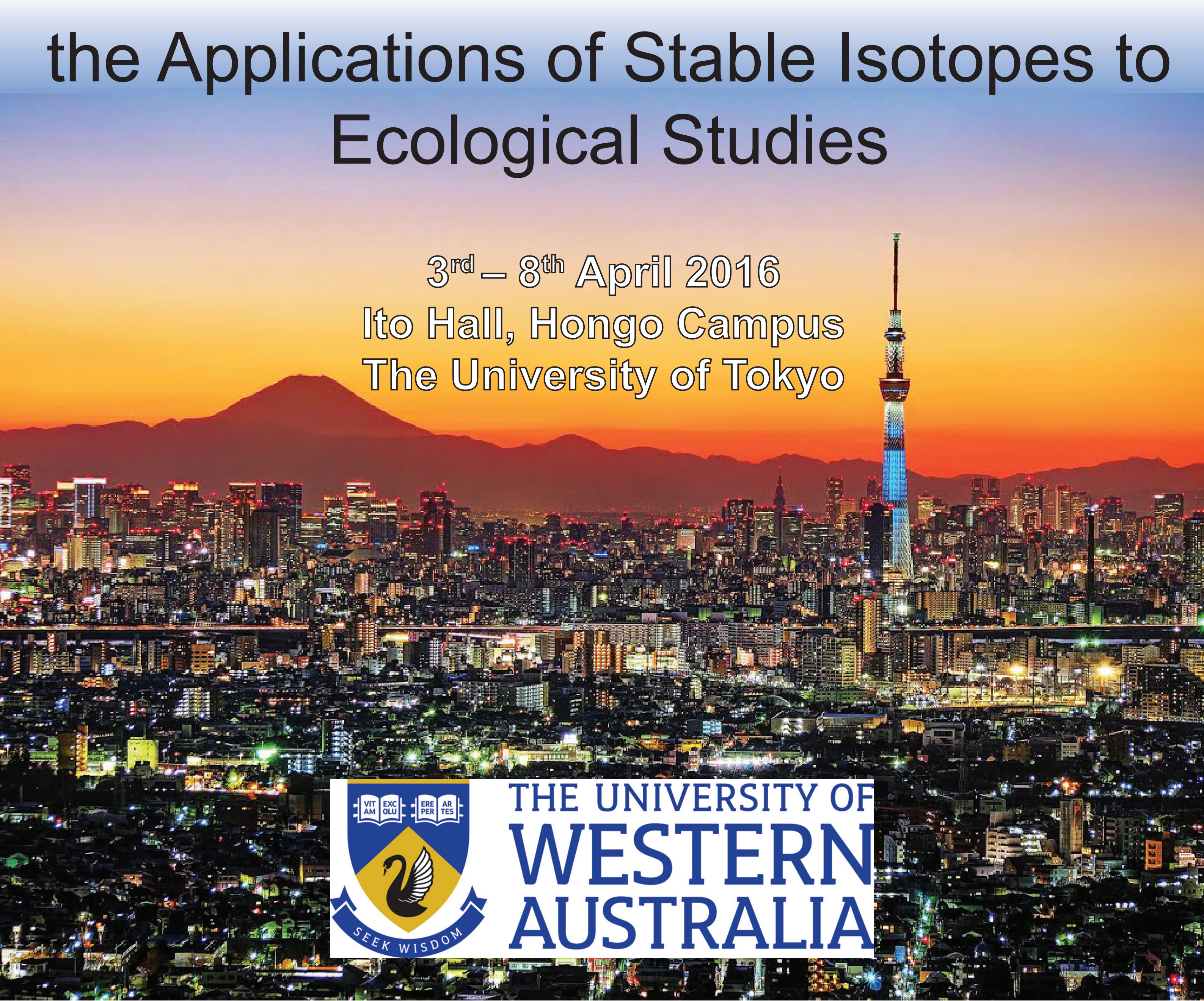
The 10th International Conference on the Applications of Stable Isotopes to Ecological Studies



3rd – 8th April 2016
Ito Hall, Hongo Campus
The University of Tokyo

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The centre is well equipped for stable isotope studies and offers a range of analytical and interpretive services.

We routinely provide analyses of $\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$, $\delta^{18}O$, $\delta^{2}H$ of solid materials, such as soils, carbonates, sediments, water and plant and animal tissues.

Our research is focused on the ecological sustainability of natural ecosystems and their response to disturbance and environmental change. We have extensive experience in the application of stable isotopes to research in both terrestrial and aquatic ecosystems; plant and animal ecology and physiology; soil and sediment chemistry; paleoclimates and groundwater-surface water interactions.

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Programme and Abstracts

3rd – 8th April 2016
Ito Hall, Hongo Campus
The University of Tokyo

Organizing Committee:
Alex Wyatt, The University of Tokyo
Ichiro Tayasu, Research Institute for Humanity and Nature
Naohiro Yoshida, Tokyo Institute of Technology
Naohiko Ohkouchi, JAMSTEC
Toshi Nagata, The University of Tokyo

Contents

Welcome to IsoEcol 2016!

On behalf of the conference organising committee and sponsors it is our great pleasure to welcome you to Tokyo for IsoEcol 2016.

We trust that you will find the 10th IsoEcol, and the first in Asia, as stimulating and inspiring as past IsoEcol. We have more than 150 delegates and over 130 oral and poster presentations spread across 5 sessions. The high quality of the submitted abstracts made it difficult to assign the limited oral slots, but we trust that delegates will benefit from the traditional concurrent oral presentation format, and take full advantage of the two poster sessions to interact with colleagues. We are looking forward to hearing about the application of isotope techniques to a wide array of ecological systems and study organisms throughout the week.

Traditionally IsoEcol meetings include a mid-conference field trip day, and we know participants will enjoy their excursion to the natural areas surrounding the iconic Mount Fuji. We also hope that you can take time to enjoy the beauty of Tokyo during the spring, and perhaps experience a hanami party under the cherry blossoms.

Thank you for your participation and helping to continue the great IsoEcol tradition. We trust that you will find the conference productive and stimulating, enjoy your time in Tokyo, and return home with renewed vigour for isotope ecology.

1 Scientific Program

1.1 Venue

The venue for the scientific program is Ito Hall on Hongo Campus of The University of Tokyo. Please refer to the website for a Google Map of relevant locations, or consult the figure overleaf. The conference space is located in the 2nd basement floor (B2F) and can be reached by stairs or lifts from the ground floor.

1.2 Oral presentations

All oral presentations will take place in the Ito Hall Auditorium. Each oral is allotted 20 mins, which includes approximately 5 mins for questions, discussion and speaker change-over. Speakers should prepare to speak for no longer than 15 mins.

For the scheduling of each session and talk, please consult the back cover of this book.

1.3 Poster Presentations

All poster presentations will take place in the General Purpose Space (多目的スペース) adjacent to the Auditorium.

Odd poster numbers will present on Monday and even poster numbers on Tuesday. Please refer to the poster number in the following pages to determine when the poster will be presented.

1.4 Student Awards

Student Presentation Awards will be made during closing remarks on Friday afternoon. Awards will be given for the Best Oral and Best Poster, as well as runners-up for each. A panel of attending scientists will judge those student presentations that nominated for award consideration during registration.

1.5 Lunch Seminar

A seminar will be presented by Thermo Fisher Scientific in the Ito Hall Auditorium during lunchtime on Tuesday. All delegates are encouraged to collect their lunch and enjoy it in the hall during the seminar.

EA-IRMS: Basic Principles and Advances

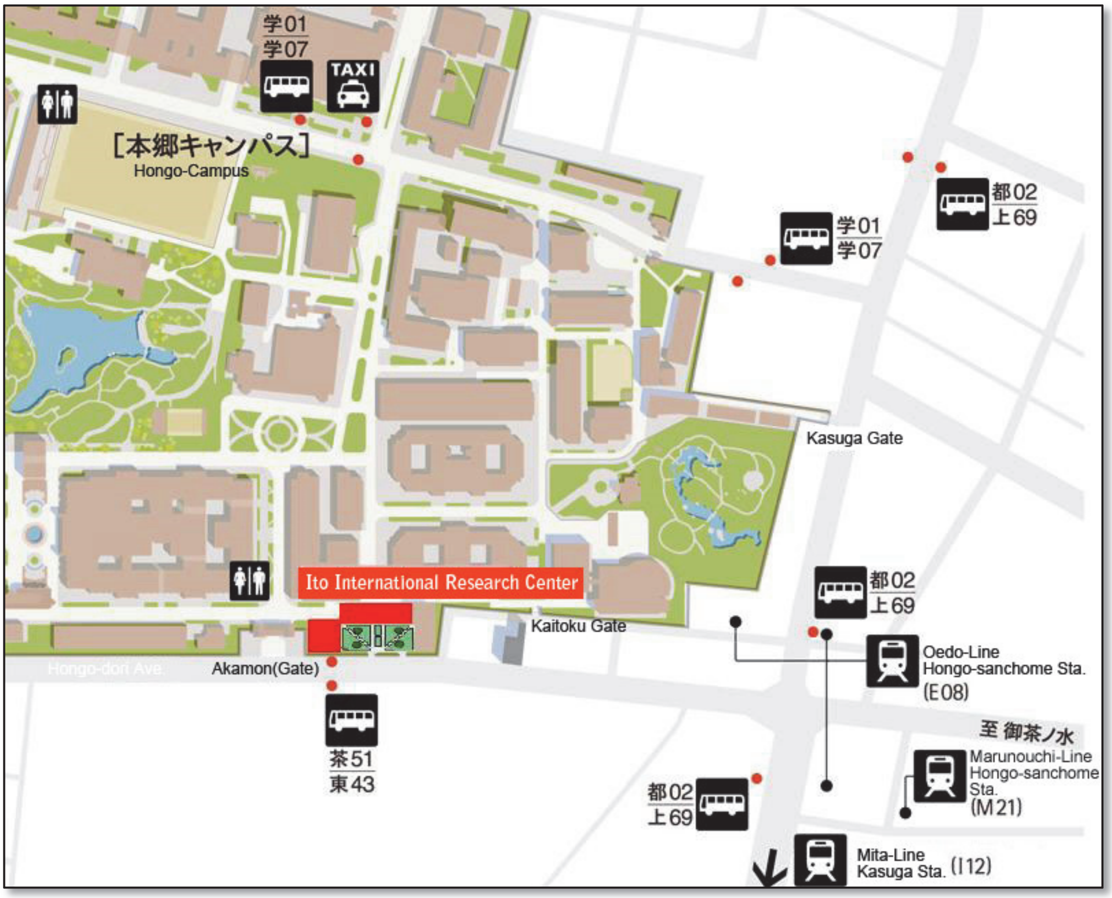
Charles B. Douthitt, Jerome A. Johemko and Andreas W. Hilkert – Thermo Fisher Scientific (Bremen) GmbH, Germany

Continuous flow applications have revolutionized Isotope Ratio Mass Spectrometry (IRMS) within the last 25 years. Today continuous flow techniques can be found in all fields of application with improved performance on sample size, throughput, multiple isotope methods, overall precision and ease of use.

Bulk stable isotope analysis (BSIA) is the most widely used continuous flow technique for all type of organic and a wide range of inorganic samples as well as water. By designing elemental analyzers for combustion and for high temperature conversion coupled to the IRMS the isotope ratios of H&O and N&C&S can be analyzed in the µg range.

Above: Location map for Ito Hall on the Hongo Campus of The University of Tokyo. The Hongosanchome Metro Station (Marunouchi Line; also services the Toei Oedo Line) is shown at the bottom of the figure. Todaimae Station (Nanboku Line) is to the north. For more details, please consult the Google Map via the webpage.

Below: A campus map with Ito International Research Center shown in red.



The need for better and more multi-element analysis with BSIA requires the constant availability of up to five reference gases. The unequal distribution of elements in a substance as well as differences in sample weights demand a quick and flexible response

to varying signal intensities by better dilution and amplification techniques. Moreover, 24/7 operation for maximum utilization of the IRMS systems requires automatic determination of system parameters like isotope ratio linearity and H_3^+ factor at run time improving the daily laboratory routines.

Examples for multi-element isotope analysis, throughput and dynamic range together with the requirements on sample size, precision and accuracy in BSIA will be discussed.

2 Social Events

2.1 Opening Ceremony

The Opening Ceremony will take place in the General Purpose Space (多目的スペース) of Ito Hall from 18:00 – 20:00 on Sunday the 3rd of April. A meal and drinks will be provided. All registered delegates are welcome to attend.

2.2 Poster Sessions

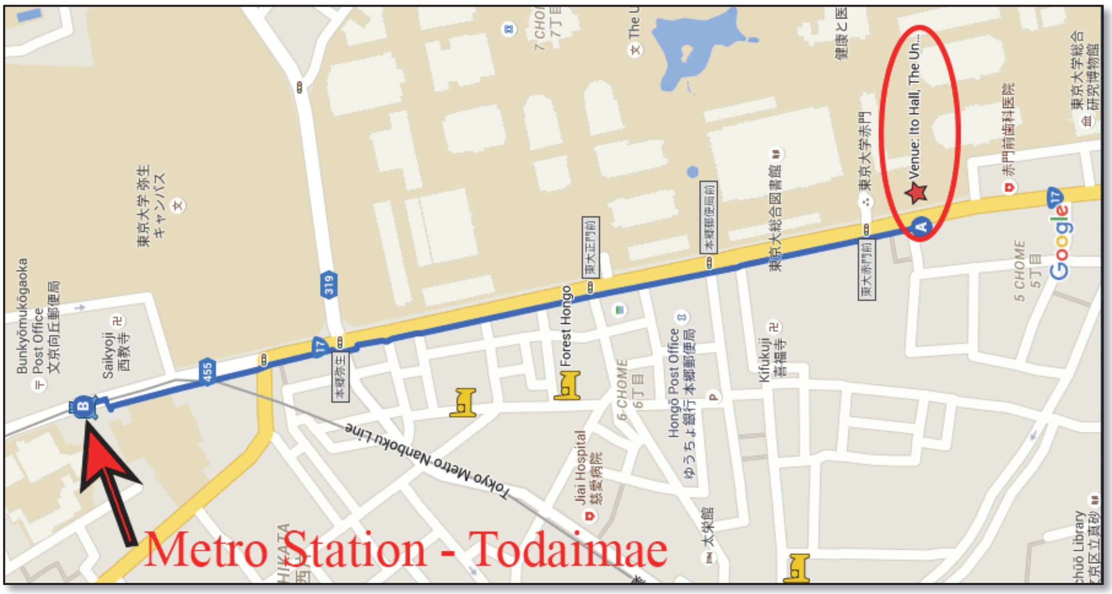
Poster sessions will take place from 18:30 – 20:30 on Monday and Tuesday the 4th and 5th of April. Light snacks and drinks will be provided. Please take the time to interact with colleagues in an enjoyable and informal setting.

2.3 Thermo Fisher Scientific Banquet

The banquet, kindly supported by Thermo Fisher Scientific, will take place at Happo-en in the evening of Thursday April 7th. Tickets for the banquet were available during registration for delegates and up to one guest.

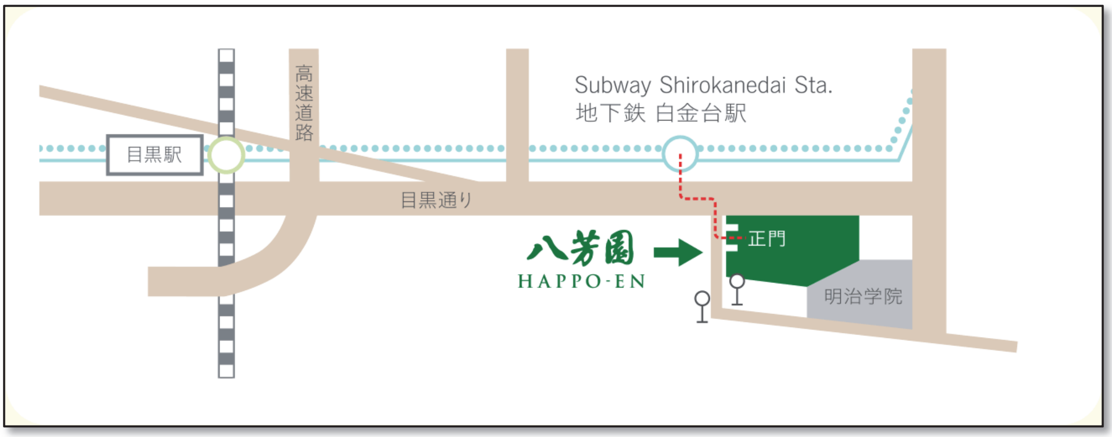
Guided transfers to Todaimae subway station on the Namboku Line will be available in groups starting from 17:00. Please get off the subway at Shirokanedai station, the stop after Shirokanetakanawa. It takes around 22 mins (10 stops) from Todaimae to Shirokanedai. At Shirokanedai, take Exit 2 and you will the imposing Front Gate of Happo-en diagonally across the intersection adjacent Exit 2. It should be no more than a minute’s walk from Exit 2. Please wear your name badge and use the gate with an “IsoEcol 2016” sign to the side.

Banquet ticket holders are encouraged to tour the Happoen garden before the banquet.



Above: The best station for accessing the banquet venue, Happo-en, from Ito Hall is Todaimae on the Namboku Line, about 10 mins walk directly north along Hongo Dori.

Below: Location of Happo-en, in relation to major train and subway stations. The most convenient station is Shirokanedai on the Namboku Line. The main gate (正門) is less than two minutes' walk from Shirokanedai Exit 2.



3 Keynote Speakers

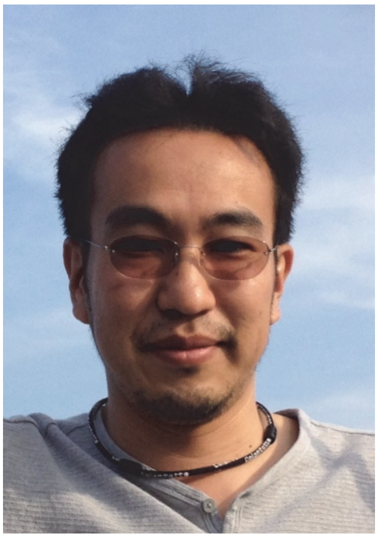
3.1 Gabriel Bowen

Gabriel Bowen is an Associate Professor of Geology and Geophysics and member of the Global Change and Sustainability Center at the University of Utah, where he leads the Spatio-temporal Isotope Analytics Lab (SPATIAL) and serves as co-director of the SIRFER stable isotope facility. His research focuses on the use of spatial and temporally resolved geochemical data to study Earth systems processes ranging from coupled carbon and water cycle change in geologic history to the movements of modern and near-modern humans. He has coauthored more than 100 peer reviewed papers and book chapters, and his work has been supported by grants from the US National Science Foundation, other US government agencies, and private foundations. In addition to fundamental research, he has been active in developing cyberinformatics tools and training programs supporting the use of large-scale environmental geochemistry data across a broad range of scientific disciplines, including the waterisotopes.org and IsoMAP.org web sites and the Inter-University Training for Continental-scale Ecology training program (http://itce.utah.edu).

Generalizing the application of isoscapes in ecology: Thinking across scales and systems

Isotopic heterogeneity in the environment is the template on which ecological studies of resource use, movement, and mass and energy flow are based. This heterogeneity can be conceptualized as a multidimensional isotopic landscape, or isoscape, with dimensions of space, time, and substrate commonly describing the majority of the variation observed. Environmental isoscapes exist for a wide range of systems and substrates and over several orders of magnitude in spatial and temporal scale. Although they have been exploited in many ecological applications, consideration of the spatiotemporal structure of environmental isoscapes is often implicit in the analyses conducted. This presentation will explore ecologically relevant hydrogen and oxygen isoscapes spanning scales from thousands of kilometers to tens of centimeters. Data from each scale illustrates strong spatial and temporal structure that informs our understanding of isoscape-generating processes and provides a basis for predictive understanding of isotope distributions. In addition to the practical utility of such predictive isoscape models, the explicit decomposition of environmental isotope variation within the space-time-substrate reference frame elucidates potential opportunities and limitations of ecological applications. The isoscape framework is thus of general utility in the development and pursuit of isotope-based ecological research, as evidenced by a growing number and scope of studies leveraging this paradigm.

3.2 Yoshito Chikaraishi



Yoshito Chikaraishi is a Senior Scientist in the Department of Biogeochemistry at the Japan Agency for Marine-Earth Science and Technology (JAMSTEC). His research interests are:

1. an understanding of factors controlling molecular and stable isotopic compositions of organic compounds in nature, and
2. development and application of new analytical methods for isotope analysis of organic compounds.

More recently, he has employed Compound-Specific Isotope Analysis of Amino Acids (CSIA-AA) as a new method in ecological food web studies, particularly for understanding trophic linkages and energy flow among plant and animal species in ecosystem networks. He has demonstrated the high applicability of CSIA-AA for viewing food web structures in marine and terrestrial food webs and is dedicated to better understanding of isotopic discrimination mechanisms in auto- and heterotrophic organisms.

High-resolution food webs viewed via compound-specific isotope analysis of amino acids

For better understanding of trophic linkages and energy flow in complex networks of ecosystems, we have recently employed compound-specific stable isotope analysis (CSIA) of amino acids as a relatively new method. In particular, CSIA has been used to estimate trophic position (TP) among animal species, using the following equation:

$$TP = [(\delta^{15}N_{Tr} - \delta^{15}N_{Src} + \beta)/TDF] + 1$$

where $\delta^{15}N_{Tr}$ and $\delta^{15}N_{Src}$ denote stable nitrogen isotopic compositions of trophic and source amino acids (most commonly represented by glutamic acid and phenylalanine), respectively, β denotes the isotopic difference between trophic and source amino acids in primary producers (e.g., aquatic and terrestrial plants), and TDF denotes trophic discrimination factor at each shift in the trophic level. However, the validity of such estimates is highly dependent on the accuracy and consistency of both β and TDF values, which are not yet fully understood.

In this presentation, I would like to show the potential of the CSIA method to illustrate food web structure in natural ecosystems, and will discuss factors controlling the β and TDF values of amino acids, based on experimental results from a number of my own investigations and previously published literature.

3.3 James Ehleringer

James Ehleringer is a Distinguished Professor of Biology at the University of Utah and director of Utah's Global Change and Sustainability Center and the Stable Isotope Ratio Facility for Environmental Research. His research has spanned from ecological studies of natural and urban systems through atmospheric trace gas studies and forensic science. Throughout his academic career, Jim has also focused on creating the facilities and organizational structures to advance interdisciplinary science and provide new opportunities for others. He launched IsoForensics, a spinoff company focusing on applications of stable isotope analyses. Jim's recent research projects have melded stable isotope analyses with process studies to understand carbon and water cycles, air quality in urban zones, humans and our foods, and biomarkers in climate studies.

Diet, location, travel, and socioeconomic status: what stable isotopes reveal about you

It is now well established that there are variations in the stable isotope abundances of light elements within our foods and our bodies and that these variations provide extremely useful information to anthropologists, biologists, ecologists, forensic scientists, and health scientists. While carbon, nitrogen, and sulfur isotope abundances have been applied to examine foods and reconstruct human dietary patterns, hydrogen and oxygen isotope abundances have been applied to examine human region-of-origin and travel movements. Over the last decade, we have witnessed a staggering expansion of stable isotope applications to understand the composition of our foods as well as humans as environmental recorders (through analyses of hair, teeth, bones, and fingernails). In this presentation, we first highlight some of that progress and then focus on three of several emerging frontiers. The first emerging frontier is glocalization and our food supply. That is, how does the global expansion of food products intersect with and adapt to the local cultures in which food is sold? Two global foods will highlight this glocalization process: hamburgers and soft drinks. A second frontier is dissecting observed variations of stable isotopes in humans to better understand the roles of both socioeconomic status and age in influencing dietary choices. Here we explore the interactions among wealth, the balance of plant versus animal proteins in our diets, and food production (commercial concentrated animal feeding operations to produce cheap food). A third emerging frontier is sequential fingernail analyses as a linear recorder of an individual's diet and travel history. Collectively, these three emerging frontiers indicate vibrant and expanding opportunities for new insights into human behavior based on stable isotope observations.

3.4 Michael Richards

Professor Michael Richards (D.Phil. Oxford, MA, BA, Simon Fraser University) is an archaeological scientist who applies methods such as isotopic analysis to determine past human and animal diets and adaptations. His research interests include the evolution of human diets, developing new isotope systems for dietary and migration studies, and using isotope analysis to explore and catalogue the range and nature of human dietary adaptations throughout the Holocene. He has published over 200 research papers, including in the journals Nature, Science, and PNAS, and he has supervised over 50 graduate students and post-docs. He is a professor of Anthropology at Department of Anthropology, University of British Columbia in Vancouver, Canada and holds honorary research positions at the Max Planck Institute for Evolutionary Anthropology in Leipzig, Germany, and Durham University in the UK. He is a Fellow of the Society of Antiquaries of London, and a Fellow of the Royal Society of Canada.

An overview of the stable isotope evidence of human dietary adaptations over the past 30,000 years

In this paper I will review the work of my group and others on the C and N isotope measurements of archaeological human and animal bone collagen for reconstructing past human diets over the past 30,000 years. There are now significant numbers of measurements (i.e. over 50,000) of archaeological humans from around the world, and we are now able to begin looking for large-scale patterning in this dataset. For much of the time modern humans have existed we were hunter-gather-foragers, and for those archaeological contexts a stable isotope ecological approach works well to understand how humans adapted their diets in response to their ecosystems. However, at approximately 12,000 years ago (at the start of the Holocene) humans began to intensify the use of individual wild resources like marine foods and more dramatically alter their environments through the domestication of plants and animals. Archaeological human palaeodietary isotope studies then show that human diets are no longer predictable based on their local ecosystems and environments, as through time they increasingly rely on the foods they have domesticated, imported or transported as they move into new landscapes.

4 Abstracts - Orals

4.1 Session 1

New developments in applying mixing models and understanding fractionation

Food-web structure and isotopic niches within deep-sea canyon and slope habitats: insights revealed through stable isotope analysis and applications of SIBER-Stable Isotope Bayesian Ellipses in R

***Demopoulos**, Amanda – US Geological Survey*

Sandra Brooke – Florida State University

Steve Ross – University of North Carolina, Wilmington

Michael Rhode – University of North Carolina, Wilmington

Jennifer McClain-Counts – US Geological Survey

Submarine canyons can experience dynamic flow and turbulence, where canyon morphology, current regime, and nutrient conditions can influence the quality and quantity of food fluxing to the seafloor. However, few studies have examined the trophic pathways of deep-sea canyons relative to adjacent slope environments. Stable isotope analysis (SIA) and Bayesian standard ellipses (SEAB) were used to examine food web structure and trophic niches in Baltimore Canyon and the adjacent slope along the U.S. Atlantic margin. Various gear was used to sample fauna, sediments and water for SIA. Results from canyon and slope environments revealed that fish and invertebrate communities were composed of isotopically diverse feeding groups, encompassing ~ 5 trophic levels, with the baseline carbon source derived from photosynthetic material. Canyon consumers were significantly depleted in 13C and nominally depleted in 15N relative to consumers on the adjacent slope. Statistical comparisons between slope and canyon environments suggest isotopic niche separation by feeding groups. Omnivores and bottom-feeders (epi- and infaunal) were enriched in 13C and 15N relative to suspension feeders. Deposit feeders exhibited a large range in stable isotope values, indicative of occupying large trophic niches. Stable isotope values and niche widths also varied among suspension feeding coral species, potentially related to differences in food selection, feeding habits, and habitat association. For all invertebrates, isotopic niche area (SEAB) was 2x greater in the canyon than on the adjacent slope, with no overlap between the ellipses. This wider isotopic niche for canyon invertebrates indicates the presence of a broad group of taxa, with many serving as generalists, and possibly exploiting both marine phytodetritus and terrestrially-derived organic matter. The large spread in $\delta^{13}C$ values for consumer groups indicate that the isotopic composition of particulate organic matter changes, which may be function of location within the canyon and slope environments. Thus, food availability, substrate type, and resuspension processes influence the food-web structure in these environments.

Addressing the complexity of diet reconstruction using Bayesian mixing model

Fernandes, Ricardo – Cambridge and Kiel universities

*Thomas **Larsen** – University of Kiel*

Bayesian mixing models are increasingly used by ecologists for quantifying source contributions. However, ecologists often neglect the complexity of consumer dietary physiology during the modelling process. We will demonstrate, with the Bayesian mixing model FRUITS, how parametrization of certain aspects of dietary physiology such as dietary routing, nutritional requirements, nutritional stress, and inter-individual physiological differences improve model accuracy.

FRUITS was designed to allow a characterization of the nutrient composition of each source and the incorporation of multiple physiological priors. This provides model estimates which include contributions from each source and levels of nutrient intake (e.g. protein vs. carbohydrates vs. lipids). FRUITS also introduced the concept of weighted contribution given that different food nutrients may contribute, in weighted proportions, to a consumer isotopic signal. This is observed, for instance, in the synthesis of proteinaceous tissues which includes contributions from essential and non-essential amino acids, as well as carbohydrates and lipids. Furthermore, with FRUITS it is also possible to apply priors to all model parameters and estimates allowing for the imposition of limits on the intakes of macro- and micro-nutrients. Such priors, defined from experimental dietary studies, constrain model estimates to a higher precision. Following a similar approach, priors defining diet-to-consumer isotopic offsets which are dependent on the levels of nutrient intake can be defined. Finally, the model allows for uncertainties in all model parameters, including isotopic values of individual consumers. The latter aspect is particularly relevant given the isotopic variability observed in single consumers as a result of inter-individual physiological differences.

Model performance, in terms of accuracy and precision, was evaluated through several isotopic dietary studies employing animal consumers of different species having well-known diets. The goal was to assess the importance of including existing knowledge from dietary physiology into the modelling process.

The controversy about trophic enrichment factors of stable isotopes in invertebrates – a study about the crustacean *Dikerogammarus villosus*

Hellmann, *Claudia – Institute of Integrated Natural Sciences, University Koblenz-Landau, Germany*

Rene Gergs – Federal Environmental Agency, Berlin, Germany

Eva Mehler – Institute of Integrated Natural Sciences, University Koblenz-Landau, Germany

Carola Winkelmann – Institute of Integrated Natural Sciences, University Koblenz-Landau, Germany

In trophic ecology, stable isotope analyses (SIA) of nitrogen and carbon are commonly used to identify the trophic function and feeding pathways of consumers in the food web. To obtain realistic results in this approach it is mandatory to know the trophic enrichment factors (TEF's) of the stable isotopes during the metabolic processing in the studied organism representing the fractionation processes of the heavy isotope in the consumer tissue to its diet. The TEF's were determined for a variety of species in the past and are widely accepted to be in average 3.4 ‰ for nitrogen and 0.4 ‰ for carbon across all species. However, it is also known that the specific factors vary strongly between species of different taxon group, habitat or trophic level, and within species depending on diet, tissue or stage. Despite this high variability in invertebrates, standard TEF's are used in trophic studies because specific values are often not available for the studied species. We measured the specific TEF's for carbon and nitrogen for the aquatic invertebrate Dikerogammarus villosus, an invasive crustacean that is currently spreading in freshwaters around the world. In an 8-week laboratory experiment, individuals were exposed to two types of diet, animal diet (chironomids) and plant diet (conditioned leaves), and differences between the consumer tissue at equilibrium and the diet were analyzed. The experimental results showed large differences in TEF's between the diet types and varied strongly from published mean values. While for nitrogen the enrichment was calculated to be 3.56 ‰ (plant diet) and 2.29 ‰ (animal diet), for carbon an enrichment of 3.27 ‰ for plants and a depletion of -1.75 ‰ for animal diet was measured. The following trophic analyses of D. villosus in a river food web showed how the function of this species can be misinterpreted by using the standard values for trophic enrichment instead of specific factors. The species was more predaceous using the standard TEF's in diet calculation than it was shown with the measured TEF's. Thus, the measurement of specific and diet-typical TEF's for handling a specific trophic study is highly recommended.

A framework for testing competing stable isotope mixing models: a bear necessity

***Hopkins**, John – Division of Biological Sciences, Ecology, Behavior, and Evolution Section, UC San Diego*

Jake Ferguson – National Institute for Mathematical and Biological Synthesis

Daniel Tyers – United States Forest Service, Northern Rocky Mountain Science Center

Carolyn Kurle – Division of Biological Sciences, Ecology, Behavior, and Evolution Section, UC San Diego

Researchers frequently use stable isotope mixing models (SIMMs) to estimate the assimilated diets of animals. Despite their increasing complexity, little effort has been dedicated to developing approaches for selecting the most appropriate SIMMs for a given dataset. We used multiple stable isotope systems (expressed as $\delta^{13}C$, $\delta^{15}N$, δ34S) and a new analytical approach to estimate the diets of grizzly bears (Ursus arctos) in the Greater Yellowstone Ecosystem. Although we learned that different combinations of stable isotope values derived from bear hair and their major food sources yielded similar proportional dietary contributions using IsotopeR, we found that the 3-isotope, 3-source models were the most parsimonious SIMMs for these data. We learned that grizzly bears sampled in Cooke City Basin, Montana foraged primarily for plants (~55±7%) and whitebark pine seeds (~32±7%), and to a lesser extent, ungulates (~13±6%) in 2007-2009. Our new analytical approach will help inform the conservation of grizzly bears in Yellowstone and aid researchers in estimating the importance of different food sources to free-ranging animals.

Resolving trophic links within temperate eelgrass meadows – use of fatty acids and stable isotopes in MixSIAR mixing model

***Jankowska**, Emilia – Institute of Oceanology Polish Academy of Sciences*

Maria Wlodarska-Kowalczuk – Institute of Oceanology Polish Academy of Sciences

Marleen de Troch – University of Gent

Michel Loic Niels – University of Liege

Seagrass beds can act as ecosystem engineers, i.e. organisms that modify availability of resources to other organisms and as a result enhance the biodiversity of benthic fauna, thus play key role in coastal food webs. The trophic links within seagrass meadows are usually very complex and indicating the major food sources for benthic fauna is challenging. In the Gulf of Gdańsk (Baltic Sea) the severe decline in Zostera marina meadows took place in the second half of the 20th century. Recently, the natural recovery of underwater meadows is observed, so that can influence the pool of the available food sources and energy pathways in benthic food webs. The aim of the present study is to compare the food sources of benthic species inhabiting eelgrass vegetated and unvegetated bottom with a use of mixing models. Sampling of meio- and macrofauna as well as potential food sources (POM, SOM, epiphytes, microphytobenthos, macroalgae, seagrass) was conducted in summer 2014, in the southern Baltic Sea. The samples of consumers and food sources were analysed in terms of total fatty acids and stable isotopes composition ($\delta^{13}C$, $\delta^{15}N$, δ34S). Before running mixing model, few treatments have been applied to obtain dataset with the most determined design. Differences in relative fatty acid composition of consumers at two habitats (vegetated vs. unvegeated) was tested on log transformed data and Bray-Curtis similarities among samples by two-way PERMANOVA test. SIMPER procedure was applied to indentify fatty acids responsible for differences between two habitats, C:15n5 has been chosen to include in mixing model. Five sources MixSIAR mixing model with three isotopes and one fatty acid was used to estimate the relative proportion of each food source to the diet of a single species. Results indicate higher variability of both meio- and macrofauna diet within seagrass meadows, whereas diet of unvegetated species was more focused on one source. Model output indicates the usefulness of using three isotopes together with fatty acid in resolving complex food webs. The obtained results highlight the importance of seagrass meadows as habitats increasing food availability and diversity.

tRophicPosition, a new R package for the Bayesian estimation of consumer trophic position

***Quezada-Romegialli**, Claudio – Instituto de Ciencias Naturales Alexander von Humboldt, Universidad de Antofagasta, Chile*

Andrew Jackson – School of Natural Sciences, Trinity College Dublin, Ireland

Chris Harrod – Instituto de Ciencias Naturales Alexander von Humboldt, Universidad de Antofagasta, Chile

Stable isotopes have proven ability to capture the complexity of trophic interactions, to identify energy sources and to infer consumer trophic position. Trophic position is an important concept used to describe the ecological role of secondary and higher consumers in food webs. However, current methods for estimating trophic position using $\delta^{15}N$ stable isotopes are limited and do not fulfil the full potential of the isotopic approach. For instance, researchers typically use point estimate for key parameters including trophic enrichment factors and isotopic baselines, and often do not explicitly include within-population variation i.e. that shown by individual consumers. There is a marked need to include such variation both in the calculation of trophic position, but also to provide robust estimates of trophic position (e.g. measures of central tendency and variance).

We present an R package 'etRophicPosition', incorporating a Bayesian model for the calculation of trophic position using stable isotopes with one or multiple baselines within the powerful approach of Markov Chain Monte Carlo simulations provided by JAGS and the statistical language R. We modelled consumer and baseline observations using relevant statistical distributions, allowing them to be treated as random variables. The calculation of trophic position – a random parameter – for one baseline follows standard equations linking $\delta^{15}N$ enrichment per trophic level and the trophic position of the baseline (e.g. a primary producer or primary consumer). In case of two baselines, a simple mixing model incorporating $\delta^{13}C$ allows for the differentiation between two distinct sources of nitrogen, thus including spatial heterogeneity derived from alternatives sources of $\delta^{15}N$.

We demonstrate the core of the package, the theory behind it, and the functions created to estimate trophic position and produce figures. Finally, we demonstrate the capabilities of tRophicPosition using a range of marine and freshwater consumers from Europe and South America.

CQR was funded by Fondecyt 3160659; CH by Fondecyt 1151515

Quantifying the trophic consequences of parasitism through the application of stable isotope metrics and mixing model

***Sheath**, Danny – Bournemouth University*

D Andreou – Department of Life and Environmental Sciences, Faculty of Science and Technology, Bournemouth University, United Kingdom

J Pegg – Department of Life and Environmental Sciences, Faculty of Science and Technology, Bournemouth University, United Kingdom

J Grey – Lancaster Environment Centre, Lancaster University; The Wild Trout Trust, Waterlooville, United Kingdom

JR Britton – Department of Life and Environmental Sciences, Faculty of Science and Technology, Bournemouth University, United Kingdom

The inclusion of parasites in topological food webs of aquatic ecosystems has revealed their high ecological and evolutionary significance. Compared with networks comprising free-living species only, these infectious food webs have revealed parasites increase food web connectedness and linkage density, but decrease robustness. These qualitative approaches, however, rarely account for the pathological and ecological consequences incurred by some parasites on their hosts. They do not incorporate the substantial phenotypic modifications that can occur in the behaviour, habitat utilisation and diet of the parasitized component of populations. Here, using of examples of freshwater fish communities parasitized by a range of specific native and invasive parasites, we demonstrate that the application of stable isotope ecology to fish-parasite studies provides considerable insights into the consequences of parasitism via the use of more quantitative approaches. Use of stable isotope metrics (e.g. standard ellipse areas) and mixing models (to estimate diet composition), we highlight that parasitism can lead to substantial trophic niche specialisation in the host component of populations, potentially resulting in altered competitive interactions in the community and modifications to food web structure. Application of these methods to both native and invasive parasites indicates that the extent of trophic modification is a function of their pathological and ecological impacts on hosts, rather than relating to parasite origin. Correspondingly, the application of these stable isotope metrics and mixing models to the parasitized component of fish communities indicates that parasites can have considerable trophic consequences for host populations that are unable to be detected by network analyses alone.

Using stable isotopes to investigate ecology of sharks and turtles: what can evidence from multiple tissues tell us?

***Vanderklift**, Mat – CSIRO*

Richard Pillans – CSIRO

Tony Tucker – Department of Parks & Wildlife

Scott Whiting – Department of Parks & Wildlife

Andy Revill – CSIRO

Ecologists are getting better at analysing data about natural abundances of stable isotopes in ways that yield more robust inferences. For marine wildlife, we need spatial and temporal information about habitat use that improves population models and directly influences management. Mixing models now incorporate error and we understand that the data reflect processes that occur over varying lengths of time. However, we are yet to take full advantage of the fact that different tissues of animals can yield different kinds of information, because they have different biochemical composition and different rates of elemental turnover. We use data from different tissues with different turnover rates to test hypotheses about the ecology of large sharks and turtles at Ningaloo, Australia. $\delta^{15}N$ of blood components (plasma, red blood cells) are highly correlated, but deviate from simple predictions about the slope and intercept. $\delta^{15}N$ of blood components are also correlated with tissues that turn over more slowly (skin, cartilage), but $\delta^{13}C$ of all tissues are poorly correlated. The data predict that sharks and turtles are largely resident within relatively small areas (less than a few km^2) at Ningaloo, and we test these predictions with known patterns from individuals tagged with satellite and acoustic tags.

Isotopic niches of deep-sea fishes along a depth gradient

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Understanding the mechanisms supporting community structure is a major topic in ecology. Incorporating information of population dynamics and species-specific traits with stable isotope information enables us to test ecological hypotheses.

Continental slopes present highly diverse marine ecosystems, which are influenced by strong environmental depth-related gradients. Food availability is among the factors responsible for depth-related trends in community structure and patterns of biomass and diversity along continental slopes. Deep-sea fishes are supported by food provided by both physical (sinking fluxes) and biological (controlled by vertically migratory organisms) processes, but the relative importance of these pathways, and their roles in shaping trophic niches remain poorly known.

In this study we report results of an extensive survey of demersal fish communities recovered between 500 and 2000m water depth on the North East Atlantic (Scottish and Irish) continental slope. We used simple bulk carbon and nitrogen data and Bayesian-based models to track nutrient pathways and trophic niches for 48 deep-sea fish species in relation to depth along the continental slope. We show that both benthic and pelagic nutrient pathways fuel fish communities; at 500m, benthic-pelagic coupling is remarkably strong, driven by impingement of vertically-migrating fauna on the seafloor, followed by an increased separation between these pathways with increasing water depth.

The effect of increasing depth (and decreasing benthic-pelagic coupling) on isotopic niche space reflects how depth-trends of trophic specialisation vary across functional groups. Benthic species have broader isotopic niche breadth but a decline in the proportion of total biomass with increasing depth, indicating a gradual competitive release after 1500m. In contrast, we found that benthopelagic fishes tend to show a competitive release within a shorter bathymetric range (1500-1800m), but an increase in the proportional biomass with depth.

Finally, we show how information on isotopic niches provides key insights into the ecological mechanisms driving deep-sea ecosystem functioning.

Stable Isotope Ratios of Hydrogen Separate Mammals of Aquatic and Terrestrial Food Webs

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Hydrogen stable isotope ratios (δ2H) are increasingly used as endogenous markers to quantify the relative importance of allochthonous input of organic material into aquatic ecosystems. Yet, it is unclear if differences in δ2H values between aquatic and terrestrial food webs translate into corresponding differences of δ2H values of consumers. Based on a multiple-isotope approach, we observed that δ2H and $\delta^{15}N$ values of fur keratin differ between species that trawl insects in aquatic habitats and those that glean terrestrial insects. Indeed, δ2H values of fur from trawling Myotis species was lower by about 33 to 56‰ in relation to that of gleaning species. We then assessed the relative association of aerial hawking bat species to the terrestrial food web using trawling and gleaning bats as representatives of consumers in aquatic and terrestrial ecosystems respectively. Isotopic niche dimensions, as estimated by standardized ellipse areas using δ2H and $\delta^{15}N$ values, varied largely among study species, with largest overlaps of isotopic niches among members of the aerial-hawking bat ensemble. We conclude that δ2H values of fur keratin is a suitable parameter for evaluating the relative membership of mammals to aquatic and terrestrial food webs and to evaluate niche packing of species within consumer ensembles.

4.2 Session 2

Emerging techniques and integrative applications – multiple elements, Compound Specific Isotope Analyses (CSIA), isotopomers, and radioisotopes

Stable and Radioisotopes Reveal Food Web Impacts of the Deepwater Horizon Oil Spill in the Northern Gulf of Mexico

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Effects of the Deepwater Horizon Oil Spill (DWH) on the northern Gulf of Mexico (nGOM) food web have been documented from plankton to fishes to marine mammals. Here, we report an extensive stable and radioisotope data set compiled for red snapper, *Lutjanus campechanus*, an ecologically and economically valuable reef fish in the nGOM ecosystem, to examine the temporal distribution of DWH-related food web impacts. Red snapper are generalist mesopredators that feed on a diversity of prey from swarming pelagic zooplankton to other fishes. There was a significant increase in red snapper muscle ($n = 338$) $\delta^{15}N$ following the DWH, which corresponded to a diet shift ($n = 1{,}255$ stomachs analyzed) away from lower trophic level pelagic prey to more benthic and demersal fishes following the spill. Red snapper diet composition and trophic position estimates resembled pre-spill values by year-5 post-DWH. During the first two years following the DWH, estimates of basal $\delta^{13}C$ (red snapper muscle $\delta^{13}C$ corrected for trophic position and fractionation) decreased relative to increasing $\delta^{15}N$ values in red snapper muscle, which is counter to expectation unless a new, lighter source of carbon had entered the system. Others have reported the transfer of bacteria-assimilated petrocarbon to zooplankton following the DWH, in turn making this lighter C available to higher order consumers. A significant positive correlation was observed between basal $\delta^{13}C$ estimates and depleted 14C in muscle samples ($n = 10$), indicating lower basal $\delta^{13}C$ estimates following the DWH were most likely due to assimilated petrocarbon that was passed through the food web to red snapper. Muscle petrocarbon signatures were ephemeral due to tissue turnover, but analysis of otoliths (earstones) of age-0 red snapper suggests that 14C-depleted organic C in the food web was conveyed to these CaCO3 structures. Otoliths are inert once formed and lay down annual growth rings, thus may serve as permanent, time-referenced recorders of food web impacts observed following the DWH.

Compound-Specific δ^{15}N of Amino Acids as a Tool to Understand Invasions in Complex Freshwater Food Webs

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The analysis of bulk stable isotopes has proven to be an extremely valuable tool for the elucidation of food web structure in various ecosystems. However, in complex food webs and in particular with abundant omnivores, the resolution of the food web structure via the classical bulk stable isotope analysis is strongly limited. To gain a higher resolution in both basal resource and trophic level estimates, the advanced technique of compound-specific stable isotope (δ^{15}N) analysis from amino acids is increasingly utilized in marine ecology, but has yet rarely been applied in freshwater ecosystems. Here, we apply this emerging method in a complex freshwater food web in order to understand the trophic position and food web interconnections of the invasive mysid crustacean *Limnomysis benedeni*. Analysis of amino-acid specific δ^{15}N allowed very precise trophic level estimates for the whole food web, which were generally consistent with those from bulk δ^{15}N analyses. Our data demonstrate that the complex food web of the investigated gravel pit lake is mainly driven by the import of allochthonous production, rather than autochthonous production within the system. Amino-acid specific δ^{15}N clearly indicate an omnivorous/detritivorous feeding behaviour of *L. benedeni*, which is different from previous classifications of this invasive species as a benthic herbivore or planktivore based on bulk isotope data. This case study highlights the immense potential of compound-specific stable isotope analysis from amino acid δ^{15}N to elucidate trophic levels and general food web structure not only in marine, but also in complex freshwater ecosystems.

Using multiple tracers across landscapes to guide coastal conservation and development

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Humans are modifying catchments and estuaries of the coastal zone worldwide, and ecologists have begun measuring coastal ecosystem responses in an attempt to conserve natural ecological conditions and health. Case studies from the eastern coast of Australia show how a combination of stable isotope and elemental tracers can be used to help guide these conservation efforts. Isotopes have helped over the last two decades in identifying and cleaning up sewage inputs to Australian coastal systems. Research is now focusing on sediments as non-point pollution sources; these sediments are moving through river and floodplain systems to fill in and fertilize downstream estuaries. We are studying effects of sediments on metabolism and growth rates of bioindicator shrimp and prawns collected in landscape-level surveys, and we are developing high-resolution position-specific isotope analysis (PSIA) of carboxyl groups of amino acids to help in these metabolic studies. Our overall goals are to use the coupled sediment and bioindicator studies to guide expensive anti-erosion measures in upper catchments, to better preserve overall ecological functioning and integrity of coastal systems.

Mechanisms responsible for high N_2O emissions from bare surfaces of sub-Arctic permafrost peatlands

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Soils make up for about 60% to the global annual emissions of N2O, with most of the N2O emissions originating from tropical and agricultural soils. Typically N2O emissions from pristine terrestrial ecosystem on arctic soils have been neglected due to the nitrogen-limited nature of these ecosystems. Nevertheless, recent findings have shown that there are habitats in the sub-Arctic region [□gso-called peat circles□h] emitting N2O at high rate and that permafrost soils have the potential for the N2O emissions after thawing. This puts N2O high on the agenda in Arctic soil research. In this study in situ N2O emissions from peat circles in subarctic permafrost peatlands (Seida, NW-Russia; 62‹57'E, 67‹03'N) were characterized by stable isotope techniques. The observed range in δ^{15}Nbulk, δ18O and SP of the N2O emitted by the peat circles soils was -17‰ to -3‰, -6‰ to 30‰, and -92‰ to 52‰, respectively, with an emission-weighted average value for δ^{15}Nbulk of -13‰ ± 2 ‰, within the range of the values reported from other natural ecosystems, and δ18O of 16 ± 3‰ (mean ± s.d; n=9). The individual SP values observed over the season fluctuated between values indicative of denitrification and nitrification. The emission-weighted average SP value for N2O of -13 ± 8‰ (mean ± s.d; n=9) is the lowest one reported so far from in situ studies and is overlapping with SP values for nitrification/nitrifier denitrification (|17‰ ± 8‰) obtained by Perez et al. [2006] in soil incubation experiments, suggesting that nitrification/nitrifier denitrification was the main pathway of N2O production in the relatively drier study year. But due to variable published SP values for these processes this interpretation has to be taken with caution. On the other hand, the SP values in the peat circles profiles (-0.7‰ to 23.0‰) suggested that in deeper soil layers denitrification-related mechanisms were the main N2O production pathway. Our analysis also showed that up to 70 % of the produced N2O was reduced to N2 within the soil. This study revealed important mechanisms behind the factors controlling N2O release from the Arctic, but also highlights the complexity in interpreting 15N results in N2O studies.

Trophic Position Adjustment of Bird Contaminant Levels Using Amino Acid-Specific $\delta^{15}N$ Analysis

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Levels of biomagnifying contaminants in wildlife reflect proximity to contaminant sources, ecosystem processes regulating contaminant availability, and organism trophic position. Many studies evaluating spatial and temporal trends in levels of biomagnifying contaminants in wildlife use stable nitrogen isotopes to evaluate organism trophic position and then adjust contaminant levels based upon $\delta^{15}N$ values. This may be appropriate when comparing individuals or species at one location during the same period. However, when comparing wildlife $\delta^{15}N$ values across space or time it is necessary to consider spatial differences and/or temporal changes in $\delta^{15}N$ values associated with nitrogen being incorporated into the base of food webs even at a single location. One way to do this is by measuring $\delta^{15}N$ values in primary consumers. However, studies that focus on wildlife often do not include collections of food web samples either because of logistical difficulties associated with working in isolated locations, e.g. Arctic, or because archived samples are being used for which no food web samples exist. Here, we investigate the utility of amino acid compound-specific nitrogen isotope analysis (AA-CSIA) to circumvent this issue.

Results from recent controlled feeding studies (McMahon et al. 2015, Hebert et al. 2016) indicate that birds exhibit similar amino acid-specific $\delta^{15}N$ patterns as those reported for other taxa (McClelland and Montoya 2002; Popp et al. 2007; Chikaraishi et al. 2009). In light of these results, we apply the AA-CSIA approach to generate baseline and trophic level $\delta^{15}N$ values using eggs from 5 seabird species collected over 13 sites and 15 degrees of latitude. Trophic position estimates are generated and subsequently utilized to remove the influence of food web position on bird contaminant levels. These trophic position-adjusted contaminant levels are more suitable for deducing the factors, e.g. proximity to sources, ecosystem attributes, landscape-scale influences, that regulate spatial differences in contaminant levels in wildlife. The AA-CSIA approach continues to open new research avenues in many fields of ecology, including ecotoxicology.

Temporal variability in trophic and foraging ecology of North Atlantic leatherback turtles based on compound-specific isotope analysis of amino acids

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The evaluation of long-term trends in trophic and foraging ecology is essential for effective management of highly migratory, endangered species. We used bulk and compound-specific isotope analysis (CSIA-AA) on a time series of leatherback turtle (Dermochelys coriacea) blood samples from St. Croix, US Virgin Islands to evaluate interannual variability in diet and trophic ecology. Leatherbacks are a vulnerable species composed of several populations throughout the world's oceans. Contrary to the precipitously-declining Pacific populations, the North Atlantic population has been steadily increasing since the 1990s. Although much of this can be attributed to nest protection, the effects of oceanography on foraging ecology has remained elusive. Our bulk stable isotope analysis of 201 samples from 1992-2010 indicated an overall decrease in $\delta^{15}N$ values over time. These results could be indicative of changes in leatherback trophic status, or of physical forcing, which alters $\delta^{15}N$ values at the base of the food web, and subsequently propagates up to consumers. We found an inverse relationship between bulk $\delta^{15}N$ values and the Atlantic Multidecadal Oscillation, suggesting that isotope values may be driven by large scale physical ocean-atmosphere processes. We are using CSIA-AA to determine the extent to which physical forcing and biogeochemical cycling and diet fluctuations are driving the observed variability in $\delta^{15}N$ values. Preliminary results indicate that leatherback trophic position has remained stable, and the decrease in bulk $\delta^{15}N$ values was due to physical forcing and biogeochemical cycling. In addition to trophic status, our results contribute to a better understanding of leatherback foraging strategies. The $\delta^{15}N$ values of source amino acids, which are used as representatives of baseline $\delta^{15}N$ values, were highly variable, indicating that leatherbacks nesting at St. Croix may be originating from multiple foraging areas. Ongoing analyses will continue to elucidate the mechanisms driving the variability in bulk $\delta^{15}N$ values in leatherback tissues, which will provide crucial information about diet, foraging, and potentially migration of an endangered species.

Estimation of freshwater fish consumption by compound-specific isotopic analysis of amino acids for Neolithic Hunter-Gatherer and Farmer in Near East

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For reconstruction of freshwater fish consumption by prehistoric human, we have applied the nitrogen isotopic compositions of individual amino acids in addition to bulk isotopic analysis of collagen from archeological bones. In this study, we analyze humans and fauna from Hasankeyf Höyük, an early Neolithic hunter-gatherer site and Tell Ain el-Kerkh, a late Neolithic farmer site. The interest here is the potential use of freshwater foods, as well as terrestrial resources, by Neolithic community in Near East. In the result, it was difficult to distinguish the contributions of freshwater and terrestrial resources to human diets by the conventional nitrogen and carbon isotopic compositions of bulk collagen, because they were estimated to have consumed mainly terrestrial resource, not only freshwater resources. By contrast, we observed that the nitrogen isotopic compositions of amino acids, especially for glutamic acid and phenylalanine, are useful for distinguishing the contribution of freshwater resources to terrestrial resources in human diet at these sites. The results suggest that a part of individuals at Hasankeyf Höyük seem to have consumed a significant amount of aquatic (presumably freshwater fish) resources, whereas the diets of inhabitants at Tell Ain el-Kerkh suggest dependence on the terrestrial resources among the members. However, these humans were unobservable to have the different diets only by carbon and nitrogen isotopic compositions of bulk collagen. By isotopic compositions of bulk collagen, even if the individuals show similar values, the nitrogen isotopic composition of amino acids of each individual can be different according to the contributions of several food resources. The isotopic analysis of individual amino acids is able to isolate consumers with the combined diet of freshwater and terrestrial resources from those depending solely on terrestrial resources, not just only a consumer exclusively on freshwater resources.

New insight into lake food webs from bulk radiocarbon and stable isotope fingerprinting of amino acids

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Determining whether aquatic food webs are fueled by autochthonous and allochthonous resources is important for assessing ecosystem changes. Reaching a consensus on this important question is difficult because different methods often yield contrasting results. We tested a novel combination of dietary proxies, radiocarbon (14C) and stable isotope fingerprinting of amino acids, to determine the importance of different basal resources in a highly eutrofied lake, Lake Schwerin in northern Germany. Radiocarbon has the advantage of being a pure source signal with a much greater dynamic range than bulk stable isotopes allowing for greater differentiation between dietary sources.

Fish (eel, roach and bream) Δ14C values were highly variable (-110 to -31) and tightly correlated with bulk $\delta^{13}C$ values ($R^2 = 0.72$, n = 20), but not with sampling season or fish size. For example, bulk $\delta^{13}C$ values of eels caught in October ranged from -27 to -14‰ (n = 6). To test whether fish with lower 14C concentrations were supported by terrestrial or bacterial resources, we applied isotope fingerprinting. This method can accurately distinguish between algal, bacterial and plant derived essential amino acids (EAA) in spite of highly variable baseline values. We found that algal derived EAA were the most important resource for fish regardless of 14C concentration. This result indicates that neither bacterial nor terrestrial food channels were important in spite of sediment trap samples from late fall and early spring being transitional between algal and plant derived EAA. Thus, bacterial reworking of particulate organic matter deposited on surface sediments does not provide a satisfactory explanation for the depleted fish Δ14C values. Instead, the most likely explanation for this observation is that DIC sources for autochthonous production varies with season and water source resulting in variable Δ14C and $\delta^{13}C$ values among consumers. In conclusion, we find with this novel combination of dietary proxies that autochthonous production makes the most important contribution to this particular food web in spite of different feeding preferences among fish specimens.

Contrasting differences in cetacean trophic position estimates derived from amino-acid specific δ^{15}N values and stomach contents

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Compound specific stable isotope analysis of amino acids (AA-CSIA) offers strong potential for determining consumer trophic position (TP). The δ^{15}N values of 'source' amino acids, whose amine bonds are not broken during typical metabolism, retain primar'y producer δ^{15}N values, while 'trophic' amino acids undergo trophic enrichment associated with animal diet. While AA-CSIA allows for separation of trophic and baseline influences on consumer δ^{15}N values, recent studies have shown the difference between δ^{15}N values of trophic and source AA in higher marine consumers is lower than predicted from empirical studies of zooplankton and fish. We compared source and trophic AA δ^{15}N values of multiple tissues (skin, baleen, and dentine) from four cetacean species representing a range of trophic positions: zooplankton specialist bowhead whales (Balaena mysticetus), common dolphins (Delphinus delphis), sperm whales (Physeter macrocephalus), and marine mammal-eating killer whales (Orcinus orca). TP was estimated using different empirically-derived equations and trophic enrichment factors (TEF). Estimates derived from the most commonly applied trophic-source AA pairing, glutamic acid (Glu) and phenylalanine (Phe), were 1-2 trophic steps lower than stomach content-derived TP estimates for all cetacean species. Further, application of a small TEF determined empirically in a marine mammal also resulted in unrealistically low TP estimates. However, the difference between Glu and Phe 15N values ($15N_{Glu-Phe}$) followed the TP order of the four species: bowhead whales had the lowest values (12.6 ‰), while killer whales had the highest values (15.0 ‰). Our results confirm that, even in the absence of accurate TEF values and TP estimating equations for cetaceans, AA-CSIA can be reliably used in assessments of trophic position using 15NGlu-Phe. Important ecological applications include retrospective analyses of structures like teeth, when baseline SI shifts cannot be independently measured over the timeframe of tissue growth, and when migratory species integrate different regional baseline SI values that can confound trophic interpretations of bulk SI values.

Embracing variability in amino acid $\delta^{15}N$ fractionation: Mechanisms, implications, and applications for trophic ecology

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Compound-specific stable isotope analysis (CSIA) of individual amino acids (AAs) has become a powerful analytical tool in trophic ecology. Differential 15N enrichment of individual AAs with trophic transfer is at the heart of the CSIA approach. Heavily fractionating 'trophic' AAs (e.g., glutamic acid: Glu) provide a robust indicator of trophic transfer, while minimally fractionating 'source' AAs (e.g., phenylalanine: Phe) closely reflect the $\delta^{15}N$ value at the base of the food web ($\delta^{15}N$baseline). Together, the CSIA approach provides an unprecedented ability to deconvolve the influences of $\delta^{15}N_{baseline}$ values and trophic fractionation on consumer nitrogen isotope values. Perhaps the most important assumption underlying CSIA applications to trophic ecology is that trophic fractionation of Glu and Phe, and thus the trophic discrimination factor $TDF_{Glu-Phe}$ ($\delta^{15}N_{Glu} - \delta^{15}N_{Phe}$) is effectively constant. We conducted a comprehensive meta-analysis of controlled feeding experiments, including 274 individuals across 56 species and 81 distinct species-diet combinations, revealing substantial, patterned variability in $TDF_{Glu-Phe}$ values driven by two dominant variables: diet quality and mode of nitrogen excretion. Consumers feeding on high quality diets (small diet-consumer AA imbalances) tend to have significantly lower $TDF_{Glu-Phe}$ values than consumers feeding on low quality diets. Similarly, urea/uric-acid producing consumers also exhibit significantly lower $TDF_{Glu-Phe}$ values than their ammonia-producing counterparts. There are several notable exceptions, including the remarkably consistent $TDF_{Glu-Phe}$ values reported in insects, despite highly variable Glu and Phe $\delta^{15}N$ values, and isotopically "invisible" trophic transfers in microbial food webs. These areas of active research may hold keys to understanding biochemical details of AA fractionation. We argue that to realize the full potential of CSIA approaches in trophic ecology, we must now embrace the variability in $TDF_{Glu-Phe}$ values. This likely requires developing new models of trophic transfer dynamics for some applications, including multi-$TDF_{Glu-Phe}$ equations that directly incorporate variability in $TDF_{Glu-Phe}$ value.

Nutrition sources of deep-sea meiofauna at hydrothermal vent and adjacent non-vent areas revealed by stable carbon and nitrogen isotope ratios and natural radiocarbon abundances

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Deep-sea hydrothermal vents host unique marine ecosystems which largely rely on organic matters produced by chemolithoautotrophic microbes. The dependences on such autochthonous organic matter varies between organisms; some megabenthos exclusively gain their nutrition from their autotrophic symbiont, while some megabenthos mainly rely on phytodetritus derived from the ocean surface. Although there are abundant meiofauna at the hydrothermal vent field, studies on their nutritional sources are still limited due to their small body sizes. In this study, we investigated dietary sources of meiofauna at hydrothermal vent fields of three submarine volcanos using stable carbon and nitrogen isotope ratios (13C, 15N) and natural radiocarbon abundances (14C) to evaluate how much do the hydrothermal vent meiofauna gain their nutrition from the chemolithoautotrophic microbes. Bacterial mats of the hydrothermal vent chimney typically exhibited heavy 13C values (up to -10‰) and depleted 14C values (~600‰). The 13C and 14C values of Dirivultidae, an endemic copepod family inhabiting hydrothermal vent chimney, exhibited similar values to the bacterial mat but distinct from those of sediments at surrounding area or water column plankton, suggesting that they exclusively rely on bacterial mat at the vent chimney. To the contrary, 13C values of nematodes at vent chimneys were -26.6 and 23.2‰, which were similar ranges to those at non-vent sites, suggesting vent nematodes did not gain their nutrition from the chemolithoautotrophic microbes. Those nutritional facts obtained from isotopic compositions are consistent to the distributional patterns of these meiofauna. Our results demonstrated that the combination of stable and radioisotope analyses on organisms serves valuable information on their nutrition and hence their adaptive ecology to hydrothermal vent area.

Uncertainty in isotopic trophic biomarker estimates: intra-individual variability in amino acid carbon and nitrogen isotopic values

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Isotopic values of body proteins have been used to investigate individuals' dietary intake across a range of fields, particularly archaeology and (palaeo)ecology. Analyses of bulk tissues have proved informative, yet compound-specific work at the amino acid level potentially offers even greater precision, particularly using nitrogen isotopic values as a trophic level marker.

Work has shown that various factors influence the isotopic values of each amino acid, and the degree to which their isotopic values change with increasing tropic level (the trophic enrichment factor or TEF), particularly at the inter-species level. So far, less attention has been paid to intra-individual factors, although intra-individual variability has been observed in different bulk tissue and amino acid isotopic values (e.g. Gaebler et al. 1966).

Here we present bulk and compound-specific carbon and nitrogen isotopic data of multiple tissues from four model species raised long-term on isotopically controlled diets. We show that there are significant intra-individual isotopic differences between collagens extracted from different tissues (skin, heart, muscle, bone) in both carbon and nitrogen. The isotopic ranges observed for each species and tissue are variable, yet there was some consistency of patterning. Similarly, we show that there is considerable variability in isotopic values of different amino acids across different tissues within an individual, for all four species.

We explore the potential implications of these observations for isotopic trophic biomarker estimates, as well as for insights into the underlying mechanisms by which such isotopic variation occurs.

Gaebler et al. 1966, Can. J. Biochem 44:1249-57.

Compound-specific nitrogen isotopic composition of chloropigments as a tool to access the nitrogen cycle in the ocean

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Compound-specific isotope analysis (CSIA) provides a clear and ""to the point"" research method, to understand chemical and/or biological processes in the complex nature. Acquiring isotope information of a certain compound, which is directly involved in the targeted process, made the CSIA to be a powerful tool in the field. In this study, nitrogen isotopic composition of chloropigments was measured to reconstructing the nitrogen cycle in photic zone. The particulate matter (PM) and water samples were collected from photic zone of western North Pacific (Station K2, 47˚00'N, 160˚00'E) during the summer blooms. Chloropigments (chlorophyll a and pheophytin a) were extracted from PM and purified by high-performance liquid chromatography with fraction collector. Nitrogen isotopic compositions (δ^{15}N) of chloropigments (δ^{15}Nchl) were determined by a sensitivity-improved EA-IRMS, which was capable of determining δ^{15}N of as small sample as 1.5 nano molar of chloropigments. The δ^{15}N of nitrate (δ^{15}NNO3) was determined by GC-IRMS.

The observed δ^{15}Nchl and δ^{15}NNO3 values were -10.5~+1.9‰ and +6.6~+9.9‰, respectively, With the δ^{15}N of phytoplankton cells (δ^{15}Nphy, -5.7~+1.0‰) estimated from δ^{15}Nchl, up to 8.3‰ of isotopic difference between phytoplankton and nitrate was estimated. The difference suggested either larger isotopic fractionation during nitrate assimilation by phytoplankton, or the significant contribution of inorganic nitrogen other than nitrate, such as ammonia, as a substrate for the photosynthetic nitrogen assimilation. The possibilities were evaluated by a nitrogen isotope model based on δ^{15}Nphy and δ^{15}NNO3 data. Considering the evaluation and relating data, it was concluded that the large (>50%) contribution of ammonia assimilation was the case. Since chlorophylls are also buried and preserved in the sediment trap samples and sediments for long, this method provides potentials to reconstructive studies of nitrogen cycle in the past environments, as well.

Applying Amino Acid-Specific Stable Isotope Analysis to Examine Facultative Herbivory in a Marine Fish, *Lagodon rhomboides*

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Marine herbivory is a widespread, specialized feeding ecology thought to be evolutionarily advanced in fishes. Most herbivorous fishes are obligate herbivores, but facultative herbivory has also been reported in a range of species, including the pinfish, Lagodon rhomboides, from the southeastern United States. It is unclear, however, to what degree these facultative herbivores derive nutrition directly from plants or macroalgae. In this study, we applied bulk and amino acid-specific stable isotope analysis (AA-SIA) of C and N to examine whether pinfish assimilated carbon directly, or even indirectly, from a range of primary producers sampled in two coastal estuaries of the northern Gulf of Mexico. Pinfish (n = 20) age was determined by counting opaque zones in sectioned otoliths, and ranged from 0 to 3 years. Fish white muscle tissue and primary producers were dried and pulverized prior to SIA. Measurement of bulk δ^{13}C and δ^{15}N values revealed it was unlikely that sampled fish assimilated C directly from either plants or macroalgae, given muscle bulk δ^{15}N values were 6-11 ‰, or two to three trophic levels, higher than δ^{15}N of algae or seagrass. Non-essential versus essential AA-specific δ^{15}N (i.e., δ^{15}Nglu versus δ^{15}Nphe) confirmed that pinfish fed at least one trophic level higher than direct grazing on either plants or algae. Estimating the basal members of pinfish food webs in each study estuary proved to be problematic with bulk C isotope analysis due to trophic fractionation and the diversity of primary producers that exist in each system. However, results from AA-specific δ^{13}C and δ^{15}N analysis clearly indicated that 100% of pinfish C was derived from algal, not plant, sources. Therefore, not only did pinfish not consume seagrass directly, but 100% of their diet was ultimately derived from non-seagrass basal sources. The combination of bulk and AA-specific SIA proved to be a powerful approach to examine pinfish trophic ecology, and could be applied to examine facultative herbivory in pinfish, or other purported facultative herbivores, more broadly.

Nitrogen and carbon isotope values of individual amino acids in bowhead whales (*Balaena mysticetus*) in Disko Bay, Western Greenland

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Climate change is causing physical and biotic changes in the arctic marine environment. Among the various changes faced by bowhead whales, the only mysticete endemic to the Arctic, are the reduction of sea ice and increased competition for food and habitat by non-endemic species of baleen whales that are expanding their range to the north. Compound-specific stable isotope analysis (CSIA) in amino acids (AA) is increasingly being recognized as a prevailing tool in ecological studies providing key information on food web structure, energetic pathway and ecosystem biogeochemistry. Here, bulk and amino acids carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope ratios were analysed in bowhead whale skin samples (2007-2013) from Disko Bay, West Greenland. Bulk $\delta^{15}N$ signatures were consistent across the seven years of sampling and were similar between sex classes. Bulk $\delta^{13}C$ and average essential AAs $\delta^{13}C$ values displayed a small but significant temporal decreased between 2008 and 2013. A significant positive linear relationship was found between bulk $\delta^{13}C$ and essential-AA $\delta^{13}C$ signatures. This correlation suggest that some of the observed isotopic variation in bowhead whale between years reflect some changes in the carbon baseline values (producers). There were no correlations found between bulk SI or CSIA of carbon and nitrogen with sea ice concentrations. Results from our study indicate that the trophic position of bowhead whales was stable over time despite large inter-annual variability in sea-ice cover. Our study suggests that the novel approach of CSIA in amino acids can be effectively used to reconstruct temporal trend in bowhead whale food web structure.

Midwater Zooplankton Response to Seasonality in Export Flux in the North Pacific Subtropical Gyre

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The ocean's deep midwaters (~400-1500 m) constitute the largest living space on the planet and are home to diverse communities of microbes and animals that are generally poorly described. Despite the importance of the mesopelagic food web, our meager understanding of this system has recently been highlighted by our inability to balance the input of food (acknowledged to be principally sinking particles) with carbon demand (estimates of respiration). Carbon demand exceeds supply by as much as 2-3 orders of magnitude. Measurement problems exist but it is also clear that alternative food resources for the mesopelagic food web must be evaluated. In particular, slowly settling or small suspended particles may comprise a substantial component of the missing carbon supply. Zooplankton in midwaters can play a particularly significant role in export fluxes to the deep ocean, yet their dynamics also remain poorly understood. We sampled particles and zooplankton throughout the upper 1500 m at Station ALOHA in the North Pacific Subtropical Gyre in 2014 at times when 234Th normalized particulate nitrogen export flux varied by a factor of ~4, with higher fluxes observed in the late summer. Using amino acid compound-specific stable isotope analysis, we find that the midwater zooplankton community responds rapidly to this summer export event. 'Source' amino acid $\delta^{15}N$ values for mesopelagic and upper bathypelagic zooplankton are lower in late summer than in winter, indicating a summer food web more reliant on large rapidly sinking labile, surface-derived N. In contrast, we find that refractory midwater small suspended particles become a more important food resource for the midwater zooplankton community in winter. This seasonality is primarily observed in the larger (1.0 – 2.0 mm) zooplankton size fraction and in target taxa such as small copepods and chaetognaths. The implications of these seasonal trends on midwater food sources and controls on the $\delta^{15}N$ values of sedimentary organic matter will be discussed.

Compound Specific Amino Acid Nitrogen Isotopes Elucidate Key Primary Producers and Trophic Position of an Elasmobranch in a Seagrass Dominated System, Shark Bay, Australia

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The brown bamboo shark (*Chiloscyllium punctatum*) is a small elasmobranch found in tropical shallow waters of the Indo West Pacific. In Shark Bay (Western Australia) brown bamboo sharks inhabit seagrass beds dominated by *Amphibolis antarctica* (Walker et al. 1988). Knowledge of an organism's trophic position (TP) is one of the most fundamental concepts in ecology as it facilitates the detection of changes in food web structure (Hairston & Hairston 1993, Branch et al. 2010). The increasing need to understand long term changes in trophic ecology requires estimates of TP that are as accurate as possible but also require an understanding of which primary producers are key to the food web of interest. In this study, bulk SIA was initially used to investigate the TP of the brown bamboo shark. Results from bulk SIA were inconclusive leading to the use of compound specific nitrogen isotopes in individual amino acids to more accurately elucidate TP. In addition, this technique allowed further investigation of the assumed importance of seagrass to the food web but analyses indicated that other epiphytic algae were likely to be more important. Thus, compound specific amino acid techniques allowed for both a more accurate TP estimates for the brown bamboo shark as well as a better understanding of trophic interactions in Shark Bay.

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Combining molecular and isotopic tools to study the trophic ecology of exotic species and their biological control by native predators

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Exotic species are mostly introduced without their coevolved predators, parasites or pathogens. Identifying top-down controls on these invaders by native predators in the recipient ecosystem is a key issue for regulating the invasive process.

Trophic interactions between a harmful invader in France, the red swamp crayfish *Procambarus clarkii*, and native fauna were studied under varied temporary and permanent freshwater habitats. C and N stable isotope analyses were run on samples to describe energy pathway through food webs, in combination with environmental DNA barcoding on gut contents to identify native aquatic animals preying on crayfish.

C in crayfish muscles mostly originates from the algal trophic pathway in seasonally flooded habitats (meadows), whereas detrital pathway is prevalent in permanent waterbodies (canals, reedbeds and ponds). Trophic levels of crayfish ranged 2.2 to 3.1 on average, depending on sites. Omnivory of crayfish was confirmed by the presence of animal DNA in their gut but at low occurrence (<10%) as compared to plants.

Isotopic models reveal that for most fish species, crayfish is the dominant food source over other benthic invertebrate species and zooplankton in meadows, reedbeds and canals. Fragments of crayfish DNA were found in the gut of almost all fish species. Remarkably, molecular analyses on the gut of aquatic invertebrates predators in ponds also show evidence of crayfish consumption.

Our study highlights some facets of interest for combining molecular and multi-isotopic tools. Low occurrence of animal DNA fragments in the gut contrasts with the relatively high trophic level of crayfish obtained from stable isotope models, suggesting that even slight portion of animal food in the invader's diet is favourably assimilated. The invasive crayfish appears to be the major food resource for fish, and it has become a key component of the current detrital trophic pathway in the invaded habitats. Finally, unexpected consumption of crayfish by native invertebrate predators underlines their possible role of controlling the invader in habitats where the conservation of remarkable aquatic species is an issue.

Unpacking 'brown' food webs: Animal trophic identity reflects rampant microbivory

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Detritivory is the dominant trophic paradigm of terrestrial ecosystems, yet accurate empirical measurement of consumer trophic position within 'brown' (= detrital) food webs has remained relatively impenetrable. Measurement of detritivore trophic position is complicated by the fact that detritus is suffused with microbial consumers and thus is actually a complex of both living and non-living biomass. For animals consuming a detrital complex, pure detritivory (consumption of only non-living material) should be exquisitely rare, perhaps nonexistent in nature. Given that microbes and animals are trophically interchangeable within a food-chain, a decomposing detrital mass should represent an elevated, non-integer trophic group. When animals consume such detritus, the animals effectively ingest multiple trophic groups (a food web microcosm). Animal trophic identity, therefore, should elevate predictably as a function of the community structure within the detritus. Here, we address this hypothesis using compound-specific isotopic analysis of amino acids, revealing the mechanisms underlying animal trophic position inflation within detritivore communities.

4.3 Session 3

New insights into ecophysiology, microbial ecology and biogeochemical cycling

Understanding the mechanisms driving variation in oxygen isotopes in tree rings in the Amazon Basin

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The water and carbon cycles are closely coupled in the Amazon Basin, so perturbations in one system can have significant consequences for the other, particularly important given the scale of the Amazon region in terms of global biogeochemical cycling. Recent evidence suggests that the hydrology of the region has been changing since approximately 1990, yet the reasons for this remain unclear. Oxygen isotopes can possibly shed some light on this as they are tightly related to the hydrological cycle. Oxygen isotope ratios in tree ring cellulose (δ18OTR) from a single site are a proven record of precipitation δ18O, and correlate strongly with precipitation over the entire Basin. Spatial coherence between δ18OTR records several hundreds of kilometers apart, as well as GNIP and ice-core δ18O series, provide further support for a large-scale control on the isotopic signal. It has been proposed that rainout of heavy isotopes during moisture transport across the Basin is the predominant control on the isotopic composition of precipitation, however, the mechanisms behind these large-scale relationships are not yet fully understood. Here we present a new δ18OTR chronology from nine Cedrela odorata trees from northern Bolivia which spans 146 years (1865-2010) and use novel techniques to explore this question further. A Lagrangian atmospheric transport model was used to calculate back trajectories for the period 1980-2010 to relate δ18OTR to air mass history in an attempt to resolve the relative contributions of Rayleigh rainout and evaporative recycling. In addition, an isotope enabled General Circulation Model (HadCM3) was used to investigate the impact of deforestation on the δ18OTR signal. Overall, our record reflects a strong signature of change in the Amazon climate system over the last one to two decades, consistent with an intensification of the hydrological cycle.

Nitrogen isotopic compositions of liver amino acids reveal insights into discrimination

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Despite a recent burst of research activity focused on compound specific isotopic analysis of nitrogen in amino acids (AA-CSIA), few studies have focused on controlled feeding experiments that examine the physiological causes of observed patterns. In order to fully exploit this cutting edge approach in ecology and eco-physiology, more of such studies are needed to better define its capabilities and limitations. Amino acid (AA) nitrogen ($\delta^{15}N$) isotope values yield different patterns than carbon ($\delta^{13}C$) because carbon groupings (essential vs non-essential) are related to an organism's ability to synthesize the carbon skeleton of the amino acid, while nitrogen groups (source vs trophic) are associated with trans- and deamination processes with many exceptions. We measured AA $\delta^{15}N$ values of liver tissue collected from mammals, birds, and fish in both wild and captive settings to examine distribution and discrimination from diet. A critical organ in metabolism, isotopic data from the liver reveals information about the synthesis of AAs and the processes contributing to nitrogen discrimination. While patterns of source and trophic amino acid groupings emerge, absolute values of all AAs vary between liver and muscle. As $\delta^{15}N$ trophic and source distinctions are based upon distributions measured in muscle tissue and whole body samples, tissue-specific isotopic discrimination in AAs indicates that attributing such classifications to trans- and deamination processes, most of which happen in the liver, may be in error. The amino acids phenylalanine (Phe) and lysine (Lys) are of particular interest as they are essential AAs and fall into the source ($\delta^{15}N$) classification. We couple these data with analyses from AA-CSIA of carbon and hydrogen to further elucidate patterns of synthesis and metabolism. Amino acid $\delta^{13}C$ data reveals clear evidence of amino acid synthesis by the microbial gut community, bringing into question the true nature of the "source" amino acid. The complete use of AA-CSIA in ecological studies to trace nutrients through communities and ecosystems will rely upon our ability to clearly classify the physiological drivers of amino acid isotopic discrimination.

Coupling SIBER analysis and isotope tracer experiments reveals variation in coral-algal symbioses

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The symbiosis between corals and Symbiodinium algae has allowed corals to thrive and diversify in oligotrophic reef habitats, helping to fuel the high productivity and diversity observed on reefs. The full extent of this symbiosis, however, remains poorly understood. Both the type of nutrients shared and direction of sharing (host to symbiont or symbiont to host) varies across species and environmental conditions. Recently, it has been speculated that the interaction between corals and Symbiodinium can range from mutualistic symbiosis to parasitism. We used a combination of natural abundance stable isotope analysis and tracer experiments to investigate how this relationship varies across 6 coral genera in Hong Kong. We used SIBER (Stable Isotope Bayesian Ellipses in R) analysis of natural abundance isotope data to compare the isotopic niche placement and area of each host and symbiont pair. Our results support the hypothesis that the relationship between coral hosts and Symbiodinium varies across coral species: in some genera (Acropora and Goniopora) the host and symbiont had nearly 100% overlap of their isotopic niches suggesting both C and N sources shared, while in others (Platygyra and Favia/Favites) there was no overlap, suggesting only C sources are shared. Finally, some genera have partial overlap suggesting a flexible symbiosis. To further investigate how carbon and nitrogen are obtained and shared by these two organisms, we conducted a stable isotope tracer experiment exposing each coral genus to seawater enriched with labeled NaH13CO3 and 15NO3-. These data confirm that there is variation across genera in the extent to which inorganic carbon and nitrogen sources are shared between host and symbiont.

Does the isotopic niche represent consumer trophic niche?

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A number of powerful tools have been recently developed to characterise and compare consumer isotopic niches (e.g. the so-called Layman statistics, SIBER). These indices are easy to calculate have become almost standard in studies of trophic ecology, and now challenge more traditional approaches, e.g. stomach content analysis. Although the isotopic niche reflects variation in a number of different, and potentially co-varying ecological and environmental influences, it is increasingly assumed to provide a robust measure of the trophic niche of a population of consumers (including by the authors of this abstract). However, to our knowledge, this assumption has not been empirically examined across species/habitats.

We compared measures of population trophic niche based on the analysis of stomach contents with various measures of the population isotopic niche estimated from δ^{15}N-δ^{13}C values from fishes and lizards inhabiting marine and freshwater habitats from Europe and Latin America. Least squares linear regression showed no evidence for a relationship between measures of niche width (e.g. SEAc v Levins B': $R^2 = 0.02$, $P = 0.2$). Hence, the assumption that the isotopic niche provides a useful indicator of population trophic niche is not supported.

Here, we extend the analysis to include more species and locations and examine the reasons for the lack of relationship between the measures of isotopic and trophic niches (e.g. consumption v assimilation, diet-switching, primary productivity, baseline isotopic variation).

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Early life sexual segregation: ontogeny of isotopic niche differentiation in the Antarctic fur seal

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Investigating the ontogeny of niche differentiation enables to determine at which life-stages sexual segregation arises, providing insights into the main factors shaping segregation at the individual and sexual levels. For instance, in dimorphic species, does niche divergence mirror size divergence? Does sexual segregation arise only when individuals reach sexual maturity? We investigated the ontogeny of the foraging ecology in Antarctic fur seals (*Arctocephalus gazella*), a highly dimorphic species with contrasting breeding strategies between sexes. The sequential 13C and 15N values of whiskers provided a longitudinal proxy of the foraging niche throughout the whole life of seals, from weaning when size dimorphism is minimal (<15%) to the age of 5 when seals have reached sexual maturity. Lactation was characterised by high 15N but not 13C values in all individuals, followed by an abrupt drop of 13C and 15N values. Females exhibited an early-life ontogenetic shift, from a total segregation during their first year at-sea, to a similar isotopic niche as breeding females as early as age 2. Variation in isotopic niche occurred exclusively along the 13C axis, indicating weaned pups fed at a similar trophic level as adults. In contrast, males showed a progressive change in isotopic niche throughout their development such that 5-year-old males did not share yet the same niche as territorial bulls. Interestingly, males and females segregated straight after weaning with males feeding in southern habitats than females. Spatial segregation was of similar amplitude as observed in breeding adults and was maintained throughout development. Such early-life niche differentiation is an unusual pattern and indicates size dimorphism and breeding constraints do not directly drive sexual segregation contrary to what has previously been assumed in otariid seals.

Nitrate and zooplankton stable isotopes together with plankton taxonomy reveal the importance of nitrogen fixation by *Trichodesmium* for the biogeochemistry of the Red Sea

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Traditionally the name Red Sea connotes to the coloration of surface waters caused by aggregations of the N-fixing cyanobacterium Trichodesmium, which typically strives in stratified and N nutrient depleted seas. The spatial and seasonal variation in the availability of N in the open Red Sea can be attributed primarily to oceanographic, atmospheric and biological processes. These processes include the monsoon-related intrusion of nutrient-rich Indian Ocean water in winter, and biological fixation of atmospheric N2. Considering the significance of diazotroph microbes at a global N scale, *Trichodesmium* and diazotroph-eukaryote symbiosis were, therefore, tentatively used to explain the positive net export of N toward the Gulf of Aden. Despite the anecdotal perception of the importance of diazotrophs for the Red Sea N cycle, empirical evidence is yet limited. The biogeochemistry of the central Red Sea was assessed during 2 multidisciplinary research cruises (at the end of the stratified period, October 2014, and during spring, April 2015). It was hypothesized that the abundance of *Trichodesmium* varies over season and manifests in changing geographic patterns of δ^{15}N values. Remote sensing and towed undulating Scanfish CTD surveys provided insight into oceanographic features, such as mesoscale eddies and upwelling, and were used for the selection of sampling sites. Assessing N and O stable isotopes of dissolved nitrate, nutrients, C and N isotopes of particulate organic matter (POM) and zooplankton, phyto- and zooplankton taxonomy, this study provides original insight into the N inventory of the Red Sea. Vertical profiles revealed low NO3-δ^{15}N throughout the Red Sea (range 1.5-3.5‰) and these are significantly lower than typically measured elsewhere. Stratification led to 10-fold increased counts of *Trichodesmium* and microzooplankton when compared to the spring survey. Across all size-fractions, zooplankton δ^{15}N values were approx. 1.7 ‰ higher in April than in October, concomitant to a shift from mostly negative to predominantly positive N' values. The large importance of N fixation by diazotrophs for the Red Sea N biogeochemistry and its food web are discussed.

Marine derived nutrient subsidies drive gigantism of endangered iguanas in the Bahamas

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Significant body size differences observed in insular vertebrates, namely tendencies toward gigantism or dwarfism, are known as "The Island Rule" and its universality and causality have long been debated. We examined four Cays in the Bahamas to study effects of seabird subsidies and invasive mice (*Mus musculus*) on island food webs and the body size and foraging ecology of endangered Allen Cays Rock Iguanas (*Cyclura cychlura inornata*). Iguanas on the cay with nesting Audubon's shearwaters (*Puffinus ihermineiri ihermineiri*) and invasive mice were 6 times heavier and nearly two times longer than those on other islands. We analyzed stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopes from iguanas and items representing all food web levels on each cay to test the predictions that 1) the food web on the seabird island would exhibit the influence of marine subsidies from seabird guano, whereas those from non-seabird cays would not, and 2) size differences in iguanas among cays were due to either a) supplemental food availability from mice and/or seabird carcasses, and/or b) access to more nutrient rich and denser vegetation as a result of regular fertilization of plants by seabird guano. Food web components from the island with seabirds had ~5 to 9‰ higher $\delta^{15}N$ values than those on the other cays. In addition, plants on the seabird island had 1.6 to 1.9 times more nitrogen in foliar tissues than those on the other cays, indicating seabird guano increased plant nutrients. The $\delta^{13}C$ and $\delta^{15}N$ values from iguana tissues and their potential diet items indicated iguanas on all cays ate primarily C3 plants and there was no evidence that iguanas consumed significant amounts of animal matter. Iguanas were moved from the seabird/mice cay to another island for one to two years during an invasive mice eradication and most starved to death presumably as a result of inadequate nutrient availability to support their body size. Our results underscore the importance of marine subsidies as ecosystem drivers on islands and serve as a cautionary tale for conservation biologists working to restore islands and protect native species from the effects of invaders.

Facultative paedomorphosis, a mechanism promoting niche differentiation in newts: Evidence from stable isotope analysis

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Facultative paedomorphosis is a heterochrony that allows a part of the individuals in a population to retain larval traits at the adult stage. In species with complex life cycles such as amphibians, this process can lead to radical differences in the morphology of adults without the need of extensive genetic modifications. In newts, paedomorphs retain external gills at the adult stage, allowing for a fully aquatic life, while metamorphs undergo complete metamorphosis, adapted for a terrestrial life-stage. These morphological differences affect feeding mechanisms and microhabitat use during the aquatic life stage of newts. Such process raises important questions concerning its adaptive and evolutionary implications. One hypothesis is that facultative paedomorphosis may be maintained in some populations as a trophic polymorphism, with the consequence to lessen intraspecific competition in environments devoid of competitors. We explored this hypothesis in facultatively paedomorphic populations of two species occurring in contrasting environments: Ichthyosaura alpestris, inhabiting deep alpine lakes and Lissotriton helveticus, inhabiting small permanent ponds. We determined the trophic niche and regime of the alternative morphs using SIBER and SIAR mixing model on carbon and nitrogen stable isotopes. Our results show that paedomorphs and metamorphs occupied different trophic niches in both species and environments. Interestingly, we observed different patterns of niche differentiation between morphs and sexes that could be linked to differences in food resources and microhabitat use. Our results support the role of facultative paedomorphosis as a trophic polymorphism promoting niche differentiation in newts.

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Microbial utilization of 13C-labelled substrates in boreal forest soils

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Boreal forest soils are the single largest terrestrial carbon storehouse in the world. Consequently, changes in boreal carbon stocks and fluxes could significantly affect the global carbon cycle. These northern, high-latitude forest soils are also highly susceptible to global warming, and in the coming century are expected to face large increases in temperature and transformative vegetation shifts. Northward migration of the entire boreal biome is predicted and, within the main boreal forest, deciduous trees will replace evergreens. The main objective of our research is to assess how this vegetation shift may affect the overall storage and stability of boreal soil carbon.

Our work focuses on the boreal mixedwood landscape of western Canada, which consists of a mosaic of deciduous and coniferous stands. Soils sampled under both aspen and spruce were incubated in the laboratory to follow how different 13C-labelled substrates (glucose, leaves and roots) were processed by the microbial communities present in the two soil types. Specifically, incubation of 13C-labelled aspen litter (leaves and roots) in spruce soils mimics the effects of future vegetation shifts. Carbon assimilation by microbes was tracked by isotope probing of phospholipid fatty acids, and evolved 13C-CO2 measurements allowed us to quantify the percent of CO2 coming from each added substrate.

The two soils maintained distinct microbial communities throughout the 67-day incubation. Both microbial communities rapidly incorporated and respired the labelled glucose, but the aspen community was more efficient in recycling the added 13C label. In addition, for aspen, the $\delta^{13}C$ values of CO2 were higher, and a greater part of the respired CO2 was derived from the added substrates (glucose, leaves, and roots) when compared to spruce. On the other hand, 13C enrichment following aspen litter addition was greater for the spruce microbial biomass. Hence, shifting from spruce to aspen in the boreal forest may increase microbe-driven carbon stabilization.

Foraging strategies utilized by the silky pocket mouse (*Perognathus flavus*) to maximize fitness in a seasonally variable, nutrient limited, desert ecosystem

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Desert ecosystems are characterized by high stochasticity in temperature and precipitation resulting in low and variable primary production. Organisms that survive in these environments must engage in a variety of foraging strategies to compensate for widely fluctuating resources. Optimal foraging theory states that organisms will forage on the most energetically available resources (C3), expanding their dietary niche when these are limited. We quantified the proportion of assimilated carbon derived from C3 versus C4 primary production in silky pocket mice (*Perognathus flavus*) plasma, examined isotopic variation both across the species and within individuals over time, and measured percent body fat, percent lean and total water in individuals as proxies for fitness. Based on optimal foraging theory, mice should specialize on energetically rich C3 resources when available, this is not what we found. We analyzed ~ 1,500 plasma samples from 60 individuals trapped 4 or more times over an 8 month period, and compared their foraging strategy to their body fat. We found that this generalist population is a mixture of both generalists (foraging across the landscape) and specialists (foraging on select items), and that specialists accrued more body fat than generalists, a potential survival mechanism. The population as a whole expands its foraging breadth (13C) from spring to fall, tracking resource availability; however when resources were limited, many individuals maintained their specialized foraging strategies, which could ultimately result in higher fitness.

Differences in changes in plasma $\delta^{15}N$ values in pinnipeds during natural fasting vs. starvation events.

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Many species of seals and sea lions (pinnipeds) undergo natural fasting during their annual life history cycle due to physical separation of their breeding and foraging habitats. During these natural fasting periods pinnipeds conserve energy and critical protein reserves efficiently through metabolic adaptations that decrease overall metabolic rate and enhance utilization of stored lipids for energy. These protein sparing adaptations result in consistent decreases in blood urea nitrogen (BUN) concentrations in the plasma within days of the initiation of fasting. When animals reach their limit of adaptive fasting they revert to catabolism of protein stores and starvation ensues. Plasma samples archived from previous studies of naturally fasting northern elephant seals (M.a.; *Mirounga angustirostris*, n=12) and grey seals (H.g.; *Halichoerus grypus*, n=4 fasting and n=14 starvation) and from studies of Steller sea lions (E.j.; *Eumetopias jubatus*, n=9) fasted in captivity were analyzed for $\delta^{13}C$ and $\delta^{15}N$ to investigate isotopic changes due to catabolic processes.

During natural post-weaning fasts of up to 86 days and 21 days respectively, M.a. and H.g. (phocid seals) exhibited minimal change in plasma $\delta^{15}N$ values (0.08 ± 0.01 and 0.04 ± 0.03 ‰ wk-1, respectively) compared to significantly higher rates in less fasting-adapted juvenile and subadult otariids (E.j. fasting up to 14 d; 0.28 ± 0.06 ‰ wk-1; $p = 0.016$). Weaned H.g. pups of normal mass showed significantly lower $\delta^{15}N$ values (15.68 ± 0.11 ‰) at the end of the 3-week post-weaning fast than did abandoned pups of unknown age when found emaciated (16.96 ± 0.81 ‰, $p < 0.001$). Finally, in emaciated H.g. pups there was a significant correlation between the plasma $\delta^{15}N$ and the plasma BUN measured in samples collected up to 21 days post-capture ($p < 0.0001$; $r^2 = 0.696$). These 3 lines of evidence suggest that the greatest changes in $\delta^{15}N$ values occur when animals mobilize primarily protein body reserves during times of food deprivation. When fasting-adapted pinniped species are able to spare protein by catabolizing lipid stores to maintain homeostasis during fasting, very little change in isotopic ratios occur.

Just how carnivorous are carnivorous plants? Investigating the dependence of carnivorous plants on heterotrophic nutrition using stable isotope techniques

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Background: True carnivorous plants (CPs) are able to attract, trap, and absorb nutrients from prey. Although numerous studies describe the biology and ecology of many carnivorous species, few in situ studies quantify the degree to which these plants are reliant upon heterotrophic nutrition (i.e. carnivory). To address this knowledge gap we apply modern isotopic methodology to some of the pioneering ecological concepts developed by Charles Darwin over a century ago.

Method: The nutrition of nearly all carnivorous genera will be assessed through international collaborations with numerous research institutions, with samples from genera including Drosera, Utricularia, Byblis, Cephalotus, Aldrovanda, Drosophyllum, and Nepenthes collected from natural populations. This presentation will focus particularly on the comparison between two morphologically similar but geographically and taxonomically distinct carnivorous species: Byblis gigantea (Fam. Byblidaceae, Or. Lamiales) from southwest Australia, and Drosophyllum lusitanicum (Fam. Drosophyllaceae, Or. Caryophyllales) from the western Mediterranean region.

To measure the relative importance of active (the capture and digestion of animal prey) and passive (root uptake) nutrition in these two species, an end-member mixing model will be used to compare their 15N/14N isotopic ratios with those of co-occurring non-carnivorous plants and prey insects from their natural habitats.

Results, Discussion & Significance: Results from this study will provide the first empirical evidence of the reliance upon carnivory in B. gigantea and D. lusitanicum, enhancing our understanding of the efficiency of prey capture for both species. This study will shed light on the role of carnivory as a convergent ecological driver in two Mediterranean-climate carnivorous plants, both of which occur in globally significant biodiversity hotspots. Empirically determining the reliance on carnivory in plant nutrition is important not only from an ecological perspective, but also in developing an understanding of the biotic relationships that these highly specialised – and often sensitive species – have with other organisms.

Unique isotope signature of large cyclonic events as a tracer of ecohydrologic processes in the arid subtropics

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The dynamics of ecological processes in arid environments are overwhelmingly driven by spatial and temporal availability of rainwater, groundwater and soil moisture. However, clear identification of the origin and ecological significance of different water sources for both stream and terrestrial ecosystems remains challenging. Here, we investigated patterns in water distributions across landscapes and within ecosystems in the Pilbara region of subtropical NW Australia by tracing the fate of infrequent but large-volume cyclonic precipitation events. We sampled water stable isotope compositions of rainfall of two large events (Heidi and Lua) and multiple smaller events between 2010 and 2015. Large events (>200 mm/day) have very negative but also highly variable spatial and temporal stable isotope signatures. The δ18O value progressively decrease from coast to central Pilbara. However, the δ18O value of inland rainfall also varied significantly at a single location. For example, at a site ~300 km from the coast, rainfall δ18O decreased from -13.7‰ to -19.3‰ over 5 hrs of rain event, resulting in floodwater δ18O of -13.4 to -16.5‰. These signatures are distinct from those of regional groundwater in alluvial aquifers (δ18O -8.0±0.8‰). Wetting fronts and downward movement of infiltrating floodwater were clearly discernible from shifts in soil moisture δ18O. For example, pre-cyclone soils at 4 m had δ18O values of -4.1‰ but were -16.1‰ at four weeks after flooding. However, the distribution of moisture in soil profiles was strongly influenced by landscape position and lithology, and varied considerably with clay and calcrete content. δ18O values at 4 m ranged between -1.0‰ at more upland sites to -6.0‰ at lower elevation sites across a 1.5 km transect. Soil stable isotope composition did not reflect moisture contents, which at 4 m depth were consistently at 13-17% v/v. Our study reveals considerable spatial and temporal complexity in determining the fate of rainwater in arid environments, and thus its significance for different types of vegetation or the maintenance of aquatic ecosystems.

Long-term effects of drought on tree-ring growth and carbon isotope variability of Scots pine in an inner-Alpine valley in Switzerland

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In recent decades, summer droughts tend to occur more frequently not only in Central Europe and the Mediterranean basin, but also in different parts around the globe. They might enhance tree decline and lead to increased forest mortality. Thus, it is important to study in more detail ecosystem responses to extreme weather conditions and changing climate. Here, we apply a combined approach of tree-ring growth and stable isotope analysis of carbon to gain a more profound understanding of tree growth and plant function responses to climate change as well as the underlying mechanisms of tree decline and mortality. In a mature Scots pine (*Pinus sylvestris* L.) forest located in one of the driest parts of the Alps, in Switzerland (Pfynwald, Valais), we compared trees that have recently died with still living trees from a 10-year irrigation experiment where annual precipitation of 657 mm has been doubled.

Our analyses revealed that tree-ring growth of the dead trees started to decline in the mid-1980s, but it was already to some extent reduced several decades before. Both mean temperature and vapor pressure deficit (VPD) strongly increased during the period of growth decline. Interestingly, intrinsic water-use efficiency derived from cellulose $\delta^{13}C$ (WUEi) was already higher for the dead trees before the decline, indicating a more conservative water-use strategy compared to the surviving trees, possibly at the cost of low carbon uptake and long-term weakening. Based on climate correlation analysis for 1960-2003, tree-ring growth of the dead trees was highly sensitive to spring and summer mean temperatures and to VPD. Based on $\delta^{13}C$ analysis, the plant function response of the dead trees to these climate variables, i.e. reduced photosynthetic rates and impaired stomatal regulations, differed from the response of the living trees, also indicated by reduced WUEi in the final stage of decline. Irrigation almost doubled tree growth compared to control trees. Therefore, we conclude that insufficient soil water supply and elevated atmospheric evaporative demand (increased VPD) might be the primary drivers for tree decline and mortality in Pfynwald.

Accounting for Ecophysiology in Predicting Animal δ2H and δ18O Values

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Hydrogen and oxygen stable isotopes are important tracers in migratory, food web, and paleoecological applications. Unlike carbon and nitrogen stable isotopes, for which only dietary sources contribute to tissue isotope values, hydrogen and oxygen can be derived from diet, free water in food, drinking water, and (for O) O2. We leveraged several published studies to develop a mass balance model accounting for these input and output fluxes in order to predict organismal δ2H and δ18O tissue values. The model permits the evaluation of ecological and physiological conditions on tissue isotope ratios, including characteristics such as body mass, total water flux, feeding guild, and dietary composition. Thus, using basic ecological information about a species, the H and O tissue isotope values can be predicted for a range of organisms. We explore sensitivity in model parameters to determine whether our refined model can explain empirical observations from the literature on animal tissue H and O isotope ratios. As paired measurements of H and O isotopes are becoming more common, there are mounting examples of weak relationships between tissue and environmental water isotope composition in one or both isotopes, which complicates predictions of migratory origin and paleoecological interpretations. We expect this model to be useful in predicting the controls on these isotopic systems and how observed H and O isotopic □disequilibrium may offer insight into the diet and physiology of individuals.

Sources, Transformation, and Linkage of Dissolved and Particulate Organic Nitrogen in the Oligotrophic Ocean Indicated by Compound-Specific Nitrogen Isotope Analysis of Amino Acids

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Dissolved and particulate organic nitrogen (DON, PON) together play the roles in N cycling and ecosystem in the upper ocean. This study explores the use of compound-specific nitrogen isotope of amino acids (δ^{15}N-AA) of coupled DON and PON samples as a new approach to examine relative sources, transformation processes, and potential coupling of the main detrital organic nitrogen form in the ocean water column. We measured δ^{15}N-AA distributions in high-molecular-weight dissolved organic nitrogen (HMW DON) and suspended PON in the North Pacific Subtropical Gyre (NPSG) from surface to mesopelagic depths. A new analytical approach achieved far greater δ^{15}N-AA measurement precision for DON than earlier work, allowing us to resolve previously obscured differences in δ^{15}N-AA signatures, both with depth and between ON pools.

First, the δ^{15}N-AA signatures of both surface and mesopelagic HMW DON suggest mainly heterotrophic bacterial sources, with the mesopelagic HMW DON bearing signatures of far more degraded material. These results contrast with a previous proposal that DON δ^{15}N-AA patterns are essentially “pre-formed” in the surface ocean, undergoing little further change with depth. Second, different δ^{15}N-AA values and patterns of HMW DON vs. suspended PON in the surface NPSG suggest that their sources and cycling are largely decoupled. We propose a new idea for production of surface HMW DON, in which ON is ultimately derived from subsurface nitrate, while PON in the mixed layer is strongly linked to N2 fixation and N recycling. In contrast, the comparative δ^{15}N-AA values of HMW DON and suspended PON in the mesopelagic depth suggest a potentially important role of PON in the production of HMW DON at mesopelagic depths. Overall, these new δ^{15}N-AA data have provided an unprecedented window into the sources, cycling, and likely biogeochemical linkage of DON and PON pools in the oligotrophic ocean.

4.4 Session 4

Ecological processes across time and space using isoscapes

Elucidating trophodynamics at hydrothermal vents using bulk stable isotope analysis

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Sweeting, CJ & Wigham, B

Hydrothermal vents are highly dynamic, ephemeral deep-sea habitats that occur along seafloor spreading centres, noted for localised, high biomass. The discovery of faunal assemblages associated with hydrothermal venting challenged the paradigm that autochthonous primary production was absent from the deep-sea, and fauna collected at these sites were among the first in the deep-sea to exhibit stable isotope values indicative of a source of chemosynthetic primary production. Here we review the current isotope derived knowledge of trophodynamics at 85 sedimented and hard substrate hydrothermal vent fields from over 200 published datasets with the aim of providing an integrated view of geochemical-microbe-metazoan interactions at multiple ecological scales, and developing sampling guidelines for future studies. The review explores: (1) stable isotope values of inorganic substrates; (2) fixation pathways utilised by microbial primary producers and their associated stable isotope fractionation values; (3) utilisation of stable isotopes to understand metazoan trophic structure; (4) how trophic structure varies with vent fluid chemistry; and (5) a global biogeographical synthesis of trophic structure utilising existing bulk stable isotope data sets. We find evidence for multiple structuring processes in a range of inorganic and organic components of the food web, including geologic conditions, fluid chemistry and biogeographic differences. Whilst many hydrothermal vent fields have been studied isotopically, the vast majority of fields are missing one or more lines of evidence that are important for accurately determining trophodynamics. In particular, some aspects are routinely overlooked; for example dissolved inorganic nitrogen, studied at just 5 vent fields worldwide. It is clear that in order to understand differences in trophic structure among hydrothermal vents, an “end-to-end” approach is required, which integrates geochemistry, microbiota and metazoa from basal sources to consumers. This will ultimately allow the development of models that examine to what extent different host systems cycle nutrients in different ways.

Combination of stable isotopes and biologging documents where and when seabirds molt

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Key facets of the ecology of seabirds during the interbreeding period still remain poorly understood because of the difficulty of studying them at sea, including during the energy demanding molting stage. Here, the extent to which three sympatric petrels from the subantarctic Kerguelen Islands modify their foraging ecology during the interbreeding period was investigated using a combination of complementary tools, namely stable isotopes and miniaturized saltwater immersion geolocators (GLS). The molt ecology was first characterized in the blue petrel, a reference species that is known to molt right after breeding. Feather stable isotopes ($\delta^{13}C$ as a proxy of the birds foraging habitat within the well defined isoscapes of the Austral Ocean) and GLS indicated that the post breeding molt of blue petrels occurred in oceanic Antarctic waters. Importantly, activity recorders showed that molt was marked by a strong peak in time spent daily sitting on water, which thereafter declined to lower values during the remaining winter months. Secondly, the peak in time spent sitting on water was used as a proxy to characterize the contrasted molting strategies of two closely related prion species. As blue petrels demonstrated, thin billed prions molted during the post breeding period in cold Antarctic waters where they fed primarily on low trophic level prey, most likely Antarctic krill ($\delta^{15}N$ as a proxy of the birds' diet). By contrast, Antarctic prions presented an unexpected pre breeding molt of longer duration that took place further north, in warm subtropical waters. The stable isotope method on sequentially sampled flight feathers nicely documented species and individual specific molt strategies, with primary $\delta^{13}C$ values depicting latitudinal movements. A comparison between the isotopic values of flight and body feathers indicated that, unlike primaries, body feather molt occurred during the whole interbreeding period. Hence, the study of feathers provides a unique opportunity to trace movements through analyses of intrinsic markers, and the method is at its best when combined with biologging to investigate the ecology of cryptic stages of birds life.

IsoBank – Stable Isotope Ecology in the Age of 'Big Data'

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Stable isotopes ratios provide valuable information to scientists working in a diverse range of fields: e.g. ecologists, hydrologists, geochemists, oceanographers and archeologists. Ecologists take advantage of stable isotope ratios to provide information on the diet and migration history of consumers or when assessed across multiple species to characterise food web structure and ecosystem function and to see how these change over time and space. However, most studies using stable isotopes are conducted by independent research groups working in relative isolation, and the resulting data are not available to researchers outside of these groups.

ISOBANK is initial result of an international collaboration of isotope ecologists and data managers who are developing a data federation resource for stable isotope scientists. This data-repository, housing stable isotope data from organisms around the globe, represents an entirely novel resource with which to portray local, regional and global patterns in food web structure. As data are geo-archived they can be related to land use, temperature or productivity gradients providing a novel insight into the factors determining spatial and temporal patterns in biodiversity, biological responses to climate change and ecosystem function at a global scale.

Using data drawn from IsoBank we present two case studies as a 'proof of concept'. The first of these is a comparison of sexual variation in isotope ratios throughout the animal kingdom. Secondly, we re-analyse food web data obtained through IsoBank to examine continental scale variations in food chain length. The IsoBank can be found at www.isobank.org and on Twitter @iso_bank for more information.

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Small-scale isoscape for marine benthic habitats – Isotope mapping of the honeycomb-worms Sabellaria alveolata diet

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The tubiculous gregarious polychaete species Sabellaria alveolata builds extensive bioconstructions all over the European coast. In some instance – such as the bay of Mont-Saint-Michel – this species engineers reefs over several hectares in the intertidal zone. Those reefs largely affect hydro-sedimentary processes, hence modifying sedimentary features and creating mudflats in place of coarse sandy sediment. We used those bioconstructions to test the concept of 'gardening' in which engineer tubiculous suspension- or deposit-feeding species enhance the production of autochtonous food source, namely biofilm and microphytobenthos (MPB). We tested whether this biofilm is part of the worms' diet and more precisely if spatial differences in the worms' diet can be evidenced from isotopic compositions (13C and 15N) within the reef habitat. We systematically collected worms every 75 meters on a 100 hectares reef (300 stations). Data related to reef structure (sedimentary features, fragmentation, percentage cover etc.) were collected at the same gridscale. Additionally, multispectral satellite imaging covering the whole reef was interpreted to produce normalized vegetation index (as a proxy of MPB abundance). Isotopic composition of the worms were krigged and revealed a 2‰ difference at the reef scale (analytical uncertainty 0.1 ‰). Within-reef differences were interpreted in the light of habitat fragmentation and mud occurrences, as satellite imaging revealed higher MPB biomass in muddy areas. Statistical analyses revealed that the geographical position inside the reef (latitude and longitude) and the reef fragmentation are important explanatory variables to take into account in order to explain the worm's isotopic compositions. Satellite images showed weak links between MPB biomass and worms' isotopic compositions, hence revealing that MPB production is available over the whole reef habitat. This investigation also showed that meter-scale variability is of primary importance when interpreting isotopic composition of marine habitats. This model is then used to provide guidance to better encompass variability in suspension-feeders isotopic composition.

Golden Eagle Fatalities Demonstrate the Continental-Scale Environmental Consequences of Local-Scale Renewable Energy Development

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Renewable energy is rapidly growing in prominence throughout the world, largely because its use produces few greenhouse emissions and it is seen as environmentally friendly. Nevertheless, renewables have environmental effects of unknown scope for wildlife and habitats. To help to understand the geographic scope of the ecological consequences of renewable-energy development, we used stable isotope (δ2H, $\delta^{13}C$, $\delta^{15}N$) and genetic data from Golden Eagles killed at a large wind-energy facility, in conjunction with the same data from known-origin reference samples, to test the hypothesis that mortality is compensatory (which would imply a limited role for immigration in population stability). In contrast, if mortality is additive, then the number of mortalities due to wind energy would occur in addition to other mortalities, and population stability would be maintained by immigration. Mean δ2H values obtained from feathers of 66 eagles killed at the Altamont Pass Wind Resource Area (APWRA) near Livermore, California, varied between -121.6 and -13.2‰. Geospatial analyses of these data suggested that ~25% of these birds were recent immigrants to the population, most from long distances (>100km) away. The genetic data suggested that the birds were from a single, panmictic population in western North America. The age structure of killed immigrant eagles was not random. Because the majority were young (2-year old birds), our results suggest that some of the older eagles killed may have immigrated to the region when they were younger. Incorporation of these results into a demographic model indicates that golden eagle mortality at the APWRA is not compensatory, implying that populations at this renewable energy facility are sustained by long-distance continental-scale migration. These results (1) indicate that management decisions concerning the impacts of renewable-energy development on wildlife populations at the local scale could have continental-scale impacts and (2) illustrate the ability of new tools for geospatial analysis of δ2H data to help address key questions about the ecological consequences of renewable-energy development.

Marine animal assignment to UK shelf sea isoscapes – validation and implications for conservation and fisheries policy

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In the marine environment carbon and nitrogen isotopes have been shown to vary spatially at the base of the food web, allowing the use of isoscapes for geolocation. Assignment to these isoscapes has been well studied, however quantitative validation of these techniques is still in its infancy. I will show how after accounting for uncertainty associated with the development of an isoscape and extension to animal tissues, geoassignment of marine animals using carbon and nitrogen stable isotopes has comparable accuracy and precision to light- and tidal stream based archival tagging approaches.

Sample based carbon and nitrogen isoscapes were created from jellyfish combined with 10 year spatially averaged environmental data for the UK shelf seas. Accuracy and precision was assessed by assigning independently sampled and analyzed scallop tissues from known locations using multivariate normal probability continuous assignment approaches with Monte Carlo simulation methods used to account for variance and uncertainty. When all recognized sources of variation are taken into account, geolocation accuracy and precision is comparable to light based and tidal stream geolocation.

Subsequently, herring and seabird (puffin, guillemot and kittiwake) samples with known catch locations and (in the case of seabirds) individual data logger-based geolocations were also assigned using these isoscapes. In this talk I will show the power of this methodology in reporting accurate and precise locations for marine organisms, discuss the sensitivity of geolocation assignments to different sources of variance, and outline areas where marine isoscape development and assignment methods could be improved.

Use of multiple isotopes in watershed ecology

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Watershed (catchment basin) is a unit to study material flow from mountain to coastal area. Primary producers in streams consume elements dissolved in water. Animals consume elements via trophic relationships, thus, isotopic signature in the organisms contains information what they subsist on. Living organisms in streams utilize materials of autochthonous and allochthonous resources, and stream food web is characterized by the mixing of both origins. Food web structure can be revealed conventionally by carbon and nitrogen isotope ratios, however, new isotope tools have been applied to watershed ecology. Recent studies indicated that 14C values and nitrogen isotope ratios of individual amino acids can separate these two contrasting organic sources, and thus, revealed the relative contribution of the resources in a food web. We show the utility of the methods in streams of Lake Biwa watershed in Kansai region.

Isotope ratios of heavy metals, e.g. Sr, are promising tools to trace material flow in watersheds. Since the isotope ratios of heavy elements are less dependent on isotope fractionation but variable on geological materials, the values of terrestrial water vary geographically in accordance with the catchment geology and are reflected in organic materials. The information can be used in investigating the movement of organisms if the distribution of isotope ratios is provided. In order to develop the "ISOSCAPES" of the elements, we have studied the distributions of the isotope ratios from multiple watersheds in Tohoku region. We demonstrate the utility of multiple isotope approach in watershed ecology.

Desktop migrations in isospace: coupling isoscapes, bioenergetics and movement models to generate isotopic hypotheses

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Kirsteen MacKenzie – IMR Tromso

Stable isotopes provide one of the most versatile and effective natural geolocation tags and have been used to great effect in the last 30 years. As our spatial isotope models improve, so our understanding of their limitations becomes clearer. In particular, temporal variability is often poorly constrained within isoscapes, and indeed in other areas of stable isotope ecology.

Theoretical model isoscapes offer the potential to test the effects of temporal variability in baseline stable isotope compositions on likely tissue compositions. Temporally explicit in-silico isotope models can be coupled with individual-based models of foraging, and bioenergetics models to allow model organisms to forage through isospace and grow tissue as they forage. Coupled models can then be used to generate isotopic hypotheses against which real tissues can be compared.

Here we use a recently developed temporally-dynamic model of global phytoplankton carbon isotope compositions to explore the implications of temporal variability in baseline isotopic compositions for food web and migration analyses. As an example, we link our isotopic model with basic migration and energetic models to predict the isotopic composition of whale baleen for rorqual whales foraging in the Atlantic Ocean. We allow whales to migrate based on a small number of simple rules, and generate hypothetical baleen isotope profiles. We demonstrate the effect of seasonal and between-year variation in baseline isotopes on predicted tissue isotopes. Finally, hypothetical isotope profiles will be compared to historic whale baleen samples to infer likely migration pathways.

Multiple habitat use strategies of juvenile North Pacific loggerhead sea turtles (*Caretta caretta*) revealed in stable isotope patterns of sequential bone growth layers

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The application of ecogeochemistry through stable isotope (SI) analysis of organic tissues allows for reconstruction of foraging ecology and habitat use of marine vertebrates. Tracking animal migration using SI is possible when geographic variations in isotope patterns are used to produce isotope maps, or isoscapes. Stable nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) isotope ratios vary predictably and create geographic patterns given source nutrients, productivity, and consumer trophic position, among other factors. While detailed isoscapes exist for terrestrial systems, comparable isoscapes remain less developed for more dynamic marine systems. In these instances, regional isoscapes can serve as comparative guides to detect animal habitat use. Next, the use of regional isoscapes with SI analysis of consumers' accretionary tissues, such as otoliths, teeth or bones, can generate a multi-year time series of habitat use. Combining these techniques, one can identify timing of ontogenetic habitat shifts and access life history patterns of difficult-to-track species. We used this approach to reveal habitat use patterns of the cryptic juvenile life stage of endangered North Pacific loggerhead sea turtles (*Caretta caretta*). Born in Japan, these loggerheads spend their juvenile stage in the central North Pacific (CNP) and/or the eastern Pacific, and return to the western Pacific as adults. We sampled annual growth rings from humeri of 45 juvenile loggerheads from the eastern Pacific near the Baja California Peninsula, Mexico (BCP). Using $\delta^{15}N$ values from known-locations bone samples, we created a regional isoscape to distinguish two distinct habitats 1) oceanic CNP, and 2) neritic BCP. The CNP $\delta^{15}N$ values were significantly lower ($p < 0.01$) than those from the BCP (10.7±1.9‰ vs. 15.7±1.1‰). The $\delta^{15}N$ values for the 258 sequentially sampled rings ranged from 8.8 to 20.6‰, increasing with body size and age. Using the regional isoscape to assign location to individual growth layers, we identified timing of recruitment to the BCP for 33 of the 45 turtles. We show a bimodal distribution in habitat shift timing, suggesting alternate life history patterns for these juvenile loggerheads.

Exploring habitat reconstructions of *C. acoupa* with $\delta^{13}C$ of essential amino acids in otoliths

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Biogenic carbonates, such as fish otoliths, grow during the lifetime of an organism and often display age bands. In combination with the (in)organic chemical composition of the carbonate, this trait is of great use when reconstructing fish movement. The chemical record stored in the otolith can be linked to the ambient chemistry of habitats it has encountered during its lifetime. Only recently has the organic part of otoliths, the proteins and their amino acid (AA) constituents, been used for this same purpose. Compound specific isotope analysis of $\delta^{13}C$ in essential amino acids (EAAs) of otoliths could allow a detailed view of where an individual fish has spent its different life stages in a particular ecosystem. However, conventional methods for derivatization of AAs in biogenic carbonates, necessary for gas chromatography, are complicated. By applying a slightly modified methoxycarbonyl (MOC) ester method, we avoid these complications and improve the chromatography. We further refute the assumption that MOC AA esters have to be measured within a short time, as we measured them after a month without isotopic difference.

The method was applied to otoliths of *Cynoscion acoupa*, a large fish with high commercial relevance in Brazil. Juveniles of this species reside in mangrove ecosystems and migrate to the ocean as an adult. As coastal and oceanic PPs are generally distinguishable in their isotopic signature, they can provide an isoscape to detect the ontogenic habitat use of C. acoupa. By measuring the outer edges of otoliths from fish with increasing size, we detected an enrichment in $\delta^{13}C$ of EAAs in adult otoliths compared to the juveniles. This trend complies with the $\delta^{13}C$ of EAAs in local PPs and suggests which mangrove PPs are important for juveniles. Utilizing structures in whole otoliths could thus provide information on habitat and resource use by individual fish.

4.5 Session 5

Paleoecology and the ecological impacts of humans

Calcium Isotopes and Trophic Diversity of Cetaceans in Modern and Ancient Marine Food Webs

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Kenneth W. Sims – University of Wyoming

Cetaceans are one of the few mammal groups to have fully adapted to the marine environment, having diversified into two major clades that exploit distinct ecological niches: the Mysticeti, which filter-feed at the base of marine foodwebs, and the Odontoceti, which forage at higher trophic positions. Looking at living species alone, it is difficult to understand the evolution of these disparate feeding methods, which are traced back to the earliest appearance and diversification of these groups during the Eocene-Oligocene Transition. Interpretations based on morphology, body size, or comparisons with extant taxa can give a rough approximation of trophic position, but these methods cannot be tested when dealing with extinct species or clades that have no modern analogues. Here, we explore how stable isotope analysis of biogenic hard parts, specifically calcium isotopes of bone, is one method that may shed light on the trophic positions and feeding habits of early cetaceans and other marine consumers.

Recent work has shown that calcium isotopes are fractionated during mineralization of biogenic hard parts. To test the effectiveness of this isotopic offset for defining trophic levels, calcium isotope values of bone from several modern species of cetaceans were measured and compared with values for Oligocene odontocetes and mysticetes from the Ashley and Chandler Bridge Formations. Among modern species, mysticetes yielded higher values than odontocetes, with those species of myticetes that favored smaller zooplankton typically yielding the highest calcium isotope values. This pattern was also evident among the fossil specimens sampled; mysticetes exhibited much higher calcium isotope values than odontocetes, with the observed range in values (~1.5‰) being of similar magnitude to that determined for modern species of cetaceans. Overall, these results indicate that calcium isotope values of modern and fossil bone can serve as a proxy for trophic position of cetaceans. More importantly, they suggest that a trophic diversity similar to that of present-day was established by at least the early Oligocene, shortly after the appearance of the Neoceti.

Same place, different times: Five million years of small mammal diet and environmental change in the Great Plains, USA

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The Miocene-Holocene deposits of Meade Basin in SW Kansas contain abundant small mammal fossils, and paleosols interbedded with local faunas. These materials provide a 5 Myr archive of ancient environmental and biologic change. The Meade faunal record is noteworthy since rodent species richness was relatively invariant over the last 5 Myr, despite major environmental changes, including the rise of a C4-dominated grassland ecosystem; local cooling and/or increased available moisture coincident with global cooling; the onset of glaciation and variation in the Laurentide ice-sheet; and large ashfall events. The Meade area provides a unique opportunity for a longitudinal study of small mammal community evolution, because of both the density of fossil faunas within a highly localized region, and the presence of a comparable modern ecosystem.

First, we investigated relationships among C, N, and O isotopic values of multiple tissues (hair, collagen, bone and enamel bioapatite) from a sub-set of modern voucher specimens. The majority of the modern sample (from 11 spp.) is hair, whereas the fossil sample is teeth. The relationships among tissue $\delta^{13}C$ values are robust, even in light of the numerous biological factors controlling tissue values. Hair $\delta^{13}C$ and $\delta^{15}N$ values reveal the influences of both seasonal changes, and dietary category, on small mammal diets.

Second, we used LA-IRMS to generate $\delta^{13}C$ and δ18O datasets of tooth enamel from 70 small mammal taxa (6 lineages) from 16 faunas over the past 5 Myr. $\delta^{13}C$ values indicate utilization of C3 and C4 resources throughout the record, as C4 biomass in the region increased. C4-dominated diets are not present until 3 MA, and $\delta^{13}C$ values of both Pliocene and modern faunas span the full C3 to C4 range. Combined $\delta^{13}C$ and δ18O datasets suggest patterns of resource partitioning within faunas, and variation in the isotopic niche space of some lineages over time. These faunal records allow us to test hypotheses about the relative roles of environmental vs. biotic factors on the evolution of small mammal communities over long timescales.

Late Pleistocene paleoecology of carnivores: dramatic discrepancies in dietary interpretations and extinction implications with different tissues and proxy methods

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The La Brea Tar Pits in southern California is arguably the richest late Pleistocene fossil locality in the world, with over 3.5 million fossil specimens of extinct organisms represented by ~600 species. Representing the past ~42,000 years, the La Brea Tar Pits are most famous for their high abundance of fossil carnivorans. Prior isotopic studies suggest that many of the large carnivorans were competing for similar resources as evidenced by overlapping $\delta^{13}C$ and $\delta^{15}N$ bone collagen values, specifically the American lion (*Panthera atrox*), the saber-toothed cat (*Smilodon fatalis*), and the dire wolf (*Canis dirus*). While increased competition and human presence may have made these carnivores more vulnerable to extinction, the idea of direct competition has not been fully vetted and may benefit from a broader dietary analysis. As the La Brea Tar Pits is an active tar seep, diagenesis and taphonomic alteration must be considered when analyzing bone collagen after degreasing fossils with harsh solvents and heat. Thus, we here examined the hydroxylapatite portion of molar tooth enamel of both carnivorous and herbivorous mammals to infer their dietary ecology. This method, less commonly applied to fossil carnivores, demonstrates significantly different and nearly non-overlapping isotopic distributions between *C. dirus* and *S. fatalis*. Specifically, *S. fatalis* consumes prey within denser C3 vegetation (likely denser scrub or forests) than the more generalized *C. dirus*. Further, *S. fatalis* was unlikely to consume significant proportions of the abundant grazers and mixed-feeders, in contrast to *C. dirus*. While both dire wolves and saber-toothed cats exhibit generalized consumption of hard and tough food items (as inferred from accompanied dental microwear texture analysis) the isotopic composition of prey consumed (and areas in which they were hunting) were disparate – questioning the idea that direct competition for similar food resources resulted in their demise. Broad disagreement between tissues also emphasizes the need for sampling of disparate tissues and further study regarding diagenetic effects of fossil cleaning treatments.

Prehistoric diets on Easter Island show environmental adaptation and resilience

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Rapa Nui is frequently used as an exemplar of human behaviour and social competition leading to an avoidable ecological disaster, in which rapid destruction of the native palm forest had devastating consequences for the island's environment and human population. Recent archaeological research has brought such Malthusian claims into question. Revised chronologies indicate settlement of Rapa Nui centuries later than previously supposed, with suggestions of a more balanced interaction with the environment than the 'ecocide' model purports. Knowing past diets is crucial for understanding the impacts of human occupation on Rapa Nui, yet prehistoric diets of the native islanders have received remarkably little attention. Here we examine prehistoric human and faunal bone using carbon and nitrogen amino acid compound-specific isotope analyses, resulting in new estimates of marine input into human diets. We used $\delta^{15}N$ values of glutamic acid and phenylalanine to estimate TP for marine and terrestrial ecosystems. Using isotope mass balance, we estimated the fraction of marine protein in human diets for our human samples of 0.5 ±0.2. Independent evidence obtained from $\delta^{13}C$ EAA fingerprints supports high seafood consumption by humans. Multivariate analyses quantified using relevant marine and terrestrial food sources and a Bayesian stable isotope-mixing model that shows that humans got more than half of their protein from marine sources (58.3±11.0%, n = 7). This is markedly different from previous estimates based on bulk tissue isotope analysis suggesting seafood comprised a minor part of human diets. Our results also point to consumption of terrestrial plants with highly elevated $\delta^{15}N$ values, which could only have come from crops grown in substantially manipulated soils. These findings are in stark contrast to the 'ecocide' model and show that the Rapa Nui prehistoric population exhibited successful adaptation and resilience in a constrained environment with nutrient poor soil conditions. Further, our refined estimates for marine protein consumption show the considerable benefit of using amino acid isotopic data for marine reservoir corrections of radiocarbon dates.

Intertidal foragers: an isotopic comparison of marine-foraging chacma baboons and archaeological humans on the Cape Peninsula, South Africa

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In recent times stable isotope analysis has been used to investigate diets of extant and extinct non-human primates, as well as early humans. In studies of omnivorous primates, interpretation of isotopic data requires prudence as patterns are complicated by physiological processes (e.g., protein routing) and different methodological approaches. We investigated aspects of the behaviour and diet of the last remaining natural-foraging troop of Chacma baboons on the Cape Peninsula near Cape Town, South Africa. The study troop ranged across an area of ~45 km^2 (largely within the Cape of Good Hope section of the Table Mountain National Park) and travelled a little more than 6 km per day on average. Behavioural observations suggested that the troop's diet consisted mainly of terrestrial C3 foods, but included a small proportion of marine foods during all seasons. Faecal $\delta^{13}C$ and $\delta^{15}N$ values ranged from -29.3‰ to -25.6‰, and from 0.9‰ to 6.3‰, respectively. Faecal $\delta^{15}N$ values were positively correlated with a composite measure of marine foraging calculated for the appropriate dietary integration period. Hair $\delta^{13}C$ and $\delta^{15}N$ values ranged from -22.1‰ to -21.3‰, and from 3.5‰ to 5.3‰, respectively. Output from SIAR models based on isotope ratios of hair and faeces are in accordance with estimates based on behavioural observation, indicating that marine foods comprised <10% of the diet. These results have important implications for assessing consumption of small amounts of marine foods in humans. Studies of Holocene Later Stone Age hunter-gatherers in this region report bone collagen $\delta^{13}C$ ranging from -19.4 to -12.1 (corrected for the fossil fuel effect) and $\delta^{15}N$ from 10.2 to 17.3. Using the baboon data, we conclude that even humans with the most negative $\delta^{13}C$ values consumed small amounts of marine food. Also, there were differences in the types of marine organisms (i.e., consumers at different trophic levels) consumed by baboons and humans, as evidenced by $\delta^{15}N$ values and archaeological food waste. In ongoing work, we will use the baboon comparison to explore quantitative reconstructions of past coastal human diets.

Compound specific amino acids as paleo-proxies: Archives, potential, and emerging challenges

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Elizabeth Gier – UC Santa Cruz

Fabian Batista, Danielle Glynn, and Tom Guilderson

In recent years, compound-specific isotope analysis of amino acids (CSI-AA) has developed rapidly as a tool with applications crossing trophic ecology, ecosystem connectivity, and biogeochemical cycles. One of the most exciting emerging oceanographic CSI-AA applications is in paleoceanography. Early work in sediments, fossil shell, and deep-sea proteinaceous corals has demonstrated new δ^{13}C and δ^{15}N CSI-AA proxies which can not only reconstruct past stable isotopic baselines, but also trophic structure, changes in plankton community structure, and inter-relationships with changing nutrient regimes, linked to natural or anthropogenic change in ocean conditions.

This talk will briefly review the emerging potential of CSI-AA as new paleoceanographic proxies across a range of archives, and will then focus on new data from deep-sea proteinaceous corals. CSI-AA work in central Pacific corals has now demonstrated the ability to reconstruct both N fixation and detailed algal community structure on multi-millennial timescales. New CSI-AA work using Pacific margin corals now suggests a wider potential to understand relationships between oscillating climate regimes (ENSO, PDO) and nutrient/ plankton regimes, at nearly annual resolution. However, a number of unexpected results have also emerged. These suggest the need for potentially altered theory and a new interpretational framework for some CSI-AA data in paleoarchives, as well as the need to better understand linkages between primary production source signals and CSI-AA patterns preserved in bioarchives. I will highlight and then briefly discuss the implications of three main challenges going forward: 1) understanding potential constraints of isotope mass balance in interpreting source AA values and trophic position estimates over long time scales; 2) the potential for structural tissue-related differences in trophic AA incorporation, and 3) the puzzle of unexpectedly large variation in essential AA δ^{13}C values, vs. expectations for possible change in export production (plankton source) bulk δ^{13}C values.

Impacts of unusually high sea ice cover on Antarctic coastal benthic food web structure

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Antarctica currently undergoes strong and contrasted impacts linked with climate change. While the West Antarctic Peninsula is one of the most rapidly warming regions in the world, resulting in sea ice cover decrease, the sea ice cover of East Antarctica unexpectedly tends to increase, possibly in relation with changes in atmospheric circulation. Changes in sea ice cover are likely to influence benthic food web structure through modifications of benthic-pelagic coupling, disruption of benthic production and/or modifications of benthic community structure (i.e. resource availability for benthic consumers). Here, we studied shallow (0-20 m) benthic food web structure on the coasts of Petrels Island (Adélie Land, East Antarctica) during an event of unusually high spatial and temporal (two successive austral summers without seasonal break-up) sea ice cover. Using stable isotope ratios of C, N and S and the SIAR mixing model, we examined importance of several organic matter sources (benthic macroalgae, benthic biofilm, sympagic algae, suspended particulate organic matter and penguin guano) for nutrition of over 20 taxa of benthic invertebrates (sponges, sea anemones, nemerteans, sessile and mobile polychaetes, gastropods, bivalves, sipunculids, pycnogonids, amphipods, sea stars, sea urchins and sea cucumbers) spanning most present functional guilds. Our results provide insights about how Antarctic benthic consumers, which have evolved in an extremely stable environment, might adapt their feeding habits in response to sudden man-driven changes in environmental conditions and trophic resource availability.

Stable isotope variability in extant and extinct faunivores: implications for understanding mammalian paleoecology after the Cretaceous-Paleogene extinction event

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The end Cretaceous extinction of non-avian dinosaurs provided mammals the opportunity to flourish. This radiation set the stage for the dominance of mammals in most ecosystems. Many early Paleocene mammals were faunivores and omnivores but little is known about how isotopes reflect dietary differences in living faunivores and omnivores in this trophic niche. We measure carbon and oxygen stable isotope ratios in 5 extant faunivorous British mammals and 9 faunivorous Paleocene genera from the San Juan Basin of New Mexico. We use dietary variability in non-herbivorous extant communities to better constrain isotope variability in the fossil record.

Preliminary data (n=16) from modern British mammal species show distinct separation of oxygen and carbon isotope ratios. Carbon isotope ratios are significantly different between badgers and hedgehogs (p=0.002). Means range from -16.91 per mil (American mink, n=1) to -14.36 per mil (hedgehog, n=7), reflecting isotope values of prey species, which reflect primary vegetation isotope values of habitats within the temperate ecosystem. Oxygen isotope ratios are significantly different between badgers (n=6) and hedgehogs (p=0.002; mean= 26.62), with means ranging between 24.86 per mil (badger) to 27.62 per mil (least weasel, n=1).

Carbon isotope ratios from fossil specimens (n=41) lack any strong indications of dietary partitioning between pairs of genera but overall are significantly different across all genera (p=0.03). The carbon isotope values are similar to mean values of contemporaneous herbivore tooth enamel. Oxygen isotope ratios are fairly homogeneous across the faunivore genera (p=0.21), which may indicate homogeneity of local water reservoirs in what was probably a humid ecosystem. The oxygen isotope ratios among the fossil genera are not as distinct as those in modern temperate environments. Future directions, such as correlation of oxygen isotope ratios with variables such as body size, physiology, and ecology, will be explored.

Human diets viewed from amino acid nitrogen isotope analysis

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Yuichi Naito – Fukushima University

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A method for precise estimation of trophic position of the organism was recently developed using the nitrogen isotopic composition of amino acids. This method is based on the empirical observations that the difference in the $\delta^{15}N$ values of glutamic acid and phenylalanine from a given organism is a simple function of trophic position. The method has an advantage over the commonly used “bulk” isotope method in that it is not influenced by the temporal variations in $\delta^{15}N$ value of primary producer or substrate in the ecosystem. In principle, the method is applicable to organisms both from the terrestrial and aquatic environments, but the isotopic relationship among amino acids is different between them. Therefore, diets of omnivores like human cannot be uniquely resolved even in this methodology. Nevertheless, we thought it meaningful and have applied the method to the nail of modern humans (including 26 people from various countries). We also measured terrestrial protein source of modern human diet for constraining the isotopic signature of modern humans. Before chemical fertilizers were heavily used, 15N values of phenylalanine from the terrestrial organisms excluding human range from 3 to 10‰ in many cases. Coastal humans during the Jomon Period (13,000 to 2,300 years ago), a prehistoric era in Japan strongly relied their protein source on marine animals. In contrast, inland Jomon humans predominantly relied on terrestrial ecosystem with apparent trophic position of 2.7 on average. As expected, our results of modern humans suggested that their diets are much less relied on marine organisms relative to coastal ancient humans. The apparent trophic position of modern humans is ~0.5 unit higher than that of ancient inland humans. Furthermore, reflecting denitrification in the modern over-fertilized soil, modern humans is plotted in the range that phenylalanine is somewhat 15N-enriched relative to the line connecting between ancient coastal and inland human populations. Among modern humans, phenylalanine from modern Japanese is somewhat depleted in 15N while glutamic acid is enriched in 15N relative to European people on average, reflecting more fish in their diets.

Evaluating Long-Term Shifts in the Distribution and Diet of North Pacific Albatrosses with Bulk Tissue and Amino Acid Isotope Analysis

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North Pacific (NP) albatross species utilize a broad range of nearshore and pelagic waters for breeding and feeding. Short-Tailed and Black-Footed Albatross are considered endangered and vulnerable, respectively, while Laysan albatross' population is stable and has even recently expanded its breeding range by several 1000s of kilometers. Before the turn of the 20th century albatross were heavily hunted for their eggs and feathers, but have struggled to rebound to pre-exploitation population levels. Studying the modern distribution, diet, and breeding habits of NP albatross is critical, however little is known on their historic ecology before anthropogenic pressures, which must be researched by means of paleoarchives such as bones found in archaeological sites. Here we analyze ancient (~10 to 2kya), historic, and modern remains of albatross using carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotope analysis of bulk tissues and individual amino acids to reconstruct albatross ecology to understand their ecological and evolutionary plasticity through time. Environmental pressures, while mainly caused by top-down control due to over-harvesting, could also be attributed to climatically-driven (bottom-up) changes in baseline ocean biogeochemical cycles in preventing albatross population recovery. With amino acid analysis, we can disentangle baseline signals from trophic enrichment that is confounded in bulk tissue isotope patterns. We use the mean $\delta^{13}C$ value of Essential Amino Acids and $\delta^{15}N$ values of Phenylalanine (Phe) as proxies for baseline production to identify the distribution of each albatross species and potential long-term biogeochemical changes in algal production over time. We estimate trophic position (TP) using the $\delta^{15}N$ of "Trophic" and "Source" AAs. TP of modern albatrosses fall within expected values (~3-3.3), and we found significant differences in $\delta^{15}N_{Phe}$ among species indicating that these modern seabirds have spatially segregated foraging areas. Beyond understanding this key species and past environmental controls, these results also have substantial implications for conservation in an era when climate change is rapidly impacting oceanic food webs.

Tracing the lives and loss of Maori dogs

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Karyne Rogers – GNS Science

Robert Van Hale – University of Otago

Ian Barber – University of Otago

Domesticated species have travelled with humans throughout the world and are integrally woven into the development of culture. In this research we examine the impacts of rapid change on the decoupling of these human-ecological linkages. The relatively recent human history of New Zealand allows unprecedented opportunity to investigate patterns of human-induced change, following the arrival of Maori c.800 years ago, and European settlers c.200 years ago. New Zealand is thus an exemplar of social and environmental upheaval, with its tight chronology of archaeological data and a rich Maori oral and material tradition. The kuri (Canis familiaris), or Maori dog, was the only domesticate brought by Maori to successfully establish in New Zealand. Cultural and archaeological evidence indicates a rich tradition connecting Maori and kuri before the disappearance of these dogs sometime in the mid-19th century. This loss is both intriguing and important given the high cultural value of kuri: as a companion, whose skins were also sewn into cloaks for chiefs, and as a food source. We have constructed isotopic niches for kuri at a range of time-scales and locations, by sampling bones from kuri (and their likely prey) from 14th and 15th C South Island sites, and hair from dog-skin cloaks made in the 18th C and 19th C. We use carbon and nitrogen stable isotopes to examine kuri diet changes that could be indicative of environmental change, and decoupled socioecological relationships. We compare our findings with historic accounts of kuri diet from early explorers and missionaries, and discuss the results in relation to changing bio-economics and cultural worldviews.

Changes in food web structure and trophic niche of estuarine fish assemblages in response to a gradient of ecosystem eutrophication

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Victor Evrard – Water Studies Centre, Monash University

Ralph C. Mac Nally – Institute for Applied Ecology, The University of Canberra

Paul Reich – Arthur Rylah Institute for Environmental Research

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Perran L.M. Cook – Water Studies Centre, Monash University

At intermediate levels of eutrophication there are often changes in the relative amounts of different producers rather than turnover in producer assemblages in estuaries. It is unknown these gradual changes translate to linear changes in the contribution of each producer to consumers and how these changes affect consumer trophic niche dimensions. We used stable isotope-based approaches to investigate structural and functional measures of fish-centric consumer food webs from eight estuaries that differ in total primary production and the relative ratios of seagrass, macroalgae and unvegetated habitat area. At the habitat scale, we found that food web structure differed between fish assemblages from vegetated areas and unvegetated areas. The distribution of biomass across trophic positions was approximately 50% higher in consumers from seagrass and macroalgae habitats than consumers from unvegetated habitats. At the whole-of-ecosystem scale, the biomass of primary and secondary consumers increased with increasing coverage of total demersal vegetation. Functional changes in consumer trophic niche, as estimated by standardized ellipse area in isotope space (niche area) and mixing model estimates of primary producer contributions (trophic pathways), were also present. Trophic niche area was 60-90% higher among consumers inhabiting seagrass and macroalgae than consumers from unvegetated habitats. Model output indicated that the contribution of seagrass increased with increasing seagrass area despite concomitant increase in macroalgae area, but the seagrass contribution declined in estuaries with the largest macroalgae coverage. Our findings highlight the presence of identifiable and consistent changes in food web structure in response to intermediate eutrophication. Importantly, we found that low-to-intermediate levels of nutrient loading served to increase biomass of primary and secondary consumers (i.e., a fertilization effect), a process apparently associated with the functionally similar, but not identical, trophic roles of macroalgae and seagrass habitats for shallow-water estuarine fish communities.

5 Abstracts - Posters

5.1 Poster Session 1

New developments in applying mixing models and understanding fractionation

#1-1: What to expect when you're expecting... isotope results

Dubois, *Stanislas – Ifremer LEBCO*

Obviously, isotopic tools have brought a lot to ecological studies in general and trophic-related investigations in particular. Scientists relying on isotopic compositions to explore food webs and trophic interactions has increased exponentially. So has the number of stable isotopic facilities (SIFs). Many university laboratories – and private platforms – are now offering dual 13C and 15N sample analyses. But each SIF has a different material, different internal standards, procedures and processes to ensure precision and reproducibility in results. But scientists sending tin caps to SIFs often forget that with every result comes an analytical uncertainty. To investigate the analytical error between SIFs, a set of 45 samples from the same home-made standards were sent to 11 SIF all over the world. All SIFs received the samples with the same standardized information without prior knowledge of the ring-test. Those samples originated from marine animals and plants to create a dummy food web (primary producers and consumers). Some standards were made with pure muscle tissues, other with whole animals and one included pelagic microalgae collected on glass-fiber filters. Animals were analyzed individually or as pool of several individuals. All SIFs received the samples with the same standardized information without prior knowledge of the ring-test. While all samples were received at the same time, the turnover was highly variable and not linked to the price per sample. Overall, SIFs provided sound analysis of the samples. Analytical error were often announced between 0.10 and 0.20‰ by SIFs, as calculated with standard deviation in internal in-house standards (e.g. animal or plant material). The blind test conducted here revealed however much higher standard deviations, in some instances over 0.60‰. Numerous reasons can explained those results, involving heterogeneity in internal standards or error associated with linearity corrections. This blind-test also showed that a batch of samples should never be split into subsets and send to several SIFs

#1-2: Food spectrum of the Chinese mitten crab (*Eriocheir sinensis*) in the Lower Rhine: New insights comparing stable isotope mixing models with genetic gut content analyses

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Invasive species impair community and food web structure via changing habitat conditions and available food resources, thereby posing a serious threat to native biodiversity, particularly in freshwater ecosystems. The catadromous Chinese mitten crab (*Eriocheir sinensis*) is listed as one of the 100 world's worst invasive species. Originating from South East Asia *E. sinensis* was first recorded from Central-Europe in 1912 from northern Germany. Nowadays the Chinese mitten crab is common in various rivers throughout Europe and high densities of the species can cause serious economic damages in water management and fishing industry. During their natural migration from the estuary downstream subadult individuals are able to reach river section several hundred kilometers away from the delta. The ecological impacts of *E. sinensis* are less considered and their diet under natural conditions is rarely studied. Macrophytes are known to be an important food source of *E. sinensis* in tributaries feeding the San Francisco Bay. However, in the Lower Rhine macrophytes are broadly missing. Initial stable isotope analyses showed that *E. sinensis* occupied a trophic level comparable to carnivorous fish. Thus, a strong predatory impact of *E. sinensis* on the fauna is likely. Here we aimed to investigate the diet of the Chinese mitten crab qualitative and quantitative in order to estimate their impact on the food web. Bulk stable isotope analyses of $\delta^{13}C$ and $\delta^{15}N$ of *E. sinensis* and potential food resources were conducted and genetic gut content analyses via group-specific primers for common macroinvertebrates were used to determine prey organisms. Using the results of those genetic analyses as a priori information we determined the proportion of all potential resources via stable isotope mixing models. Our results show a high contribution of some easily accessible macroinvertebrate species to the diet of *E. sinensis* indicating an impact of the crab on the benthic fauna of the Lower Rhine.

#1-3: Trophic ecology of eight deep-sea fishes from the continental slope of the Celtic Sea

Kopp, *Dorothee – IFREMER*

Robert Marianne – IFREMER

Pawlowski Lionel – IFREMER

In marine ecosystems, the study of trophic relationships has extensively benefited from the development of stable isotopes analysis (SIA) as dietary tracers. SIA are particularly useful in elucidating the structure of deep sea food webs given the constraints to obtain gut-content data from deep trawling. We used carbon and nitrogen stable isotopes analyses as well and SIBER and SIAR routines, to determine the trophic ecology of eight deep-sea fishes from the upper continental slope of the Celtic Sea. For that purpose, 33 species were sampled during the French EVHOE survey (part of the annual International Bottom Trawl Survey) in November 2014. Trophic positions were determined and then aggregated into functional groups using clustering analysis coupled to a bootstrap procedure. This analysis provides a simplified description of the food web structure (reduced number of potential source pools) that could be directly used as input in trophic modelling and isotopic niche determination. The deep sea food web of the Celtic Sea formed a continuum of four tropic levels and is characterized by 5 main functional groups. SIAR mixing models revealed that all considered deep sea species are omnivorous and are able to feed on all the sampled taxa. No clear differences could be detected from one species to another except for I that present benthic affinities. Three species, *Helicolenus dactylopterus*, *Phycis blennoides* and M*alacocephalus laevis* presented overlap in their isotopic niche.

This study is the first attempt to describe the trophic ecology of deep sea species the Celtic Sea continental shelf. SIA allowed deducing some general tendencies in food-web structure and species trophic interactions and confirmed diet determined by gut-contents analysis for the same species, in other eco-regions.

#1-4: Trophic interactions between a neustonic gastropod and its hydrozoan prey: insights from Bayesian stable isotope data analysis tools

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The cnidarian By-the-wind sailor *Velella velella* Linnaeus (1758) and the violet snail *Janthina globosa Swainson* 1822 are marine neustonic organisms (i.e. organisms that live upon the upper surface of the ocean and inland waters or beneath its surface film). By-the-wind sailors are zooplankton feeders, preying actively on diverse planktonic taxa. Janthinids are strictly specialised in the consumption of neustonic cnidarians. Both species are hyperspecialised in their habitats and their life mode but differ in their trophic ecology as V. velella has probably a wider trophic niche than *J. globosa*. Bearhop et al. (2004) have postulate that that a diet specialist population (or species) composed of individual feeding on the same unique food source should display almost no isotopic variability, compared to generalist population. This has been demonstrated experimentally by diet-controlled experiments. However, to our best knowledge, this basic assumption has not been tested to date in a natural population, because such theorical case is infrequent in Nature. Isotopic niche parameters were computed using SIBER (Stable Isotope Bayesian Ellipses in R;), a part of the R package SIAR. As predicted, isotopic variability of violet snail is very low (S.D. 0.3 and 0.1 for delta 15N and 13C values, respectively, n=74) and equal to analytical S.D on replicated measurements. TEF values for *J. globosa* were 2.3 ± 0.3‰ and 0.2 ± 0.1‰ (mean ± S.D., n= 74) for Delta 15N and Delta 13C, respectively. There was no overlap between isotopic niches of V. velella and that of *J. globosa*. Standard ellipse area (SEA) of V. velella was greater than that of *J. globosa* (0.574 vs. 0.106 ‰2), which is very small. This study confirms the hyperspecialised diet of violet snails, the prediction of Bearhop et al. (2004) and applicability of SIBER approach to describe an animal population composed of diet specialist feeding on the same food item.

#1-5: Constructing robust food webs using gut contents, $\delta^{13}C$ and $\delta^{15}N$ isotopes and predator-exclusion experiments

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Food webs and studies of trophic interactions are among the most central aspects of community ecology, and, while complex to resolve, can interrogate ecological phenomena such as extinction, biological invasion, and disturbance among others. In this study, we used traditional gut content analyses of consumers in four tropical reservoir communities heavily influenced by urbanisation and exotic taxa to inform subsequent source-partitioning Bayesian mixing models (SIAR) of bulk $\delta^{13}C$ and $\delta^{15}N$. We used the latter to resolve high-resolution quantitative food webs by determining relative interaction strengths among taxa, and the robustness of the resulting conceptual food webs was tested with predator-exclusion experiments. We found that changes in the community observed from the experiments were largely consistent with top-down cascading effects predicted by our conceptual food webs. This suggests that mixing models using bulk $\delta^{13}C$ and $\delta^{15}N$ isotopic profiles informed by gut content analyses can be a reliable food web construction method which may prove to be a useful approach for conservation and management efforts in human-dominated landscapes.

#1-6: Shift in food source for mysid *Neomysis awatschensis* in a river-estuarine gradient, stable isotope evidence

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Relative importance of potential food sources for the mysid *Neomysis awatschensis* was evaluated along the Yura River estuary, central Japan. Riverine particulate organic matter, marine phytoplankton, and mysid samples were collected monthly from March 2014 to July 2015 for stable isotope analysis. Major findings are as follows: (a) the $\delta^{13}C$ values for marine phytoplankton (-22.6 to -18.9 ‰) and riverine particulate organic matter (-29.3 to -24.4 ‰) were significantly different ($p<0.01$), (b) mean mysid values at estuarine (-21‰) and riverine sites (-24 ‰) differed significantly ($p<0.01$). The observed $\delta^{13}C$ and $\delta^{15}N$ values indicated that relative importance of riverine particulate organic matter and marine phytoplankton to the diet of mysid varied seasonally and spatially. Application of a dual isotope mixing model revealed that the contribution ratio of each food source ranged from 0% to 100%. We concluded that the mysid uses either riverine particulate organic matter or marine phytoplankton in response to the surrounding environment along the estuary throughout the year.

5.2 Poster Session 2

Emerging techniques and integrative applications – multiple elements, Compound Specific Isotope Analyses (CSIA), isotopomers, and radioisotopes

#2-1: Diet preference of long line cultured oyster using bulk stable isotope and compound specific isotope of amino acid

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Pacific oyster, *Crassostrea gigas* is wildly cultivated by long line culture system in southern coast of Korea. In long line culture system, as the cultured oysters submerged for 24 hours, suspended particulate organic matter is major diet source for cultured oyster. Also various studies have suggested that size of particle can be important factor to determine diet of oyster. Hence, we investigated diet preference of cultivated oysters among the size fractionated particles using carbon and nitrogen isotope analysis of bulk tissue in high dense oyster farm. In addition, we estimated trophic position of cultivated oyster and size fractionated particles via CSIA of amino acid. Oysters and size fractionated particles were sampled from start season (May) of the oyster culture to harvest season (Feb.) at 3 stations in Gamak bay in southern coast of Korea. The suspended particles were fractionated for 3 size fractions; up to 100 μm, 20~100 μm, 2~20 μm. Assuming that carbon isotopic trophic discrimination factor (TDF) of bulk tissue for oyster is 1.75 (by Dubois et al., 2007), $\delta^{13}C$ value in the predicted diet source of oyster matched with large size POM (larger than 20 μm) while small POM was not overlapped. This result can be interpreted that the major diet source of cultivated oyster derived from large size POM. However $\delta^{15}N$ value in the oyster was not significantly different compared to large POM. In addition, exclusively high POC/ chl-a ratio of POM indicating low diet ability were observed for all seasons. Moreover, based on the 15N of individual amino acids (glutamic acids and phenylalanine), the trophic positions of oyster and the POM larger than 100 μm were close to 2 as primary consumer, and that of POM between 20~100 μm was 1.3 indicating mixture of between primary producer and other organic matter. Consequently, we found that the long line cultured oyster should preferentially feed larger POM (>20 μm), but feed phytoplankton as main diet indicating little ingestion of faunal organic matter, even though phytoplankton biomass showed constantly lower contribution to POM pool in oyster farm coastal environment.

#2-2: Employing amino acid $\delta^{13}C$ analysis to quantify environmental change in a Late Holocene nearshore ecosystem

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Kelp forests are among the most productive but vulnerable marine ecosystems on earth. These regions serve as biodiversity hotspots and CO2 sinks, but are also highly sensitive to human impacts such as overexploitation and shifting ocean chemistry. Understanding their dynamics is crucial for effective conservation and management. Here, we employ $\delta^{13}C$ analysis of individual amino acids from two top marine consumers, sea otters (*Enhydra lutris*) and sheephead (*Semicossyphus pulcher*) to evaluate the importance of kelp forests in the late Holocene (~3500 years before present). Since only primary producers and microbes synthesize essential amino acids (AAESS), consumers typically directly route them into tissues and thus AAESS are minimally altered as they move up food chains. Moreover, different producers (e.g., phytoplankton and macroalgae) in nearshore marine ecosystems have highly distinct $\delta^{13}C$ values and so the $\delta^{13}C$ values of AAESS in top consumers can provide a 'fingerprint'of the dominant producers in the local foodweb. We analyzed bone collagen from late Holocene sea otters and sheephead from two islands (San Nicolas and San Miguel) off the coast of southern California. We also characterized the baseline amino acid $\delta^{13}C$ profiles for modern producer groups: kelp (Laminaria and Nereocystis), green algae (Ulva) and red algae (Neorhodomela). We used mixing models to quantify the contribution of each algal group to ancient sea otter and sheephead AAESS $\delta^{13}C$ values. As expected, kelps had significantly higher $\delta^{13}C$ values than red and green algae for all amino acids measured. We found remarkable consistency between ancient sea otters and sheephead, both between individuals and among sites. Both top consumers were predominantly feeding in ecosystems driven by kelp production; in some instances, an estimated 99% of consumer essential amino acids were derived from kelp. These findings suggest that, at these sites, kelp forests may have been more extensive in the late Holocene than they are today. Our study demonstrates the utility of amino acid $\delta^{13}C$ analysis in investigating historical ecological problems that hold relevance for modern conservation biology.

#2-3: Life history of Indian catfish reconstructed from otolith geochemistry

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Understanding life history of catfish is of ecological and economical importance because catfish constitute one of the largest fish stocks in the coastal Indo-Pacific regions. Additionally, abundantly available otoliths, ear stones of teleost fish, excavated from the coastal ruins of the Indus civilization are expected to be a paleoclimatological proxy during the Holocene. To understand life history of the Indian catfish (Siluriformes, Ariidae) and evaluate potential of otolith geochemistry as a paleoenvironmental recorders, we measured both oxygen isotopes (δ18O) and Sr/Ca ratios along the growth direction of otoliths. We used otoliths of catfish that lived in the two different gulfs in western India, the Gulf of Khambhat and the Gulf of Kutch. We employed high-resolution micro-drilling device (GEOMILL326) to obtain CaCO3 subsamples for δ18O analyses and laser ablation ICPMS to measure Sr/Ca. Both sets of data suggest that the catfish migrated from a brackish environment to the sea during its lifetime. Migration history of the catfish was estimated with a monthly resolution aided by a numerical modeling. Otolith δ18O clearly record seasonal temperature variability with a precision of 1 °C when the catfish dwelt in the sea, which means that they can be used as a paleothermometer of seawater in this region. Therefore, it is expected that δ18O measurements of fossil otoliths excavated from the ruins likely reveal water temperature history during the Holocene when the Indus civilization repeated the rise and fall with abrupt climate changes.

#2-4: Radiocarbon measurements for marine fish to understand their ecology

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Yusuke Yokoyama – Atmosphere and Ocean Research Institute, The University of Tokyo

Naohiko Ohkouchi – JAMSTEC

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Radiocarbon is produced in upper atmosphere and distributed around the surface of the Earth including ocean. It provides the “time” thus different water mass has different values. The deep water aged after isolation from the surface so depth information of water can be deduced using radiocarbon. Surface variation of radiocarbon concentration also can be found where different water masses meet. Off Japan is such a location where radiocarbon depleted Oyashio cold current (Δ14C?50‰) and radiocarbon less depleted Kuroshio warm current(Δ14C50?70‰) is converged. Thus we corrected fish off Japan to better understand their ecology using radiocarbon measured by newly installed single stage accelerator mass spectrometry (AMS) at the Atmosphere and Ocean Research Institute, the University of Tokyo.

Fish that was collected from off Suruga (Shizuoka prefecture) shows relatively constant value as Δ14C+50 to +70‰, whereas fish collected from off Iwate varies inΔ14C from -40 to +40‰. This might be reflecting the ecological signatures where they had been obtaining nutrients. We will discuss also theΔ14C variations in different part of the fish body to further support our biological interpretations."

#2-5: Blade-order-dependent radiocarbon variability in brown seaweed *Undaria pinnatifida*: A new tool for assessing the change in current regime during the growth period of kelps

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Kelps, large brown algae, are highly productive and important primary producers in coastal ecosystems and provide valuable ecological services for humans. Growth and distribution of kelps along the coastline of temperate regions are thought to be influenced by the current regime, which is variable over multiple spatiotemporal scales. However, the relationship between oceanographic conditions and kelp physiology has yet to be clarified fully. This study investigated the use of radiocarbon in kelp blades as a new tool for examining the shift in current regime during their growth. Specifically, we examined whether radiocarbon contents (14C) of kelp *Undaria pinnatifida* blades reflect the Oyashio intrusion event in a small embayment of the Sanriku coast, northeastern Japan. Kelp sporophytes were cultivated between November 2013 and April 2014 and 14C values of individual blades were determined by the accelerator mass spectrometry. The data revealed that 14C of younger blades tended to be more depleted in 14C relative to older blades. This trend was explained by the change in the current regime of the bay during the investigation period, which was characterized by the shift from the regime prevailed by Tsugaru warm current [14C of dissolved inorganic carbon (DIC) = 15.7 ‰] to that prevailed by Oyashio current (DIC 14C = |57.1 ‰). Thus, kelp blades formed after the intrusion of Oyashio water were generally more depleted in 14C relative to the blades formed before the intrusion event. These data indicate that blade-order-dependent 14C variability of *U. pinnatifida* reflects the Oyashio intrusion event in the bay, suggesting that radiocarbon approach can be potentially useful for examining oceanographic conditions that kelps experienced during their growth.

#2-6: Examining the intricacies and seasonality of the base of the mesopelagic food web using compound specific isotope analysis and ^{234}Thorium measurements for sinking and suspended particles

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Export of carbon and nitrogen to the deep sea can be largely influenced by the mesopelagic food web, yet the intricacies of the mesopelagic food web and its influence on the biologic pump remain for the most part unconstrained. In addition, the processes controlling the enormous attenuation of particle flux by mesopelagic consumers and thereby the strength of carbon sequestration to the deep ocean are not understood. Here we examine seasonal variation in particulate organic matter export to the mesopelagic zone in the subtropical North Pacific Ocean, north of Hawaii. We sampled the upper 400m of the water column at station ALOHA and used the combination of 234Thorium (234Th) measurements with amino acid concentrations and compound specific nitrogen isotope (δ^{15}N) analysis to better understand how upper water column processes seasonally impact mesopelagic dietary substrates. We measured the concentration and nitrogen isotopic compositions, as well as the 234Th-normalized export flux of bulk C, bulk N and total hydrolyzable amino acids (THAA) of small particles (1-53 μm), large particles (>53 μm) and sinking particles caught in sediment traps in the winter and summer of 2014. We found 234Th normalized particulate export flux varied by a factor of up to ~10, with higher fluxes observed in the late summer. In winter, C, N and THAA flux was dominated by small particles, but in summer, flux was dominated by particles >53 μm. As previously observed, small and large particles are isotopically distinct, with differences in the δ^{15}N values of individual source amino acids of up to 10‰ at mesopelagic depths. This distinction in δ^{15}N values allows not only for the relative seasonal contribution of each particle type to the higher food web to be tracked, but also provides evidence of the biogeochemical mechanisms by which large and small particles degrade as they descend to depth. Identifying the inputs of organic matter to the base of the mesopelagic food web is of prime importance to understanding its function, both in regards to carbon sequestration and forage production for commercially exploited species.

#2-7: Amino acid and radiocarbon insights from captive whale sharks

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Stable isotope analyses (SIA) have the potential to provide novel insights into spatial and temporal patterns in the trophic ecology of poorly understood organisms like whale sharks *Rhincodon typus*. However, interpreting SIA depends on accurate diet-tissue discrimination factors (DTDF) to quantify diets and trophic positions, with experimental derivations of DTDF rare for such large-bodied organisms. Captive *R. typus* have provided a unique opportunity to validate a range of SIA, compound-specific isotope analyses (CSIA) and radioisotope approaches in the world's largest fish and one of three planktivorous sharks. Diet records over the past five years revealed a diet dominated by North Pacific and Antarctic krill, 44% and 49% of weighted diet for *Euphausia pacifica* and *E. superba*, respectively. Despite the well-known diet, SIA of fin tissue from three captive *R. typus* (7.1, 7.2, and 8.4 m in length) proved hard to reconcile, especially for bulk carbon. In contrast, CSIA of amino acid (AA) nitrogen in the sharks' tissue was relatively stable over time, despite evidence of variation in AA compositions and δ^{15}N-AA of diet components. Tissue radiocarbon further suggested either long turnover in fin tissues (27 months), or the preferential assimilation of the smaller E. pacifica (Δ14C of 3 ‰ compared to -112 ‰ for *E. superba*). Daily-scale analysis of radiocarbon in *R. typus* faeces may support the preferential assimilation hypothesis, faeces generally being depleted relative to diet. Together, CSIA-AA and radiocarbon analyses add multiple additional axes to our isotope space and may alleviate some of the complications involved in interpreting bulk SIA in ecological studies.

#2-8: Effects of different sample preparation methods on stable oxygen isotope values of phosphorus in soil

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Zhang Xian, Chen Zhigang, Jiang Chaohua

The possible use of stable oxygen isotopes of soil phosphate as a tracer for P cycling has gained attention lately. As in nature conditions, the δ18Op will not influenced by inorganic process, while enzyme-mediated reaction will modify the composition of oxygen isotope ratio. The δ18Op value might reflect the activity of soil ecosystem. Due to different soil phosphorus sources have different reaction activity, which might provide different nutrient supply mechanism to plants and soil microorganism. phosphate oxygen isotope makes it possible to clarify the role of organic phosphorus and inorganic phosphorus in soil on plants and microorganisms. Due to the composition of soil is complex, containing a variety of organic matter, phosphate in soil has multiple oxygen sources. Extraction of phosphate form the soil is quite complicated. For a successful extraction method, we should avoid strong pH adjustments or high temperature treating condition to preclude hydrolysis of organophosphorus or oxygen exchange process, moreover the organic matter adsorbed in Ag3PO4 during precipitation process should be removed. A reliable sample enrichment and purification technique is foundation for δ18Op research. Here two types of soil sample were extracted sequentially by H2O, NaHCO3, NaOH and dilute HCl, phosphates in each solution was extracted by multi-step precipitation. Results show that extraction and purification procedure do not change δ18OP. the δ18OP values decrease from H2O to D.HCl solution, which is identical to the bio-availability trend of phosphate in soil. Results also show that microbe activity can increase δ18OP, so soil sample should be stored at low temperature (-20Ž) or dried as soon as possible after collection. Although the effects of drying temperature and sieve mesh are not intensive, the freeze drying or 80Ž drying and 100 mesh sifting were recommended to reduce the effects of microbe and organic material.

5.3 Poster Session 3

New insights into ecophysiology, microbial ecology and biogeochemical cycling

#3-1: Sediment sources of a Tropical Freshwater River: Applications of Stable Isotopes and Heavy Metals Analysis

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The upstream of Perak River Basin, Malaysia is the main water supply for locals and is developed as a water resource management area under the Integrated River Basin Management by the Perak State Government. Knowing the source of carbon and nitrogen in the sediment can be an important tool in determining these elements between natural and anthropogenic origins and whether the elements in the sediment may pose risk to the water quality of the area. Total organic carbon, total nitrogen, C:N ratio, $\delta^{13}C$ and $\delta^{15}N$ were determined from surface sediments of three selected sites of Perak River Basin; Gadong River, Cueh Village and Iskandar Bridge to assess the sources of carbon and nitrogen in the sediments. Heavy metals analysis (Cr, Pb and Zn) of the sediments were also measured to indicate anthropogenic induced contaminations. Total organic carbon and total nitrogen were analysed using CHN analyzer, $\delta^{13}C$ and $\delta^{15}N$ were measured using elemental analyser-isotope ratio mass spectrometry and heavy metals were analysed using flame atomic absorption atomic spectroscopy. Sediments of Iskandar Bridge has the highest C:N ratio and the most enriched $\delta^{15}N$ implying a higher ratio of terrestrial derived contributions and higher sediment erosion. $\delta^{13}C$ were the most depleted at Gadong River, reflecting sediment organic matter from forested streams. Overall, our result shows that the sediment in the upstream part of Perak River Basin were mostly derived from terrestrial input. Further study should incorporate the forest leaves and particulate organic matter to elucidate better the organic matter sources of each particular area. This study provides a baseline understanding of the sources of organic matter which is important in managing the water resources effectively.

#3-2: Liverwort-Fungal Associations: Novel Symbioses in Polar Regions?

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Kevin Newsham – British Antarctic Survey

Will Goodall-Copesake – British Antarctic Survey

Symbioses between plants and fungi are ubiquitous and play key roles in terrestrial ecosystems. The plants provide the fungi with carbon and in return the fungi provide their host plants with limiting nutrients such as nitrogen and phosphorus. These associations, often referred to as mycorrhizas, have been hypothesised as a key innovation that helped facilitate the colonisation of Earth by land plants. However, we still know very little about fungal symbioses in one of the most ancient groups of land plants – the liverworts – and even less still about liverwort-fungal associations in Polar Regions. This study provides the first large-scale survey of fungal colonisation of Antarctic liverworts (from South Georgia), increasing our knowledge of these symbioses in the region from three to 34 liverwort species. Fungal DNA present in the 12 most frequent liverwort species was also sequenced to establish the identities of the mycobionts present. Furthermore, the relative abundance of $\delta^{15}N$ isotope was analysed for the first time in polar liverworts to explore whether the fungal species present, and their frequencies of colonisation, are associated with liverwort N nutrition. Of the 34 liverwort species surveyed, fungal colonisation was prevalent, with Sebacina being one of the most abundant fungal taxa. It was shown that dark septate endophyte coils, blue staining coils and rhizoid colonization all correlated positively with plant N content, suggesting that fungi provide nutrients to their liverwort hosts. This supports previous assertions that liverwort-fungal associations are symbiotic in nature. There are, however, limitations to this approach, as relative abundance alone can only provide correlative information. To confirm whether the mycobionts are responsible for higher $\delta^{15}N$ signatures and % nitrogen contents, 15N isotopic label will be applied in Koch's Postulates experiments, as has been previously described by Field et al. (2014, Nature Comms.,Doi: 10.1038). The plant material will then be examined with NanoSIMS to visually confirm if the isotope is transferred via hyphae from mycobiont to plant.

#3-3: Sub-tropical Asia is a hotspot of mycoheterotrophic orchid diversity – Combining stable isotope natural abundance and molecular approaches provides novel insights

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Most fully mycoheterotrophic orchids investigated to date are mycorrhizal with fungi that simultaneously form ectomycorrhizas with forest trees. Only a few mycoheterotrophic orchids are currently known to be mycorrhizal with saprotrophic, mostly wood-decomposing, fungi instead of ectomycorrhizal fungi. This study (Lee et al., 2015) provides evidence that the importance of associations between mycoheterotrophic orchids and saprotrophic fungi is currently under-estimated.

Using microscopic techniques and molecular approaches, mycorrhizal fungi were localized and identified for seven mycoheterotrophic orchid species from four genera and two subfamilies, Vanilloideae and Epidendroideae, growing in four humid and warm sub-tropical forests in Taiwan. Carbon and nitrogen stable isotope natural abundances of mycoheterotrophic orchids and autotrophic reference plants were used in order to elucidate the nutritional resources utilized by the orchids.

Six out of the seven mycoheterotrophic orchid species were mycorrhizal with either wood- or litter-decaying saprotrophic fungi. Only one orchid species was associated with ectomycorrhizal fungi. Stable isotope abundance patterns showed significant distinctions between orchids mycorrhizal with the three groups of fungal hosts. Thus, this is the first report on significantly different carbon and nitrogen stable isotope natural abundance patterns in mycoheterotrophic orchids mycorrhizal with wood- and litter-decomposing fungi.

Mycoheterotrophic orchids utilizing saprotrophic fungi as a carbon and nutrient source are clearly more frequent than hitherto assumed. On the basis of this kind of nutrition, orchids can thrive in deeply shaded, light-limiting forest understoreys even without support from ectomycorrhizal fungi. Sub-tropical East Asia appears to be a hotspot for mycoheterotrophic orchids mycorrhizal with saprotrophic fungi.

Reference:

Lee Y.-I., Yang C.-K. and Gebauer G. (2015) Annals of Botany 116: 423-435

#3-4: Use of ^{13}C to identify the primary drivers of crop residue decay in diverse Canadian agricultural soils

***Gregorich**, Edward – Agriculture & Agri-Food Canada*

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The rate of plant litter decay affects the functioning of ecosystems in several fundamental ways: it governs the amount of carbon stored in the soil, it reflects the rate of solar energy transfer to soil organisms, and it controls the rate of nutrient release. To identify the most important variables governing crop residue decay across a broad scale, we established in 2007 a long-term study at 10 sites across the agricultural regions of Canada, spanning a wide range of climate and soil properties. Barley was grown to maturity in a greenhouse and labelled with 13C to an abundance of about 10 atom%. This residue was applied, in the fall of 2007, to four replicate microcosms. After 0.5, 1, 2, 3, and 5 years the microcosms were destructively sampled at each site and analyzed for recovery of remaining residue-derived 13C. The results showed rapid initial decay of the residue about 50 to 70% was lost after 1 year followed by gradually-diminishing rates of loss, so that about 10 to 20% remained after 5 years. Temporal patterns of decay varied among sites, but a single two-pool exponential model effectively described the kinetics of decay for all sites when time was adjusted for temperature, using the concept of 'thermal time', defined as cumulative degree-years (calculated from measured soil or air temperature above a baseline of 0 degrees C). Soil properties, such as clay or sand content had minimal discernible influence on decay kinetics. These findings demonstrate that temperature is the primary driving variable for residue decay in cool climates like those in the agricultural regions of Canada. They show, further, that projected increases in temperature under various climate change scenarios may exert a strong and lasting influence on residue decay, microbial activity, and storage of carbon in soil.

#3-5: Use of $^{13}CO_2$ and $^{14}CO_2$ to estimate organic matter decomposition in the boreal forest soil

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In ecosystem models, the decomposition of soil organic matter (SOM) is estimated using temperature and moisture as main controlling parameters. However, there is increasing evidence that the decomposition is significantly affected by easily available carbohydrates, in a process called priming. The C assimilation by the boreal forest trees will increase in the future due to climate change. As trees allocate large part of assimilated C to roots and soil microorganisms, particularly to ectomycorrhizal fungi, the priming effect is assumed to get stronger in the future. The aim of the experiment was to identify and quantify the priming effect in the field conditions.

We established a three-year long dual experiment in a boreal Scots pine forest where we performed soil respiration experiments in two scales in a parallel manner. In the smaller scale, we used mesh bags of different mesh size filled with approx. 30 g DW of SOM from the same forest. The larger scale consisted of a trenching experiment where the belowground C flow from standing pine forest was controlled using root-exclusion with mesh fabrics. In both experiments, the mesh size of 1 μmm excluded both tree roots and fungal hyphae and served as priming controls with decreased C supply. In treatments with mesh of 50 μmm the fungal hyphae could penetrate the plots or the bags. The largest C input entered the bags with 1 mm mesh size and the non-trenched field plots.

Carbon isotope (14C and 13C) concentrations of the respired CO2 were measured in all treatments. Using the 13C abundance of respiration of washed roots, as well and the separation of autotrophic and heterotrophic respiration by trenching, we calculated the priming effect in SOM decomposition caused by plant-derived C flow to different treatments. The SOM mass loss data was used to quantify the amount of increased decomposition.

Based on the different isotopic signals in the treatments and the observation that the mass loss was significantly higher in 1 mm and 50 μmm bags compared to 1 μmm bags in the third year, we are able to show in a quantitative manner that plant-derived C flow into the soil increases SOM decomposition.

#3-6: Effects of thinning on leaf carbon isotope composition and stem growth in hinoki cypress plantation forests in Japan

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Thinning is an important method in confer plantations to promote stem growth. Stem growth of remaining trees is expected to increase due to higher light, water and nutrient availability. Carbon isotope ratio ($\delta^{13}C$) in leaves is used as an index of water use efficiency of plants and is expected to decrease after thinning. We investigated changes of leaf $\delta^{13}C$ and stem growth of hinoki cypress plantations in Kochi, Shikoku Island. Ten forests were selected and were thinned with a range of different intensity. Leaves were collected for three years and their $\delta^{13}C$ was measured. Growth of height and diameter at breast height was measured for 5 years after thinning. For leaf and growth properties, response of thinning was calculated as a ratio of the value in the thinned forest to that in the unthinned forest. The response of $\delta^{13}C$ is higher when $\delta^{13}C$ of the thinned forest decreased due to lower water use efficiency. The response of$\delta^{13}C$ of strongly thinned forests (75% removal) was lower than that in moderately thinned forest. At strongly thinned forests, increase of $\delta^{13}C$ indicates lack of adaptation to new environment after thinning. The response of $\delta^{13}C$ was positively correlated with that of height growth but was not related with DBH growth. The results suggest that change of water use efficiency is strongly related with height growth of trees and should affect the shape of trees.

#3-7: Does forest fragmentation alter resource use of apex predators: a test in an experimental landscape

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Removal of native trees from tropical forests, and planting of agricultural crops, homogenize communities and alter biotic interactions. Continued conversion and coincident habitat fragmentation necessitate understanding of how landscape-level processes affect resource use of predators, a group sensitive to perturbation at this scale.

Bats as voracious insectivores provide a system to explore altered resource use in novel homogenized communities at the Stability of Altered Forest Ecosystems (S.A.F.E) experimental landscape, Borneo. The objectives of our project are i) to ‘map’ trophic groups, and ii) analyse variation in intra- and interspecific trophic niche width over a gradient of landscape modification.

Forest replacement with oil palm has taken place throughout 2015/2016, whilst leaving 6 experimental fragments intact. These provide a gradient of landscape forest cover. With decreasing landscape quality we hypothesise greater decoupling of the primary and detrital energy channels. We also expect the isotopic niche of resilient species to contract owing to a reduced resource base, but expansion at the community level as generalists prevail.

‘Pre-conversion’ data was collected in 2015 by harp-trapping 1 control plus 5 future fragments. From over 500 individuals we sampled wing tissue, representing diet over c.40 days. We also collected samples of putative insect prey and basal resources (leaves, litter, fungi and soil).

We will use circular statistics, SIAR and SIBER to analyse changes in food web architecture, energy derivation and niche width respectively. Baseline correction of delta values allows meaningful comparison across the gradient of landscape quality.

We have found preliminary indications of significant interspecific variation within sites, differing between our control and experimental fragments. For example, at our control the standard ellipse area (SEA) of the dominant species across S.A.F.E, Kerivoula intermedia, was significantly smaller than other abundant species, and > 90% overlaps those species’ SEAs, whilst neither observation is true for fragments (<70% overlap).

#3-8: Using stable isotopes for tracing the organic carbon dynamics in storm disturbed mangrove forests: a case study from Can Gio Mangrove Biosphere Reserve, Vietnam

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As a consequence of climate change, the frequency and intensity of storms are increasing in the tropical regions, leaving strong impacts as destroyed and degraded mangrove forests. A number of abiotic (e.g. temperature, light intensity, sedimentation rates) and biotic (e.g. primary producers, benthic fauna composition, organic carbon accumulation) factors could be subsequently altered in deforested areas. These evidences suggest that stable isotope signatures of carbon and nitrogen can be applied to figure out the alterations of biogeochemical processes following the storm disturbance of mangrove forests. Therefore, we compared the δ^{13}C and δ^{15}N values of organic carbon sources and benthic invertebrates in growing and storm disturbed mangrove forests in Can Gio Mangrove Biosphere Reserve, Vietnam for tracing the alterations of organic carbon dynamics. The δ^{13}C of surface sediments significantly increased from -28.6 to -26.4‰ in growing and disturbed forests, reflecting that the sedimentary organic carbon (SOC) was shifted from mangrove organic matters (Ma) to suspended organic matter (SPOM). The δ^{13}C and δ^{15}N values (mean ± S.E) of mangrove crabs in growing forest were -25 ± 0.3 ‰ and 5.4 ± 0.5 ‰, respectively and significantly lower than those of disturbed forest (-20.3 ± 0.4 ‰ and 7.3 ± 0.2 ‰). Similarly, mean δ^{13}C and δ^{15}N values of gastropods significantly shifted from -25 ± 0.3 to -20.2 ± 0.3 ‰ and 3.5 ± 0.8 ‰ to 6.4 ± 0.1 ‰ in growing and disturbed forests, respectively. The stable isotope compositions of the benthic invertebrates showed that the food sources of the benthic invertebrates were significantly shifted from a mixture of Ma, SOC, and benthic microalgae (BMA) in growing to a mixture of SPOM and BMA in disturbed forests. Consequently, the faunal abundance and diversity, organic carbon sequestration and accumulation could be altered following forest disturbance. Our results highlighted that the stable isotopes can effectively record the alterations of organic carbon dynamics following storm disturbance, which provide valuable information for management and conservation of mangrove forests.

#3-9: Nitrate dynamics perceived by triple oxygen isotopes of nitrate in Lake Kizaki

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Nitrate is one of dominant forms of nitrogen in aquatic ecosystems and has several sources and sinks such as atmospheric deposition, nitrification, uptake, and denitrification. Revealing nitrate dynamics leads to understand biological activities in the ecosystems. Nitrogen and oxygen stable isotopes of nitrate are useful tools to study biogeochemical processes in natural ecosystems. In addition, mass-independent 17O enrichment of nitrate has been used to distinguish atmospheric and microbiological nitrate. In semi-closed system such as a lake, using triple oxygen isotopes of nitrate as a natural tracer can allow us to quantify gross production and consumption of nitrate (Michalski and Thiemens 2006, Tsunogai et al. 2011).

Water samples were collected at the deepest in a dimictic lake, Lake Kizaki, on March, May, June, and July 2013. Nitrogen and oxygen isotopic compositions were determined using the denitrifier and thermal decomposition method (Casciotti et al. 2002, Kaiser et al. 2007). The lake has snowfall in winter and atmospheric nitrate was considerable source in the lake. Previous studies showed both nitrification and denitrificaion has occurred in the lake (Yoh et al. 1990, Sasaki et al. 2011). However, nitrification and denitrification can be active simultaneously in the same water and it is difficult to quantify those rates by traditional methods. Mass-independent 17O enrichment of nitrate was expected to shift due to new production of nitrate in the lake.

Nitrate was uniformly distributed in the water column by water circulation on March. From spring to summer, once thermocline was formed and transfer of materials was disturbed, nitrate has accumulated with the decrease of dissolved oxygen. The shift in concentration and mass-independent 17O enrichment of nitrate suggested that nitrification became active from bottom to upward and more than half of nitrate newly produced by nitrification in the deep water had been consumed by denitrification.

#3-10: The role of ^{15}N isotope for improving agricultural nitrogen use efficiency in Latin America

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Isotopes have been used as an important tool in agricultural research, especially after the establishment of International Atomic Energy Agency (IAEA), which has been providing isotopes to be used in scientific research for developing human resource and research capacity. The technique has provided opportunities to obtain important information on soil-plant relationship particularly on plant physiology and crop nutrient use.The advancement on the application of 15N stable isotope in agriculture has helped scientist to quantify biological N fixation(BNF) in legume based cropping systems which saved billions of dollars in N fertilizer cost. Recently the interest on the use of this technique has returned, especially in Latin American countries. More than twelve countries from the region are participating in a common regional project supported by the IAEA, with the objective of enhancing the productivity and sustainability of staple food crops. In this project 15N is used by all participating countries to quantify fertilizer N use efficiency and BNF in legume based cropping systems. Results have shown that usually no more than 30 % of applied N is used by the crops. The remaining portion is almost totally lost and the utilization of residual fertilizer N by the subsequent crop is around 3%. Studies have also shown that crop N use can be increased to 50% or more only by applying it adequately. Brazil, one of project participants, is the second world largest soybean producer (100 million tons in 2015/2016). This amount of grain is produced without supplying synthetic N fertilizer, thanks to the high efficiency of BNF, confirmed by the 15N techniques, which have always been the best way for quantifying BNF.Using this technique, researches have shown that the sugarcane is also benefited by BNF. In the project this is another issue being considered for pasture crops. The project is much promising and it is expected, by the conclusion, a considerable progress in N fertilizer management in all participant countries, with their better contribution in furnishing foods not only to own population, but also to others. Results from the project will be discussed (IAEA and CNPq).

#3-11: Transformation pathways of particulate organic matter in the aphotic layer of aquatic environments: Constraints provided by the compound-specific nitrogen stable isotope composition of amino acids

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We determined the compound-specific nitrogen isotopic signatures of amino acids (delta-15N-AAs) in particulate organic matter (POM) collected in the surface and aphotic layers of coastal marine (Sagami Bay, northwestern Pacific coast) and freshwater environments (Lake Biwa, central Japan). In Lake Biwa, the extent of 15N enrichment with depth was identical for both trophic amino acids (Tr-AAs) and source amino acids (Src-AAs), whereas in Sagami Bay, the depth-dependent 15N enrichment of Tr-AAs was larger than that of Src-AAs. The data were interpreted using delta-15N isotope indicators (plots of the disparity in delta-15N-AAs between Tr-AAs and Src-AAs and the variance of delta-15N of selected Tr-AAs) and a newly developed isotopic model that quantitatively evaluates the extent of organic nitrogen reuse and resynthesis in aphotic layers. Both results indicated that the primary processes responsible for POM transformations in Lake Biwa and Sagami Bay were bacterial heterotrophy and zooplankton metabolism, respectively, although bacterial contributions in Sagami Bay could have been underestimated if bacteria used the salvage pathway instead of the de novo synthetic pathway to obtain Src-AAs. Our data demonstrate that delta-15N-AAs of POM provide constraints on the mechanisms underlying POM transformation in aquatic environments.

#3-12: Using multiple isotopes to identify factors controlling organic matter stabilization and turnover in kettle hole sediments

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Kettle holes are small, pond-like depressional wetlands less than 1ha in size, and they are interspersed in the moraine agricultural landscape of NE Germany. Kettle holes are strongly linked with the surrounding landscape, i.e. they function as catchments for terrestrial organic matter, nutrients, and soils and thus act as biogeochemical hotspots in the landscape. High autochthonous biomass production and sedimentation rates yield the potential for long-term carbon storage belowground. Kettle holes also undergo pronounced wet-dry cycles, which drive the biogeochemical transformations in the sediment. We use the isotopic footprint of sediment organic matter (SOM) to better understand how the hydroperiod (i.e. water holding period over the year) and land use impact SOM transformations and how organic matter is stabilized. We sampled sediment cores from 50 kettle holes from different land use within a 38.2km^2 wide study area that represent the geomorphological and hydrological variability. We measured the Δ14C, δ^{13}C, δ^{15}N and C:N ratio of the bulk sediment and physically and chemically separated fractions that are hypothesized to represent OM pools under different stabilization mechanisms. We expect that the hydroperiod significantly impacts C turnover, i.e. aeration in temporarily water-filled kettle holes stimulates decomposition via microbes, which drive isotopic fractionation in SOM. In addition to land use and hydroperiod (wet-dry cycles), we further relate stable isotope ratios and Δ14C to mineralogical parameters such as texture and different phases of aluminum and iron to examine possible mechanisms of organic matter stabilization via mineral interaction. From these multiple lines of analyses, we plan to characterize kettle holes as an important carbon sinks in agricultural landscapes that are potentially sensitive to the changes in land use, hydrology, and climate.

#3-13: Marking wild malaria mosquitoes using stable isotopes of carbon and nitrogen: a small-scale field demonstration in south-eastern Tanzania

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Background: Marking wild mosquitoes is important for understanding their ecological components and mitigating the diseases they transmit. Stable isotopes are gaining widespread use for non-invasive marking of arthropods, permitting greater understanding of mosquito dispersal and responses to interventions. We describe here a simple technique for marking naturally breeding malaria vectors using stable isotopes of nitrogen (15N) and carbon (13C), and describe potential applications for monitoring vector control measures.

Methods: We created man-made aquatic mosquito habitats and added either15N-enriched potassium nitrate or13C-enriched glucose, leaving non-adulterated habitats as controls. We then left wild mosquitoes to lay eggs in these habitats and monitored the resulting larvae in situ, through all larval stages. Pupae were collected promptly as they appeared and kept in netting cages. Emergent adults were pooled (~4 mosquitoes/pool) and some of the pupae were singly stored, desiccated and analysed using Isotope Ratio Mass Spectrometry (IRMS).

Findings: An. gambiae s.l and Aedes spp. from enriched 13C and enriched 15N larval habitats had significantly higher isotopic levels than controls (P=0.005). Both isotopes produced sufficient distinction between marked and unmarked mosquitoes: mean δ^{15}N for enriched females and males; 275.64±65.12, 247.95±54.55 and mean δ^{15}N in controls; 2.1±0.1, 3.9±1.7 respectively, mean δ^{13}C for enriched females and males; 36.08±5.28, 38.5±6.86 and mean δ^{13}C in controls; -4.3±0.2, -7.9±3.6 respectively. In all cases, there were variations in standardized isotopic ratios between mosquito species.

Conclusion: This study demonstrates that isotopic amendment of semi-natural larval habitat was sufficient to enrich Anopheles spp. mosquitoes with a unique marker. The stable isotopes of nitrogen and carbon can be efficiently delivered to larval habitats receiving natural colonization by mosquitoes. Marking mosquitoes in this context enables studies pursing research questions regarding eco-physiology, dispersal, and responses to vector control interventions.

#3-14: Predicting the fate of terrestrial arthropods under environmental disturbance

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Terrestrial arthropods respire through a series of spiracles, the faunal equivalent of plant stomata. Spiracles permit gas exchange and control respiratory water loss. Desiccation is a major threat to terrestrial arthropods, which is why they show a strong response to moisture gradients. Previously, Ellwood et al.[1] used the Craig-Gordon equation to show that the oxygen composition (18O) of insect blood is a function of environmental temperature and relative humidity. Evaporative enrichment of 18O within haemolymph occurs in the same way as in a body of water. Most microfauna spend their lives within millimetres of their preferred microclimatic region. We therefore propose that the 18O of an individual's blood will allow us to quantify its abiotic niche.

Bird's nest ferns (*Asplenium* spp.) occur across Southeast Asia, and are important reservoirs of arthropod diversity in tropical rainforest and oil palm plantations. The fern's ability to control its internal microclimate makes it a particularly valuable refuge for sensitive animals in oil palm plantations, which are otherwise relatively hot and dry. According to the Intergovernmental Panel on Climate Change (IPCC), Southeast Asia's climate will become more severe, experiencing hotter and drier conditions. Bird's nest ferns have the potential to ameliorate the effects of environmental disturbance and protect sensitive animals where they would otherwise perish. Human health would suffer considerably in the absence of these ecosystem engineers, which provide services such as pollination, biological control, decomposition and maintenance of water quality. By defining the climatic tolerances of these animals, and safeguarding their niches, we can maintain the services they provide.

1.Ellwood, M.D.F., et al., On the vapour trail of an atmospheric imprint in insects. Biology Letters, 2011. 7(4): p. 601-604.

#3-15: Influence of Water Quality on Isotopic Variations Between Freshwater and Estuarine Fishes of Tropical River Ecosystem

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Land use changes such as sand mining and farming activities near river bank can alter the carbon sources of primary production and the food sources or the prey available for fishes of commercial interest in a riverine ecosystem. In order to assess the relative contributions of terrestrial and aquatic sources of carbon to the food web ecosystems and whether there is any effect to the food web due to land use changes, stable isotope analysis was conducted on the muscle tissues of freshwater and estuarine fishes of Perak River, Malaysia. The objective of this study is to assess the impact of land use changes on the water quality of Perak River and if these effects cause changes to the isotopic variation of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) between freshwater and estuarine fish of Perak River. In this study, more depleted $\delta^{13}C$ of muscle tissues in freshwater fish indicates carbon sources from assimilated organic matter of primary production from C3 plants while more enriched $\delta^{13}C$ for estuarine fish implies assimilated organic matter of a mixture of C3 and C4 plants originated from both freshwater and estuarine site. The $\delta^{15}N$ values of fish muscle tissues were significantly higher in estuarine than the freshwater counterpart, which reflect more human activities and land use changes input such as the sewage effluent. This factor is also supported by lower DO, higher TDS and higher conductivity in estuarine sites compared to freshwater sites. This study aimed to provide a reference record for conducting further stable isotope analysis to determine the effects of the land use changes to the food web in tropical river ecosystem for deeper understanding and better management in conserving ecosystem services.

#3-16: Natural abundance of ^{15}N of microbial biomass as a promising indicator for in-situ nutrient-use efficiency of soil microbes

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Nitrogen use efficiency (NUE) of microbe can be calculated by how much organic-nitrogen (N) would be incorporated by microbes and how much of the incorporated N is released from microbial biomass as inorganic N. NUE is the important factor to predict the future primary production (Manzoni and Porporato 2009), because plant growth is generally limited by the supply of inorganic nitrogen (Vitousek and Howarth 1991).

Mooshammer et al. (2015) measured in-situ NUE of soil microbes for the first time. However, the addition of 15N tracer in their measurements would under- or overestimate the NUE due to the disturbance of soil N dynamics. In this study, we applied the natural abundance of nitrogen 15N ($\delta^{15}N$) which can provide in-situ information from environment without disturbance of nitrogen dynamics. Collins et al. (2008) found that $\delta^{15}N$ of microbial biomass ($\delta^{15}N$-microbe) increased in the media with low C/N because 14NH4+ would be preferentially released during N mineralization. $\delta^{15}N$ (=$\delta^{15}N$-microbe – $\delta^{15}N$-substarte N) was also increased suggesting that $\delta^{15}N$ can be related to the microbial NUE. In our study, we confirmed the mechanism of $\delta^{15}N$-microbe increase, and evaluated $\delta^{15}N$ as a new indicator for NUE. We cultured Aspergillus Oryzae in different C/N media for four days, and used only glucose and glycine for the carbon and nitrogen sources. We measured concentration of NH4+, $\delta^{15}N$-NH4+ and $\delta^{15}N$-microbe in each day.

In the media of C/N 5 and 10, considerable amount of NH4+ was released (92% and 60% of supplied glycine-N) by day 4, which means that NUE was gradually decreased. $\delta^{15}N$ was gradually increased (2.7±0.5‰ in day 4) with low $\delta^{15}N$-NH4+ compared with $\delta^{15}N$-microbe. However, in other media with C/N 30, 50 and 100, NH4+ was not released at all (under 2% of glycine-N supplied) and $\delta^{15}N$ was almost 0‰ during the study period. These results supported the mechanism of $\delta^{15}N$-microbe increase which Collins et al., (2008) proposed. In addition, we found strong negative correlations between $\delta^{15}N$ and NUE in the media of C/N 5 and 10 ($\delta^{15}N = -1.7*NUE + 1.4$, $R^2 = 0.92$), indicating that $\delta^{15}N$ can be a promising indicator for NUE.

#3-17: High resolution detection of taxa capable of polycyclic aromatic hydrocarbons degradation in a marine coastal system using SIP-tag

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Polycyclic aromatic hydrocarbons (PAHs), recalcitrant carcinogenic molecules found in nature as a component of crude oil and a byproduct of partial combustion of fossil fuels, are degradable by few microorganisms. The port of Los Angeles, one of the largest ports in the world, experiences constant inputs of PAHs in measurable mg x m-2 concentrations. Those inputs should sustain a seed of PAH-degrading microbes within the natural community in the port seawater. We are studying the potential of that community to use high concentrations of PAHs as a carbon source. Surface water from the highly impacted port of LA and the oligotrophic San Pedro Ocean Time Series (SPOT) location (ca. 15 miles from the port) were incubated over 48-88 hours with 13C- and 12C-naphthalene, the most labile PAH. Extracted DNA was fractionated by density at a high resolution (50 fractions per 5ml), and the community composition of each fraction was determined by tag-sequencing the V4-V5 hypervariable regions of the 16S-rRNA gene. This enabled us to trace shifts of DNA density in specific taxa within the natural community. *Cycloclasticus* sp. and *Colwellia* sp. were the major primary degraders and assimilators of naphthalene in the port of LA, but several other taxa displayed smaller yet discernible shifts. Despite the short distance between the port and the SPOT location, we observed utilization of added naphthalene by very few taxa at SPOT, and it was not as appreciable as the utilization observed at the port. This leads us to believe that these taxa do not specialize on PAH degradation, and thus that the potential for biodegradation of high concentration naphthalene does not extend into the San Pedro Channel.

#3-18: Eating in or eating out. Evidence for site fidelity in beaked whales?

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Beaked whales are the second largest family of cetaceans and in some parts of the world amongst the most numerous cetaceans. As such they are important top predators for marine ecosystems yet little is currently known about the ecology of the majority of the species. Feeding is an intrinsic part of a species' movement and ecology and thus knowledge of traits such as habitat use can help predict responses to environmental change and service future management measures to preserve beaked whale populations.

In this study we analysed carbon and nitrogen stable isotopes in bone samples from strandings of seven beaked whale species within the Atlantic sector of the Southern Ocean to investigate variations in long-term habitat use. Three species (*Mesoplodon layardii*, *M. hectori* and *Hyperoodon planifrons*) showed little intraspecific variation in $\delta^{13}C$ and $\delta^{15}N$ values, suggesting distinct niche or feeding site fidelity. *M. layardii* and *H. planifrons* specimens also showed the same, or very similar, intraspecific mitochondrial haplotypes indicating that a similar trophic ecology was shared by individuals that shared a genetic lineage. The other beaked whale species (*Berardius arnuxii, Ziphius cavirostris, M. grayi* and *M. europaeus*) varied widely in their habitat use and haplotype. $\delta^{13}C$ and $\delta^{15}N$ values moreover identified two trophic groupings with *Ziphius cavirostris*, *M. europaeus* and *M. grayi* making use of a distinctly different feeding niche than the other four species.

#3-19: Amino acid systematics using $\delta^{13}C$ and $\delta^{15}N$ in benthic archaeal methanotrophy at Black Sea

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Compound-specific isotope analysis (CSIA) for nitrogen isotopic composition of amino acids is a useful tool to understand biochemical pathways and ecological systems (cf. invited talk in IsoEcol 2016). Our recent analytical development of CSIA also opened up the new insight of sub-seafloor methane cycles and the central role of protein amino acids. The anaerobic oxidation of methane (AOM) in marine sediments is an important microbial process in the global carbon cycle and also greenhouse gas emission constraints. For further investigation of amino acid metabolism in archaeal methanotrophy, we conducted CSIA of protein type amino acids for carbon and nitrogen by using anaerobic methanotrophic archaea (ANME) samples from a methane seep site in Black Sea (e.g., coenzyme factor 430 [1, 2]; classification of membrane lipids [3]). We separated the ANME samples into three sections; ANME-1 dominated pink section, ANME-2 dominated black and carbonate section. Here, we discuss the two-dimensional diagram using delta13C and delta15N of CSIA to trace the metabolic pathway of bioavailable sub-seafloor methane, resulting a concurrent 13C-depletion and 15N-enrichment among the ANME-1 and ANME-2 communities.

References:

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[2] Kaneko et al., Quantitative analysis of coenzyme F430 in environmental samples: a new diagnostic tool for methanogenesis and anaerobic methane oxidation. Analytical Chemistry 86, 3633-3638 (2014).

[3] Blumenberg et al., Membrane lipid patterns typify distinct anaerobic methanotrophic consortia. PNAS 101, 11111-11116 (2004).

#3-20: Stable isotope approach for the reproductive biology of small pelagic fishes: evidences for income resource allocation to egg production in the Japanese anchovy and chub mackerel

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Temporal relationship between energy acquisition and allocation to reproduction is a common interest in the reproductive strategy of animals including fish, and has been considered as a continuum between capital and income resources. Generally, income breeding is considered to be common and more rewarding than capital breeding for multiple-batch-spawning fish species with indeterminate fecundity, such as short-lived pelagic fishes. We firstly examined the breeding strategy (income-capital continuum) directly using the carbon and nitrogen stable isotope ratio (SIR) as a tracer for Japanese anchovy *Engraulis japonicus* and chub mackerel *Scomber japonicus* under experimental condition. Diet-switching experiments were conducted in which feed with low (or high) SIRs was provided initially for more than a month and then switched to feed with high (or low) SIRs, as conducted for determining the SIR turnover rate in animal tissues. The effect of the latter feed on eggs was evident for both species with the changing SIRs for both carbon and nitrogen after the diet was switched, suggesting that income resources were primarily used over a season. However, the estimated transfer rates of the resources from food intake to eggs produced varied along with the degree of prevailing temperature in the experiments of anchovy. Our findings also suggested that, although well-fed female Japanese anchovy are largely income breeders, the resource allocation strategy used for egg production might be more complicated than previously thought. Thus SIR experiments could expand our knowledge for the resource allocation strategy and reveal potential factors which control capital-income continuum for many fish species.

#3-21: Integrated approaches of N_2/Ar measurement, stable isotopic and molecular techniques and investigating dissolved organic matter characteristics to evidence the denitrification in river

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Keisuke Koba – TUAT

Midori Yano – TUAT

Akiko Makabe – JAMSTEC

Co Thi Kinh – TUAT

Akihiko Terada – TUAT

Sakae Toyoda – Tokyo Tech

Naohiro Yoshida – Tokyo Tech

Yotaro Tanaka – Kyoto University

Masanori Katsuyama – Kyoto University

Muneoki Yoh – TUAT

Nitrogen in rivers can be remove by denitrification in sediments and hyporheic zones where suitable condition for the denitrification such as high labile organic carbon, denitrifying bacteria, and anoxic condition is established. Denitrification is expected to be more active in N polluted rivers than pristine rivers because the larger N flows into hyporheic zones would raise more anoxic states. We applied several approaches such as N2/Ar measurement, stable isotope abundance, functional genes, and characteristics of dissolved organic matter (DOM). In this study, we measured concentrations (e.g. [TDN]) and stable isotope ratios of TDN, NH4+, NO2-, NO3-, and N2O, as well as N2/Ar, denitrifying gene abundances (nirK, nirS, nosZCladeI, nosZCladeII), and TOC & DOM quality in surface water of Tama river, Tokyo, Japan. [TDN] and δ^{15}N-TDN at downstream of river were much higher than that at upstream of river confirmed the water chemistry in downstream sites were effected by wastewater of plants along this river. [N2O], δ^{15}N and δ18O of N2O were close to atmospherically-equilibrated values indicating that N2O production were negligible. [N2O] in downstream sites were over- to super-saturation and δ^{15}N-N2Oproduced were lower than δ^{15}N of NH4+, NO2-, NO3-. The differences in δ^{15}N among N species, such as ?δ^{15}NNO3- – NO2-, ?δ^{15}NNO2- – N2O, and ?δ^{15}NNO3- – N2O fell in the reported ranges for denitrification. Denitrifying gene abundances, [TOC], and humic and protein components of DOM in downstream sites were higher than those in upstream sites indicating the favorable environments for denitrification in downstream sites. Although N2/Ar measurement did not directly indicate for denitrification because [N2] approached to air-[N2] denitrification, stable isotopic data, abundances of functional genes, and DOM characteristics all indicated the occurrence of denitrification in downstream sites in Tama River which would remove the N.

#3-22: Evapotranspiration partitioning: From local to global

Wei, *Zhongwang – University of Tokyo*

Yoshimura Kei – University of Tokyo

Partitioning ecosystem evapotranspiration (ET) into soil evaporation (E) and transpiration (T) is crucial for understanding hydrological processes. Recently isotopic method provides an unprecedented opportunity for ET partitioning across a variety of spatial and temporal scale which is unattainable by conventional technique. However, most of isotope-based studies show that T generally contributes more than 70% to the ET, while non-isotopic measurement generally leads to considerably smaller transpiration fractions. Thus, quantitatively understanding of ET partitioning is somewhat arbitrary and remains the necessary of a theoretical and technical change, in both in-situ measurement and global scale. Here we compare the isotopic T/ET approach with a two-layer model in a paddy field throughout a full growing season. The agreement between model and isotopic approach demonstrated that the robustness of using isotope measurements for ET partitioning. The discrepancy between isotopic and non-isotopic ET partitioning approach in previous studies may result from mismatch of measurement time scale, because isotopic method is only applicable under strong transpiration condition. As same as many previous studies, we found T/ET was strongly controlled by the leaf area index (LAI). Based on relationship between T/ET and LAI in our isotopic measurement and other previous studies, we established a quantitative relationship between ET partitioning and LAI for different vegetation systems. we developed an ET partitioning algorithm, combining ET estimated by remote sensing, land surface models and LAI regression documented by 56 previous studies. The algorithm was used to access spatial patterns and global average T/ET. Our global mean transpiration ratio (50%) is smaller than the literature complication studies but in good agreement with the value reported in GCMs. On the other hand, it was significantly smaller than that reported in isotopic approaches. Although further validation is required, our measured provided a new inspiration for partitioning global evapotranspiration and suggested vegetation plays a major role in driving the contribution of T/ET.

#3-23: Nitrogen deposition patterns and sources in the Greater Yellowstone Ecosystem, USA, determined from δ^{15}N values of epiphytic lichens

***Williams**, David – University of Wyoming*

Shannon Albeke – University of Wyoming

Jill McMurray – US Forest Service

Sarah Anderson – Washington State University

Dave Evans – Washington State University

Atmospheric deposition of reactive nitrogen (Nr) continues to be high in the Western US, and hotspots of Nr deposition are found within remote, wildland areas managed for conservation of natural biodiversity. What is critically missing is an understanding of the sources and spatial pattern of Nr pollution in these areas. Is the source of Nr deposition from industrial or agricultural activities, or some mixture of these, and what are the geographic origins of these sources? Answers to these questions will focus efforts on the best management practices for protecting sensitive ecosystems, and guide policies that regulate air quality. Epiphytic lichens often are used to monitor Nr deposition and its impacts on ecosystems. Because lichens absorb their nutrients directly from the atmosphere they potentially incorporate the isotopic signature (15N/14N) of Nr. We measured δ^{15}N values and N% content in bulk tissue of epiphytic lichens collected in the Wind River Range, Wyoming, on the southern end of the Greater Yellowstone Ecosystem (GYE), near potential N emission sources in an area of active oil and gas development, and in remote distal areas in wildland environments. Ion exchange resin samplers, which provided estimates of Nr deposition rates were collocated at lichen sampling sites. δ^{15}N values and tissue N content of lichens were strongly correlated to Nr deposition rate across sampling locations. δ^{15}N ranged from -14.0 to -6.5‰ in lichen tissue and was positively correlated with total Nr deposition rate, a patterns consistent with an industrial combustion source of Nr. In contrast, historic herbarium samples collected on the west slope of the GYE show progressive depletion in 15N, consistent with intensification of agricultural activity as a major source of Nr. Our analyses highlight the regionally complex nature of Nr sources in the GYE, and illustrate the utility of lichen δ^{15}N measurements for understanding spatial and temporal patterns of Nr deposition.

#3-24: Nitrate N and O isotope analysis of soil using ion exchange membranes and the denitrifer method

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Chieko Takahashi – Tokyo University of Agriculture and Technology

Ayumi Tanaka-Oda – Forestry and Forest Products Research Institute

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Eric Bremer – Western Ag Innovations

Keisuke Koba – Tokyo University of Agriculture and Technology

The stable isotopic composition of nitrate has been used to identify sources and infer processes in soil ecosystems. Monitoring soil nitrate using ion exchange membrane (IEM; Plant Root Simulator (PRS) probes) technique has advantage compared to traditional soil sampling because it represents an integrated measure of soil N and causes minimal disturbance. However, previous studies have reported isotopic fractionation during extraction of ion exchange resin using column, or oxygen isotope exchange between nitrate and water under low pH conditions.

In order to establish a method for measuring isotopic signatures of soil nitrate using IEM, we compared the nitrate N and O isotopes of loading solution to extracted nitrate from IEM using the denitrifer method. After conditioning, nitrogen was loaded by shaking IEM with 20ml of 0 or 100 microM KNO3 (0 or 0.11 micromol/ cm^2) in a zipper bag for 24 h. The monthly averaged nitrate supply rate of the Japanese temperate N-saturated forest soil in Gunma prefecture was 1.28 ± 1.00 micromol/ cm^2/ month (n=117). After loading, nitrate was extracted by 1 or 3 h shaking with 20ml of 0.5 M NaCl (instead of HCl) in a new zipper bag, and it was repeated 4 or 3 times for 1 or 3 h extraction, respectively. Average recovery of loaded nitrate was 123% (or 152%), 17% (or 28%), 5% (or 12%) and 4% for 1st, 2nd, 3rd and 4th extraction with 1 h (or 3 h) shaking. Although nitrate concentrations were sufficiently low after conditioning, there was possibility of contamination from atmospheric nitrate during shaking, which amounted to 1.68 ± 1.52 nmol/ cm^2/ h (mainly occurred during 24 h loading) and showed -3.6‰ for $\delta^{15}N$ and -4.6‰ for $\delta^{18}O$. Isotopic fractionation during extraction was not significant. The observed difference in $\delta^{15}N$ and $\delta^{18}O$ values between loading solution and extracted nitrate in a sample with the least contamination was 0.2‰ for $\delta^{15}N$ and 1.0‰ for $\delta^{18}O$. Values were in good agreement.

Thus, we concluded that the denitrifer method can be used with the IEM technique in case nitrate concentrations of samples are sufficiently high compared to IEM blanks, and that it is recommended to elute IEM inside an airtight container.

#3-25: Growth and calcification effects of ocean acidification on cultured bivalve, *Scaphrca broughtonii*, revealed from radiocarbon and stable carbon isotopes

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Kozue Nishida – Geological Survey of Japan, National Institute of Advanced Institute Science and Technology, Japan

Atsushi Suzuki – Geological Survey of Japan, National Institute of Advanced Institute Science and Technology, Japan

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Mizuho Sato – Geological Survey of Japan, National Institute of Advanced Institute Science and Technology, Japan

Yukihiro Nojiri – Department of Earth and Environmental Sciences, Hirosaki University, Aomori, Japan

The effects of ocean acidification on bivalve growth and calcification have been widely debated in recent years due to potential impacts on the aquaculture sector and thus human food security. This study employs radiocarbon and stable carbon isotopes in order to investigate bivalve assimilation of dissolved inorganic carbon (DIC) in seawater and organic carbon in plankton feed with changing seawater chemistry. Sixty-four specimens of the filter-feeding clam *Scapharca broughtonii* (Bivalvia: Arcidae) were cultured in aquaria of 6 different pCO2 conditions at 25 °C for 8 weeks, with a novel high-precision pCO2 control system which maintained steady CO2 levels. Bivalve shells and soft tissues, as well as the relevant water and plankton feed samples, were then analysed forδ^{13}C in an Isotope Ratio Mass Spectrometer (IRMS) and Δ14C in an Accelerator Mass Spectrometer (AMS) to examine variations in end-member contributions to the bivalve components. We will present the result in this presentation with newly obtained radiocarbon data.

#3-26: The isotopic effects of fish otoliths stored in organic solvents

***Yongwen**, Gao – Makah Fisheries Management*

Stephen Crowley – University of Liverpool

Robert Conrad – NW Indian Fisheries Commission

David Dettman – University of Arizona

Fish otoliths are important proxies for climate change and ecological studies and are typically stored in glycerin or ethanol for preservation. This study is the first attempt to assess the isotopic effects of these preservatives on carbon and oxygen isotope ratios of otolith aragonite. Experimental tests from the 4th annulus of dried Pacific halibut otoliths collected from the Washington west coast were compared to samples from the same otoliths collected after 30 day storage in either glycerin or ethanol. In addition, isotopic measurements of abiogenic pure aragonite samples soaked in glycerin, ethanol, acetone, dichlorom (DCM), and methanol were compared to the initial values following the same protocol. No 18O effect was observed for otoliths stored in glycerin or ethanol; and no isotopic effects (both 18O and 13C) were observed for abiogenic pure aragonite stored in the five organic solvents commonly used in geochemical laboratories. Although there was a significant but very small difference in carbon isotope ratios of halibut otoliths, the shift was of a barely measureable magnitude and the statistically significant difference only in 13C values may result from the inhomogeneous composition and structure of otoliths. Thus we concluded that there was no isotopic exchange during organic solvent storage or preservative interference in the isotope ratio measurements.

5.4 Poster Session 4

Ecological processes across time and space using isoscapes

#4-1: Habitat dependent differences in resource use within shark populations on a global scale

***Bird**, Christopher – University of Southampton*

Clive Trueman – University of Southampton

Ana Verissimo – University of Southampton

Sarah Magozzi – University of Southampton

Sharks play a fundamental role within many marine environments yet their continued exploitation and limited recovery potential is causing global declines in numerous populations. To effectively inform management and conservation initiatives, many studies have attempted to uncover key aspects of trophic and spatial ecology using stable isotope analysis but to date no attempts have been made to collate this information.

We compiled bulk carbon and nitrogen stable isotope information from newly collected samples, 43 published and 4 unpublished datasets resulting in a total of 3872 individual sharks. These data were examined in conjunction with a newly developed theoretical model predicting the spatio-temporal distribution of $\delta^{13}C$ values of phytoplankton on a global scale at a one-degree and monthly resolution. We compared bulk $\delta^{13}C$ values, latitudinal trends in $\delta^{13}C$ values, and isotopic variance among shelf, oceanic and deep-sea sharks.

The results from this study highlighted that although sampling biases can have a strong influence on isotopic variability, significant ecologically-relevant patterns were still prevalent. Isotopic metrics alone were able to distinguish the habitat preferences of ~80% of the species sampled, highlighting distinct ecological differences between these habitats. Latitudinal variations in $\delta^{13}C$ values in shelf sharks closely matched theoretical trends, implying limited latitudinal movements. By contrast oceanic sharks likely assimilate nutrients from broad latitudinal ranges, particularly at both low and high latitude extremes.

Although bulk $\delta^{13}C$ values in consumer tissues may be influenced by many factors including spatio-temporal variation in isotopic baselines, differences in trophic enrichments, and analytical methods, we show that ecological influences on $\delta^{13}C$ values are strong enough to overcome limitations providing sufficient data are available, and we encourage data-sharing within the stable isotope community.

#4-3: Nitrogen isotope ratios of phytoplankton in the northwestern North Pacific

***Chisato**, Yoshikawa – JAMSTEC*

Haruyo Yamaguchi – NIES

Nanako O.Ogawa, Hisami Suga, Akiko Makabe,Yohei Matsui, Shinsuke Kawagucci, Tetsuichi Fujiki, Naomi Harada, Naohiko Ohkouchi

Nitrogen is a limiting element for the phytoplankton growth in the ocean. The phytoplankton assimilates not only nitrate, but also ammonium, and nitrite as the nitrogen source. Each phytoplankton taxon has a different preference for these nitrogen sources. 15N-labeling technique with incubation has been used for estimating the contributions of these substrates to the phytoplankton. However, it may not duplicate the actual environment due to addition of labeled nutrient and artificial temperature and light conditions. In this study, we estimated the contributions of each substrate to phytoplankton using natural abundances of nitrogen isotopes of nitrate and phytoplankton and a marine nitrogen isotope model. We conducted water and particle samplings at the K2 station (47‹N, 160‹E) during cruise KH-15-J01 of R/V Hakuho Maru in July 2015. Water samples were collected using rosette-mounted Niskin bottles. They were filtered and removed nitrite and then preserved at □|20 °C. The nitrogen isotopic composition (δ^{15}N) of nitrate were measured by GC-IRMS after the samples were processed with the denitrifier method. Particle samples were collected on 0.2 and 20 μmm filters using an in situ filtration sampler from 80 L of seawater at the chlorophyll maximum depth. The collected particles were sorted for two size-fractionated phytoplankton samples (0.2-5 μmm, and 5-20 μmm), using a cell sorter. The δ^{15}N of these samples were determined by a sensitivity-improved EA-IRMS. The δ^{15}N values of phytoplankton (-6 ‰ for 0.2-5 μmm; -3 ‰ for 5-20 μmm) are significantly lower than that expected from the δ^{15}N value of nitrate at the same depth (8 ‰) and the isotopic fractionation associated with the nitrate assimilation by phytoplankton (~5 ‰). The results suggested that the phytoplankton assimilates not only nitrate, but also nitrogenous nutrients except nitrate, maybe ammonium at the K2 station in July. Our marine nitrogen isotope model supported this hypothesis.

#4-5: Developing isoscapes to underpin the description and analysis of marine food webs

***Duffill Telsnig**, Jessica – Newcastle University*

Simon Jennings – Centre for Environment Fisheries and Aquaculture Science

Aileen Mill – Newcastle University

Nicolas Polunin – Newcastle University

Food web models can be used to describe and predict relationships between species in marine environments. Stable isotopes can improve these models by establishing the source of carbon in species' diets ($\delta^{13}C$) and their trophic position ($\delta^{15}N$). This study explores differences in the structure of marine food webs across four UK seas (Irish, Celtic, North and English Channel) using 59 marine species. A large variation of up to 13.5‰ $\delta^{15}N$ and 13.2‰ $\delta^{13}C$ exists between sites and species. This is partly due to differences in the food web structure but also to spatial variations in food web baselines, the latter needing to be accounted for using a primary consumer baseline for $\delta^{13}C$ and $\delta^{15}N$. An isoscape was created across the four seas using *Aequipecten opercularis* (queen scallop), which has slow tissue turnover, is widely distributed within UK waters and has a diet consisting of a combination of material which supports benthic production and phytoplankton. Environmental variables were used to predict $\delta^{13}C$ and $\delta^{15}N$ values of *A. opercularis* by creating linear models. The complete isoscape provides a robust baseline for spatial variation in the marine species' trophodynamics and especially in the extent of benthic-pelagic coupling. These differences underpin the structure and stability of communities and fisheries yield, ultimately essential for the planning and management of diverse human uses of these dynamic marine waters.

#4-6: ^{87}Sr/^{86}Sr of whole otoliths reveal the different movement patterns between upstream and downstream populations of three-spined sticklebacks

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Ichiro Tayasu – Research Institute for Humanity and Nature

Takanori Nakano – Research Institute for Humanity and Nature

Shin Ki-Cheol – Research Institute for Humanity and Nature

Seiichi Mori – Gifu-keizai University

Manabu Kume – National Institute of Genetics

Syotaro Nishida – Gifu-keizai University

Strontium stable isotope ratio (87Sr/86Sr) of otolith has been used as one of the powerful tools in the studies about the fish movements, especially migratory fish. They have potential advantage in evaluating the habitat use of freshwater fish. So we examined the movements of freshwater-type three-spined sticklebacks (*Gasterosteus aculeatus*) in tributary rivers by 87Sr/86Sr ratios of whole otoliths and the ambient water. As a result, (1) 87Sr/86Sr ratios of otoliths of three-spined sticklebacks and those of ambient water in isolated habitats were consistent with considerable accuracy (87Sr/86Srotolith = 0.96 ~ 87Sr/86Srwater + 0.026, $r = 0.996$, $p < 0.001$). (2) 87Sr/86Sr ratios of water in the upstream and downstream in the tributary rivers were clearly different. (3) In the upstream population, 87Sr/86Sr ratios of otoliths were consistent with those of the ambient water. By contrast, in downstream population, 87Sr/86Sr ratios of otoliths were various and some otoliths had 87Sr/86Sr ratios close to those of upstream water or the main river water. Those findings clearly showed that three-spined sticklebacks had different movement patterns between upstream and downstream in the tributary rivers.

#4-7: Latitudinal distributions of stable nitrogen and carbon isotopes in the epipelagic ecosystem in the Pacific Ocean: Contribution of nitrogen fixation to grazing food webs

***Horii**, Sachiko – The University of Tokyo*

Kazutaka Takahashi – The University of Tokyo

Takuhei Shiozaki – The University of Tokyo

Fuminori Hashihama – Tokyo University of Marine Science and Technology

Ken Furuya – The University of Tokyo

This study investigated nitrogen sources for biological production and their pathway within grazing food chain in the open water of the Pacific Ocean by means of carbon and nitrogen stable isotope ratios ($\delta^{15}N$ and $\delta^{13}C$). The isotopic signatures were determined for suspended particles (0.6 – 200 μmm), two fractions of net-plankton (0.2 – 0.5 mm and 0.5 – 1.0 mm) and micronektonic myctophid fish from epipelagic layers along 170°W, ranging from Bering Sea (60°N) to southern subtropical front (40°S) during summer season in 2013 and 2014. Generally the $\delta^{15}N$ of suspended particles increased with decrease of surface nitrate concentration, however those in the subtropical area were negatively correlated with rates of in situ N2 fixation activity, suggesting significant input of biologically fixed nitrogen to primary producers. $\delta^{15}N$ of each biotic component showed similar fluctuation pattern along latitude and the trophic levels inferred from $\delta^{15}N$ in net-planktons and myctophid fish were 2 and 3-4, respectively, regardless of regions. These results indicate significant contribution of diazotrophic nitrogen to primary and secondary consumers up to micronektonic fish, which is important prey for large marine predators. Fitting lines of $\delta^{13}C$ – $\delta^{15}N$ diagram for different regions from the equatorial, the subtropical and high latitude do not have significantly different slopes, suggesting the presence of common physiological aspects of feeding processes throughout the open ocean ecosystems including the area with high N2 fixation activity. The $\delta^{13}C$ – $\delta^{15}N$ fitting line of the region between 20° and 25° north and south showed significantly lower $\delta^{15}N$ along trophic levels from that of any other region (ANCOVA test with post hoc Tukey's HSD, $p < 0.05$), indicating consistent contribution of diazotrophic nitrogen to biological production in this region.

#4-8: Movements of Individual Salmon (*Salmo salar*) in the Baltic Sea Revealed by Otolith Stable Isotopes

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Jyrki Torniainen – University of Jyväskylä

Anssi Lensu – University of Jyväskylä

Marja Keinänen – Natural Resources Institute Finland

Willian P. Patterson – University of Saskatchewan

Eloni Sonninen – University of Helsinki

Pekka J. Vuorinen – Natural Resources Institute Finland

Roger I. Jones – University of Jyväskylä

Spatial stable isotope variability (isoscapes) offer potential for various applications in migration ecology, wherein stable isotope values of animal tissues are compared to values measured from the environment. We used otolith oxygen (δ18O) and carbon ($\delta^{13}C$) stable isotope measurements to investigate seasonal movement patterns of individual Baltic Sea salmon and the migratory connectivity of one breeding population in their non-breeding areas in the Baltic Sea. Spatial variability in δ18OH2O and δ^{13}CDIC values of the water collected around the Baltic Sea was determined to generate horizontal and vertical gridded isoscapes. Salmon individuals ascending the river to spawn were collected in early summer in four sequent years. Prior to stable isotope analysis, micro-sampling of the otolith powder was conducted using a three-dimensional micromilling system. Two selected time points from salmon otoliths, the 2nd summer in the sea and the following winter, were analysed for isotope values. The differences between summer and winter in Baltic Sea δ18OH2O values were low, whereas δ^{13}CDIC values exhibited substantial seasonal variability. Preliminary tests of probable locations of individual salmon via spatial probability surface maps revealed that present knowledge about $\delta^{13}C$ values is insufficient for modelling purposes. Therefore only δ18O values were used to study seasonal and annual differences in locations of individual salmon in their non-breeding areas at the sea and locations in relation to the time of ascending the spawning river. Our results indicate that during their feeding migrations salmon move considerable distances within their non-breeding area and that migratory connectivity of the breeding population varies in spatial and temporal scales, the extent of which is poorly understood in salmon ecology. We conclude that better knowledge of movements within the non-breeding areas of migratory animals is important for understanding possible drivers (e.g. resource distribution) of animal migrations. Our observations also raise concerns over how to conserve and manage populations which are continuously moving substantial distances in their non-breeding area.

#4-9: Interaction between native and alien gammarids

***Liu**, Chien-Fan – Queen Mary University of London*

Jonathan Grey – Lancaster Environment Centre, Lancaster University, UK

Biological invasions are a significant driver of human-induced global change. Freshwater environments are particularly susceptible to invasion due to the enhanced dispersal capabilities associated with aquatic organisms. Amphipods of the family Gammaridae are an especially successful group at expanding their freshwater ranges. Changes to gammarid community composition caused by alien gammarids have important implications for ecosystem structure and functioning, yet are not well understood.

The Ponto-Caspian Dikerogammarus villosus, known as the killer shrimp, was first discovered in the Norfolk Broads, UK in early 2013. Several studies have reported D. villosus to be a destructive invader and hence, the structure of Broads communities could be extensively altered.

We carried out seasonal field surveys at four sites in the Broads, ranging from low- to high-density scenarios (based on catch effort), which reflects D. villosus time of establishment. Parallel temperature-controlled mesocosm experiments allow us to investigate the potential casual relationships between allopatric/sympatric native Gammarus pulex and D. villosus, as well as their functional impacts under different D. villosus invasion densities. We use stable isotope analysis to assess trophic position, isotopic niche and other population metrics, and diet estimation via the packages Stable isotope Bayesian ellipse in R (SIBER) and stable isotope analysis in R (SIAR).

Results from the field showed that niche space and trophic position of D. villosus remain similar across all sites, both temporally and spatially, whether they are established or establishing. Mesocosm data showed that the loss of Asellus aquaticus was significant in mesocosms populated with D. villosus, whereas survival of the native G. pulex was not greatly impacted. Leaf litter decomposition rate and chlorophyll a concentration, as an indication of functional impacts, did not vary significantly across treatments. While native shredders A. aquaticus were greatly reduced, D. villosus potentially fulfilled that functional role. Stable isotope data did not reveal a predatory superiority of D. villosus over G. pulex.

#4-11: Seasonal change of particulate organic carbon isotopic ratios and high production in anaerobic layer in the monomictic lake, waku-ike (Nagano Prefecture)

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Yuta Tan – Tokyo Institute of Technology

Sakae Toyoda – Tokyo Institute of Technology

Keita Yamada – Tokyo Institute of Technology

Naohiro Yoshida – Tokyo Institute of Technology

The stable carbon isotopic compositions of autochthonous particulate organic carbons (POC) have helped to assess their sources and cycling. Many studies have achieved on aerobic water environment and have made the carbon cycle model for estimating primary production. The carbon cycle model in aerobic condition is driven by phytoplankton primary production and the relationship between the amounts of production and environment conditions such as pH and water temperature have been also investigated (A.de Kluijver et al., 2014,). On the other hand, in the anaerobic conditions such as lake bottom and sediment, where not only oxygenic phototrophs but also anoxygenic photoautotrophic bacteria and chemoautotrophic bacteria contribute to primary production, the proportion of anaerobic biomass production to the whole have been estimated to be 3 to 90% by using carbon fixation rate in situ or pigments (Parkin and Brock, 1980; Taipale et al. 2011). In order to consider both aerobic and anaerobic ecosystem, more carbon isotopic observations of anaerobic conditions would be needed. In this study, we measured concentrations and isotopic compositions of dissolved and particulate carbon compounds in water column and sediments. The production of POC in anaerobic layer was found to be higher than that in aerobic layer by seasonal observation. We estimated the contributing factors by using isotopic mixing calculation and fractionation factors of carbon fixation pathways.

#4-12: Nutrient contributions from the ocean across a river basin using stable isotope analysis in Shiretoko Peninsula

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Yasuyuki Maruya – Koto University

Kei Matsumoto – Kitami Institute of Technology

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Shiretoko was registered as natural World Heritage Property on the 17th of July 2005. The Shiretoko World Heritage area is a small pennusla where a unique interaction occurs between the terrestrial and oceanic systems, which are linked by nutrient exchange processes. Pink (*Oncorhynchus gorbuscha)* and chum salmon (O. keta) move upstream and feed inland animals, such as bears (*Ursus arctos*) and sea eagles (*Haliaeetus* spp.). Conversely, runoff processes result in the transport of nutrients from the terrestrial system to the ocean. Shiretoko thus has a unique coupled nutrient circulation system over its 50 km long by 15 km wide peninsula. When we consider nutrient circulation between the land and ocean, nutrient flux from a river mainly occurs as dissolved and particulate nutrients. Total nitrogen may be transported in not only river channels but also across entire river basins such as via ground water and migratory animals. In a conserved area like Shiretoko, nutrient flux is dominated by particulate nutrients, such as particulate organic matter, which flow out mainly during floods. Since marine derived nutrients (MDN) are hypothesized to largely be transported in particulate form, it is necessary to investigate the contribution of particulate MDN in forest ground surface soils to the total MDN at the river ecosystem scale. In this study we investigated TN export from an entire river basin of the Rausu river. Also, the contribution of MDN to particulate nitrogen in river basin soils was investigated in the Rausu and Rusa river basins. As a result, the contribution of MDN to particulate nitrogen in river basin soils was estimated to be more than 23 % using stable isotope analysis of nitrogen ($\delta^{15}N$).

#4-14: The dark side of the black caiman: spatial and temporal trophic niche variations

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A floating scientific platform was built in the Kaw marshes (French Guiana) to study this unique and still unknown ecosystem. Preliminary research has shown this pond is seasonally colonized by a large population of black caimans and represents the most important breeding site for many rare bird species. At the top of the food web, the black caiman is the largest neotropical predator and a particularly threatened crocodilian species, which implies that they have a major structuring role within the swamp ecosystem. Our preliminary study during the dry and wet season revealed important seasonal variations in the structure of the Agami pond population and the temporal presence of large caimans during the wet season (rarely observed in the Kaw marshes). Thus, coupling isotopic analysis (controlled diet and field studies) and different monitoring approaches (behavioral, microchip marking and satellite Argos tracking), we aim to conduct a detailed study of the Agami pond black caiman population: (i) to study the structure of the population and its variation over time, (ii) to estimate the intra- and inter-annual movements of individual caimans and identify potential feeding, reproductive and nesting areas in the Kaw marsh and (iii) to estimate this top predator's role on the marsh's ecological functioning and survival throughout the year. For this purpose, we will compare different field periods during the wet and dry seasons characterized by different hydrological and ecological patterns. This new information will help predict species response to disturbance, which is a prerequisite to effective conservation plan management involving local people and wildlife administrations. Finally, by coupling Argos tracking and stable isotopes, we will collect significant and valuable data for future applications at a global scale.

#4-16: Variation of foliar 15N natural abundance in Alnus species regenerated at different altitude after a massive landslide on Mt. Ontake in central Japan

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The objective of this study is to clear the variation of foliar 15N natural abundance of naturally established Alnus species and try to evaluate the contribution ratios of N2 fixation to N absorption at the massive landslide area in 1984 on Mt. Ontake in Japan. The measurements were conducted at four plots, which were set up at different elevation to monitor the periodical changes of vegetation and soil properties during 30 years after the disturbance; 1100 m (Low site), about 1700 m (Middle site), and about 2000 m (High site). At the high elevation, two plots were used; one was set at the site remained surface soil (High site +S) and the other was set at the site without surface soil (High site -S). In August, from 2012 to 2014, we measured the 15N natural abundance in leaves of Alnus species, *A. maximowiczii* and *A. matsumurae* in two High sites and Middle site, *A. firma* in the Middle site, and *A. hirsuta* in the Low site, and associated non-N2 fixer species, Betula species, at each site. We also measured the 15N natural abundance of the surface soil and the leaf litter in organic layer at High site -S and Low site. The foliar $\delta^{15}N$ values of Alnus species were -2.4 to -1.3 and lower at Lower site than other sites. At higher altitude, *A. maximowiczii* and *A. matsumurae* showed no difference in the foliar $\delta^{15}N$ values despite of the presence of surface soil. On the other hand, the foliar $\delta^{15}N$ values of Betula species were -5.3 to -3.9 and lower than those of Alnus species except Middle site. The $\delta^{15}N$ values of surface soil was higher at Lower site, -0.4, than Higher -S site, -2.3, and was higher than those of leaf litter at each site, -2.4 and -3.4, respectively. The estimated fractional contribution of N2 fixation to the absorbed N was lower in *A. hirsuta* at Low site, which showed the faster revegetation rate after the disturbance, than those in *A. maximowiczii* and *A. matsumurae* at both High +S site and High -S site. From these results, it was suggested that higher contribution rate of N2 fixation in *Alnus* species would be maintained even after 30 years from the disturbance.

#4-17: Tracking the Serengeti migration: linking hydrogen isotopes in wildebeest hair and local water

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Effective conservation of a migratory species requires a full understanding of all aspects of the animal’s ecology, including route taken, how diet and nutritional status fluctuate along the way, and what impact this has on health overall. However, monitoring these variables in the wild can be challenging. For example, traditional methods of tracking movement come with significant limitations, typically associated with fixture/recovery of external tags. Intrinsic bio-marker methods, such as stable isotope analysis of incrementally-grown tissues, overcome some of these issues. Stable isotopes conform to predictable spatial and temporal patterns in nature and the local isotope signature in any given environment is incorporated into the tissues of the animals that inhabit it. This allows them to act as effective tracers as the animals move through environments of differing isotope ratios. For δ^2H, variation in tissues will reflect differences in drinking/dietary water, both ultimately derived from precipitation. Thus large scale patterns or contours in average precipitation δ^2H have the potential to allow reconstruction of origins and movements of animals in the wild.

This project aims to use a multi-stable isotope (H, O, C, N, S and compound specific H) approach to quantify several interlinked aspects of blue wildebeest (*Connochaetes taurinus*) ecology. Presented here are isotope data from a number of elements, alongside discussion of the verification of these isotopes as proxies for diet, stress and movement. Our primary question is: do δ^2H values in Serengeti water show sufficient spatial variation that is reliably incorporated into wildebeest tail hair to allow us to track the route of the annual migration? We test this approach by creating a seasonal δ^2H isoscape of the study area to compare with δ^2H along the length of tail hairs. If there is a correlation, a timeline of movements can be inferred and this can be assessed for accuracy using the actual route established via GPS-tracking. There are many complicating factors in African systems, so the limitations of the approach will be considered along with the applications."

#4-18: Does δ^{15}N show size-structured food webs in a coral reef-fish community?

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δ^{15}N data have shown that some major fishery food webs (e.g. North Sea, Gulf of Oman) are substantially body-size structured, indicating that predation and thus trophic position are driven by size. There are reasons for and against coral reefs conforming to such size structuring, but the idea has not been tested. In the Caribbean, ongoing coral-reef degradation includes shifts in reef fish community species abundances, size distributions, composition and diversity. We conducted underwater visual census and stable isotope analysis of fish white muscle across four reef sites at Cape Eleuthera (Bahamas) to analyze size spectra to test relationships between δ^{15}N and fish body mass and total length at community and species level. Regression analysis shows: i) on individual level, δ^{15}N increased with size for most species, exceptions including small species feeding at higher trophic levels, those with mixed diet and those changing diet with development, ii) the strength of relationship between δ^{15}N and body size or mass varied among reef sites, iii) combining all sites there were positive linear relationships with body mass and total length (body mass, $r^2 = 0.8818$, $p = 0.0003 < 0.001$; total length, $r^2 = 0.8758$, $p = 0.0038$). This is the first demonstration of positive δ^{15}N-site structuring in coral reefs, however these reefs appear to be significantly degrade in terms of Caribbean reef-fish abundances and hard-coral cover. Future researches on size and trophic structuring should focus on less damaged reefs, but the indications are that strong δ^{15}N-site relationships also exist in the coral reef ecosystem.

#4-19: Characteristics of food chain structure of the North Pacific pelagic ecosystems inferred from end-to-end profiles of carbon and nitrogen stable isotopes

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It is generally believed that nitrogen and carbon stable isotope ratios of marine organisms indicate the trophic levels and sources of organic matters, but actual ent-to-end relationships from primary production to apex predators in open ecosystems have not been validated well. To examine the structure of the pelagic food chain in the North Pacific Ocean, we analyzed carbon and nitrogen stable isotope ratios of various organisms from phytoplankton, zooplankton to top predators. We combined materials and data obtained from three different series of surveys in the western and central North Pacific; i) oceanographic survey for plankton and micronekton, ii) driftnet surveys for pelagic fish community, and iii) sighting and biopsy surveys for small cetaceans. We compared the C-N stable isotope profiles and predator species compositions in four distinct areas characterized by physical oceanography (transitional domain, transition zone, Oyashio area, and Oyashio-Kuroshio mixed area). The results provided valuable information to understand the structure of pelagic food chain and the utilization of regional productivity by migratory top predators in the pelagic ecosystems.

5.5 Poster Session 5

Paleoecology and the ecological impacts of humans

#5-1: Geochemical analyses of fossil shells of *Gafrarium tumidum* collected from Tongatapu Island and its application in paleoceanography and archaeology during the Holocene

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Molluscan shellfish skeleton is often retrieved from archaeological sites since they are important nutrient source for ancient society. The skeletal calcium carbonate is an ideal recorder of the past environment as well as understanding their ecology. In this study, we collected bivalves *Gafrarium tumidum* excavated from Holocene archaeological sites in Tongatapu Island, Tonga (21 °10'S, 175 °10'W) and measured stable oxygen and carbon isotopes and trace element. Reconstructions of the past environment on the island are attempted since the island was act as a hub island for human migration to the eastern Polynesian islands namely the information will be of key to understand anthropological questions. The island is under influence of South Pacific Convergence Zone (SPCZ) that brings rainfall in the region though past evolution of this atmospheric configuration is lacking. SPCZ variation could have affected dispersion of ancient humans to the Polynesian islands during the Mid- to Late Holocene as their migration via maritime transportations dependent on wind. We employed laser ablation IRMS (isotope ration mass spectrometry) and LA-HR-ICPMS (laser ablation high resolution inductively coupled plasma mass spectrometry) to measure respectively oxygen isotopic composition (δ18O) and trace element / Ca ratio (e.g., Sr/Ca, Mg/Ca, and Ba/Ca) of the shells along the maximum growth axis. To understand the physical property of ambient water where shells grow, we calculated local marine 14C reservoir ages (*ƒ*¢R). In this presentation, we will discuss the results of Tongatapu Island paleoenvironmental reconstruction base on geochemistry possibly related to regional to global climate changes.

#5-2: Dietary reconstruction of the extinct Ezo wolf (*Canis lupus hattai*)

Matsubayashi, *Jun – Research Institute for Humanity and Nature*

Ichiro Tayasu – Research Institute for Humanity and Nature

The presence of top predator have a great impact on the local ecosystems. In the Hokkaido Islands, Japan, Ezo wolves became extinct due to the human activities at the end of the 19th century. The ecological roles of the extinct Ezo wolf should be important information for ecosystem management and biodiversity conservation in Hokkaido. Nevertheless, almost no ecological information about Ezo wolf has been available to date. Here, we measured carbon, nitrogen and sulfur stable isotope ratios in bone collagen of Ezo wolves and reconstructed their feeding habits. Bone fragments of wolves were fairly infrequently excavated from the remains of past people, and we collected 7 bone samples from entire region of Hokkaido. Sulfur isotope ratios were used to detect the consumptions of marine prey. In western Canada, which have similar environmental condition with Hokkaido, the grey wolves frequently fed on marine diet such as salmon, herring and sea mammals. Therefore, wolves in Hokkaido may also consumed marine prey. We evaluated puerility of extracted bone collagen by concentration of each element and 1 collagen was excluded from stable isotope analysis. The mean δ^{13}C, δ^{15}N and δ34S values of Ezo wolves were -19.31‰±2.15‰ (-15.23-21.01‰), 9.36±2.29‰ (range: 7.67-13.72‰) and 6.96±3.07‰ (1.82-11.34‰), respectively. A wolf (ID: YNG-01) showed higher isotopic values (-15.23‰, 10.08‰ and 11.34‰ for δ^{13}C, δ^{15}N and δ34S) than the other individuals, which suggest great depend on marine food. Thus, our stable isotope analysis of revealed that Ezo wolves had large variation in their feeding habits and most of wolves should be depended on terrestrial mammals such as deer and rodent species, whereas some population consumed large amount of marine prey as well as wolves in western Canada. The results suggested that Ezo wolf contributed not only for population control of herbivore species, but also for nutrient transportation from marine to terrestrial ecosystems in Hokkaido Islands.

#5-3: Evaluation of a proxy model for isotope in tree-ring cellulose and its application

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Kei Yoshimura – University of Tokyo

Knowledge about past climate change is invaluable to understand the climate system and to predict the future. Climate proxies have offered wide range of information on the climate of past which we cannot directly know because there are no instrumental records. To interpret proxy data appropriately, however, a forward model which predicts the proxy (proxy model) is necessary. Up to date, several proxy models for stable water isotope in tree-ring cellulose have been developed, but they have not been globally evaluated. In this study, the models are evaluated using the output of the isotope enabled GCM. The proxy model and data are compared on the interannual timescale. The result shows that the reproducibility for tree-ring cellulose is comparable with that of isotope ratio in precipitation with the metrics of temporal correlation; around 70% of the proxies are well reproduced by the models. Even though the reproducibility is somewhat limited by the quality of the input and/or the poor representation of the proxy models, the model seems to represent the key mechanisms in the proxies.

In the presentation, the feasibility of proxy data assimilation as an application of the proxy model will be also shown. In this study, the annual mean isotopic compositions are assimilated into GCM simulation albeit in an idealized way. The results shown are promising. By assimilating the isotopic information, temperature, precipitation, and pressure fields are well reconstructed. The reason is that the isotope ratio is sensitive to major climate modes (e.g. ENSO, and annular modes) which have great impact on the interannual variability of those variables. The findings of this study makes a case for proxy data assimilation and acts as a milestone for future paleoclimate data assimilation research.

#5-4: Isotope Ecology of Ancient Rice Paddy of Prehistoric Japan

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The transition from hunting-gathering-fishing societies to farming societies was recognized as the ending of Jomon period and the beginning of Yayoi period of ancient Japan. Generally speaking, a cultural complex consisting of wet rice agriculture with irrigation system, animal husbandry including pig and chicken, and new type of pottery production was imported from Korea and China around 2800 year ago. A drastic increase of archaeological sites and the change in facial morphology of people in the Yayoi period suggest a massive human migration and/or drastic population increase supported by rice farming. However, the impact of rice in diet is a challenging task not only for archaeology but also for isotope ecology, because the plants exploited by hunter-gathers (mainly chestnut, horse chestnut and acorn) and rice both are C3 plants. We are expecting the impact of denitrification in the wet condition might elevate delta15N in rice crops but the modern agriculture with fertilizer is not suitable reference for prehistoric rice. Hence, we collected and analyzed a series of rice crops from the experimental field for archaeobotanical research, where fertilizer never applied and some ancient lineage of rice was cultivated in the fashion reconstructed from archaeological evidences. Our preliminary results suggested the increase of 15N in wet rice might occasionally occurred and this isotopic signature can be traced to human collagen data.

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Time	Monday, April 04	Tuesday, April 05		Thursday, April 07	Friday, April 08
09:00	Welcome	Housekeeping	Field Trip	Housekeeping	Housekeeping
09:10	Opening: **Wada**	Keynote: **Ehleringer**		Keynote: **Bowen**	Keynote: **Richards**
09:20					
09:30	Keynote: **Chikaraishi**				
09:40					
09:50		Popp		Trueman	McCarthy
10:00					
10:10	McMahon	Steffan		St John Glew	Ohkouchi
10:20					
10:30	O'Connell	Mercier Quideau		Turner Tomaszewicz	Jarman
10:40					
10:50	Matthews	Bradley		Bell	Fox-Dobbs
11:00					
11:10	*Coffee Break*	*Coffee Break*		*Coffee Break*	*Coffee Break*
11:20					
11:30					
11:40	Nomaki	Timofeeva		Jones	Clementz
11:50					
12:00	Revill	Skates		Vane	Woodland
12:10					
12:20	Ogawa	Skrzypek		Cherel	Michel
12:30					
12:40	Hetherington	Baker		Nelson	Vokhshoori
12:50					
13:00	*Lunch*	ThermoFisher SCIENTIFIC *Lunch Seminar*		*Lunch*	*Lunch*
13:10					
13:20					
13:30					
13:40					
13:50					
14:00					
14:10	Fry	Kernaleguen		Tayasu	Montanari
14:20					
14:30	Hebert	Rea		Hayden	Grawe DeSantis
14:40					
14:50	Barnette	Kurle		Hopkins	Wehi
15:00					
15:10	Fink	Vander Zanden		Quezada-Romegialli	Lewis
15:20					
15:30	Roussel	Noble		Fernandes	Sheath
15:40					
15:50	*Coffee Break*	*Coffee Break*		*Coffee Break*	*Coffee Break*
16:00					
16:10					
16:20	Pomerleau	Harrod		Hellmann	Demopoulos
16:30					
16:40	Patterson III	Lejeune		Voigt	Vieira
16:50					
17:00	Larsen	Yamaguchi		*Banquet travel and free time in Happo-en*	Jankowska
17:10					
17:20	Itahashi	Kuerten			Vanderklift
17:30					
17:40	Gil	Conti-Jerpe			Closing Remarks & Student Awards
17:50					
18:00	Poster Session (odd poster numbers)	Poster Session (even poster numbers)		ThermoFisher SCIENTIFIC Thermo Fisher Scientific Banquet (Happo-en)	Free time in Ueno Park for Hanami (cherry blossom viewing party)
18:10					
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