE 2004. After training in Longyearbyen, the M/V Polarsyssel transported us north to the Bockfjord Volcanic Complex (BVC), followed by field work at the Ebbadalen formation (EDF) on the return to Longyearbyen. Source: courtesy of H. Amundsen.

For the instrument engineers, there is every land form and slope imaginable, often within easy walking distance from shore. With many rock types, cold weather, and remote electronic access, the Svalbard environment put lab-designed instruments to a realistic test. All of the team was intrigued by the presence of glaciers, permafrost, Arctic rivers, and sea ice. The polar regions of Mars contain water ice year round. Learning how to look for signs of life in snow and ice became a major focus in subsequent years. Svalbard is cold enough to contain ice caps, laminated ice that has existed for hundreds if not thousands of years. Two geothermal environments, Troll and Jotun Springs are the most northern thermal features on land (Jorge-Villar *et al.*, 2007). Troll Springs built significant calcium carbonate (travertine) deposits forming terraces that extended over several 100 m. All of these features were within a distance from

Figure 11.4 The AMASE crew at Pyramiden, Svalbard 2004. Bottom row: Libby Hausrath, Marylin Fogel, Maia Schweizer, Liane Benning, Elisabeth Cooper, Eammon Shaw, and Lonnie Lane. Top row: Kjell Ove Storvik, Pamela Conrad, Ellen Mahlum, Andrew Steele, Jake Maule, Allan Treiman, Hans Amundson, Jan Toporski, Ivar Mitkandal, Susana Jorge-Villar, Torstein Jossang, and Chris Swarth. Photo credit: AMASE and H. Amundsen.

our ship that allowed for relatively easy sampling and collection of specimens as well as field deployment of instruments.

Target areas for investigation were set before each year's expedition. In 2004, our first sample site was the Bockfjord Volcanic Complex (BVC) including Sverrefjell volcano which rose up from sea level to over 500 m (Skjelkvåle *et al.*, 1987). Vertical lava conduits, some which are filled with magnesite-dolomite cemented lava breccias were part of the draw to go to this remote area. Previously, Hans and Allan Treiman found Mg-Fe-Ca carbonate globules in these rocks (Amundsen, 1987), which are nearly identical in appearance to those in the martian meteorite ALH84001 (Treiman *et al.*, 2002). Work in 2003 hinted that there was microbial activity on the stromatolite-textured Mg-carbonate coatings on the lava conduit walls. Our stable isotope data on carbonates suggested that the coatings were deposited by low temperature glacial meltwater. Across the inlet from the BVC were the high, steep Devonian red beds that looked strikingly like the iron-rich red rocks of Mars.

Near the end of the expedition we hiked at midnight in the land of 24 hour daylight to the Ebbadalen Formation in Billefjorden, an area that included Carboniferous sediments (*ca.* 320 Ma) with Ca-sulphate bearing evaporites

deposited from a shallow marine setting (Fig. 11.5). Outcrops contain mixed sulphate and clastic lithologies analogous to evaporite sediments studied by the Mars rover Opportunity. Though dominated by a carbonate mineralogy these spheroidal Fe-rich concretions appear to be analogues to the "blueberries" discovered by Opportunity. Our team of 20 reached the outcrop at 2 am and swarmed over the layered rocks, rock hammers out and tapping. We found many sedimentary deposits hosting structures similar in appearance to the "blueberries" found on Mars (Jorge-Villar *et al.*, 2011). Clearly this was another example of a Mars analogue site (Fig. 11.5).

Figure 11.5 Ebbadahlen outcrop where the AMASE team found gypsum-containing "blueberry" rocks, 2004. Photo credit: M. Fogel.

11.2 Microbial Life in Svalbard

It is generally presumed that if we are able to detect life on another planetary body, it will most likely be similar to our simplest forms of life – the microbes. Therefore, NASA's concentrates on looking for microscopic signs of life. From the 2002 NRC report:

> *"The detection of extremely low levels of microorganisms after spacecraft sterilization involves increasing refinement of laboratory techniques designed to detect known types of terrestrial organisms. It also requires research into the possibilities and*

consequences of failure to detect unknown or poorly known microorganisms that exist within Earth's environment. The detection of extant life on samples returned from another planet, or analyzed in situ, is a much less well-defined venture. It requires a set of assumptions about the fundamental nature of life that might exist on another planet. It also requires selection of techniques that will work well in difficult quarantine laboratory environments or in miniaturized and automated form on an extraterrestrial body."

The NRC quote defined how we searched for "signs of life" during the 2004 AMASE trip as we sampled along the flanks and waters of Jotun Hot Springs. On the travertine terrace several tens of metres high, the water from three spring outflows had temperatures around 30 °C (cover photo and Fig. 11.6). At each outflow, a small pool 1 to 5 cm deep supported a dense covering of microbes and algae, green in colour, and often filamentous. As the effluents passed down the travertine slope, they cooled and were replaced by larger filamentous organisms and diatoms. I was drawn to study the thermophiles there and the scene brought back great memories of fieldwork in Yellowstone National Park. Now working with the Carnegie and JPL groups, we developed a completely different sampling and strategy plan.

We took a step "backwards" so to speak and walked away to refrain from immediately collecting the obvious organisms in the springs and devised a coordinated sampling plan on the terraces adjacent to the springs hoping that they would present a greater life detection challenge. The Carnegie team of Andrew Steele, Jake Maule, Jan Toporski and Maia Schweizer were determining the microbial composition and biomass using field-based PCR and an ATP-based bioluminescence and LAL instrument manufactured by Charles River Laboratories. LAL stands for *Limulus* amebocyte lysate, an extract from the horseshoe crab. My task was to measure ammonium and nitrate extracted from these samples as well as to determine the absorption spectrum of microbial pigments. The JPL group, Pamela Conrad, Lonny Lane, and Rho Bhartia, were testing a UV-fluorescence prototype instrument. We argued for several hours on the outcrop before taking the first sample and making measurements. The microbiology group sampled first so as to avoid contamination that could come from our activities. I sampled next, since my sampling was simple: a scoop of travertine adjacent to the microbiology sample. Last came the UV-fluorescence team who took their time troubleshooting their instrument along the way. They encountered numerous problems including computer screens that literally froze, telephone type cable connections that shrank in the cold and made no contact, and light contamination from sunlight.

Nitrogen concentrations for both nitrate and ammonium in travertine samples were 10-15 mg/gram of sample, values more than three times greater than volcanic rocks nearby (3-5 mg/gram of rock). In effluent waters downstream from the springs' sources, ammonium concentrations were relatively high (40 μM) then decreased to 5 μM, while simultaneously nitrate concentrations increased to 40 μM. These data clearly indicate microbial nitrification. Pigments were extracted in the field at 4 °C (ambient air temperature). In these

"off axis" samples, the absorption peaked at about 400-450 nm, indicative of the Soret band of chlorophyll. In many samples, light absorption in the near UV (360-400 nm) indicated the presence of the pigment scytonemin, a microbial pigment synthesised to protect organisms from UV damage. The absorption bands were especially strong in what we called "black" and "white" sediments.

Figure 11.6 Jotun Hot Springs, Svalbard. Our team spent several hours discussing how to take samples in this fascinating and complex area, 2004. Photo credit: AMASE and H. Amundsen.

Back in the lab at the Carnegie, I analysed total nitrogen as well as the carbon and nitrogen isotopic compositions of the organic matter of these samples. Total nitrogen in these sediments was >1200 mg/gm of sample *vs.* 250 mg/gram for adjacent volcanic rocks. Compared to the extractable, inorganic nitrogen measured on board ship, the total nitrogen was overwhelmingly organically bonded nitrogen. The $\delta^{15}N$ of biomass and rock samples from Jotun Springs varied from -7 to +5 ‰, with $\delta^{13}C$ values of -28 to -35 ‰. The variation in the $\delta^{15}N$ values measured over only several tens of metres reflects microbial isotope fractionations in effluent waters, primarily uptake and nitrification reactions. If there had been no microbial activity, I would have found uniform $\delta^{15}N$ values.

The $\delta^{13}C$ values of microbes were quite negative, and we surmised that the concentration of dissolved CO_2 coming from the hot spring sources must be high. Again, if we were measuring the $\delta^{13}C$ of abiogenically derived organic

carbon, we would expect the values to be higher and much more uniform. On Earth, the "background" value measured in what is supposed to be "clean" materials is -26 ± 1 ‰, a measure of slight oil contamination that is ubiquitous. I concluded that 1) based on changes in nutrient concentrations over the spatial extent near the springs, 2) elevated concentrations relative to igneous rocks, 3) the absorption of light in the visible and near UV region (Fig. 11.7), and 4) the variable isotopic compositions that these represented biosignatures of life in these samples.

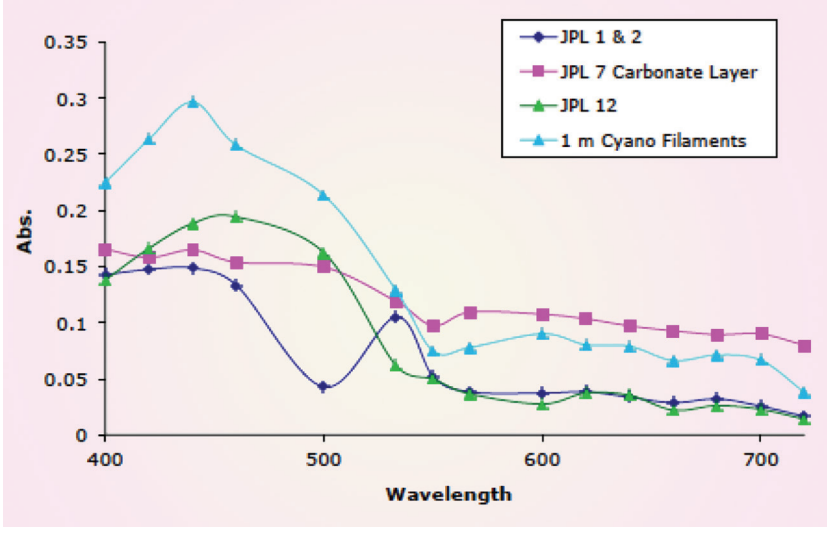

Figure 11.7 Absorption spectra of acetone extracts of pigments from Jotun Thermal Springs sediments. Chlorophylls absorb light in the 400-500 nm range. Carotenoids absorb light in the 500-600 nm range. Unpublished graph of M. Fogel.

Over the years, we made many trips to Troll Springs, which are located about 6 km from the shoreline. The hike there circumnavigated Sverrefjell Volcano, crossed a marshy area where we had to step on clumps of moss to prevent sinking in quicksand, before reaching a rushing, cold Arctic river about 100 m wide. There were few comfortable or safe options for crossing the river. Option one, encase your hiking boots in trash bags and run quickly through the water, hoping the bags stayed on your boots. Option two, put the trash bags over your feet, then back in your boots. Wade more surely through the river channel, but you'll have wet boots on the other side. Option three, run as fast and as fleet as you can and hope that your boots remain as dry as possible. All three options generally resulted in cold feet and wet boots. Having a dry set of socks in your backpack was a good idea.

Troll Springs spread out on the landscape for about 500 m. Some of the springs had dried completely, while others were going full blast. Those with active flow supported robust communities of microorganisms (Starke *et al.*, 2013). Gases emitted from the springs had high concentrations of CO_2 (Jamtveit *et al.*, 2006). I measured nutrients and pigments in soils, sediments, and springs from this area for many years. Particularly interesting were the rock samples in which the cyanobacteria and other microbes were found mainly within cracks and crevices (*e.g.*, endoliths).

In 2004, we discovered a relatively small area at Troll Springs littered with hundreds of small bird bones. Upon closer inspection, we realised the bones were clustered around an Arctic fox den, which was situated on the side of a dormant spring where the soil was warmed year round. Immediately, I collected bones and soils from around the den. During repeated trips to Troll Spring over the next five years, it appeared that a fox inhabited this den continuously. Based on the isotopic composition of the soils, I found that the fox's "influence" on the landscape through its feeding and excreting activities extended 60 m in three of the cardinal directions (*i.e.* North, South, and East) from its den. Plants growing on those soils had distinctive isotope values for both carbon ($\delta^{13}C = -32 \pm 2$ ‰) and nitrogen ($\delta^{15}N = +13 \pm 5$ ‰). Isotopic analyses of the small bird bones showed the fox diet consisted of both terrestrial (*e.g.*, ptarmigan; $\delta^{13}C = -18$ ‰; $\delta^{15}N = +8$ ‰) and marine birds ($\delta^{13}C = -16$ ‰, $\delta^{15}N = +15$ ‰). About 80 % of the bones were from marine seabirds, such as kittiwakes. Accordingly, the fox was responsible for bringing nutrients from the ocean into a nutrient-starved terrestrial environment creating a green, "hot spot" that could be seen from some distance. Although "foxes on Mars" is a ridiculous notion in itself, this discovery was a robust example of how biological processes of organisms can physically and chemically alter the landscape in which they live.

The second focus of 2004 AMASE was to sample the ice and rocks in a small cave near the top of Sverrefjell Volcano. It was an exciting climb to near the top of this 525 m volcano. Perched precariously on a ridge top, we deployed the microbial instruments, the UV fluorescent probe, and I collected samples for nutrients and isotopes (Fig. 11.8). Hans Amundsen hacked into the ice with a sterilised ice axe, and the team bagged a substantial chunk of ice to be shipped back frozen to the United States, apropos of sample return from Mars. The microbe team found elevated levels of ATP and LAL-luminescence in the ice relative to surrounding rocks. Their PCR results indicated eubacterial and Archael genes. In the breccia surrounding the ice, we identified genes amplified from sulphate reducing bacteria. Similar genes were not found within the ice. These samples were particularly interesting because they surrounded the layered carbonate found on earlier trips by Amundsen and Treiman. Organic carbon in these samples was <0.1 % with a $\delta^{13}C$ of -24 ‰. They also contained carbonate carbon with $\delta^{13}C$ values of -5 ‰ indicating that these carbonates were precipitated cryogenically under glacial ice, perhaps with mantle-derived CO_2.

Figure 11.8 High ridge top on Sverrefjellet volcano in the Bockfjord Volcanic Complexin 2004. We sampled a small ice cave in this location (lower left side of the ridge), as well as surrounding rocks and sediments. JPL scientists tested their instruments there. Photo credit: M. Fogel.

Although we were focused primarily on the ice cave-carbonate location, we were also excited to collect mantle xenoliths, rocks produced deep in the Earth's interior then propelled to the surface during eruption. Xenoliths are found in great abundance on the surface of Sverrefjell. For a biogeochemist, I was at first unaware of how special it was to find rocks like these. I collected almost 50 xenoliths, each about 8 to 10 cm in diameter. The amount of "organic" carbon in xenoliths was typically 0.01 %, with $\delta^{13}C$ ranging from -25 to -33 ‰. Nitrogen was undetectable. Usually with these low organic carbon concentrations, we would suspect contamination, however, Steele's further investigations with Raman spectroscopy confirmed the indigeneity of the organic carbon.

Steele *et al.* (2007) compared the the Bockfjorden Volcanic Complex (BVC) carbonates from Sverrefjell Volcano to similar carbonates in the Allan Hills 84001 meteorite. Using freshly fractured surfaces, Steele rastered the Witek Raman instrument's beam across the sample interrogating it at the micron scale. Optical microscopy confirmed that the carbonate globules were in the form of magnesite between rims of magnetite. Raman spectroscopy revealed some zoning in the carbonates with trends going from siderite ($FeCO_3$) to calcite ($CaCO_3$). Both the meteorite and the BVC carbonates contained haematite in close proximity to macromolecular carbon (MMC), measured as ordered and disordered graphite. Others had found MMC in ALH 84001 (McKay *et al.*,

1996; Becker *et al.*, 1999) and had trumpeted their finding as evidence of life. That said, the distribution of graphite bands in the meteorite and the BVC carbonate are very different from that of biologically-derived kerogen (Fig. 11.9). Using the BVC sample, which was generated in mantle rocks deep in the Earth, Steele *et al.* (2007) argued for a similar mechanism of formation for the meteoritic organic carbon through reaction series with iron oxides, graphite, and CO_2. Because the carbon phases were imaged and analysed *in situ*, this work confirms the indigeneity of the organic carbon in xenoliths as well as that of the ALH 84001 meteorite.

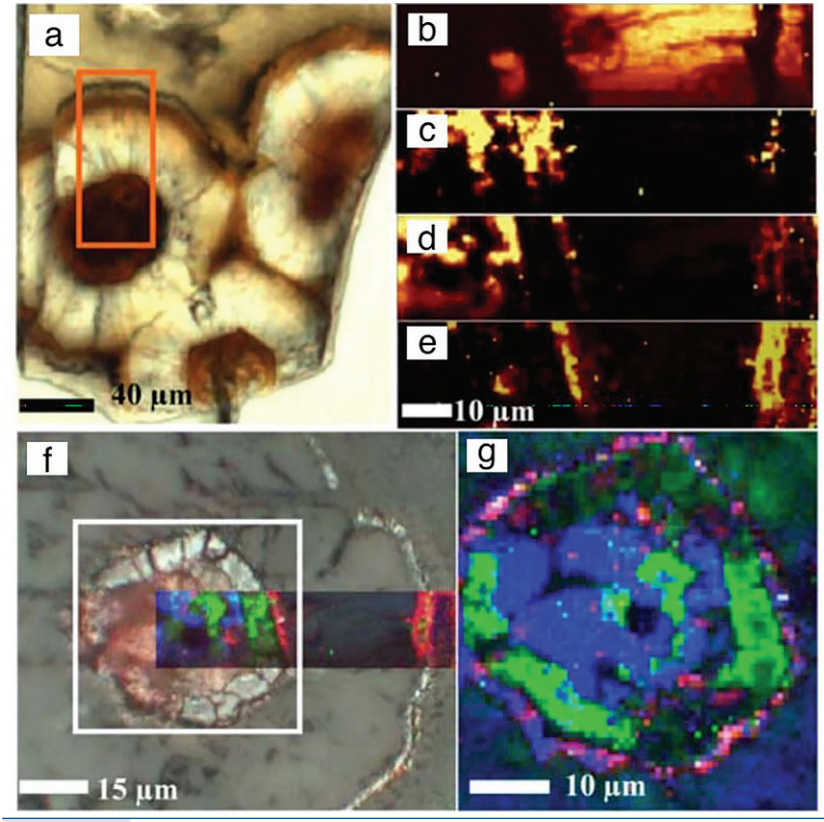

Figure 11.9 **(a)** Light microscopy imaging of carbonates from the Bockfjord Volcanic complex. **(b)** Carbonate, **(c)** Hematite, **(d)** Magnetite, and **(e)** Graphite maps from Raman spectroscopy. **(f)** and **(g)** Reflected light with carbon showing as red. From Steele *et al.* (2007) with permission from John Wiley and Sons.

Whether this MMC was biological in origin was the next question. The $\delta^{13}C$ of the BVC carbonate was -5 ‰, supporting its formation from mantle CO_2. Because of the tight physical relationship between mineral phases and the macromolecular carbon, we know that these reactions occurred without the intervention of biological processes. It follows that a similar mechanism is favoured for the martian sample. Arguments supporting or refuting the original McKay *et al.*, (1996) paper have targeted the group's conclusion that organic matter in the meteorite was of biological origin. Steele *et al.*'s (2007) paper put a nail in that argument.

11.3 ASTEP Project

By the 2006 expedition, the priority for AMASE trips shifted towards testing new instruments in the field prior to their being selected for space flight on upcoming Mars missions. In a proposal to the Astrobiology Science and Technology for Exploring Planets programme, we asked the following questions:

1. How do we access suitable samples?
2. How do we identify, sample and detect molecules of interest at suitable spatial and detection sensitivity scales?
3. How do we ensure sample integrity and control for cross-contamination by organic, biogenic and inorganic molecules?
4. How do measurements from laboratory and field instrumentation compare in terms of analysing terrestrial samples from a Mars relevant environment?

Each year we worked with a JPL crew that brought along a sophisticated rover that was put to the test on slopes and terrains similar to those found on Mars. JPL scientists Terry Huntsberger, Ashely Stroupe, Paulo Younse, and Michael Garrett took turns operating the Cliff-Bot rover. This team was given 2-3 days of special time to test their rover. Many of us envisioned the rover swiftly covering the landscape in a matter of minutes, reaching out its robotic arm, scooping up sediment and returning faithfully to its base. Unfortunately, sending a rover over a complex landscape, as though it were on a remote planetary body, was a much slower, hour-by-hour and inch-by-inch process that tested the patience of many a crew member.

The project now included two potential instruments that were ultimately chosen to fly on Mars Curiosity: CheMin and SAM (Sample Analysis on Mars). CheMin's instrument PI is David Blake, a scientist at NASA Ames. Blake, a US Navy veteran and an expert in designing and testing field X-ray mineralogy instruments, is also quite a character. Dave sang navy songs laced with profanity, told jokes and funny stories of all types, and laughed with a distinct pirate-like "*Har har*". To say he brought some "colour" to the expeditions is an understatement. SAM's PI, Paul Mahaffey, brought a crew of scientists

from NASA Goddard including Pamela Conrad, Jennifer Eigenbrode, and Inge Loes ten Kate. SAM is a combination gas chromatograph-mass spectrometer (GC-MS) equipped with the capability of high temperature pyrolysis GC-MS and a tunable diode laser for measuring methane and its isotopic composition. CheMin was fully portable and field deployable; SAM was not.

In addition to instrument teams, Steven Squyres, the PI of the Mars Exploration Rover mission with Opportunity and Spirit, was invited to observe sampling in the field and to conduct mock Mars sampling exercises (Science Operations Working Group: SOWG – pronounced "Sahg") based on his experience "roving Mars" from Earth. The exercises were designed so that scientists and engineers, required to work together in teams during real missions, would learn as a group how to answer the four technical questions posed above. The question of how to access suitable samples had to be tackled separately with specialised practice with the rover team. Our second question – how to identify, sample and detect molecules of interest at suitable spatial and detection sensitivity scales? – took up most of our time.

For many of the last AMASE expeditions, about three SOWG exercises were held each year. Travelling with AMASE was a German camera crew led by Nicole Schmitz, who was testing a camera that she hoped would fly on a future Mars mission. She joined AMASE expedition photographer Kjell Ove Storvik, Steele, and Amundsen, who chose an outcrop for investigation. The two photographers then provided PanCam like photos that were sent back to the team "on Earth" – meaning inside a room on the ship – for them to analyse. Photos were in black and white and then pieced together to form a mosaic of the outcrop. The CheMin, SAM, UV fluorescence, and Life Marker Chip instrument teams were assigned an energy budget. For each measurement requested, the team needed to use up one or more of its energy allotments to "pay" for the analyses.

After the teams finished arguing about where on the outcrop the samples should be taken and how they would use their precious energy resources, the crew on land sampled the outcrop with hammers and delivered the samples to the instruments. CheMin, UV fluorescence, and the Life Marker Chip instruments were deployed in the field; SAM on board ship. When the analyses were completed, data were "downlinked" from "Mars" to "Earth" for inspection and analysis. At this point, teams argued as to whether they were able to detect molecules of life on "Mars". The discussion then shifted to whether or not a sample should be cached for future return to Earth for more sophisticated sampling.

These exercises were intense: periods of high drama and discussion, followed by periods of restless inactivity, cooped up on the ship or lounging on a rock outcrop. All samples were brought back to the ship and analysed by the full AMASE crew with a summary report for each SOWG exercise. For a flavour of this process, detailed steps are presented in Text Box 11.1.

Text Box 11.1 – The SOWG Exercise

1. Science Downlink Assessment

- Receive the data downlink
- Request and listen to the Mission Manager's report of the rover state of health
- Each Science Theme Group (STG) reviews all the data that is pertinent to their theme
- Each Payload Downlink Lead (PDL) reviews the data received from their instrument

2. Science Operations Working Group

Led by the SOWG Chair

- Make introductory statement:

"Welcome to the SOWG Meeting that will generate the uplink plan for Sol XXX"

[Sol is the generic term…used because this whole operations process was developed for rovers on Mars where a day (diurnal cycle) is called a 'Sol'.

Mission Manager (MM) reports on available Rover resources.]

Request and hear the payload (P/L) status from each PDL. The intent is to hear about the most recent data received form the instrument, and the status of the instrument as pertains to using it.

Pancam and/or Engineering Cameras
Micro Imager
Raman
UV Fluorescence
Life Marker Chip
CheMin
SAM

- Request and listen to each STG's intentions and inputs to the plan
- Each STG has this chance to state their arguments for their parts of the Sol's plan
- Read though the existing draft of the plan for Sol XXX
- Make edits to the plan for Sol XXX
- Adjust the plan to properly reflect the consensus science intentions
- Review and modify the targeting of the various observations
- Final walk-through of the plan for Sol XXX, confirming that it is ready to submit to the Command Approval Meeting (CAM)

3. Command Approval Meeting

Run by the Mission Manager

The idea is to check all parts of the plan to make sure that resource limitations are respected and that the requested commands and target designations are clearly understandable by the rover.

4. End-of-Sol Science Meeting (EOS)

This is the fun part of the meeting when the science team should throw out their ideas about the data. Freewheeling ideas are the order of the day.

11.4 Roving on Mars

Curiosity Rover on Mars Science Lab was launched on November 11, 2011, and landed safely on Mars August 6, 2012. It's been roving every day since then without a hitch, far exceeding NASA's original goals for its lifetime. The mission goal is not to look directly for signs of life, but rather to determine whether or not Mars was ever able to support microbial life. The Rover has examined sedimentary deposits, visited the bottom of possible lake beds, and scratched away at the surface oxidised, thick dust to sample more interesting rocks beneath.

Jennifer Eigenbrode, my former postdoctoral fellow, used her organic geochemistry training to focus on data coming from SAM. In a recent paper (Eigenbrode *et al*., 2018), she and colleagues, including Andrew Steele, detailed results from evolved gas analysis (EGA) of 3 billion year old sedimentary rocks from the bottom of Mars' Gale Crater. They found complex sulphur compounds, known as thiophenes, that could only have come from the complexation of sulphide with organic matter during diagenesis. Thiophenes were released at temperatures between 550 and 820 °C in the EGA system, in which solid material is heated gradually and the evolved gases are swept into a simple mass spectrometer system that monitors certain masses: mass 45 for CO_2; mass 60 for COS; and mass 84 for C_4H_4S, a thiophene fragment (Fig. 11.10). For this work, they were able to take a cut of the volatile gases and direct them into SAM's GC-MS system, which confirmed the presence of thiophene, 2-methylthiophene, and 3-methylthiophene, as well as various aromatic compounds. Because of the diversity of molecular structures, the nature of this organic material is consistent with complex synthesis, whether from meteoritic or geological interactions.

Another interesting observation from many of the SAM analyses is that the chemical structures of the organic compounds are variable between samples. These rocks were lacustrine mudstones formed when Mars had liquid water on its surface. If SAM were only measuring background contamination, we would expect a more uniform composition. Organic matter, which has been found to be widespread among martian sedimentary rocks, could fuel living organisms, if they occurred, and might support a biological carbon cycle. The authors stopped short of declaring that they found evidence of past life on Mars. They concluded, however, that given that sediments found near the surface contain detectable amounts of complex organic material, drill cores from greater depths might provide even more definitive samples of organic matter, traceable to that formed by living organisms. Ultimately, Mars sample return remains the single most important goal for confirming if martian organic matter was formed as the result of biological processes.

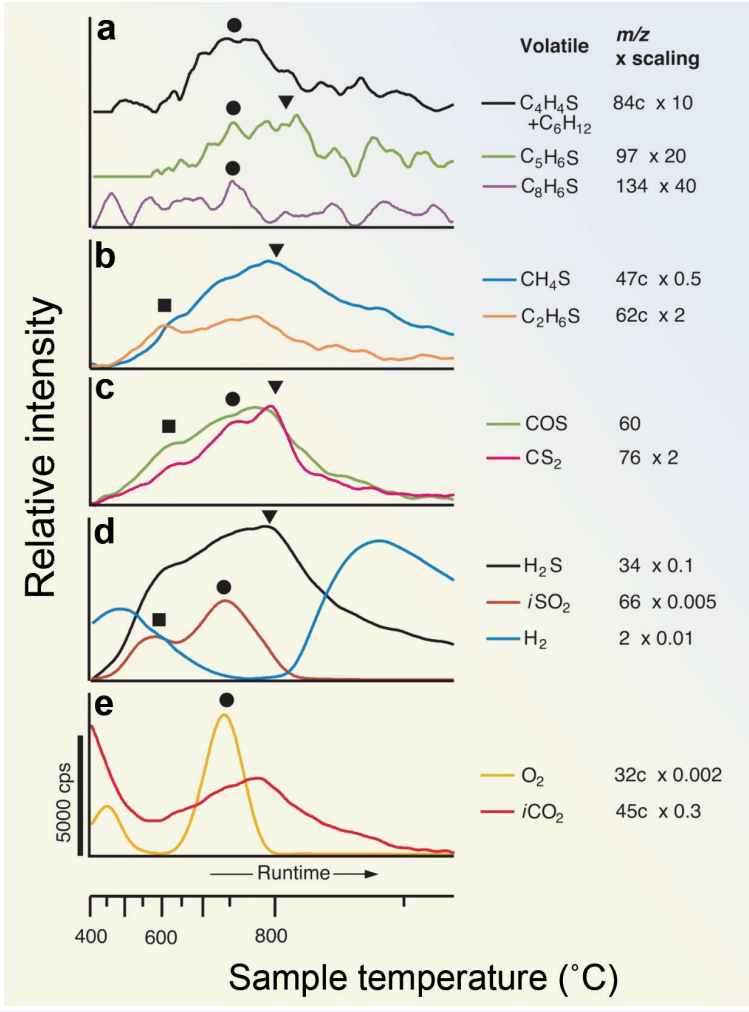

Figure 11.10 Evolved gas analysis (EGA) of volatile gas profiles from the Mars Mojave site. Profiles for thiophenes **(a)**, thiols and sulphides **(b and c)**, other volatiles **(d)**, and O_2 and CO_2 **(e)** are shown. Symbols mark correlations between panels in peak maxima within an error of ±25 °C due to signal smoothing: squares, 625 °C; circles, 750 °C; and triangles, 790 to 820 °C. Axes and the placement of symbols relative to the temperature are the same in Fig. 2 and Figs. S1 to S6. The x axis is scaled linearly relative to the run time, and the corresponding sample temperature is shown. The y-axis scale bar in counts per second (cps) is for all panels. Profiles in **(a)** are shifted along the y axis to show peaks clearly. From Eigenbrode *et al.* (2018) with permission from The American Association for the Advancement of Science.

Steele and colleagues (Steele *et al*., 2018) followed up on Eigenbrode *et al*.'s (2018) paper, once again taking full advantage of the martian meteorites that we have on hand that can be fully analysed by sophisticated instrumentation. Using confocal Raman imaging spectroscopy and transmission electron microscopy, they were able to examine the intimate details of organic matter formation. Their findings, consistent with Mars Curiosity measurements, show that it is the interaction of brine-fluids with sulphides and spinel minerals by an electrochemical mechanism that results in the deposition of MMC in the martian samples. They conclude "*The hypothesis developed from our observations on martian meteorites has profound implications for our understanding of other martian phenomena, including the presence of methane in the atmosphere and the origin of the refractory organic material in ancient sedimentary rocks found in situ by the SAM instrument*." Steele and his colleagues who participated in AMASE (Eigenbrode, Benning, Fries, Siljesrtom, Conrad, and McCubbin) "cut their teeth" on the samples from Sverrefjell volcano, demonstrating the power of collaborative inspiration often related by field investigations.

AMASE team member Ashley Stroupe sometimes "drives" Curiosity from Mission Control at JPL. She posted this blog on August 13, 2018:

> *"In today's plan, Curiosity begins analysis of the long-awaited Pettegrove Point drill sample at the "Stoer" target, which was successfully collected last week (and I had the pleasure of helping to sequence as a Rover Planner). Our main activity is the drop-off of sample to CheMin, based on the characterization of the drop-off portion size done in the weekend plan. There is still a good bit of wind, so the drop-off is around noon, during the calmest time. Overnight, CheMin will be busy analyzing the sample; we'll have the results down late Wednesday, which can then inform decisions about dropping off sample to SAM as early as this weekend's plan for analysis early next week.*
>
> *On both sols of the plan, we're continuing our atmospheric observations to monitor the dust storm as it continues to abate, with dust devil surveys, and zenith and horizon opacity imaging. We'll be collecting additional ChemCam and Mastcam images of the drill hole, to look for vertical variability, and of the tailings, for change detection."*

Mars Science Lab remains a very active mission, daily engaging many of AMASE's scientists and engineers.

12. INTERSECTION OF BIOGEOCHEMISTRY WITH THE STUDY OF METEORITES

As an isotope biogeochemist with a fully working laboratory, I am often included in studies far outside of my range of expertise because I can make reliable and precise analyses of organic materials. Fortunately, at the Geophysical Laboratory this happened frequently! Collaborating on a diversity of scientific projects is one of the most satisfying and challenging aspects of my chosen field. My interest in carbonaceous chondrites started when staff scientist George Cody began a collaboration with Carnegie's Department of Terrestrial Magnetism (DTM) staff member, Conel Alexander. George, an organic geochemist who specialises in the analysis of complex organic matter, is an expert at using solid state NMR to interrogate the structure of kerogen and coal. He's also developed a unique talent for using soft X-rays, XAFS (X-ray Absorption Fine Structure), at several of the U.S. beamlines to learn intimate details about the chemical bonds in geochemical materials. It was a natural extension of this work that he applied his analytical skills to the study of meteoritic organic matter. Conel, a cosmochemist, has an encyclopaedic knowledge of carbonaceous meteorites and a knack for obtaining significant quantities of rare meteorites for destructive analysis.

Conel's colleague, Fuod Terra at DTM, had developed a new, chemically-gentle method for dissolving the rock matrix of chondrites using a CsF-HF buffer at pH 5-7. Cody and Alexander (2005) examined the molecular structure of the insoluble organic matter (IOM) by NMR spectroscopy and found that this method and traditional HF-dissolution produced IOM with nearly identical functional groups and composition. After Conel took the lead on purifying IOM from a great variety of chondrites, we were off and running with isotope studies. Over time, I gained a real appreciation for this kind of work for the following reasons. After studying terrestrial organic matter of all ages, meteoritic IOM was the perfect medium for understanding almost everything about isotope effects that are catalysed by non-biological reactions. IOM could be formed under high temperature conditions; it could be heavily metamorphosed; it could have been formed at ultra-low temperatures; or a combination of all these could be responsible. Although the soluble organic compounds in meteorites could readily include contamination from Earth, the IOM phase did not.

Our first study (Alexander *et al.*, 2007) with 75 different meteorites measured % C ranging from 0.002 to slightly >2 % organic carbon. The IOM residues contained 8 to 75 % carbon with H/C ranging from 9 to 95 showing at a first glance that we were dealing with a great diversity in synthesis and molecular structure. Conel had hoped we'd have a diversity in IOM chemical composition based on his informed choice of so many different meteorites types. The $\delta^{13}C$ of IOM varied from -7 to -34 ‰, just about the full range of bulk organic carbon found in Earth materials. Although the $\delta^{13}C$ of specific compounds in meteorites (*e.g.*, Murchison; Engel *et al.*, 1990) has values that

are quite positive (>+20 ‰), bulk meteoritic IOM did not. While others had measured $\delta^{13}C$ in IOM previously and found similar values, ours was the first study to include such a diversity in meteorite types and weathering conditions.

The $\delta^{2}H$ values were spectacular: -100 to >+5000 ‰! For me, the challenge of making measurements with such a huge range was very instructive. We learned about "blanks" and "memory" especially with the meteorites that had very positive $\delta^{2}H$ values. Conel and I developed a suite of reference materials with $\delta^{2}H$ of +1000 ‰, +2000 ‰, and +3000 ‰ that we carefully weighed and analysed with each batch. After every isotopically heavy measurement, we ran a "blank" that was typically too small to capture, but cleared the slight "memory" from the previous sample. The $\delta^{15}N$ values of IOM were frequently outside the range of anything found on earth: -47 to >+400 ‰.

Our analyses included meteorites from many classes: CI (*e.g.*, Orguiel), CM (*e.g.*, Murray and Murchison), CR (*e.g.*, Al Rais), CO, OC, EH, and EL, as well as some that were labeled "ungrouped" (*e.g.*, Tagish). Meteoritists assume that each type of meteorite comes from a single parent body formed from a uniform reservoir in the solar nebula. This may not be the case. We measured an extreme range in the isotopic and elemental compositions within one meteorite class alone, which means that there are different processes taking place during the formation of IOM in parent bodies. Our results could not predict whether the IOM was formed in the solar system or an interstellar location. For a biogeochemist, this means that within a solar nebula, there are different abundances of volatile gases. The isotopic compositions, therefore, could represent the formation of IOM at different stages of the formation and reaction in the nebula. We concluded the following: "*Taken together, the presence of large isotopic anomalies in the IOM and the higher abundance of IOM-like material in comets compared to chondrites require that if IOM is solar it formed in the outer rather than the inner Solar System (Alexander et al., 2007).*" It still humbles me today to analyse material that could have originated from outside our solar system.

One of our findings contributed significantly to the ongoing controversy about the source of water to the terrestrial planets (Alexander *et al.*, 2012). Previously, it had been thought that water-rich comets delivered water as the planet passed through cometary tails. We proposed that meteorites and their parent asteroid bodies, rather than comets, were the primary source of planetary water. After analysing the IOM $\delta^{2}H$ compositions, we back tracked and compared that data to the $\delta^{2}H$ of bulk, whole rock meteorites. The thermochemolysis system (TC/EA) we used for analysis evolved water and converted it to H_2 gas at 1450 °C. Because we knew the IOM $\delta^{2}H$ values and concentrations, we could correct for the contribution of IOM to the bulk value. Based on spectral measurements of the $\delta^{2}H$ of cometary water, our results showed that Earth's water was closer in isotopic composition to the water in chondrites than it was to the water in comets. Further, we concluded that the chondrites originated from the region of the solar system between Mars and Jupiter and came from a variety of different parent bodies.

The third meteorite endeavour I worked on began with Andrew Steele. He had shifted his career from microbiology to planetary sciences after working at NASA's Johnson Space Flight Center and developing expertise on the AMASE expeditions. Steele pioneered the use of the WiTek Ramen instrument for examining the relationship of macromolecular carbon with minerals on a nano to micro scale. While his work on ALH 84001 showed that MMC was indeed indigenous, it was a "one off", and he wanted to learn if what he found in that meteorite could be found in others. Mars sample return missions, originally scheduled for 2005, then 2008, then not at all, were going to provide material for all of us to examine with sophisticated lab instrumentation. Our Geophysical Laboratory stable isotope lab was prepared and equipped to handle C, N, H, S, and O isotopes in all types of organic and inorganic phases, if we had the chance to get our hands on returned samples.

Instead of returned martian rocks, Steele obtained 12 known martian meteorites. I began work with Roxane Bowden, our isotope Lab Manager, to measure % C and $\delta^{13}C$ of very small quantities of powdered "Martians" that Steele passed to us as though they were precious gems. We developed a protocol that was enforced by Roxane's strict adherence to quality control. Anything that touched the samples was muffled in a furnace at 500 °C for two hours. All utensils were rinsed with distilled, deionised water, because even an ethanol wash leaves a carbon residue. The elemental analyser autosampler was cleaned, the combustion and reduction columns were renewed, and the mass spectrometer was checked out with blanks, boat blanks, and procedure blanks. This ensured that we were able to measure reliably the % C and $\delta^{13}C$ of less than 1 microgram of carbon. While this might not sound like a feat, even though less than a microgram of carbon is routinely measured in compound specific analyses, it is extremely difficult to control contamination from air, surfaces, and natural exposure. Such stringent measures are needed because most meteorites are found many years after they came to Earth (*e.g.*, ALH 84001), exposing them to colonisation by microbes or contamination by organic matter in soil or dust.

To determine the $\delta^{13}C$ value of indigenous martian carbon, we subjected each sample to a series of analyses. Our first included all carbon: both inorganic and organic. We assumed that a meteorite might have picked up terrestrial carbon. The second step was combustion in air at 550 °C, which removed all simple carbon molecules like oils or amino acids, and left high molecular weight MMC. Then we acidified the sample to remove inorganic carbonate and measured values again to give us total martian organic carbon (TOC). Percent TOC in the final fraction was 0.0019 to 0.0095 % with only a half a microgram of carbon or less in the sample. The $\delta^{13}C$ ranged from -16.9 to -24.7 ‰, with some of the samples overlapping potentially with terrestrial carbon. We were able to pick out discrete olivine crystals from the meteorite DAG 476 to compare with the bulk meteorite. Samples of basalt and Allende meteorite were measured as controls. The basalt (0.003 % TOC) allowed us to run multiple analyses to

calculate standard deviations and ranges for these small samples. The standard error in measuring basalt (n = 9) was 0.46 ‰ for $\delta^{13}C$, and we could measure % C within 0.001 % on 0.3 micrograms of carbon.

Together with Steele's microscopic investigations, we assembled a pathway for MMC synthesis on Mars. MMC was found in association with high temperature mineral phases in 11 out of 12 martian basaltic rocks. Based on the location of MMC next to these minerals, we concluded that MMC precipitated from reduced carbon phases inside melts hosted by olivine (Steele *et al*., 2012). This carbon, including polyaromatic hydrocarbons formed by igneous processes, was detected in meteorites that covered most of martian geologic history from 4.2 billion to 190 million years ago. We concluded the paper with this statement: "*Therefore, a positive detection of organics (especially PAHs) on Mars by Mars Science Laboratory, even if coupled with isotopically "light" $\delta^{13}C$ values, may be detecting this abiotic reservoir.*" Recent discoveries by Eigenbrode *et al*. (2018) have been made with sedimentary rather than igneous martian rocks, but the premise in the earlier Steele *et al.* (2012) work is important. There, we sounded a word of caution on a potential conclusion that organic carbon found on Mars is not automatically considered to be the result of extraterrestrial biological activity.

My fourth major project on meteorites was a collaboration with Zita Martins and the organic geochemists at Goddard Space Flight Center. For her Ph.D. dissertation, Martins was searching for nucleobases, the building blocks of DNA and RNA, in organic extracts of Murchison meteorite, one of the most famous carbonaceous chondrites of all time. Zita was working with Danny Glavin and Jason Dworkin at Goddard, and her colleagues and Ph.D. advisor in the Netherlands. Using time of flight mass spectrometry, she could detect nucleobases in some of the Murchison extracts. Selected ion monitoring was used to zero in on these unusual and complicated structures, essentially picking the "needles out of the haystack" of other compounds. She arrived with extracts in hand at the Geophysical Laboratory, and we were confident that we could readily measure the $\delta^{13}C$ based on the chromatograms. Unfortunately, the combustion-GC-IRMS system combusts all carbon compounds in the chromatogram, which was loaded with dicarboxylic acids, as well as with trace amounts of nucleobases (Martins *et al.*, 2008).

We were able to devise a GC ramp programme that separated the acids from the nucleobases without any serious overlap. During chromatography, the isotopic composition of the head of the peak can differ quite a bit from the tail of the peak. The $\delta^{13}C$ of the dicarboxylic acids were very positive (+20 ‰ or more) and similar to measurements others had made. We measured a $\delta^{13}C$ of +44.5 ± 2.3 ‰ for uracil in the Murchison, relative to a $\delta^{13}C$ value of -10.6 ‰ for uracil from the soil surrounding the location in Australia where it was found. Xanthine from Murchison had a $\delta^{13}C$ of +37.7 ‰ (Martins *et al.*, 2008), confirming that these nucleobases originated from meteoritic organic carbon. This work supports one of the many theories about the origins of life. One

of these theories holds that meteorites delivered organic molecules to Earth during its formation and the period when Earth was continually bombarded by incoming asteroids. Although it was known that amino acids (*e.g.*, Engel and Nagy, 1982; Martins *et al.*, 2007) were common monomers in some chondrites, no one had ever found nucleobases.

For the decade or more that I worked on meteorites it impressed me how precious these exceedingly rare samples are and what a boon for planetary scientists to have them in their hands. Each year, NASA selects a team of scientists to travel to Antarctica to search for meteorites that might have fallen in previous years. Dark rocks on icy, white surfaces will melt the snow around them making them easy to see. The other major source of meteorites is from the Sahara desert, where alternatively, there is little to no vegetation to obscure the meteorite falls. NASA's strategy of funding the study of cosmochemistry with samples in hand (meteorites), as well as through complex and expensive missions, has advanced our knowledge about the solar system to a remarkable degree in the past few decades. Given what we know now about organic carbon on Mars, in the coming 10 to 20 years the question of whether life arose elsewhere in the universe, I believe, will be answered.

13. EARTH'S EARLIEST SIGNS OF LIFE: IF WE FOUND IT, COULD WE RECOGNISE IT?

Since the early 1970s, scientists have been measuring the $\delta^{13}C$ of organic carbon from Precambrian rocks (*e.g.*, Schopf and Klein, 1992). Geologists were soon searching the world over for older and older rocks resting exposed on the surface that might contain evidence of the first signs of life. Greenland's Isua rocks were first scrutinised in the 1980s by Cyril Ponneperuma and his student Cliff Walters. Walters was given the task of examining the organic geochemistry of the Isua rocks. His professor wanted him to discover something revolutionary, perhaps Earth's earliest evidence of life. Cliff struggled at the University of Maryland to find any molecules that did not look like contamination, but his professor pressured him to "discover" something big. Fortunately for Cliff, he sought out the wisdom of Tom Hoering. Hoering's reputation for careful, exacting work was well known in the community, particularly after he debunked an earlier study on "Precambrian" hydrocarbons, which turned out to be ink from the newspapers wrapping the rock specimens (Hoering, 1965). Walters struggled for years before concluding that any molecular signals in these samples were modern contamination. He went on to become a very successful organic geochemist at Exxon Mobil, having learned from Tom Hoering about stringent lab procedures.

A decade later, UCLA scientists, including Stephen Mojzsis, used more sophisticated instrumentation to measure $\delta^{13}C$ *in situ* with an ion probe. The ion probe was promoted as the answer to answering the question of whether the carbon in ancient rocks was indigenous to the sample or was caused by contamination. An ion beam was focused on a fresh surface of the sample to ionise carbon atoms which were then detected by sensitive ion counting methods. The UCLA group measured $\delta^{13}C$ of -30 ± 2 ‰ (Mojzsis *et al.*, 1996) in Isua samples, concluding that these quite negative $\delta^{13}C$ values matched those of other younger, firmly established Precambrian stromatolite samples.

The problem with the ion probe measurements was that there were no comparable working standards. The ion beam was first rastered onto a graphite chip of known $\delta^{13}C$ composition, and was then focused on the sample. The carbon sputtered out of the rock but did not always come out with a uniform isotopic composition. Repeated measurements of the sample were used as proxies for measuring a similar standard material. If results varied by ±5 ‰, then averages were taken and considered to be representative of the $\delta^{13}C$ of the sample. As time went on, ion probe users realised this approach was not valid. Dominic Papineau, a postdoctoral fellow at the Geophysical Laboratory, compared "conventional" elemental analyser methods with ion probe methods to learn more about the standards needed for accurate and precise $\delta^{13}C$ analyses (Papineau *et al.*, 2010b) using Akilia rocks, from southwestern Greenland. Several papers were published on the Akilia "rocks" (*e.g.*, McKeegan *et al.*, 2007), however, there are only a handful of specimens from this location and no real outcrop that can be studied by the community. Therefore, few samples can be shared among labs. Speculation and debate about what type of rocks these are and how they were formed abounds.

Dominic obtained a couple of the Akilia specimens from his Ph.D. advisor Stephen Mojzsis, who was now at the University of Colorado. Our first paper based primarily on microscopic analyses using Raman spectroscopy, transmission electron microscopy, and synchrotron X-ray based microscopy (Papineau *et al.*, 2010a). Papineau studied graphite inclusions in association with apatite crystals (Fig. 13.1). Andrew Steele and I encouraged him to quantify the occurrences of graphite-apatite pairs rather than loosely describing them. Were these common features? Were there only one or two within a thin section? Did they all present the same appearance? About one sixth of the apatite crystals was associated with a graphite coating. The graphitic carbon was primarily ordered graphite, with a much smaller amount of disordered carbon. Raman spectroscopy was also used to determine that the graphite in these rocks was crystallised at very high temperatures during metamorphism (>650 °C). The carbon was severely re-ordered making it impossible to determine if it was originally biogenic or abiogenic carbon.

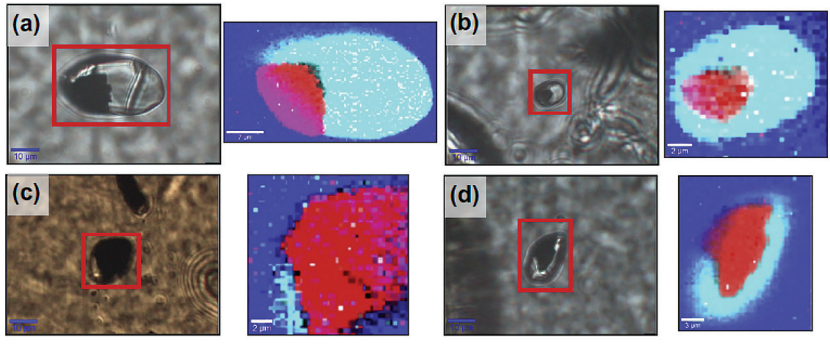

Figure 13.1 Transmitted light photomicrographs **(a–d)** of apatite grains associated with graphite and their corresponding Raman hyperspectral images. In the hyperspectral images the intensity of the main Raman vibrational excitation mode in quartz is represented in blue; apatite and graphite are represented in turquoise and red, respectively. From Papineau *et al.* (2010a) with permission from Elsevier.

It is important for scientists to debate and ultimately come to an agreement on the first conclusive evidence of life on Earth. Many researchers use the carbon isotope compositions of graphite from Earth's oldest rocks as firm evidence that photosynthesis was an active process 3.85 billion years ago. Others argue that owing to metamorphic processes, graphitic carbon in ancient rocks could result from numerous types of abiogenic reactions that show carbon isotope compositions similar to photosynthetic ones. This distinction is important because we want to know how to identify very old signs of life after we have sufficient samples from Mars and other planetary bodies.

In a companion paper (Papineau *et al*., 2010b), we compared the $\delta^{13}C$ of some banded iron formations that were slightly younger than the Akilia rocks. The Vichadero banded iron formation (BIF), 2.3-1.7 Ga, and the Bijiki banded iron formation, 1.8 Ga, were clearly formed when living organisms thrived on Earth. Their $\delta^{13}C$ values were -28.6 ± 4 ‰ and -24 ± 0.4 ‰ for the Vichadero and Bijiki BIFS, respectively. On the other hand, using both elemental analysis-IRMS and Nano-SIMS, the $\delta^{13}C$ of the Akilia rocks had values of -17.5 ± 2 ‰ (EA-IRMS) and -13.8 ± 5.6 ‰ (Nano-SIMS). These samples included more than half of the carbon as carbonate with much lower amounts as graphite. For EA-IRMS analysis, we measured the samples before and after acidification, which was not possible using the Nano-SIMS. The variation we measured by Nano-SIMS can easily be explained by the inclusion of some amount of carbonate carbon in the sample. The $\delta^{13}C$ of the carbonate itself was -4.0 ± 1 ‰. Nano-SIMS also determined that the graphite included O, H, and N, elements that are typically associated with biogenic organic matter. However, these elements are also common in metamorphic fluids and could be sourced from fluids rather than decomposed organic remains. Additionally, because of my experience working with meteoritic organic matter, we knew that IOM is without question abiogenic yet contains C, O, H, and N. We still struggle to find an unambiguous signal of first life on Earth.

The work with Papineau took me to several locations around the globe to examine Precambrian rocks in the field. We travelled to Ontario and Quebec to study BIFs on a NAI-sponsored field trip (Ohmoto *et al*., 2008) where a diverse team of scientists argued in the field about the levels of oxygen on early Earth, formation of BIFs, and isotopic compositions of Proterozoic and Archean rocks. One memory I have of this trip is of Dick Holland, Harvard University, and Hiroshi Ohmoto, Penn State, standing on a BIF and speaking with a bullhorn to young astrobiologists, to give their perspectives on all of these topics. My next trip with Dominic was to Rajasthan, India, where we sampled stromatolitic phosphorites from the Aravalli Supergroup. My trip to India and my first in-depth field trip to examine stromatolites in a natural setting was a remarkable experience.

Standing on outcrops that extended for several kilometres and that had been formed almost entirely by the actions of microbes was a highlight for me as a biogeochemist who was brought into the field by the early work of Barghoorn and others from the 1970s. My challenge was to inspect the rocks in the field and couple observations with my more reductionist approach based on isotopic measurements in the laboratory. We travelled with two Indian specialists, Professor Roy and Professor Ritesh Purohit, who had studied the geology of these formations for many years. Field work in India, as opposed to other places I had worked, was never conducted without close watchfulness from local people. At the end of a 1 to 2 hour sampling, our field area would be lined with about 20 to 30 men, women, and children along with goats, water buffalos, and cows observing our activities.

Based on the samples we collected from India, we published a series of papers on the putative development of the nitrogen cycle during an increase in primary productivity associated with an input of phosphorus derived from erosion within closed marine basins (Papineau *et al.*, 2013) (Fig. 13.2). Based on these 2.15 billion year old samples, we linked the carbon cycle to a robust nitrogen cycle at the time when atmospheric oxygen increased 2.4 billion years ago. Microbes, primarily cyanobacteria, were the producers of oxygen *via* Photosystem II at that time. Not only did we measure high concentrations of organic carbon in these rocks, but their $\delta^{13}C$ values ranged from -33 to -11 ‰. Extreme variability in carbon isotopes is indicative of swings from low to high productivity. In a subsequent paper (Papineau *et al.*, 2015), we measured many more $\delta^{15}N$ values and developed the use of V_2O_5 as an addition to samples to promote quantitative combustion of the samples. C/N values varied

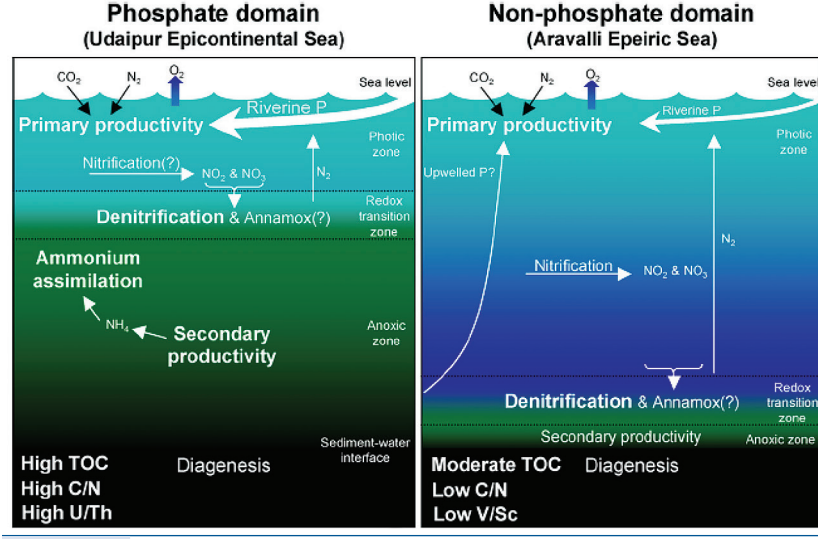

Figure 13.2 Nitrogen isotope data of ancient sedimentary rocks have the potential to describe ancient biogeochemical processes. We developed this schematic diagram of the possible water column profiles including redox states where turquoise indicates the photic zone; blue, the moderately oxic waters; and green is anoxic zones. Nitrogen and carbon cycling are indicated where bold characters show stronger pathways for the phosphate domain of the Udaipur Epicontinental Sea and the non-phosphate domain of the Aravalli Epeiric Sea. In the model for the phosphate domain, the input of phosphorus from rivers (and/or upwelling) is larger, the redoxcline is shallower, and biological ammonium assimilation is more important in the anoxic zone compared to the non-phosphate domain. Nitrogen isotope fractionation factors for these reactions have been summarised in Fogel and Cifuentes (1993) and Papineau *et al.* (2009). From Papineau *et al.* (2009) with permission from Elsevier.

from 10 to >300 in phosphate domain samples and from 5 to 25 in non-phosphate domain samples (*e.g.,* black shales). Although some phosphate domain samples had high C/N values and some of the most positive $\delta^{15}N$ values (+30 ‰), others had $\delta^{15}N$ values around +5 ‰.

Based on the lithology of the samples, we developed conceptual models of primary production, denitrification, ammonia oxidation, and N_2 fixation that compared the Udaipur epicontinental sea where phosphorites were formed with the Aravalli Epeiric Sea, where non-phosphate stromatolites were formed. Both models relied on the depth of the anoxic zone: shallower in the epicontinental sea and deeper in the epeiric sea. A more extensive anoxic zone results in higher preservation of organic matter, with higher TOC and TON concentrations. The formation of phosphate domain rocks also relies on external inputs of phosphate to the ecosystem, primarily from riverine transport. Without an external source of phosphorus it is difficult to explain the precipitation of large deposits of phosphorite. For the non-phosphate domain rocks, phosphorus is cycled *via* microbial decomposition and upwelling, regenerating the phosphorus to the euphotic zone, but not adding a new source of this nutrient. In both cases, we see evidence of "modern" nitrogen cycles which have been confirmed by studies published subsequent to ours (*e.g.,* Stüeken *et al*., 2016).

My second major field trip with Dominic Papineau was fascinating for its spectacular geology, the remoteness of the location, and the chance to interact with native people of northern Quebec. From a small village on the eastern shore of Hudson Bay we chartered a fishing boat, the Kakivak, in July 2011, that was crewed by Inuit men. My husband, Chris Swarth, accompanied me and 13 other scientists along with five Inuit crew for a two week adventure on Hudson Bay. We set sail from Umijaq on a Sunday afternoon, making our way across the Bay to the Belcher Islands. These islands are special for several reasons. First, they are very remote and scientists have visited them only sporadically over the past 100 years. Robert Flaherty described the geological formations in 1918. Our target samples were 1.875 Ga stromatolites that had first been found in the early 20th century. Scientists at that time realised how special these rocks were and found evidence for the remains of microorganisms that lived on the early Earth. We returned to several of these sites, spending three days at one of the most spectacular stromatolite sections that I have ever seen (Fig. 13.3). Second, the islands are special because they are biologically pristine. This was the second time I was able to study and sample tundra vegetation. As the temperatures of Arctic and tundra areas increase due to climate change, plants will respond with longer growing seasons, making it important to develop records of present day communities and the processes that influence them. I was able to collect about 75 specimens from the Belcher Islands for my herbarium collection that may—some day—serve as an historic record of what the plant life was like in the early 21st century. People, other than the Inuit, rarely visit the Belcher Islands, as there is little to no support for ecotourism in the area. We were fortunate to be able to experience Inuit culture including native fishing. Periodically, the crew fished while we were out examining rocks.

They caught Arctic char which they shared with us: the muscle, Canadian sushi, went to the scientists and the rest of the fish – tongue, liver, intestines, skin, heart – was consumed raw with great relish by the crew. The Inuit understand in a very fundamental way about the ecosystem in which they live.

Figure 13.3 Belcher Islands stromatolites, 1.8 Billion years old, Hudson Bay, Canada. Photo credit: M. Fogel.

The 2nd week of our expedition took us back towards the mainland. We travelled to the Nastapoka Islands that form an arc parallel to the coastline, a part of the Hudson Bay considered by some to be a remnant crater from a meteorite impact. Our scientific party scoured several of these islands looking for evidence of shocked rock strata indicative of such an impact. We were unable to find samples of this nature, but could see correlations between these rocks and those on the Belcher Islands. Our 3rd destination was the Richmond Gulf, an unbelievably beautiful body of water with high mountains, cliffs, and crystal clear waters. Our team scoured at least 7 different sites with numerous outcrops to compare the stratigraphy here with that on the Belcher Islands. Canadian Geological Survey scientist, Dr. Wouter Bleeker, took samples for dating, as there are only a handful of dates from this whole area.

In the Richmond Gulf, we were treated to a sighting of beluga whales, small white whales considered a delicacy by the Inuit. The pod of about 20 belugas swam into the inlet where we were moored, diving, jumping, and hunting for the abundant Arctic char. Our Inuit crew watched them carefully, but decided not to hunt owing to the fact that we had 15 people on one small boat.

Almost 600 kg of rocks were shipped back to the United States and Ottawa for further analysis at Boston College, the Geophysical Lab, and the Canadian Geological Survey. The expedition was a lifetime experience for all of us, as we were privileged to seeing places, rocks, and people that very few people will ever have the opportunity to experience. The results from this trip are currently being written up for a publication, spear-headed by Papineau, on the nature of concretions found in Palaeoproterozoic rocks and what they mean in terms of organic carbon cycling.

Studies on isotopic compositions of Earth's earliest sedimentary rocks are going to feed into studies that will consume the astrobiological community when samples from Mars are returned to Earth. It is vitally important for the scientific community to continue carefully to study biosignatures on the Earth weighing what is a definite biosignature *versus* an ambiguous one. The personalities that study Earth's oldest rocks are quite strong; individuals hold strong opinions. There is a constant push and pull to announce the first evidence of life on Earth, similar to the desire to find the signs of life on Mars.

Bell *et al.* (2015) published a paper with $\delta^{13}C$ of a single graphite inclusion from the Jack Hills Formation, Australia, 4.1 Ga. They found only one graphite inclusion, as opposed to the many that we found in the 3.8 Ga Akilia rocks. The UCLA team's paper generated a lot of controversy. The $\delta^{13}C$ of the graphite inclusion they studied had a value of -24 ± 5 ‰, in the range of C_3 photosynthesis, and was measured with an ion microprobe, similar to earlier measurements by Mojzsis *et al.* (1996) that were subsequently found to be analytical artefacts. In comparison, a study by Papineau *et al.* (2011) of the >3.8 Ga Nuvuuagittuq BIFs had $\delta^{13}C$ of -22.8 ± 1.9 ‰. Although these $\delta^{13}C$ values are similar to biologically-derived carbon, the graphite structures were re-precipitated from metamorphic fluids before deposition. We could not be sure whether the carbon in this graphite had passed through the rump of a microbe. $\delta^{13}C$ values (-16 to -24 ‰) from martian meteoritic organic carbon were not definitive enough for us to conclude whether the origin was biological (Steele *et al.*, 2012). As much as I like carbon isotopes, they are not, by themselves, conclusive biosignatures. The concept of isotope fractionation (*i.e.* comparing isotopic values of a product relative to a substrate) is probably a better way to determine whether the isotope compositions could result from reactions carried out by living organisms.

Recently, Papineau's graduate student, Matthew Dodd, University College London, described micrometre-scale haematite tubes and filaments that could be the remnants of microbes in hydrothermal systems in the Nuvuuagittuq samples (Dodd *et al.*, 2017). Organic carbon associated with the microtubes has a $\delta^{13}C$ of -19.7 to -25.7 ‰. Konhauser *et al.* (2017) remarked that if these are in fact microbial in origin, it implies that iron oxidising bacteria were living in a microaerophilic environment at this time, which would further imply that extant cyanobacteria were producing photosynthetic oxygen. If and when we finally have samples returned from Mars, the community researching ancient rocks on Earth will need to pull out all the stops in looking at the samples with a nano- and microscopic view.

14. GEOECOLOGY

As my career developed, I became more engaged in straight-out ecological research because stable isotope biogeochemistry was becoming increasingly accepted by the ecological community. My goal as a stable isotope ecologist is to learn as much as I can about modern ecosystems on a global scale so that I can use that knowledge to interpret fossil ecosystems where direct observation of the plants, animals, and microbes is not possible. I have worked in most of the major biomes: deserts, temperate deciduous forests, grasslands, alpine, arctic, taiga, tundra, temperate rain forests, woodlands, freshwater wetlands, estuaries, coastal wetlands, lakes, marine ecosystems, and coral reefs. From my first forays in South Texas bays in 1974 to my last trip to Arctic Svalbard in 2015, the unity of nature in combination with all of the variables that shape ecosystems and influence their isotopic composition has been a learning experience. My work took me to almost every continent: North America, South America, Central America, Europe, Asia, Australia, and Africa, only missing out on Antarctica. Doing ecological field work internationally requires extensive preparation, sampling permit applications, import and export permits, advance planning, and being nimble on your feet when conditions change – but it is worth it all. In relationship to field work, Mat Wooller, University of Alaska, trumpeted the "Seven Ps": "*Prior preparation and planning prevent piss poor performance*."

The term Geoecology, as it pertained to my research, surfaced around 2009. For the first two thirds of my career, the impetus for my research was primarily in sync with the biogeochemical community, and secondarily the geochemical community. Much of my early work established isotope fractionations for certain biochemical pathways, information which proved useful for others who were interpreting isotopic measurements that were more applied to a particular problem or ecosystem. The major incentive for me to begin to concentrate more on ecology came from two fellowships (Loeb and Mellon Fellow) awarded to me by the Smithsonian Institution's Environmental Research Center (SERC) in Maryland. I was awarded unrestricted funds to develop collaborations with Smithsonian Institution scientists at SERC, as well as to provide access to my stable isotope lab at Carnegie since the Smithsonian did not have an IRMS facility at that time.

My first collaboration was with Anson Tuck Hines, Associate Director of SERC and a fish and crab biologist. Seining for estuarine fish and other field sampling took place weekly on the Rhode River, Hines' lab study site on the Chesapeake Bay. We measured the $\delta^{13}C$ and $\delta^{15}N$ of over 800 samples of fish, invertebrates, zooplankton, phytoplankton, and seaweeds in three Chesapeake Bay tributaries: Rhode River, Nanticoke River, and Muddy Creek. Ecologists are interested in identifying the source of primary production at the base of food webs and how it changes over space (habitats) and time. In terrestrial river systems, organic carbon that supports food webs can come from sources

produced in the river-estuary system (*i.e.* autochthonous) or from terrestrial plant material washed in from land (*i.e.* allochthonous). For my first project with SERC, we determined that autochthonous sources were most important for zooplankton and the larger fish, such as the striped bass, whereas allochthonous sources influenced the diets of benthic organisms, like clams. In the Chesapeake Bay, the striped bass is an important and threatened fish species which once supported a plentiful, commercial fishery. Now the catch is strictly regulated and open only to recreational fishing.

With SERC plant ecologist Jess Parker, we rented a crane that took us to the tops of 30-40 metre trees to collect leaves in SERC's temperate deciduous forest. We documented that leaves growing higher in the canopy have more positive $\delta^{13}C$ values (-26 ‰) than those lower in the canopy (-31 ‰) (Graham *et al.*, 2014). Our results were paired with Penn State's results on tropical rain forests, which were used to bolster the interpretation of $\delta^{13}C$ values from fossil leaves. In a sedimentary deposit, leaf fragments often persist millions of years (*e.g.*, Schweizer *et al.*, 2006), and their $\delta^{13}C$ values serve as proxies to construct palaeoenvironmental conditions such as palaeo-CO_2, temperature, or rainfall.

14.1 Biocomplexity of Mangrove Ecosystems

By far the most engaging research I started in the late 1990s was joining an interdisciplinary group of ecologists led by Candy Feller at SERC. Feller is a plant and insect ecologist, who teamed up Myrna Jacobson, a geochemist, then at Georgia Tech. Candy and Myrna attended a workshop where the research concept was introduced, swapped research interests and a collaboration was born. Candy Feller along with Catherine Lovelock, plant physiologist, and Karen McKee, USGS ecologist, were already studying the effects of nutrient pollution on the growth and health of mangrove forests (Feller, 1995; Feller *et al.*, 1999). Mangroves encompass a wide variety of salt tolerant tree species that grow in coastal zones in the tropics and sub-tropics worldwide. The mangrove ecosystem hosts a diverse community of organisms in the trees' canopies, underlying soils, and in the adjacent waters. They are some of the most productive ecosystems on the planet, fixing carbon at fairly high rates and providing fuel for important fisheries. Because they grow at the land-water interface, mangrove ecosystems protect inland communities from flooding, hurricanes, tsunamis, and sea level rise. Unfortunately, because they are found along the coast, these forests are often targeted for destruction to make way for development. Understanding how the mangrove ecosystem functions in a natural state, and because of their proximity to growing human populations, it is critical to learn how they tolerate and adapt to changing global conditions (Fig. 14.1).

Our research team included scientists from six institutions and was, at that time (1999), one of the larger projects I ever worked on. After an All Hands meeting at SERC, we travelled to Smithsonian's research station on Carrie Bow Cay, Belize, for orientation in our research site on nearby Twin Cayes.

Going from Washington, DC, we boarded a plane at National Airport bound for Miami, where we joined a group of largely tourists heading south to Belize City. After passing through Belizian customs with large, plastic containers of our supplies and gear, we made our way south to the small city of Dangriga. Sometimes, we hired an old station wagon to take us from the main airport 60 km south; more frequently than not it broke down along the way. If we managed to jump on a small plane, often our luggage was too big and was delayed in Belize City until a truck could be found.

Figure 14.1 Twin Cayes, Belize, 2003. White patches on the islands were clear cut for potential resort development, which never happened. Vegetation on the islands is primarily three species of mangrove trees. Photo credit: M. Fogel.

In Dangriga at the Pelican Beach Resort Hotel, our gear was loaded onto an open boat, tied down, and we motored across 20 km of coastal waters to reach Smithsonian's research station at Carrie Bow Caye. Carrie Bow is a small, 50 metre wide sand atoll on the edge of a coral reef. During hurricanes, the island is covered with water. Electricity is generated by solar power, and food and fresh water are brought in once a week by boat. Scientists share communal space and meals prepared by a Belizian cook who always prepared healthy, delicious meals using local foods. The laboratory consisted of a wet lab, complete with running seawater, and a dry lab, where we set up our small equipment for nutrient and pigment analyses.

After breakfast each day, we hopped into a 3.5 metre boat with an outboard motor and headed to Twin Cayes, a pair of islands several kilometres to the west. Twin Cayes were originally preserved and protected for scientific

research, but with time, local fishermen built small huts, docks, and outhouses in several areas. In general, our field sites were kept safe from interference by others, including tourists, but we were required to find remote areas of the islands to set up more permanent experimental plots. Feller and her colleagues established three study areas in 1995: The Dock, Boa Flats, and The Lair. At each of these sites, they had established a transect between mangrove trees beginning at the fringe of the island extending through a transition zone at higher tide level and ending in an interior region. Trees at each zone (*i.e,* fringe, transition, and interior) were marked with plastic flagging. Their experimental design included three treatments: control, nitrogen fertilisation, and phosphorus fertilisation. Twice a year, Feller and colleagues travelled to Twin Cayes to fertilise the trees, to collect leaf samples for isotope analysis, and to measure productivity and other biological parameters.

I made many trips to Belize over the next twelve years, working on three different projects. On my first collecting expedition, I was joined by Matthew Wooller, postdoctoral fellow at Geophysical Laboratory, Myrna Jacobson and Barbara Smallwood (University of Southern California) and Mandy Joye and Rosalind Lee (University of Georgia). My main tasks for this project were to sample the leaves, stems, and roots from different species of mangroves, and to sample detritus, surface sediments, seagrasses, POM, and any animals we could catch. In the lab in Washington, we were to measure $\delta^{13}C$ and $\delta^{15}N$ of each of these organic matter reservoirs, as well as to determine the weight percent of carbon and nitrogen. The data were to be used in an ecosystem network model crafted by Bob Ulanowicz and Ursula Scharler of the University of Maryland. The isotope values would allow the modellers to connect the branches of the food webs, and the elemental concentrations were to be used for determining nutrient flows in different ecosystem zones and as a function of fertilisation treatment. This seemed like a very straight-forward task. Mangroves are C_3 plants; seagrasses have much higher C_4-like $\delta^{13}C$ values. Twin Cayes were small islands. We figured there would be very small variations in $\delta^{15}N$ values. We would come to realise that this system was much more complex than we'd initially imagined.

Wooller and I planned to collect our samples at gridded locations across the cayes. We came armed with GPS coordinates, a couple of older GPS units, and a lot of sample bags and tubes. Jacobson, on the other hand, shipped a portable gas chromatograph to Belize, which was held up in customs for many days. The instrument required a clean source of helium to operate. There was no compressed helium of suitable purity in the entire country. So we invited Jacobson and Smallwood to come along on our grid sampling. Adventures awaited us at nearly every "station" that we occupied. The locations of Feller's experimental transects were readily accessible, after you climbed over some serious mangrove roots and waded maybe 10 metres onshore. Our grid stations provided even greater challenges. GPS units in 1999 were not as accurate as they are today. Under the mangrove canopies, we often lost signals and had to approximate our location. Along the way we encountered sharks, boa constrictors hanging from low branches, crocodiles, stinging jellyfish, and deep holes in

the mangrove peat that swallowed up our legs and banged our shins. In a day, we could sample about 6 to 7 stations and by the time we returned at night to Carrie Bow, filthy, sunburned, covered with mangrove muck and the microbial soup that flourished in the interior ponds. After a shower, a couple of beers, and dinner, we were refreshed and headed up to the lab to prepare samples, write up our field notes, and analyse nutrients.

When we analysed our first set of samples from the grid stations, we were surprised at the variations in $\delta^{15}N$ values we observed in mangrove leaves. If only I had a portable IRMS in Belize, we would have analysed every mangrove tree on Twin Cayes. About one third of our samples were from tall, fringing trees, another third from medium height mangroves in transition zones, and the remaining third were dwarfed trees, no higher than 1-1.5 metres tall growing in the island's interior. Fringing mangroves, primarily the red mangrove (*Rhizophora mangle)*, had mean (± standard deviation) $\delta^{15}N$ values near 0.0 ± 1.7 ‰ (Fogel *et al.*, 2008). Red mangrove leaves from the transition zone were slightly more negative with mean $\delta^{15}N$ of -1.9 ± 3.0 ‰. Dwarf trees, which can be decades old, had mean $\delta^{15}N$ of -6.8 ± 4.7 ‰ with a very wide range from -21.7 to 1.4 ‰.

As our study investigated biocomplexity, each of us thought about these data in a slightly different way. In biocomplexity theory, an emergent property is an observation that is non-linear and may explain organisational properties of a system. The $\delta^{15}N$ of mangrove leaves was our emergent property. As far as we could tell, these were some of the most negative $\delta^{15}N$ values measured in a naturally-growing plant. At this point, there was no easy explanation as to why there was such great variation from a single species on two very small islands. Fringing mangroves grow at the edges of the ecosystem, whereas the dwarf mangroves with their sculpted morphology found growing in interior ponds were excellent examples of self organisation. The hunt for an explanation ensued and consumed Wooller, Jacobson, John Cheeseman (University of Illinois), and I for the next four years of the project.

At the same time, Feller, Lovelock, and McKee had been collecting mangrove leaf samples from their prior fertilisation areas. Their results showed that the $\delta^{15}N$ values of dwarf trees were strongly influenced by phosphorus fertilisation and the control and nitrogen fertilised trees had more similar and slightly negative $\delta^{15}N$ values. We started collecting leaves from all of the experimental trees to compare with samples collected several years prior. Our $\delta^{15}N$ data for the nitrogen fertilised trees were more negative than those of McKee *et al.* (2002) with values as low as -12.4 ‰. Control samples had $\delta^{15}N$ ranging from -8.9 to 0.7 ‰. The $\delta^{15}N$ of the phosphorus fertilised dwarf trees ranged from -1.6 to +1.1‰. How phosphorus changed the $\delta^{15}N$ of a mangrove leaf was not immediately intuitive.

We began a phosphorus fertilisation experiment with dwarf trees that we guessed had very negative $\delta^{15}N$ values, then collected newly emerged leaves periodically over the next two years. Within 2-3 months, the $\delta^{15}N$ increased

from -13 ‰ to -8‰ and then leveled out at -2 ‰ after one year. We found interior mangrove trees distributed around the islands that had been fertilised by Feller with phosphorus almost a decade earlier, but with no subsequent phosphorus additions. The $\delta^{15}N$ of these plants was similar to recently fertilised trees in the experimental plots. Our conclusion was that once a tree was provided a slug of this limiting nutrient it held onto it for a very long period of time. McKee *et al.* (2002) reached the same conclusion.

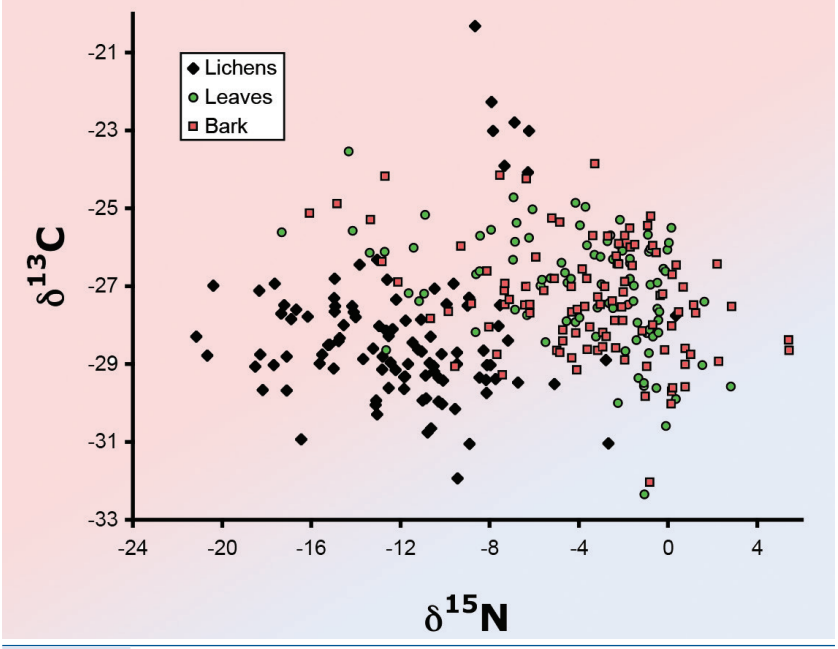

Figure 14.2 The extreme range in $\delta^{15}N$ values of mangrove leaves and bark was a puzzle for several years until we analyzed a few lichens. Lichens, which use nitrogen from the air, were important for helping us understand the processes summarise in Figure 14.3. The $\delta^{15}N$ of bark, leaves, and lichens growing on mangrove trees (*R. mangle)* at Twin Cays. The data set is for paired sets of leaf:bark:lichen collections sampled from fringe, transition, and dwarf regions around the islands. Modified from Fogel *et al.* (2007).

Phosphorus is important for many biochemical reactions in a plant, in particular for making ATP, an organism's energy storage compound. The enzyme that transports nitrogen into a plant's roots requires several molecules of ATP for each nitrate molecule transported. We concluded that the phosphorus effect on $\delta^{15}N$ related to more efficient uptake of nitrogen (Fig. 14.2). We expanded our work by developing field methods to collect and measure dissolved ammonium in sediments and porewaters. The NH_4 in surface sediments had mean (± s.d.)

$\delta^{15}N$ values of +4.3 ± 4.0 ‰. Microbial mats, actively fixing N_2, had values of +1 to -1 ‰, in line with the source of nitrogen to the ecosystem coming from microbial input. Why the $\delta^{15}N$ of leaves were so negative continued to challenge us.

Later, we learned about a paper by Erskine *et al.* (1998) who had found depleted ^{15}N values in plants collected downwind of a marine bird rookery on a subantarctic island. They postulated that isotopically light NH_3 volatilised from bird guano was incorporated into plant tissues. Could there be volatilised ammonia in our mangrove ecosystem? Wooller and I found a nifty way to detect and measure NH_3 concentrations in the air. We purchased disposable NH_3 "badges" that could be tied to tree branches to absorb NH_3 and change a colour-sensitive dye embedded in the badges. Our first deployment of these NH_3 detectors took place over a 50 cm thick microbial mat where, in midday, oxygen bubbled vigorously from the surface. After only 1-2 hours, we were able to see substantial NH_3 emissions! Following this first deployment, we measured NH_3 emissions in several locations on the islands. Emission rates were highest over microbial mats and nitrogen fertilised experimental areas. Our next test was to capture the NH_3 and measure its isotopic composition.

Large isotope fractionations have been measured between NH_3 and NH_4. In equilibrium, the nitrogen isotope fractionation is about 18 ‰, but with kinetic reactions, can be much greater (Fig. 14.3). The $\delta^{15}N$ of NH_3 in the air

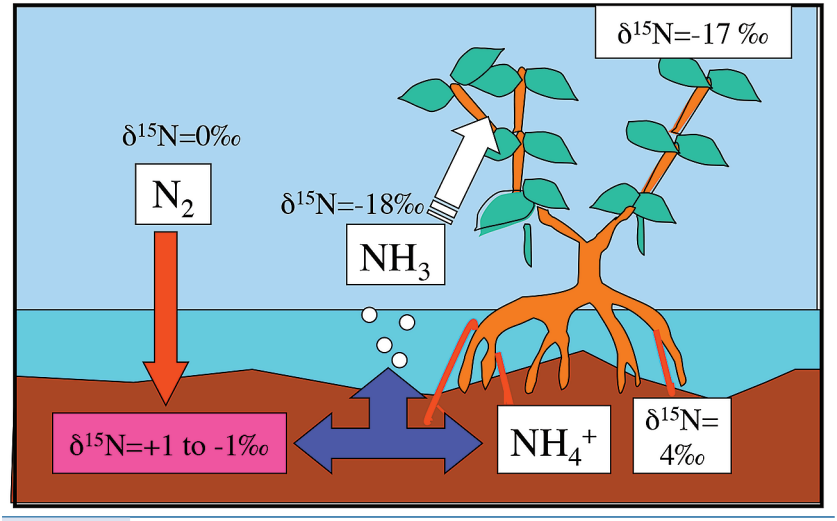

Figure 14.3 Matthew Wooller assembled this diagram for nitrogen isotope pathways in microbially-dominated mangrove ecosystems after several years of investigation. Isotopically-depleted NH_3 is passively taken up by leaves, then incorporated into biomass. From Fogel *et al.* (2007).

on Twin Cayes averaged -19.5 ‰, but varied from -30 to -7 ‰ showing the dynamic range and heterogeneity of this nitrogen source. Our final experiments showed that mangrove leaves can, indeed, incorporate gaseous NH_3 when they open their stomates to capture carbon dioxide. Last, we found that lichens growing on the bark of mangroves throughout the islands also had isotopically negative $\delta^{15}N$ values. Lichens are symbiotic combinations of algae and fungi with no roots. They take up any available nitrogen from the air, probably with little to no isotopic fractionation. The $\delta^{15}N$ of lichens ranged from 0.4 ‰ in the fringe zone to –21 ‰ in the microbial mat and dwarf zones. Our subsequent sampling of the entire community took advantage of the fact that dwarf mangrove tissues had unusual $\delta^{15}N$ that could be readily traced into marine invertebrates in this ecosystem (Figs. 14.4 and 14.5).

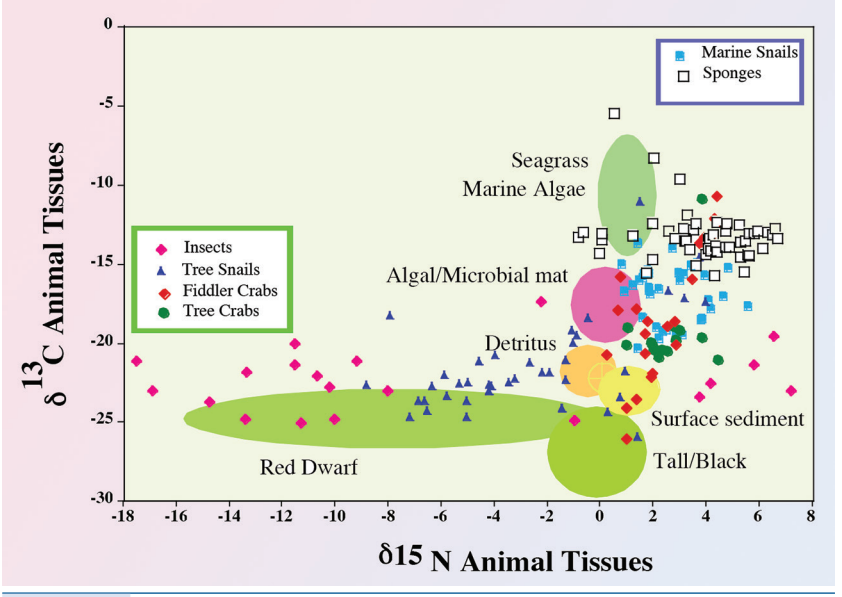

Figure 14.4 Isotopic composition of marine food web on Twin Cayes. The colored ellipses show the isotopic compositions of the primary producers. Insects and tree snails incorporated the very negative $\delta^{15}N$ from red mangrove leaves influenced by foliar uptake of ammonia. Unpublished graph of M. Fogel.

Mat Wooller went on to establish palaeo-proxies of sea level and conditions using peat cores from Twin Cayes and Spanish Cay in Northern Belize (Smallwood *et al.*, 2003; Wooller *et al.*, 2003, 2007; Monacci *et al.*, 2009). Using the $\delta^{15}N$ of leaf fragments plucked from peat cores, we were able to determine whether each leaf was from a dwarf, transition, or fringing mangrove tree. Coupled with accurate dates from the leaf fragments and pollen records, Wooller

and team assembled a climate and environmental record of the Holocene in tropical Belize. The Belize biocomplexity project was one of the favourite studies of my career. It began with simple concepts, but soon moved into a complex realm that was challenging in the field, in the laboratory, and with the interpretation of the data.

Figure 14.5 Matthew Wooller and Quinn Roberts are seining for fish adjacent to Twin Cayes in nearshore waters, 2002. Photo credit: M. Fogel.

14.2 Coral Biogeochemistry

In 2009, after accepting a position at the National Science Foundation, I wrangled a postdoctoral fellowship for David Baker. Baker, a graduate student from Cornell University, visited the Geophysical Lab a couple of times asking for a postdoc so he could continue his work on isotopes in corals. Finally, I recognised that he was a determined, smart young man who had a clear vision of how he wanted his career to unfold. He managed to get a matching fellowship at the Smithsonian, which opened up the possibility of going back to Twin and Carrie Bow Cayes.

Baker was interested in studying the effects of nutrient additions to coral symbionts. His field areas included Australia's Great Barrier Reef, Panama, Florida, and Mexico (Baker *et al.*, 2013a, 2017). He was able to trace pollution

from sewage into coral skeletons adjacent to crowded tourist destinations in Mexico and Bermuda. At the Carnegie, he extended his studies to include investigations on coral symbionts called zooxanthellae, photosynthetic dinoflagellates living inside the coral host. The coral symbiont, *Symbiodinium sp.,* are genetically very diverse. The genetic variants, called clades, are associated with particular coral species and with the environmental conditions of the coral reef. For example, clade D is more resistant to heat, whereas clade C, which is much less tolerant to fluctuations in temperature, is more commonly found in a greater variety of corals.

In a set of experiments conducted at Australia's Great Barrier Reef, the coral *Acropora tenuis* was infected with either clade C or clade D symbionts (Baker *et al.*, 2013b). Using enriched ^{13}C and ^{15}N tracers at two different temperatures (28 °C and 30 °C), we found that clade C outcompeted clade D at 28 °C in terms of carbon and nitrogen uptake. At 30 °C, clade D was able to fix more CO_2 than clade C, but clade C continued to take up more nitrogen, often a limiting nutrient in oligotrophic environments. We concluded that clade C symbionts will dominate, even though they are less tolerant of thermal stress, owing to their nutrient gathering ability.

Warming of the world's oceans destabilises the association of corals and their symbionts and reduces the ability of corals to survive climate changes. Dave wanted to test the popular hypothesis that, when coral reefs warm in polluted waters, *Symbiodinium* symbionts will sequester more resources for their own growth and become parasites on their coral hosts. In 2011, Baker, Dartmouth graduate student Derek Smith, Chris Freeman, a graduate student at the University of Alabama, and I travelled to Carrie Bow to carry out our investigations.

Baker and Freeman were certified scientific divers trained to collect corals in a variety of environments. Every few days, we would go off shore or near another coral reef, so Baker and Freeman could collect samples for experiments and isotope measurements. Derek and I were responsible for staying in the boat and figuring out when and where the divers would surface. One particularly choppy day just off shore of Carrie Bow, we waited anxiously for the dive "balloons" to surface so that we knew where they were. Divers attach these balloon to their wrists so that people on the surface can see where they are. That day Freeman forgot to do this, and the balloon floated away undetected. The water was rough, we were drifting onto the reef, and we needed to take action fast. I worried that our propellers would hit the divers as they surfaced. Derek was concerned that as we drifted, we'd end up on the reef. After drifting 100 m or so away from the divers, we turned on the engine and circled around. Baker and Freeman were pretty surprised when they surfaced and saw the boat quite a distance from them. Derek and I learned some important boat safety that day and gained some confidence.

After they collected the coral specimens, we set up a series of experiments with small 5 cm pieces of a couple of coral species, placed them in bottles, and incubated them in temperature-controlled plastic pools on Carrie Bow. Each morning and evening dissolved oxygen levels were measured in the experimental bottles to determine respiration and photosynthetic rates. At night we extracted chlorophyll from the corals and measured inorganic nitrogen nutrients. The coral *Orbicella faveolata* hosted one of two *Symbiodinium* clades and was held at ambient (26 °C) or sub-bleaching (31 °C) temperatures. We added excess nitrate with an enriched ^{15}N content, as well as enriched ^{13}C. The presence of enriched ^{13}C in the symbiont represents assimilation, whereas enriched ^{13}C in the coral host tissue represents translocation from the symbiont. Incorporation of nitrogen for both symbionts and corals allows for growth and cell division (Baker *et al.*, 2018).

Compared to ambient temperature measurements, the symbiotic dinoflagellate variants (A3 and C7) fixed more carbon and nitrogen than at the higher temperatures. However, warming to 31 °C decreased net primary productivity by dinoflagellate symbionts by 60 % due to increased respiration. Furthermore, the coral host received 15 % less carbon and substantially less nitrogen at the higher temperature of 31 °C. While the symbiont was "happy" with higher temperatures, it cost the coral host substantially. This work has major implications for the resilience of coral reefs under threat from global change.

Baker and Freeman also targeted rouge lionfish (*Pterois volitans)*, an invasive species that escaped from aquaria over the past 25 years. Lionfish are native to the Indo-China region where they are top carnivores. In the Caribbean, they are decimating native fish populations, thereby altering the trophic structure of coral reefs. As Dave and Chris collected their coral samples, they speared lionfish if they could, collecting over 100 fish. Lionfish have very poisonous spines, which we carefully removed before preparing them to eat. The carbon and nitrogen isotopic compositions of lionfish showed them to be major predators, even potentially cannibalistic, on the reefs.

15. GEOECOLOGY AT THE CARNEGIE AND THE RETURN TO HYDROGEN ISOTOPES

15.1 Geoecology of Wombats

In 2006, postdoc Seth Newsome was hired at the Geophysical Laboratory to work on the Australia project, in particular to set up the Gas Bench for carbonate analyses as well as to study the geoecology of the Southern Hairy Nosed Wombat (*Lasiorhinus latifrons*). In a recent paper (Miller *et al.*, 2005), we had compared the $\delta^{13}C$ values of fossil emu and *Genyornis* eggshell with those of the wombat, an herbivorous grazer. The Southern Hairy Nosed Wombat is still extant in South Australia, so it seemed logical to study this modern organism in order to interpret a sizable collection of fossil specimens. I contacted Rod Wells at the University of Adelaide, who is the world expert on the Southern Hairy Nosed Wombat, and set up a field trip to his wombat preserve. My husband and son, Evan Swarth, joined the trip and provided valuable assistance.

Wombats live in burrows they excavate into the red, hard-pan soil. They aren't strictly colonial, but you often find their burrows within 100 metres of one another. I'd read that wombats were slow – travelling at a top speed of 100 metres *per* hour – and they foraged during the early morning and late afternoon. I also learned that if a predator (*e.g.*, dingo) tried to enter their burrow, the wombat wedged its substantial rump in the burrow essentially blocking access. One evening, we went "wombat hunting" to observe them foraging on a local ranching station. Several wombats were grazing far from their visible burrows. Seth and my son Evan wanted to see how close they could get to them, so they started slowly walking then sprinting towards them. Within seconds, the wombats raised their heads, saw the impending danger, and ran towards for their burrows. Quickly the pace increased until the wombats were running at full tilt, easily outrunning the humans. As they neared their burrows, the wombats took a dive and disappeared neatly underground. Definitely faster than 100 m/hour!

We were able to collect many wombat skulls, teeth, and bones because of their interesting, fastidious, and unique behaviour. When a wombat is nearing death, it goes into its burrow to die. Later its younger relatives push the deceased animal just outside of the burrow leaving the skeleton conveniently available for sampling. We collected hair, teeth, skeletons, faeces, and dietary plants from their range south of Adelaide all the way across the Nullabar Plain.

Wombats have continuously growing teeth, so the teeth can be subsampled and analysed to determine the variation in diet and drinking water across several seasons. We were interested in using $\delta^{13}C$ and $\delta^{18}O$ values in their teeth to examine seasonality in this region of Australia. Fossil wombat teeth collected in the Murray-Darling basin showed distinct seasonal trends providing us with a record of how influential the Intertropical Convergence

Zone has been in fueling the summer monsoon in this region over time. Unfortunately, we were unable to date accurately our fossil wombat teeth using radiocarbon methods, because apatite is contaminated during processing with modern carbon. When teeth are compared with eggshell material from the same location, we could use $\delta^{13}C$ as a record of whether grasses were C_3 or C_4, a parameter than remains important in interpreting the $\delta^{13}C$ of eggshells and inferring the strength of the summer monsoon, and by extension ecosystem structure (Fig. 15.1). Analyses of wombat teeth from specimens older than the megafaunal extinction >45,000 years ago had 60 ± 5 % C_4 plants in their diet. After the major extinction event, carbon isotope results from teeth showed only a 18 ± 2 % contribution from C_4 plants, similar to the shifts we measured in the $\delta^{13}C$ of emu eggshells.

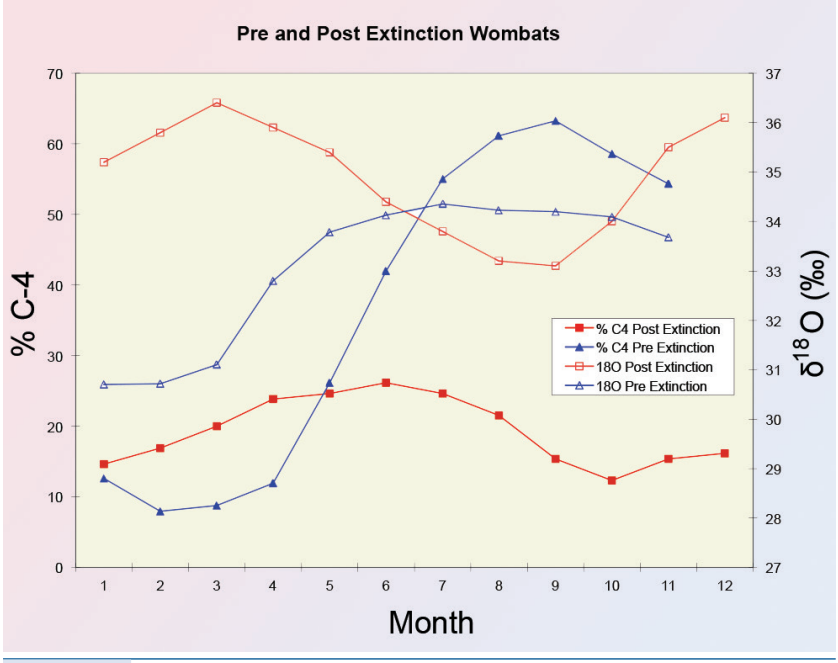

Figure 15.1 Wombat tooth carbon and oxygen isotope compositions. Wombats have continuously growing teeth which record their diet and water sources throughout the year. The seasonal swings in isotopic composition demonstrate these changes. Prior to the megafauna extinction at 55 kA, between 45-65 % of the wombat's diet originated from C_4 plants, however afterwards only 10-20 % of the diet was C_4 plant material. Oxygen isotopes show that seasonality was greater prior to extinction. Unpublished data of M. Fogel and S. Newsome.

Newsome also continued to work with colleagues at UC Santa Cruz on sea otters and their diets. Because the stable isotope lab at the Carnegie was "open" to all good ideas, Seth analysed thousands of samples from all over the world in his quest to spread the utility of isotope biogeochemistry into studies of ecosystems. This led to collaborations with scientists from Argentina, California, Wyoming, the Smithsonian, and Canada (Newsome *et al.*, 2009b, 2010a,b,c). Of particular interest, now that the TC/EA (thermo-chemolysis elemental analyser) was operating routinely, was a new collaboration with Gary Graves, Curator of Birds, at the Natural History Museum of the Smithsonian.

Seth and I had read papers by Hobson and Wassenaar (1997) and Rubenstein *et al.* (2002) and had become intrigued by their results on bird migration. Graves had collected (*i.e.* killed and stuffed) thousands of Black-throated Blue warblers in a decade long study of their breeding habits and ecology. Our collaboration with Graves has lasted over 10 years. We began with a series of feathers collected from warblers that were sampled along a latitudinal gradient from northern Georgia into southern Canada. Collaborating with Matthew Betts (Oregon State University), we analysed hundreds of δ^2H values from 22 sites ranging from 34 to 46 ^oN latitude. There was a clear influence of latitude on the δ^2H of feather keratin with more positive values measured in the south and more negative values from the north (Fig. 15.2). The samples included adult birds and juveniles in their second year of life. Black-throated Blue warblers migrate each fall from the continental United States and southern Canada to tropical islands in the Caribbean, where they spend the winter. They fly north again in spring to breed and molt their feathers. Year after year, biologists who band Black-throated Blue warblers found that adult birds returned to the same breeding grounds, often to within 100 metres of where they had nested the previous year. Our initial research question was the following: did Black-throated Blue warbler juveniles return to where they had been born or did they strike out for new territory? We found that, with very few exceptions, juvenile Black-throated Blue warblers returned to the same general region where they were born. This work prompted further studies with δ^2H.

Graves had colleagues at Chicago's Field Museum with freezers full of Great Gray Owls, one of the largest birds in North America. For unknown reasons, these birds leave their territories in Canada and Alaska and invade the lower 48 states periodically, probably in response to a lack of prey. Once there, they still face dangers. During the winter of 2004-2005, 265 owls were killed in Minnesota alone by vehicular collisions. We sampled tissues from this unprecedented collection of avian specimens and published our work on $\delta^{13}C$ and $\delta^{15}N$ values of muscle, liver, and feathers (Graves *et al.*, 2012). Muscle tissue $\delta^{13}C$ ranged from -27 to -20 ‰ with the more positive values from birds that showed greater nutritional stress and a poor body condition. The $\delta^{15}N$ of muscle was quite variable and ranged from 3 to 9 ‰, essentially spanning three trophic levels, but were not related to nutritional stress or body condition.

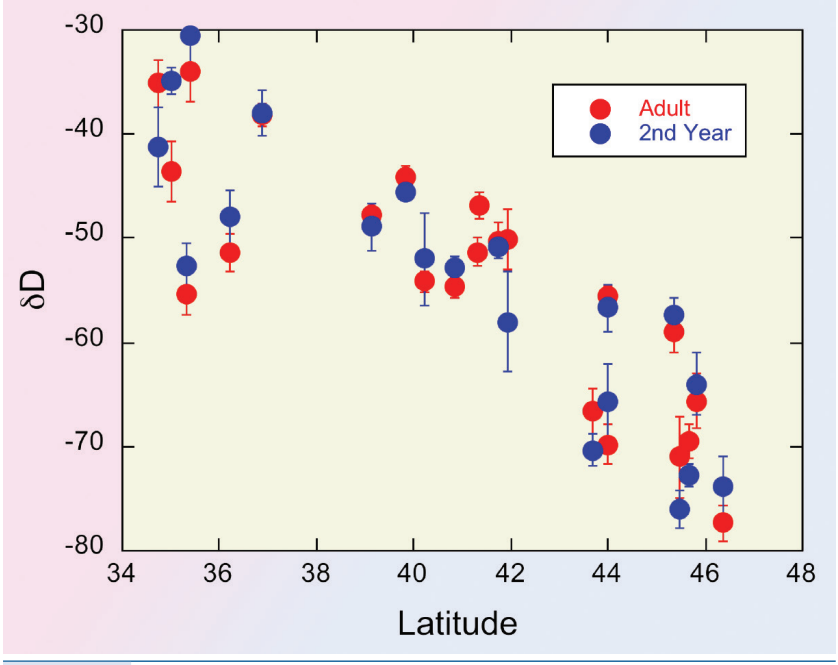

Figure 15.2 Hydrogen isotope ratios of Black-throated Blue Warblers varies as a function of latitude as the base of the food web is influenced by the isotopic composition of precipitation which is taken by plants. The data shows that from low to high latitudes, one year old (2nd year) birds returned to their natal homes throughout their entire range. Unpublished data of Graves, Betts, Fogel, and Newsome.

The carbon and nitrogen data were interesting, but the δ^2H data on feathers revealed much more. The δ^2H of animal tissue is determined from the hydrogen coming from an animal's diet (*i.e.* organically bonded H) as well as drinking water (Estep and Dabrowski, 1980; Hobson *et al.,* 1999; Newsome *et al.,* 2017). Drinking water δ^2H is generally considered to be similar to that of precipitation, and the δ^2H of precipitation is dependent on latitude, altitude, and distance from the ocean. δ^2H in plants is also primarily influenced by precipitation. Herbivores show an enrichment of 20-30 ‰ relative to plants. The δ^2H in diet is a combination of lipids (isotopically more negative), carbohydrates (isotopically more positive), and proteins (variable in δ^2H). Carnivorous birds, two to three trophic steps above plants, have much more positive δ^2H values in their feathers than do herbivorous birds. Great Gray Owls eat small mammals, placing them at least two and perhaps three trophic levels, above plants. To estimate the δ^2H of plants and precipitation, we can subtract 30 ‰ for each trophic shift. A Great Gray Owl feather with a δ^2H of -50 ‰ would

have consumed prey with a δ^2H of -80 ‰, which would have eaten a plant with a δ^2H of -11 0‰. Plant δ^2H values are about -40 ‰ more negative than precipitation, therefore, the δ^2H of precipitation in the region where the owl lived and grew its feather should be about -70 ‰. Obviously, if the owl were preying on omnivorous animals (*e.g*, voles), the δ^2H of estimated precipitation would be slightly more negative: δ^2H of -85 ‰.

Great Gray Owls invaded northern tier of states south of Canada in late October, were found on roadsides, and were collected throughout the winter and spring until mid-May. We questioned whether owls arriving earlier were from nearby regions of Canada *vs*. those coming from colder, more mountainous western regions. We measured the greatest range in δ^2H (-20 to -120 ‰) (Fig. 15.3) from owls collected in February. Estimating *via* the method above that in February owls arrived from parts of Canada with precipitation δ^2H varying from -40 to -155 ‰. This range in precipitation δ^2H values corresponds to a geographical range from maritime Canada in the east to the Canadian Rocky Mountains and Northwest Territories to the west. Only 5 owls had δ^2H less than -100 ‰, but the bulk of the δ^2H owls varied by 80 ‰ which meant that the origin of the owls was very widespread.

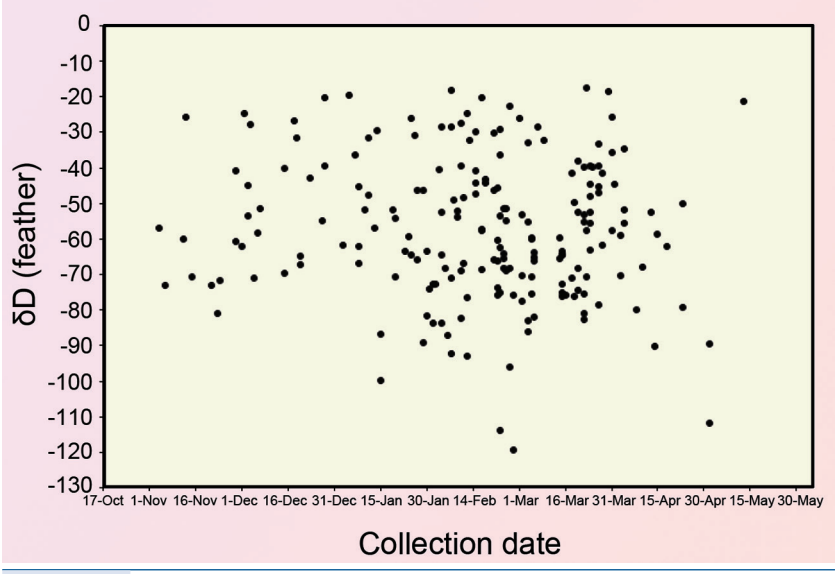

Figure 15.3 Hydrogen isotope ratios of Great Grey Owl feathers as a function of the date in which they were found and collected in Minnesota. Unpublished data of Graves, Fogel, and Newsome.

15.3 δ^2H in Amino Acids

In 2008, I received a grant from the W. M. Keck Foundation that provided funding for a new GC-C-IRMS system and the ability to measure the δ^2H of individual compounds. All of the papers published with δ^2H in compounds were on lipid biomarkers or hydrocarbons. I wanted to try my hand at measuring δ^2H in amino acids.

Although hydrogen is one of the major elements in living organisms, it is not fully understood how organisms incorporate hydrogen from their surroundings into the biomolecular compounds that comprise them. Living systems derive their hydrogen from two primary sources – food and water – and a full understanding of how hydrogen moves through an organism or an ecosystem must consider studies of both sources. To determine how hydrogen from water is incorporated into living matter, post-baccalaureate student, Patrick Griffin, cultured *E. coli* in nutrient solutions composed of waters of differing isotopic compositions. To determine the influence of the dietary source on hydrogen, experiments were conducted with a glucose-based medium, as well as a medium based on the protein digest tryptone. Together with Patrick and Seth Newsome, our experiments showed that roughly 25-35 % of the hydrogen in *E. coli* biomass reflects the composition of the water in which it is grown, and the remainder transfers directly from the diet to cellular biomass (Fogel *et al.*, 2016). More hydrogen originates from media water in *E. coli* grown on the glucose-based medium than from organisms grown on tryptone. We found that hydrogen isotope compositions in the single protein, tryptone, varied by up to 250 ‰, encompassing nearly the entire natural range of hydrogen isotopic compositions of living organisms on Earth. Amino acids extracted from these *E. coli* cultures showed even more extremes with some amino acids having identical isotopic compositions to those in tryptone and others being enriched or depleted in deuterium.

Our results from these simple experiments with *E. coli* grown on tryptone provided the basis for examining complex organisms, such as birds, mice, and fish. Tryptone is essentially food for the microbes, and our data showed that the more complex branch chained amino acids (isoleucine, valine, and leucine) could be incorporated, or routed, directly into cellular protein. Less than 10 % of the hydrogen in these amino acids was sourced from water. Models suggested that ~40-50 % of the hydrogen in alanine was sourced from media water. Thus, by analysing δ^2H values in a suite of amino acids, we had discovered a possible way to estimate the δ^2H of an animal's diet as well as the δ^2H of its drinking water.

The next experiments entailed growing *E. coli* solely on glucose as a food source. The microbes would need to synthesise all of their amino acids *de novo*. We found that 36-75 % of the hydrogen in individual amino acids came from water, and the remaining 25-64 % originated from hydrogen in glucose. Not only does the hydrogen in proteins rout directly into organic tissue, but

some of the hydrogen in carbohydrates does as well (Fig. 15.4). For organisms consuming complex diets containing a mix of macromolecules, the hydrogen in all of the dietary macromolecules seemed to be important in determining the δ^2H of bulk tissue.

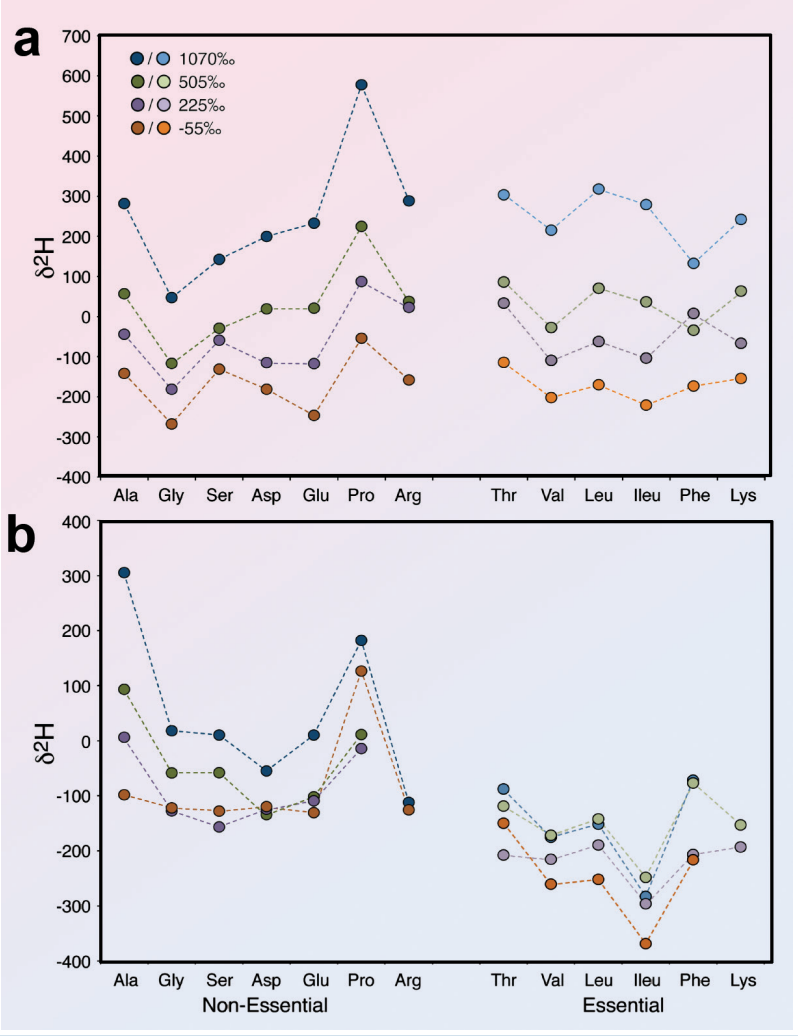

Figure 15.4 Hydrogen isotope ratios of individual amino acids from *E. coli* grown on glucose **(a)** or tryptone **(b)**. From Fogel *et al.* (2016).

We went on to measure the δ^2H of amino acids in bird feathers. Using some of the Black-throated Blue warbler samples measured previously, we found that there were similar patterns of discrimination between the δ^2H of individual amino acids. Amino acid δ^2H values from feathers in warblers from North Carolina were 30-50 ‰ more positive than those amino acids in feathers from warblers collected in Ontario, Canada. For fun, we analysed some of the Great Gray Owls and were surprised to find that the δ^2H of proline had values as positive as +500 ‰. As we measured more and different organisms, we found an enrichment in deuterium within the proline molecule that mirrored the trophic position of the organism: the higher on the food web, the more enriched the deuterium content of proline. This finding holds importance for the ecological community in establishing new tools for determining complex food web structures.

Seth Newsome, then at the University of Wyoming, carried out a defined diet experiment in which tilapia fish (*Oreochromis niloticus*) were grown in tanks where the δ^2H of environmental water and the δ^2H of each of the major diet macromolecules of carbohydrates, proteins, and fats was known. We used cornmeal from various parts of the United States and ^2H-labeled glucose to further vary the δ^2H of dietary carbohydrates. For the protein (casein), the δ^2H was invariant, but the δ^2H of the water in the tank varied by about 100 ‰. We had nine experimental tanks: 3 different δ^2H diets, each tested with three different δ^2H waters. The δ^2H of the bulk tissues were analysed as well (Newsome *et al.*, 2017). About 25 % of the hydrogen in fish tissues originated from water. Of the remainder, 34-44 % came from dietary protein, 25-30 % from cornmeal and sucrose, and <1 % from lipids.

The δ^2H of the individual amino acids was more complicated. Interpreting the hydrogen isotope composition of individual amino acids required us to measure the carbon isotope composition of the amino acids as well. Since corn is a C_4 plant, and the casein was derived from C_3-based milk protein, we could use the $\delta^{13}C$ values to estimate roughly which proportion came from cornmeal and sucrose and which from casein. We separated the amino acids by metabolic "class": the glycolytic amino acids (alanine, serine, and glycine), the TCA cycle amino acids (glutamate, aspartate, and proline), and the essential amino acids (threonine, valine, leucine, isoleucine, phenylalanine, and lysine). We anticipated that the glycolytic amino acids would show the strongest relationship to the isotopic composition of the water in the tank. Based on carbon incorporation proportions we expected the TCA cycle and essential amino acids to be more influenced by the δ^2H of food rather than water, specifically the δ^2H composition of cornmeal and individual amino acids in dietary protein (casein).

The δ^2H of individual amino acids was not influenced significantly by the δ^2H of the bulk cornmeal ($r^2 < 0.2$), bulk diet ($r^2 < 0.18$), total carbohydrate ($r^2 < 0.19$), or glucose ($r^2 < 0.18$). The δ^2H of the tank water significantly influenced the δ^2H of only alanine ($r^2 < 0.64$), glycine ($r^2 < 0.73$), and serine ($r^2 < 0.89$)

for all of the nine experimental tanks. The remainder of the amino acids had little to no isotope relationship between the δ^2H of water and the δ^2H of the amino acid ($r^2 = 0.24$ for glutamate; $r^2 < 0.12$ for the rest).

These findings support for the most part the findings from bulk tissue, in which the δ^2H of cornmeal supplied 25-33 % of the hydrogen to tissues. A small disparity can be reconciled by comparing the δ^2H of individual amino acids from the cornmeal and the individual amino acids in the tilapia tissue, as was done for the compound specific carbon isotopes. Although we used one source of casein for the entire experiment, we needed to use three different sources of cornmeal to produce the range in dietary δ^2H values. We found that the δ^2H of amino acids in the different cornmeal samples had somewhat different values. Although some amino acids (*e.g.*, serine) in corn always had some of the most positive δ^2H values and others (isoleucine and valine) the most negative, the range in δ^2H within cornmeal can be as large as 350 ‰. Based on our carbon isotope data, it is evident that tilapia are able to use the amino acids from both casein and cornmeal proteins to build muscle tissue.

We linked the carbon isotope data with the hydrogen isotope data to understand and possibly tease out the source of hydrogen to all of the amino acids. Alanine, glycine, and serine are exceptions because the δ^2H of these amino acids is principally sourced from water. Carbon contributions are based on mass balance calculations using the $\delta^{13}C$ of the IAA in casein and cornmeal as end members and assuming no isotopic fractionation. For δ^2H, the assignment is based on whether or not the value of the particular amino acid is similar to or different from the δ^2H of casein and cornmeal amino acids. If a δ^2H value falls completely outside of the casein and corn IAA values, it is assigned a microbial origin.

What is striking about these results is the fact that very few of the amino acids in the tilapia came from only one dietary carbon or hydrogen source. Interestingly, the origin of essential amino acids was as variable as the non-essential amino acids. Lysine hydrogen in particular is noted as microbial in origin in most of the tilapia samples, however the source of carbon is quite variable. It is likely that even though we can trace the carbon in essential amino acids to a C_3 or C_4 source, unless the H isotope pattern agrees, microbial synthesis in the gut must have been a critical component supporting protein synthesis in the fish.

15.4 The Gut Microbiome

The results of this experiment and others have led to the start of a major research project on the influence of the microorganisms in an animal's gut to the isotopic composition of their proteinaceous tissues. The 40 year old paradigm that "you are what you eat" plus or minus a couple of per mille is seriously in question when carbon, nitrogen, and hydrogen isotopic compositions of animal tissues are examined at the molecular level. Starting with experiments on tilapia (Newsome *et al.*, 2011b), we found that fish growing on high levels of protein had greater

incorporation of amino acids directly into tissues based on $\delta^{13}C$ data. Fish grown on minimal amounts of protein had essential amino acid $\delta^{13}C$ values that were very different from the $\delta^{13}C$ in corresponding amino acids in their dietary protein. By inference we concluded that gut microbes must have provided amino acids that were assimilated by the intestine and used to build muscle tissue.

A more recent study on house mice (unpublished data Newsome, Feeser, Bradley, Wolf, Taykacs-Vesbach, and Fogel) fed varying proportions of carbohydrates *versus* proteins showed that substantial amounts of essential amino acids are derived from microbial synthesis, particularly when an animal is grown on a protein-limited diet. Most herbivores and even some omnivores consume diets with limited protein, and we predict that their tissues will have the greatest influence from the gut microbiome on their isotopic compositions. With funding from NSF, we are embarking on a series of controlled growth experiments with mice and will use isotopically-labeled amino acids. With a new triple quad mass spectrometer system coupled to our IRMS, our goal is to measure intramolecular isotopic compositions of the amino acids.

16. UNIVERSITY OF CALIFORNIA RESEARCH: SOILS, RIVERS AND VERNAL POOLS

The University of California prides itself on conducting research on an international scale. That said, when I arrived at UC Merced in 2013, the new, small campus had only two other ecologists and very few earth scientists. There were very few high-tech instruments on campus, even in physics and chemistry. While UC Merced lacked the accoutrements I was accustomed to at the Geophysical Laboratory, it was a very upbeat environment and was improving its infrastructure fast. I built my first isotope lab there in a building that struggled with environmental controls, but we managed to make things work. While pursuing my interests in compound specific isotope analyses of amino acids from various experiments and organisms, the majority of my colleagues were interested in various aspects of soil ecology and biogeochemistry. Adjacent to the campus is the Merced Vernal Pools and Grassland Reserve, a 65,000 acre preserve created in 2013 and available to UC students and faculty for study. My husband served as Director of the Reserve, so we took advantage of his access and its proximity to introduce the students and faculty on campus to stable isotope biogeochemistry.

Asmeret Berhe, Stephen Hart, and Teamrat Ghezzehei, faculty at UCM, were all interested in various aspects of soil isotope biogeochemistry. As the director of the isotope facility, I became involved in many of their studies. At first glance, the work did not fully capture my interest, particularly after studying exotic meteorites and amino acids from owls. California is almost exclusively a C_3-plant based ecosystem, so the $\delta^{13}C$ of soils typically ranges from -24 to -27 ‰. Berhe and her students developed methods for separating bulk soils into density fractions, which added to the quantitative assessment of how carbon, nitrogen, and even hydrogen is cycled in soils. With $\delta^{13}C$, % total organic carbon, % total nitrogen, as well as the mass balance of the density fractions, the research became more engaging (*e.g.*, McCorkle *et al.*, 2016; Abney *et al.*, 2017).

Postdoctoral researcher Elizabeth Williams pioneered work on hydrogen isotopes in soils based on the work by Ruppenthal *et al.* (2015). One of the major questions in soil ecology today is whether the organic matter in soil comes from root exudates, litter deposition from the surface, microbial biomass, or a combination of these three sources. Ruppenthal took advantage of the difference in the δ^2H of leaf water (more positive) relative to δ^2H in root tissue. He argued that if decomposed leaf litter were the source of soil organic matter, then its δ^2H would be more positive than if it came from root exudates. He developed a protocol for measuring the δ^2H of exchangeable and non-exchangeable hydrogen in soils. His conclusion was that soil organic matter in his study site, a grassland ecosystem, was derived primarily from root exudates.

Liz Williams set up a series of experiments using soils from an altitudinal gradient in the nearby Sierra Nevada mountains. Density fractionated soils were subjected to isotopic exchange with water, then quickly measured on the

TC/EA-IRMS system. She calculated that only a small portion (5-15 %) of the hydrogen in soils was exchangeable, probably because the majority was water that was tightly adsorbed to high density minerals. Even the low density plant fragments had little hydrogen in exchangeable positions. The δ^2H of bulk soil and the heavy, mineral fraction were related to the δ^2H of local precipitation. It remains to be seen whether the δ^2H of deeper soils will carry a palaeoenvironmental signal. Because of complexities in relating δ^2H of leaf waxes to environmental parameters, Williams' approach might provide an independent and more holistic assessment than the measurement of a single biomarker.

At UC Riverside, my lab group joined efforts with Seth Newsome's lab group at the University of New Mexico to measure the δ^2H of amino acids in primary producers. When Newsome and I started our research on δ^2H in amino acids, we were thinking about microbes, birds and animals, but not plants (*e.g.* Rodriguez Curras *et al.*, 2018). Knowledge about plants and hydrogen isotope fractionation was lacking. Newsome's graduate students, Alexi Besser and Emma Elliot-Smith, came to Riverside armed with collections of plants from terrestrial and marine habitats. Joined by my lab group, Bobby Nakamoto, Jon Nye, and Kaycee Morra, we've measured the δ^2H of amino acids from 50 plant samples to understand primary isotope fractionations during photosynthesis. In terrestrial and freshwater plants, for the amino acids that can be made by any organism, serine has the most negative δ^2H value, whereas in marine plants aspartate has the most negative δ^2H. In general, glycine, serine, aspartate, proline, and glutamate, are more closely involved in an organism's central metabolism. Variation in the δ^2H of these amino acids will be influenced by fluxes of energy in plants.

Those amino acids with more complicated biosynthetic pathways, threonine, valine, leucine, isoleucine, phenylalanine, and lysine, typically have more negative δ^2H values, probably owing to the additional enzymatic steps needed for their synthesis. The ultimate goal of this work is to determine whether there are isotope fingerprints in δ^2H of amino acids in primary producers, similar to those measured with carbon isotopes (Scott *et al.*, 2006; Larsen *et al.*, 2009, 2012).

17. PERSPECTIVES ON LABORATORIES, EQUIPMENT AND GEAR

At a recent AGU meeting (2016), I sat on a panel of isotope geochemists that included John Hayes, Ed Young (UCLA), John Eiler (Caltech), Max Coleman (JPL). We each took a couple minutes to tell the audience about what we thought was important for young isotope geoscientists to consider. Eiler gave a strong pitch for knowing exactly how the instrumentation works, essentially saying that if you don't understand the finer points of mass spectroscopy, you should find another line of research. John Hayes politely inserted himself and disagreed. "*Without a strong scientific question to drive you, it's not enough to get you up in the morning to do your work*." Both Eiler and Hayes have answered major scientific questions in their careers; both, technically competent. Clearly, to be an innovative biogeochemist you need to have intellectual as well as technical expertise. Having the best possible laboratory and equipment is key.

During my career, I have set up six stable isotope laboratories, essentially from the ground up. My first major lab renovation took place in 1982 at the Geophysical Lab when it was on 2801 Upton Street. The old, ceramic-tiled lab benches were ripped out by our maintenance crew. My next step was to draw up lab plans including plumbing, electrical, air handling, and cabinetry. I was pretty proud of my effort to design a new laboratory and presented my diagrams to the Lab's chief carpenter and plumber. He looked at them in disgust, flung them on the floor, and said he they weren't good enough. I was crushed. My eyes brimmed with tears. I went down the hall to talk to Hoering and told him what happened. "*Ah!*" he said, "*He doesn't know how to read. I'll show you what to do.*" He grabbed four different coloured markers, marched into my lab, and said, "*Draw the lines on the walls!*"

Swallowing my pride, I asked the carpenter to return to see if my plans were better this time. Lines on the walls worked. Renovations happened smoothly thereafter. I learned to never assume that people, including students, know or understand everything I do.

Essential to purchasing the best equipment (see Table 17.1) is establishing a rapport with service engineers. The Geophysical Lab had in-house electronics engineers, Chris Hadidiacos and David George, who designed computer systems for automating the Nuclide instruments and repaired boards and vacuum pumps. For our Nuclide instruments, we personally knew the individuals who had built our IRMS and called them if there were problems. With Finnigan-Thermo Fisher, we worked with a United States based group of engineers. Frank Trensch led the group in the 1990s. His phrase "*Isolate the variables*" serves as my mantra for troubleshooting. When a Thermo instrument was installed, an engineer worked in your lab for two to three weeks. Roger Husted installed our 252 IRMS in 1991 and our Delta Plus XL in 1999. A taciturn, southern man, Roger arrived in the morning with two cups of coffee, worked silently all day, then left abruptly when he thought the job was finished.

Other engineers were more sociable, chatting with students, going out for beers, and joining us at lunch. The personalities of engineers were well known, and it was important that you had a good rapport with the person who installed your instruments.

Over time, Roger Husted demonstrated his knowledge and patience, and he was responsible for keeping our 251, 252, 253, and two Delta instruments in decent shape by solving the inevitable problems. In 2012, our Delta Plus XL was dead in the water. A faulty 24 volt power supply knocked out some of electronics at an unprecedented scale. Derek Smith, Dave Baker, Roxane Bowden, and I tried for several months to figure out what happened. Finally, a clandestine effort by Thermo's IRMS salesman Chuck Douthitt and Roger Husted saved our instrument. Roger drove to DC, set out his schematics, and within four hours diagnosed our problems: the mother board, high voltage board, inlet control board, and communication board had all been ruined by the faulty power supply! Talented engineers like Roger Husted are key to keeping stable isotope research going. Learning to troubleshoot instruments, take them apart, and fix them takes time and hands-on experience measured in years, not just weeks or months. Because isotope instruments are increasingly automated, we sometimes forget how complicated they really are.

Purchasing the right IRMS for your research goals is key. At the Geophysical Laboratory, we were able to eventually find the resources from public and private organisations to keep our isotope lab fairly current. Early on we relied on American technology with the Nuclide Corporation, then switched to German technology with the Finnigan MAT-Thermo, when Nuclide slowly got out of the IRMS business. At Carnegie, we kept our instruments running for 14-20 years because we had the time to take care of them and were not required to accommodate large numbers of inexperienced users. On university campuses, professors need to rely on laboratory managers and technical experts because there isn't enough time in the day to teach, serve on committees, do research, write grants, and maintain a lab to a high standard. Fortunately, there are many scientists who enjoy the role as "fixers" and thrive in situations that require them to spend their days with instruments. Tom Hoering used to say with regards to fixing mass spectrometers, "*Show it who's boss*." Over the years, I developed decent skills as a fixer, but working with other talented people with technical skills is an enormous help.

Increasingly, many of the difficult analyses we did in the past that took days are now done automatically. Brian Fry, stable isotope ecologist, helped innovate the field tremendously by spending time developing on-line combustion methods for bulk carbon and nitrogen isotope measurements (Fry, 1992). Zachary Sharp innovated the oxygen and sulphur isotope geochemistry field with his implementation of laser fluorination (Sharp, 1990). Hayes, Freeman, Robert Dias, Jeanette Jumeau and others developed the early peripheral devices for compound specific analyses that transformed the biogeochemistry and organic geochemistry fields. Willi Brand (*e.g.*, Brand, 2014) and Andreas Hilkert

at Thermo were key engineers in Bremen, Germany, who understood the needs of the research community, saw that we had the equipment we needed, and innovated within their company (*e.g.*, Gas Bench, TC/EA, GC-Isolink). My career advanced in leaps and bounds when I was able to incorporate one of these scientists' inventions into my research.

Table 17.1 Isotope measuring instruments I used during my career 1974-2019 at University of Texas (graduate years), Carnegie Institution (1977-2012), University of California (2013-2019).

Instrument Type	Source	Years in Service	Peripheral devices	Notes
6" sector IRMS Hg inlet for CO_2, N_2	Nuclide	1966-1980	None	UTMSI Parker's lab
6" sector IRMS Hg inlet for CO_2, N_2	Geophysical Lab homemade	1960-1982	None	Magnet wound by A. O. Nier
3" sector IRMS Hg inlet for H_2	Nuclide	1970-1990	None	Vibrating reed electrometer for mass 3
6" sector IRMS metal inlet for CO_2, O_2	Nuclide	1982-1996	None	Manual inlet system computer controlled gas switching
Double focusing modified Dupont 491 IRMS for N_2	Nuclide	1985-1999	None	Hg inlet computer controlled gas switching
251 IRMS with four collectors for SF_6	Finnigan MAT	1985-2001	None	Push button inlet first purchased computer system
252 IRMS with collectors for triple oxygen, H_2, CO_2	Finnigan MAT	1991-2015	Carbonate device; Gas Bench; laser fluorination line; GC-IRMS	NSF funded; multiusers
Delta Plus XL IRMS	Thermo-Finnigan	1999-2019	EA, Gas Bench, TC/EA, GC-IRMS	Shared with Smithsonian Institution
253 IRMS for SF_6	Thermo-Finnigan	2002-2017	Laser fluorinaton line	^{32}, ^{33}, ^{34} and ^{36}S isotope capability

Instrument Type	Source	Years in Service	Peripheral devices	Notes
Delta V Plus IRMS including SO_2	Thermo Fisher	2007-2019	EA, CUBE	^{32}, ^{33}, ^{34} and ^{36}S isotope capability
Delta V plus IRMS	Thermo Fisher	2014-2019	GC-IRMS, TC/EA, EA	UC Merced
Delta V Plus including SO_2	Thermo Fisher	2014-2019	GC-IRMS, Gas Bench, CUBE	UC Merced
Delta V plus IRMS with triple quad mass spec	Thermo Fisher	2017-2019	GC-IRMS, TC/EA, CUBE	UC Riverside
CO_2 laser based for C isotopes	Los Gatos Research	2017-2019	Multichannels	Mobile Lab UC Riverside
N_2O laser based for N, O, N_2O, and site specific N	Los Gatos Research	2017-2019	^{17}O capability	Mobile Lab UC Riverside

18. NEW HORIZONS IN STABLE ISOTOPE BIOGEOCHEMISTRY

My laboratory at UC Riverside is now routinely analysing carbon, nitrogen, and hydrogen isotopes in amino acids. Our philosophy is that the power of the analysis and interpretation for understanding ecosystems and ecological relationships increases exponentially as another isotope is added. Carbon isotopes provide estimates of the source(s) of primary productivity to an organism; nitrogen isotopes in amino acids can determine trophic position; hydrogen isotopes can give clues to location and diet. Ph.D. student Jon Nye is using this approach for studying the ecology and diet shifts in pinnipeds (*i.e.* seals and sea lions) from Patagonia, South America. Bobby Nakamoto, an ecology Ph.D. student, is pioneering statistical methods to determine complex patterns in particulate organic matter in riverine systems. Postdoc Kaycee Morra and Nakamoto will try out the first measurements with the new TSQ-IRMS system, which will open up a whole new field of organic isotopologues.

Katherine Freeman and others have developed the nano-elemental analyser, which will allow the analysis of ever smaller samples, down to individual microbes or cells (Polissar *et al.*, 2009). Although there were other methods (*e.g.*, the moving wire method) developed earlier, the new nano-EA relies on continuous flow methods. Freeman and Polissar are currently perfecting the pico-GC-combustion-IRMS. When samples are eventually returned from Mars, the nano-EA and the pico-GC-C-IRMS will be the instruments of choice.

Laser-based mobile instruments measuring the concentrations and isotopic compositions of greenhouse gases will make inroads into studies of climate change. At UCR, we recently outfitted a mobile Mercedes Sprinter van designed to carry four of these instruments so it can be driven on freeways, to farm fields, industrial sites, or pristine national parks around the west. An immense amount of data will be collected *via* these methods. As time progresses, how we handle large amounts of isotope data remains to be seen.

Isobank, a data storage mechanism for all isotope data, particularly for ecological studies, was started at the University of New Mexico by Seth Newsome and colleagues (Pauli *et al.*, 2015, 2017). Without question, I will never be able to publish individual manuscripts from all the isotope data I've generated in my lifetime. This year alone with just two grad students, one postdoc, and a lab manager, we've produced about 2,000 isotopic measurements of various materials. Since I started using the EA (1999) and compound specific (1991) instruments, my lab's probably produced 3,000 measurements *per* year. In 20 years that equates to 60,000 accurate and precise isotopic ratios! Hopefully, with an open access policy, others without open access to all the instrumentation that I've had might be able to access that data. Newsome writes:

> *"IsoBank will enable a diverse and rapidly growing scientific community to harness the advantages of big data analytics. At the same time, it will support and increase the efficiency of a wide range of existing isotope-based applications, which require reference data to support data interpretation. We envision that IsoBank will foster interactions across disciplines that speak a common chemical language and the fusion of diverse perspectives, a process that has resulted in some of the biggest and most creative advances in science. We anticipate that our project will catalyze the overdue conversation among disciplines regarding the standards that constitute sufficient isotope metadata, which is likely the reason why a centralized isotope database does not currently exist. IsoBank will enhance the standards for data quality assurance and control by creating a network among core isotope laboratories in the U.S. that are currently producing nearly a million new datapoints per year."*

Finally, large format mass spectrometers like Panorama at UCLA (Young *et al.*, 2016) and the Thermo version at Caltech (*e.g.*, Douglas *et al.*, 2017) are turning out isotopologue measurements on methane, nitrogen gas, and soon carbon monoxide that are allowing scientists to further understand mechanisms and processes in biogeochemistry that could never be determined with "bulk" compound specific analyses. These instruments are proliferating in the United States, China, and Europe.

19. SUMMARY

My 50 year experience in biogeochemistry has taken me down a road that was often, rather than not, less well travelled than if I had chosen a traditional career as a biologist. The Carnegie Institution of Washington provided me with complete freedom to follow any topic that I found interesting. With freedom, came competence because I learned from my mistakes throughout my career. Competence coupled with curiosity and a tenacious nature were required to finish and publish most projects and write grants for competitive funding agencies. Qualities that helped me become a successful scientist also include resilience, honesty, doggedness, a sense of humour, humility, and intuition.

I particularly enjoyed working with bright young people on complicated topics delving into new areas of research that I knew little about before starting. As I embarked on the learning experience, the postdoc or student and I often developed a true mentoring relationship. Teaching them how the equipment worked and training them to someday manage their own labs was a challenge that I relished. Watching their careers evolve at that time, and after they left my lab, remains exceptionally rewarding.

I believe I did a credible job at teaching the young people working with me that creative innovative science is fun; that it is about advancing the field and not finding fame or fortune; and that the people, friendships, and relationships that are built along the way are equally as important as the research being pursued. While the path might not have always been smooth, it was a lot of fun. If I had one piece of advice to give to younger scientists ready to embark on this career, it would be to find people you like to work with. I've worked with some of the smartest young earth scientists in the field, but there's a greater depth to each of them that made science special for me. If you share a love of science and a deep curiosity of how things function, you can't go wrong.

20. CLOSING THOUGHTS

20.1 Women in Geoscience

The Geoscience workforce was composed of only 21 % women as of 2015, a proportion that lags behind other science and math fields (Holmes *et al.*, 2015). Studies have shown that this is not a "pipeline" issue, as there are enough women interested in the field who have started their careers in earth science but don't continue. Overwhelmingly, those studying the reason for low participation in geoscience feel that it is a "chilly" climate that drives women from sticking with a life of studying Earth and planetary sciences, thus creating a "leaky pipeline". Below, I write about my personal perspective during almost 50 years of my career.

> Settles *et al.* (2006)…. "*Women scientists who perceived the department climate to be sexist reported lower levels of felt influence and job satisfaction...In contrast, women's perceptions of a positive or supportive department climate were related to higher levels of job satisfaction and productivity. A positive academic climate, as measured here, is one in which there is more collaboration and cooperation, respect, and collegiality. Factors such as collaboration are thought to be critically important for increasing positive work outcomes for women scientists.*"

20.2 Early Years as a Geoscientist

When I first started my career as a young undergraduate at Penn State, we all took a test that would help us determine what we might be interested in and suited for in terms of a career. Recently, I unearthed the old results of that test taken nearly fifty years ago in 1970. Options for careers for women at that time – college educated women – included nun, secretary, and sewing machine operator, among other women-only type careers. My results indicated a suitability for Chemist, Entertainer, and Naval Officer. One of them was spot on; the other two describe me in some sense. As a leader of a scientific laboratory for 40 years, you've got to have some "military officer" personality to succeed. As a college professor for the past 6 years, I've also had to be an Entertainer on occasion.

In 1970, there were quotas for how many men could be accepted to universities and colleges *vs.* how many women. Most of the "top" universities were just starting to accept female undergrads. 1969 was the watershed year for high ranking places like Yale and Princeton to decide to accept women for the first time. At Penn State, the ratio of men to women was 3:1; at Cornell University where I also applied, the ratio was 8:1. A male student from my high school with nearly identical test scores and grades was accepted to Cornell, but my woman friend and I were not. Luckily, I was accepted at Penn State, but owing to the fact that I was from out of state (New Jersey), I needed to start

classes in the summer. At that time, we sensed the discrimination based on quotas was unfair, but no one did anything about that. In my science classes, there were about 20-30 % women in biology, fewer in chemistry, geology, and physics classes. As a youngster, having more young men around than young women was fine with me.

In graduate school, I paid no attention to the relative proportions of women to men. I was engaged in my studies and ignored the fact that all of the faculty were men, and most of the fellow graduate students were men. As long as there were undergraduates around, it never seemed like an overwhelming number of male cohorts.

Not until I arrived at the Geophysical Laboratory, a rarefied environment on a hilltop in Northwest Washington, DC, was I aware of how rare I was at that time.

There were no other women postdoctoral fellows or staff scientists in 1977-1979. Every summer, a few women from other universities would arrive and work as interns. The Director of the Lab addressed memos to Dr. Hoering, Dr. Hare, and Mrs. Estep (my married name at the time). The only other woman scientist, on staff in the 1960s briefly, was referred to as Mrs. Donnay, who was married to Dr. Donnay, both of them crystallographers. Of course, "Mrs." Donnay was in her own right Dr. Gabriel Donnay. A senior woman professor from France, who visited frequently and had been a postdoc in the 1960s, was called Mrs. Velde, since she married another postdoc, Dr. Velde. "Mrs." Dr. Professor Danielle Velde was by far the more prominent scientist, with a long productive career as a petrologist at the University of Paris.

At this time, visiting speakers at the Geophysical Lab during the 1970s and 80s, were used to addressing a male audience. I was often the only woman attending the seminar. Male speakers took full advantage of the sex-biased audience. One prominent male professor from a California university punctuated the sections of his seminar with photos of naked women. Thinking back to the 1990s, one of my colleagues tells this story:

> *"I was recently talking with a couple colleagues about how there were postcards of women in skimpy bathing suits taped up on a wall in the laboratory where I did my graduate work. Now, this was a laboratory of someone who has high respect for all the women in his life – however, it did not dawn on him to take these postcards down until a comment by a new woman postdoc prompted him to. I suspect this being his first woman postdoc and his noting her attitude may have jarred his understanding from simply a joke amongst male colleagues and students to the reality of what it meant to all the rest of his colleagues and students."*

Off-colour jokes and swearing were not common at the Carnegie, because in general I worked with gentlemen. However, in attending conferences and interacting with outside scientists, it was clear that women in the earth sciences were not taken seriously. When I started to look for a full time position as an Assistant Professor in 1979, I am fairly certain that my applications were not reviewed as fairly as those of my male colleagues. For example, two men in the

stable isotope biogeochemistry field, all of us recent Ph.D.s, were interviewed at the Geophysical Lab, before I was even considered to be a viable candidate for a permanent position.

20.3 Mentors, Friends, and Partners

> Suzanne O'Connell (2015). *"Success in the academy is a combination of many factors. Intelligence and hard work are essential but not sufficient by themselves. Help from mentors and advisors in learning how to navigate the complex corridors of the academy is also fundamental; it is unlikely that someone will master this process unaided. Unfortunately for the outsider, multiple studies have shown that workers in any field tend to mentor and advocate for people who are similar to themselves [e.g., Chesler and Chesler, 2002; McGuoire, 2002]. To break this pattern, mentors and mentees, students and faculty, insiders and outsiders, chairs and administrators need to examine the importance of passing information between groups and make sure this transmission occurs."*

Our personal partnerships and support mechanisms have been and will continue to be important to the success of under-represented groups in science. One of the office staff at the Geophysical Lab, Marjorie Imlay, and I often discussed how women received the short end of the stick. She counselled me wisely, "*By your work, shall ye' be known.*" Throughout, I stuck to scholarly research, published, and "kept my nose clean" for the first ten years of my career. While in graduate school, I married a local Texas guy who was not a scientist. At the time I was 21 and life was fun. By the time I was awarded my Ph.D. at the age of 24, I felt the impact of having a husband who did not understand the rigours of an academic life. At the Geophysical Lab, the marriage deteriorated as I became more successful. It ended in divorce.

I had to learn the hard way that women in science need a sympathetic partner to succeed. The long work hours, the travel, and the fixation on seemingly small "problems" are things that academics are used to, but most others are not. It was a painful period for me, and I then awoke to the fact that women in science in the 1970s and 80s were either married to another scientist, and often subsumed by him, or unmarried. I was determined to be neither going forward. Today, 83 % of academic women scientists are married to an academic, science partner (Schiebinger *et al.*, 2008). The support and respect of our partners is key. Men in science have always had respect from their families. Women need that as well, but sadly I suspect some women don't get this.

Fortunately for me, I went on sabbatical starting in 1985 and met my current husband on a whale watching trip. A year and a half later, we were married, but this time I did not change my last name. Fogel is my family's name, and I am proud to be thought of as a Fogel. Two years later, my daughter Dana was born, followed in three years by my son Evan. Both my husband and I worked at full time positions. He is an ecologist who was the director of a wetlands sanctuary. We loved our jobs, and our kids were familiar with both

the places where and people with whom we worked. By this time, the pendulum was starting to swing in academia to recognise that women could be both productive scientists and mothers. Many of my women science colleagues were young mothers. We supported each other as much as possible.

In the past 25 years, I have made a point to mentor early career women as opportunities arise. I participate in programmes with the Association for Women Geoscientists and the Geochemical Society. At the Carnegie and the University of California, I have been particularly outspoken about women's rights as scientists and have spent many hours listening to and advising early career women. For most mentees, having a sympathetic ear to listen to their problems was enough. Other times, however, I needed to speak directly to Directors and Deans about abuses, in particular over sexual harassment and gender discrimination issues. These were not pleasant conversations and were outside of my scientific expertise, but in the few places where I have worked, it is essential that a woman in the profession provide guidance and advice.

20.4 Implicit Attitudes

> Mary Ann Holmes (2015). *"Our implicit attitudes influence how we evaluate people for jobs, for admission to graduate school, and for awarding fellowships, scholarships, and professional awards without our being aware of them. Implicit associations that form implicit attitudes develop by repeated contact with a given phenomenon. For example, when every nurse we've ever seen is a woman, we mentally picture a woman whenever we hear the word "nurse." Similarly, most scientists portrayed in the media are men, so most people think of a man whenever they hear the word 'scientist'."*

It was in the mid-1980s, 1985 I think, when the Geophysical Lab purchased a large format, orange-coloured 251 IRMS to measure SF_6 for all four stable sulphur isotopes. Back in those days, Finnigan made custom instruments for its users, similar to the now defunct Nuclide Corporation (State College, PA). Our other IRMS systems at that time were Nuclides and a home-made IRMS with a Nier-wound magnet. The 251 came with a first version of Isodat, which was run on one of those little Apple computers that many have kept in their basements as antiques. The valves were operated by rectangular, green-lighted push buttons. The dual inlet system and vacuum system were controlled, then, not by software, but with these buttons.

The instrument had been installed by German and American engineers in Tom Hoering's old Geophysical Lab laboratory. In those days the acceptance of a new instrument brought in Finnigan "brass"—vice presidents, Chuck Douthitt's bosses—essentially men in suits who stood at attention when the instrument was being demonstrated.

Staff scientist Tom Hoering, a modest man, was the anointed person to handle the analysis of the first sample. Doug Rumble and I, mere children in those days, stood at attention behind Tom, looking on as required by strict

German protocol. It didn't take but a few minutes for Tom to push the wrong button and vent the IRMS. Imagine the slowing whine of the turbo pumps and the disappointed looks on the Finnigan faces.

As the instrument was being restarted, the Isodat on the little Apple needed rebooting as well. While the machine was pumping down, the engineer was demonstrating the great features of Isodat. [Isodat has vastly improved since 1985, but still remains something most of us have a love-hate relationship with.] As we watched, I commented to the engineer that the software didn't look very useful or straightforward to use.

He spun around with disdain and remarked, "*This is not a mass spectrometer for a housewife!*" The room was silent for many awkward seconds. The American Finnigan team looked at their shoes; Tom and Doug frowned; and I stood flabbergasted without a quick rejoinder. I wonder what I would say today in these circumstances. I am sure I would have a rejoinder now.

Somehow, I continued to be a customer of Finnigan—now Thermo. Today's Isodat and auto samplers can handle any and all types of analyses, while the busy housewife can go home at 5 pm, cook for her family, bake bread, clean the house, and tuck her children into bed at night—all while the IRMS precisely and accurately measures isotopes in the 5th decimal place. Thermo can now proudly say, "*We make mass spectrometers for housewives!*"

20.5 How do We Dress for Success?

At the Geophysical Lab, the male scientists have a "uniform" of sorts or dress code. During the work week, old khaki pants or jeans with an L.L. Bean shirt typically weathered by 15 years or more of wear with a pair of old sport shoes are *de rigeur*. For more formal occasions, many kept a tie and a navy blue sport coat hung on the back of their office door that could be put on over the old khakis, if need be. In general, I followed the L.L. Bean code of dress for day-to-day work, but women don't hang formal clothes behind their doors when they want to dress up and look good.

In fact, women in science have puzzled about how they should look and present themselves in job interviews, during seminars, and while teaching. For young men going out in the world, I gave them simple advice: comb your hair, wear a belt that matches your shoes, and pick a pair of decent socks. (Men often cross their legs and pull up their pant legs while discussing things. Their socks should look good.) For women, they need to be concerned with skirts *vs*. pants, purses *vs*. brief cases, blouses *vs.* shirts, jackets *vs*. sweaters, colour combinations, hair styles, makeup, heels *vs*. flats, jewelry and other accessories, necklines and hemlines. As a woman if you're dressed too informally and are young, people think you're a student. If you're too stylish, you might be considered to be a member of the administrative staff. God forbid that a woman scientist should be considered sexy and bring out unwanted advances.

Early in my career, my mother sewed all of my clothes for meetings and seminars. We chose patterns and fabrics that were different but stylish and classic. In a world where clothes are ordered on-line, this seems impossibly old fashioned. During the past 20 years when I earned a decent salary, I shopped at two or three small women's clothing stores and had the sales force provide advice.

One of my colleagues who took a position in the 1980s at a large Midwestern university told this story:

> *"After receiving poor teaching scores in my first semester, my sister [a University professor] gave me a list of do's and don'ts including how to dress. I started wearing suits and high heels – spikes even. My scores skyrocketed. Can you imagine what it sounded like as I walked down the hall to my office, passing some of my most difficult colleagues. I never thought about it at that time, but those high heels made a statement in more ways than one and not just to the students. My sister was right!"*

Fortunately these days, working women have many choices for looking professional, although often women's evaluations often remain low relative to men regardless of dress.

20.6 The Wage Gap

> Cheng *et al*. (2018). *"People often justify the gender wage gap by suggesting that "Women are not doing the same amount of work" "they are opting out" or "they are working fewer hours." These justifications put the responsibility and blame on women themselves, preventing us from identifying and addressing the real root of the problem: not women's actions or inactions but systemic inequity within organizations and society. Gender discrimination occurs both subtly and overtly throughout hiring, placement, and promotion processes."*

I am proud to show my first letter of appointment as a Staff Member of the Geophysical Laboratory to the community. In 1979, I asked the Director of the Lab for a salary of $20,000 and was given $19,500 to start. No one told me that I should have bargained for more, at least $19,750. For the following 18 years, I never asked for a raise. Some years, the Director of the Lab gave a generous 5 %, but in other years, all we got was a paltry 2 % raise. Unbeknownst to me, my male colleagues regularly went into the Director's office and demanded a raise. Half the time, I think that tactic worked. By the mid-1990s, my former postdocs now at their own universities were earning a good 20 % more per year than I was.

At lunch one day, a table of "us women", including National Medal of Science superstar Vera Rubin, discussed salaries. Vera and I had never brought the subject up with our bosses and had no idea what our male counterparts at the Carnegie were paid. But both of us told the postdocs who were negotiating for positions in the 2000s to ask for and expect to receive a fair salary. It turns out that "*Women Don't Ask*", the title of a popular book on salary negotiations

(Babcock and Laschever, 2003). Prior to my accepting positions at the University of California, I read the book, asked for five salary considerations, and was given three of them.

As I transition into retirement, I can see the obvious value of getting paid what you're worth. Childcare costs are through the roof. The cost of sending children through college is increasing, even in the state of California, which for years had some of the lowest costs in the United States. Money to help out with domestic chores is also something that women need as the burden of housework often falls to them. Having enough resources to hire a cleaning service or purchase meals is often the difference between a happy balanced geoscientist and an overwhelmed one. Women need to pay careful attention to their retirement funds, since most institutions don't provide pensions. Fortunately, this generation of women geoscientists seems to be savvy with respect to wages.

20.7 Career Advancement

> Horner-Devine *et al.* (2016). *"Community and empowerment are necessary for individuals to leverage their scientific training, skills, and expertise into successful and impactful careers. To advance diversity in STEM, we must change the ways we support women scientists and scientists from other underrepresented groups as they embark on their careers, and we must develop strategies that create a sense of community and empowerment...By creating opportunities to foster community building and empowering all our early-career scientists, especially women and underrepresented minorities, we can foster a scientific culture in which all our scientists can thrive."*

Although women are now seriously considered for academic positions, several roadblocks are still in place. Many articles have been written about the problems (Holmes *et al.*, 2015) often referring to a "leaky pipeline" in which trained women scientists drop out along the career path, such that few attain senior status. I knew the dangers first hand. With my first husband, there was constant pressure to give it all up and live in a cabin in the woods. While searching for a full time academic position, the rejections were enough to convince anyone they should settle for a lab assistant's job. With motherhood, the pressure from society and family to stay home was present. Certainly, keeping up long hours at the lab were no longer possible. And although I was in a stable, understanding relationship, it was a constant balancing act. I was comfortable at the Geophysical Lab for 30 years and made little effort to further my career beyond growth in research contributions. Things changed when my son was about to leave for university and the possibility of leading the Geophysical Lab opened up in 2009.

I wonder how my career might have advanced if I were a man. In 2008, I put my name forward to be considered for the Director of the Geophysical Laboratory. I understood the operation of the Lab, knew everyone on campus and in the Institution including the President, and was reaching some prominence in my field of biogeochemistry, astrobiology, and geobiology. For final consideration, there was only one other candidate, my colleague Russell

Hemley. Rus is a member of the National Academy of Science (and I was not at the time), has published more than 400 papers, and had brought in millions of dollars from external grants. A committee was appointed to review "the candidates" and at first, it was comprised of only men. After a complaint was lodged with Carnegie's President, one woman was appointed. As candidates, we submitted a 2-3 page statement and our CV. There was one interview with the committee, and none with the staff or the administration. We did not give a special seminar. When President Meserve made his decision, he picked Rus Hemley, who on paper was eminently "more qualified" than me. To achieve what I have, I convinced that I had to pour more energy into aspects of my career than did my male colleagues. Aspects of early career disadvantages remain even today. Are there roadblocks to administrative leadership that women are more vulnerable to than men?

In response, I decided to build some administrative credentials and was selected to be a Program Director at the National Science Foundation in Geobiology and Low Temperature Geochemistry. Many researchers reach a point where they want to explore new challenges. Administrative leadership is a common line of career expansion sought in academia. After my stint at NSF, I learned a few important things about myself and about women in science. One, pure science credentials – a good reputation, lab skills, great postdocs and students, and consistent funding – are not enough for advancement into administrative leadership. Leadership is viewed as being Director, Chair, Dean, Provost, or President. Anything less than this, doesn't count. Two, I am first and foremost a scientist, not a bureaucrat, and I'm good at the creative aspects of science and research. Personnel management and paper shuffling is not my strong suit, jobs often delegated to administrative leaders. Three, I should have made the effort earlier in my career to get "leadership" experience. As a woman, this is required.

I returned to the Geophysical Lab in 2010. When my husband, a native Californian, said he was more than ready to move across the country back to his beloved natal state, I took the opportunity to cast a wide net to see where I might contribute in a university setting. By 2012 I had interviewed and obtained an offer at the University of California Merced, where I was offered the opportunity to provide leadership while doing research and formal classroom teaching. In January 2013, Chris and I "jumped ship" and headed to California. Walking into a tenured UC position, especially at my age (60) isn't something one can do – male or female – without a solid record of scientific accomplishment.

At the University of California, other challenges to women were evident. We served on the lion's share of service committees, mentored more students and faculty, and were given less glamorous teaching assignments. The most troublesome aspect for women in academia is finding a suitable position for their partners/husbands, who are also more likely than not professionals, often in the same field. Carnegie was too small and too inflexible to handle spousal hires. As a result, they lost many great women candidates for their

senior scientist positions. The University of California (UC) was much better at negotiating spousal hires, but I soon learned that even though UC has many policies on spousal accommodations, spousal hires often came without any real financial backing to them. As Department Chair, I was able to swing a couple of spousal hires, but not able with some others. When UC did offer two positions to a couple, one was often under-funded with the spouse often given insufficient lab space and more extensive teaching duties. For Earth Science departments, having women on the faculty is not just important, it is essential. Bringing in new perspectives in petrology and geochemistry, the more traditional fields, as well as current thinking in geobiology and climate science is needed to keep our science engaged in the many national and international debates on climate, natural disasters, and sustainability.

Chris and I landed in a small town on the edge of California's sprawling Central Valley at a new university campus that seemed to be on a matching wavelength for doing new innovative research and education with an under-represented student population. The shift to a professorship at the University of California was a 180° change in the direction of my career. Building my lab in temporary quarters, hiring faculty to work in the "Wild West", and helping to build the campus was both fascinating and annoying at the same time. I served for three years as Chair of a fledgling "department", taught many classes, and thought my career would end there. This was not to be. In 2017 I took a new position at the University of California Riverside.

20.8 Intersectionality

This past year, I started working with faculty in the Gender and Sexuality studies department at UC Riverside. Most of them women, when we met I often felt like a fish out of water when we discussed areas of common interest. I learned a new term "intersectionality". The Oxford dictionary defines this as "*the interconnected nature of social categorizations such as race, class, and gender as they apply to a given individual or group, regarded as creating overlapping and interdependent systems of discrimination or disadvantage.*" Women and men of colour in geoscience comprise only 6 % of total employment as of 2006. Estimating that only 25 % of geoscientists are women, this implies that only 1.5 % of geoscientists are women of colour. This past spring at UC Riverside, I taught a class on Sustainability in the Salton Sea in the Gender and Sexual Studies department. I was the only white person in the classroom, something unfamiliar to me, and I was the only scientist as well. (I may have been the first scientist this group of students had ever met. They were apprehensive of research and science in general.) Although I was a white scientist, I taught in a wheelchair, which placed me firmly in the ranks of a women, trait #1, with a disability, trait #2. I now know first hand about intersectionality.

Attending a recent Goldschmidt meeting now in a wheelchair, people that I had known for 30-40 years walked right past me. Do women of colour sometimes feel isolated in this same way when walking past thousands of

predominantly white men geochemists? The isolation of adding an extra social characterisation compounds how people are treated, often unwittingly by those without targeted social backgrounds. Although not strictly in the definition of intersectionality, mothers with infants or small children faced problems at society meetings until recently both AGU and Goldschmidt have provided spaces for nursing as well as childcare centres.

The Earth Science Women's Network for the past 16 years has provided a way for women in the geoscience field to communicate and commiserate. Today there are over 3,000 members from 60 countries. The Network holds social gatherings at conferences, supports workshops, and has published articles and books on women in geoscience. In 2016, five of their 12 leadership board members were women of colour. Several years ago, the organisation transitioned from daily email bursts to online forums. Women post questions and receive answers and support. As a group, they (we) are a powerful group that is transforming the way women in earth science are regarded.

20.9 Disability and Science

In 2016, after my ALS diagnosis, we moved again now to UC Riverside to lead a new Institute (EDGE: Environmental Dynamics and GeoEcology Institute). I had to start all over again building the lab and making personal connections. This time it wasn't as easy as it was in 2013. There were a few disappointments.

"*I thought we'd be further along than this,*" my long term friend and colleague, Noreen Tuross, said as we discussed how women fare in academic departments, particularly as we've aged. I've seen a lot in my career but nothing could prepare me for ending my nearly 50 year scientific journey being sidelined by ALS, a disease I knew about but never imagined would partly define me. As the only senior woman scientist at a major research institution for about 35 years, I grew up and was used to the trials that women face during graduate school, postdoc training, job hunting, grant writing, and lab building. Over time, I developed a sense of where I wanted to go with my scientific career, had a full family life, and once my children were out of the nest, I was ready for extra challenges.

No one can predict when health crises may strike. As a chemist working with toxic substances and solvents my entire career, I felt that cancer might be in my future, even though I was careful from day one. In 2014 on a trip to attend a conference in Germany, my ankle buckled once – then two more times, followed by months of falling over at odd times, and wondering why carrying things seemed so difficult. After 18 months, I received the ALS diagnosis. My world stopped for days to adjust to the news. At the same time, I was being recruited for a more senior position at UC Riverside and offered the opportunity to create an institute in my field and bring together interdisciplinary faculty. My husband said, "*Let's go*!" Starting any new academic position is daunting. Starting as a

newly disabled person and a member of two academic departments, as well as directing a new Institute is enough work for the youngest and healthiest. No longer able to unpack boxes of books or samples, no longer able to wield the wrenches needed to assemble a laboratory, and no longer helped by the group of students and postdocs from my former campus, the task moved at a pace I was impatient with given my new sense of time. Gradually, very deliberately, people were hired, accommodations were made for my disability, and things fell into place. In August 2018, a year's worth of planning culminated with the installation of a first-of-its-kind instrument, the purchase of a vehicle to take our instruments to remote locations, and the submission of three extramural grants.

Being offered an endowed professorship at UC Riverside was a wonderful opportunity. I am able to hire undergraduates to work on sustainability, invite guests to visit from around the world, and provide graduate students with research stipends. At this stage in my life, I shouldn't complain. Although I'd been promised a promotion to "Distinguished Professor", administrators reneged on their offer. This decision was deeply disappointing. I realised later that women in the college of science who are members of the National Academy are those accorded the distinction of "Distinguished Professor", however men in the college have been given the title without being members of the NAS.

After completing the first draft of this epilogue, I sent copies to UC Riverside's leaders and within a week heard back from my Dean, Kathryn Uhrich. She called and asked if I was amenable to trying to re-do the Distinguished Professor process in an expedited manner. Of course, I said yes, but this time it had to done right with proper letters and consideration of my whole career. By the end of April, the promotion package had been sent to the Provost's office. Based on what I read in the external letters and the department review, I was hopeful.

On Tuesday, April 30th, I had just taken my first sip of morning coffee when my cell phone started to buzz. A text message from Kate Freeman appeared. "*I am writing to say congratulations on your election to the National Academy of Sciences this morning!!*" Needless to say, I was stunned and called right back. At 6:40 am, I received congratulations from seven friends who are members of the Academy. The rest of the day went fast as phone calls, emails, and flowers came rolling in. By Friday, more good news arrived. I had, at last, been promoted to Distinguished Professor!

The timing, though, seemed to still support the uncomfortable fact that women needed to be Academy members to be Distinguished Professors. I learned, however, that the committee making the decision for the promotion had decided mere days before the NAS election! This small fact is important for busting open the opportunity for women to advance without selection by an outside organisation.

I wonder if the University would have honoured its original offer if I arrived on campus without a wheeled walker and a diagnosis of ALS. Alternatively, perhaps my recent physical disability had little or nothing to do with the

University's decision. But I would bet, and it would be a reasonable bet, that if I were a man rather than a woman, the promotion wouldn't have been delayed by three years. Women, and even men, in academia need to have the resilience to let frequent disappointments fall to the wayside and not let anger shape their outlook. I've managed to continue having fun, enjoy my colleagues, and embrace the work of teaching young students. Even with initial disappointment to expand my career, I was able to figure things out, make positive impacts, and move on and up notwithstanding a crippling disease.

20.10 Conclusion

I have been told that I've been an extremely positive force in many people's lives. For me the glass has always been half full, even when things were more difficult than they should have been. There were many events that were indeed negative, but I overcame them all in my own way and learned a tremendous amount about people. When young women geoscientists read this well into the future, I hope that I helped blaze a path for them and that there is a light at the end of the tunnel. It is my sincere wish that because of things that we older geoscientists have gone through, younger women will have it easier. I hope to continue to provide inspiration for young geoscientists, both women and men, even today. When people think of me, I hope they will think of getting science done and paving the way – be it in a lab, at a university, in a wheelchair, or at the mass spectrometer.

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GLOSSARY

Amino acid racemisation: the conversion of L-amino acids to D-amino acids over time and as a function of temperature.

Aquaporins: proteins found in cellular membranes that mediate the flow of water into cells.

Biocomplexity: the study of complex structures and behaviours that arise from non-linear interactions of active biological agents, which may range in scale from molecules to cells to organisms.

Biomarker: a measurable substance in an organism whose presence is indicative of some phenomenon or coming from a specific organism

Biosignature: a measurable parameter (*e.g.*, isotopic composition) organised into a pattern that can be produced by a living organism.

Casein: the main protein present in milk and (in coagulated form) in cheese. It is used in processed foods and in adhesives, paints, and other industrial products.

Collagen: the main structural protein found in animal connective tissue, yielding gelatin when boiled.

Conophyton: a cone-shaped microbial structure found in hot springs and other thermal areas.

Cytochrome oxidase: any of a number of compounds consisting of heme bonded to a protein. Cytochromes function as electron transfer agents in many metabolic pathways, especially cellular respiration.

Dole Effect: the enrichment of ^{18}O in atmospheric oxygen as discovered by Malcolm Dole in the 1930s.

ELISA: enzyme-linked immunosorbent assay, an immunological assay technique making use of an enzyme bonded to a particular antibody or antigen.

Epicontinental sea: a shallow sea that covers central areas of continents during periods of high sea level that result in marine transgressions.

Essential amino acid: an amino acid that is required for the growth and health of an organism and which cannot be synthesised by that organism's enzymes.

Gut microbiome: the complex community of microorganisms that live in the digestive tracts of humans and animals, including insects.

Isotopologue: molecules that differ only in their isotopic composition. They have the same chemical formula and bonding arrangement of atoms, but at least one atom has a different number of neutrons than the parent.

Lignin: a complex organic polymer deposited in the cell walls of many plants, making them rigid and woody.

Macromolecular carbon (MMC): complex organic carbon found in meteorites or metamorphosed rocks.

NADPH: nicotinamide adenine dinucleotide phosphate. Molecule that provides reducing power for biosynthetic reactions.

Non-essential amino acid: an amino acid that can be synthesised by an organism and need not be present in its diet.

Nucleobases: purines or pyrimidines that can form nucleic acids

PCR: polymerase chain reaction; a method of making multiple copies of a DNA sequence, involving repeated reactions with a polymerase.

Pentose phosphate pathway: a metabolic pathway parallel to glycolysis. It generates NADPH and pentoses (5-carbon sugars) as well as ribose 5-phosphate, the last one a precursor for the synthesis of nucleotides.

Photorespiration: a respiratory process in many higher plants by which they take up oxygen in the light and give out some carbon dioxide, contrary to the general pattern of photosynthesis.

Point Potential Evaporative Transpiration (PPET): a climate parameter that takes into account temperature, evaporation, and precipitation.

Rubisco: an enzyme present in plant chloroplasts, involved in fixing atmospheric carbon dioxide during photosynthesis and in oxygenation of the resulting compound during photorespiration.

Stromatolite: a calcareous mound built up of layers of lime-secreting cyanobacteria and trapped sediment, found in Precambrian rocks as the earliest known fossils, and still being formed in hypersaline lagoons and in thermal areas.

Symbiont: either of two organisms that live in symbiosis with one another.

Thermal pyrolysis: decomposition brought about by high temperatures.

Thermophile: a bacterium or other microorganism that grows best at higher than normal temperatures.

Thiophene: organic compound containing sulphur in a ringed structure

Tricarboxylic acid cycle: the sequence of reactions by which most living cells generate energy during the process of aerobic respiration. It takes place in the mitochondria, consuming oxygen, producing carbon dioxide and water as waste products, and converting ADP to energy-rich ATP. Also known as the Krebs cycle.

Trophic position: a term describing where an animal is within a food web. Higher trophic position means the animal depends on many smaller organisms for its diet.

Tryptone: the assortment of peptides formed by the digestion of casein by the protease trypsin.

Western blot: an adaptation of the Southern blot procedure, used to identify specific amino acid sequences in proteins.

Xenolith: a piece of rock within an igneous rock that is not derived from the original magma but has been introduced from elsewhere, especially the surrounding country rock.

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DR. MARILYN FOGEL uses a wide range of expertise —including biology, chemistry, geology and astrobiology— to study distinctive isotopes of carbon, oxygen, hydrogen, and nitrogen in order to trace various phenomena tied to modern and fossilised ecosystems. She received her B.S. in Biology from Penn State and a Ph.D. in Botany and Marine Science from the University of Texas at Austin's Marine Science Institute in Port Aransas. Fogel joined the Carnegie Institution of Washington's Geophysical Laboratory as a Carnegie Corporation Fellow in 1977. In 1979, she became a Senior Scientist and Staff Member at the Carnegie's Geophysical Laboratory where she mentored postdoctoral fellows, graduate students, and undergraduates, who became Professors, Deans, and Department Chairs. Since 1998, Fogel has worked on astrobiology as a Member of NASA's Astrobiology Institute including studies to examine the isotopic patterns in meteorites.

In 2013, Marilyn joined the Life and Environmental Sciences faculty at the University of California's newest campus in Merced and served as Chair, established a state of the art stable isotope laboratory, and increased faculty with interdisciplinary hires. Fogel joined UC Riverside in Earth Sciences and Environmental Sciences departments in Fall 2016 and is creating activities and ideas for the EDGE (Environmental Dynamics and Geo-Ecology) Institute, including building of the EDGE Institute laboratory working with colleagues to support geobiology and biogeochemistry research. Fogel holds the inaugural Endowed Wilbur W. Mayhew Chair of Geoecology and is a Distinguished Professor of Geoecology.

Marilyn is a Fellow of the Geochemical Society and the European Association of Geochemistry, the American Association for the Advancement of Science, and the American Geophysical Union, a Fulbright Scholar, and served on the National Research Council's Space Studies Board. She served as Program Director in Geobiology and Low Temperature Geochemistry with the National Science Foundation. Fogel was elected President (2013-2018) of the Biogeosciences section of the American Geophysical Union. In 2014, she received the Alfred Treibs Medal in Organic Geochemistry for lifetime achievement in the field and is the first woman to be awarded this honour. The Geological Society of America recently recognised Dr. Fogel for her Distinguished Career in Geobiology. In 2019, she was elected to the United States National Academy of Sciences.