4th International Conference on
Applications of stable isotope techniques to ecological studies

Abstracts

Abstracts in alphabetical order by **presenting** author

Section 1:
ORAL PAPERS

Off-season uptake of nitrogen in temperate heath vegetation

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Off-season ecosystem processes is becoming an area of increasing interest, being important when considering annual nitrogen and carbon budgets. The general assumption that physiological activity in soil microorganisms as well as vegetation is low during winter may not be justified. In this field study we show that northern temperate coastal heath vegetation has a significant uptake potential for nitrogen, both in the form of ammonium and as glycine, throughout the non-growing season.

We used ^{15}N ammonium and 2*(^{13}C)^{15}N glycine as nutrient tracer. This was injected into the soil two times during winter and once at spring. The winter temperatures were similar to those of average winter in the northern temperate region of Europe, with only few days of soil temperatures below zero and a winter mean of 2.4 ˚C. The vegetation, consisting of the evergreen dwarf shrub *Calluna vulgaris (L.)*, the deciduous dwarf shrub *Salix arenaria (L.)* and the graminoids *Carex arenaria (L.)* and *Deschampsia flexuosa (L.),* showed root uptake of both forms of nitrogen, both one day after labelling and after a month. Translocation of the labelled nitrogen to shoots was generally evident after one month and increased as spring approached, with different translocation strategies in the three plant functional types. Furthermore, shoot total nitrogen concentration increased in all plant types, but only the graminoids and, eventually, *S. arenaria* showed shoot growth during winter. Our study suggests that plant nitrogen uptake can cause increasing nitrogen concentration in shoot tissue from fall to spring.

To our knowledge this is one of the first studies of plant nitrogen uptake during non-growing season at a temperate heath. Our results show that the nitrogen uptake is in the same order of magnitude as summer uptake in other types of ecosystems in the temperate/boreal region. These results suggest that plant nitrogen uptake during winter should be included in the annual nitrogen budgets of heath ecosystems, and that the view of plant nutrient uptake as low in this climatic region during winter should be revised. Furthermore, these results should be taken into account when ecosystem responses to climatic changes such as warming are evaluated.

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Carbon isotope composition of organic compounds as short term integral of plant physiological processes

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The determination of carbon isotope composition in plant tissues is now widely used by plant eco-physiologists to integrate the influence of a range of environmental factors on plant performance. The main difficulties in interpreting $\delta^{13}C$ signatures in plant tissues such as leaves are the effects of carbon storage, re-translocation and related fractionation of carbon isotopes and carbon that has been assimilated in previous growing seasons. In deciduous trees, for example, foliage that is developed in spring is mainly formed from stored carbon and mixed with newly assimilated carbon. In evergreen trees, such as eucalypts, it is often difficult to determine the age of leaves and therefore the time when the bulk of the leaf tissue has been assimilated.

In this study we aimed to identify plant organical compounds whose $\delta^{13}C$ signatures can be utilized as a short-term integral of plant physiological and environmental parameters. These organic compounds should be produced or assimilated within days or hours prior to their harvest, thereby removing the uncertainty created by carbon assimilated weeks or months before.

We investigated the diurnal change in $\delta^{13}C$ composition of water-soluble compounds, soluble carbohydrates, and starch in leaves, whole leaf tissues as well as phloem sap in three year old *Eucalyptus globulus*. We also recorded leaf gas exchange and environmental parameters at the same time the samples were taken. The results indicate that these organic compounds are reliable indicators for short-term changes of plant physiological and environmental parameters and that these changes are not detected when analysing bulk leaf material.

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Stable C and O isotopes reveal the effects of land use on the carbon fluxes in mountainous ecosystems

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The aim of the EU-project CARBOMONT (Effects of land use change on sources, sinks and fluxes of **carbo**n in European **mo**u**nt**ain areas) is the analysis of the source/sink relationship of biogenic carbon fluxes and its flux components in European mountainous ecosystems. In this project, the Net Ecosystem Exchange (NEE) of carbon and water in grassland ecosystems is measured with the eddy covariance method at 13 sites in Europe.

NEE alone does not allow a detailed analysis of changes in the carbon fluxes of an ecosystem. A separation of NEE into the component fluxes (assimilation, ecosystem respiration of plants and soil) will provide a more thorough view on how land use change affects the carbon budget. At the Swiss study site Seebodenalp, we partitioned the measured NEE with the help of stable carbon and oxygen isotopes. Each CO_2 flux component has typically a specific isotopic signature. Therefore we analyzed air samples as well as plant and soil materials of the different compartments of our grassland ecosystem for their $\delta^{13}C$ and $\delta^{18}O$, and leaf water for the $\delta^{18}O$ values. Based on the assumption of Yakir (2000) and Bowling (2001), NEE is separated into net assimilation and respiration. Thus, we will get an insight into the dynamics of the carbon exchange processes with respect to the ability of the ecosystem having a carbon source or carbon sink potential.

In the presentation, the results of NEE-partitioning of two adjacent grasslands, one extensively used and one abandoned, will be compared throughout the vegetation period.

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$\delta^{13}C$ of respired CO_2 reflects $\delta^{13}C$ of recently fixed photosynthate in *Nothofagus* forests

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Analysis of nocturnal gradients in CO_2 concentration and carbon isotope composition within forests (using the Keeling 1958 approach) allows valuable insights into photosynthetic and respiratory processes at the ecosystem-scale. The carbon isotope composition of ecosystem-respired CO_2, dominated by soil respiration ($\delta^{13}C_R$; the Keeling plot intercept), has been found to vary with precipitation and atmospheric vapour pressure deficit (Bowling et al. 2002). $\delta^{13}C_R$ is expected to be influenced by environmental conditions because a large proportion of ecosystem-respired carbon is thought to have been recently fixed during canopy photosynthesis (Högberg et al. 2001), and so retains an isotopic signature related to photosynthetic fractionation. The extent to which C_3 plants discriminate against $^{13}CO_2$ is recorded in carbohydrates formed in the leaves and depends on the stomatal conductance and photosynthetic capacity at the time of fixation, both of which respond to changes in the environment.

Although several studies have reported significant variation in $\delta^{13}C_R$ over time, and explored this variation in terms of environmental effects, the direct link between $\delta^{13}C$ of recently fixed carbohydrate and $\delta^{13}C_R$ has not been clearly demonstrated. The *Nothofagus* ecosystem of New Zealand presents a unique opportunity to measure variation in $\delta^{13}C$ of carbohydrates and respired CO_2 at high temporal resolution because the presence of the phloem-tapping scale insect *Ultracoelostoma assimile* allows easy access to phloem carbohydrates of recent origin.

Phloem carbohydrates excreted by scale insects (honeydew) and within-canopy air were sampled for $\delta^{13}C$ analysis over several nights at two sites in the central South Island, New Zealand. Variation in $\delta^{13}C$ of honeydew between sites reflected effects of soil water availability on stomatal conductance, and variation between sampling times reflected environmental influences on both stomatal conductance and photosynthetic rate. $\delta^{13}C_R$ also varied considerably over the sampling period, and mirrored variation in $\delta^{13}C$ of honeydew collected from exposed canopy trees. The short lag time between a change in $\delta^{13}C$ of honeydew and a subsequent change in $\delta^{13}C_R$, just 1-2 days, demonstrates the speed of the link between carbon fixation by the canopy and release of this carbon during respiration.

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Segregation of breeding Blackcaps *Sylvia atricapilla* with respect to wintering area and its potential consequences

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A recent shift in the migratory behaviour of European Blackcaps has presented a rare chance to investigate some of the processes underlying the migratory phenomenon. However it is only now, with developments in the field of using stable isotope ratios in the tissues of animals as geographic markers, that we have the tools in place to take advantage of this opportunity. We used δD ratios in the toenails of blackcaps to infer wintering origins of birds on the breeding grounds. This has enabled us to assess whether individuals from the rapidly increasing UK winter population of blackcaps gain selection benefits over conspecifics that winter in Iberia. Moreover, since assortative mating (with respect to wintering area) on the breeding grounds (in S. Germany) has been predicted, we hope to gain an insight into the types of process that might lead to segregation of breeding populations and ultimately speciation.

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Annual and seasonal distribution of stable water isotopes in precipitation and their relevance to animal migration studies

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The stable hydrogen and oxygen isotope composition of precipitation varies widely and systematically across the globe, imparting a chemical label to local meteoric water that is propagated through hydrological and ecological systems. This label is incorporated into the tissues of organisms, providing a powerful tracer of the location of origin of plants and animals that are relocated due to natural migration or transport by humans. Efficient and accurate use of these isotopic labels requires that 1) spatial distributions of water isotope compositions exhibiting substantial and relatively simple patters of variation exist and are known, and 2) the relationships between the isotopic composition of precipitation delivered to a location and that of biological material synthesized at the location are understood.

It has been shown that for many migrant animals, the isotopic composition of body tissues integrates some part of the annual water isotope signal at the location of synthesis. For animals that derive most of their body water from food items, such as birds and insects, water isotope labels are ultimately inherited from hydrogen and oxygen assimilated by plants, and the average value of local precipitation during the growing season is commonly assumed to provide a good estimate of the source label. We have made key modifications to a validated model for the representation of mean annual water isotope fields, allowing the calculation of monthly and seasonal water isotope distributions and the creation of a global grid of growing season water isotope values, and here we use this grid to highlight the potential and limitations of deuterium and oxygen isotopes as tracers of animal origin. We find that many low-latitude regions exhibit distributions that are spatially uniform and poorly suited for this application. In contrast, the complex spatial distribution of water isotopes in mountainous regions complicates interpretations of origin in many cases but may prove useful in others, particularly if the ranges of species are accurately known. At mid- to high-latitudes, where the greatest potential exists to use hydrogen and oxygen isotopes to track migration, spatial gradients in growing season water isotope compositions in many places are diminished relative to gradients in mean annual values. In regions with strong monsoonal circulation, however, growing season isotope gradients may be as strong as mean annual gradients, making these regions ideally suited for the application of the water isotope tracer. Finally, we use two sets of data on the hydrogen isotope composition of bird feathers to investigate the assumption that growing season values provide the best estimate of the water isotope label propagated through local food webs. We find that this assumption holds true for the North America data set, but not for Europe, where feather isotope values are more strongly correlated with mean annual precipitation values. We attribute this result to incorporation of water delivered during the relatively wet European winter, through mixing in ground and surface water reservoirs, into pools available to growing plants. The incorporation of a non growing-season signal in European foodwebs increases the applicability of the water isotope tracers there, and may indicate the more general utility of the application in regions where the winter/summer precipitation ratio is high.

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Scales of carbon movement and assimilation by invertebrates in estuaries

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Carbon is generally considered to be more mobile in aquatic than terrestrial systems. It therefore cannot simply be assumed that aquatic animals derive their nutrition from plants in their immediate vicinity. The potential separation of animals and their ultimate autotrophic energy source has implications for the design of conservation measures to protect aquatic fauna, including fisheries resources. We tested the extent of movement and assimilation of carbon among estuarine habitats in subtropical and temperate Australian waters. We measured $\delta^{13}C$ values of sedentary invertebrates along transects crossing the boundaries of habitats in which the dominant autotrophs had very different $\delta^{13}C$ values. In the subtropics, we sampled omnivorous shore crabs and snails in several pairs of adjacent areas of saltmarsh grass (mean $\delta^{13}C$ -12 ‰) and mangrove forest ($\delta^{13}C$ -28 ‰). In temperate waters, we sampled carnivorous and detritivorous polychaete worms along transects hundreds of metres long, from the edge of mangroves ($\delta^{13}C$ -28 ‰), across intertidal mudflats containing benthic microalgae ($\delta^{13}C$ -21 ‰), and into subtidal seagrass meadows ($\delta^{13}C$ -12 ‰ for both seagrass and epiphytic algae).

We predicted that if carbon moves extensively and is assimilated by animals, the position of animals would not be important, and $\delta^{13}C$ values of animals would be similar at all positions. On the other hand, if carbon movement and assimilation occurs only on a small scale, the position of animals will strongly affect their $\delta^{13}C$ values.

The extent of carbon movement and assimilation by sedentary invertebrates differed in the two regions. In the subtropical surveys, animal $\delta^{13}C$ values matched those of the dominant autotrophs immediately alongside, and therefore changed rapidly across the saltmarsh-mangrove boundary. The animals evidently obtain their carbon from sources in the surrounding few metres. In temperate waters, animal $\delta^{13}C$ values were similar at all positions along the transects, and consistently matched values for seagrass and epiphytic algae. Animals here utilise carbon from seagrass meadows regardless of their proximity to the meadows. Further tests in other regions will help to elucidate the underlying factors determining the extent of carbon movement and assimilation. It will also be important to extend the work to more mobile animal species.

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Short- and long-term changes in the diet of domesticated animals from Qasr Ibrim, Egypt

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Compound-specific $\delta^{13}C$ values of individual fatty acids and amino acids, obtained *via* gas chromatography-combustion-isotope ratio mass spectrometry, are providing new insights into palaeodietary studies of humans and animals. Traditionally, bulk $\delta^{13}C$ and $\delta^{15}N$ values obtained from bone collagen and apatite have been used for human and animal palaeodietary reconstruction. For the first time, compound-specific $\delta^{13}C$ values of bone-derived fatty acids and collagenous amino acids are utilised in conjunction with bulk collagen and apatite stable isotope values, to determine the ancient feeding practices of domesticated animals.

The Nubian site of Qasr Ibrim (*c.* 1000BC – AD1800) is situated on the Nile in modern day Egypt. Due to the extremely arid environment, organic material from the archaeological site is very well-preserved. This exceptional preservation is not only observed at the macroscopic level in finds such as bones, plant remains, leather, parchments and textiles, but is also witnessed at the molecular level.

The high degree of preservation of faunal remains at this archaeological site provides an opportunity to investigate aspects of animal husbandry, such as fodder/foraging patterns. Thirty-eight domesticated animal bones were selected for analysis; comprised of cattle and sheep/goat bones, from various periods throughout the site's occupation. Utilising models of the biochemical correlations with the dietary components and their turn-over rates, bulk stable isotope values from bone collagen and apatite combined with compound-specific stable isotope values of the collagenous amino acids and fatty acids (*n*-hexadecanoic and *n*-octadecanoic acids) were used for palaeodietary reconstruction. The collagen, apatite and amino acid $\delta^{13}C$ values provided long-term indicators of the 'protein' and 'whole-diet' components of cattle and sheep/goats from the site, while the fatty acid $\delta^{13}C$ values provided short-term 'whole-diet' indicators. The results indicated significant differences in the long- and short-term diets of the sheep/goat, and less pronounced differences in the cattle's diet. Indeed, cattle appear to have preferentially fed on/been fed on C_4 plants, such as sorghum (*Sorghum bicolor bicolor* Moench.) and millet (*Panicum miliaceum* L.), during the later periods of the site. Furthermore, essential amino acids provided insights into the long-term protein component of the animals' diet, further illustrating changes in the animals' diet during the three thousand years of occupation at Qasr Ibrim.

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Isotopic variation in *Ulva lactuca* within a New Zealand fjord: interactions between physical gradients, nutrient source pools, and productivity

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Irradiance, salinity, and water flow can strongly influence isotope signatures ($\delta^{13}C$ and $\delta^{15}N$) of macroalgae by affecting composition of source pools (e.g. HCO_3 and CO_2, NH_4 and NO_3) and uptake of different chemical species (e.g. Kubler and Raven 1995). The present study aimed to identify the mechanisms that influence isotope signatures of *Ulva lactuca* in Doubtful Sound, New Zealand. Fjord-wide surveys were completed to describe patterns of isotopic variation and biomass of *U. lactuca* along gradients of irradiance, salinity, and wave exposure. Surveys included paired collections at sites that were exposed to high versus low irradiance. A consistent and distinct trend in carbon and nitrogen signatures was identified along the axis of the fjord. Carbon signatures peaked at mid-fjord locations ($\delta^{13}C \sim -12$) and were most negative at the head and tail ends of the fjord ($\delta^{13}C \sim -18$). Estimates of biomass and percent cover mirrored this trend, with highest estimates at mid-fjord locations. Nitrogen signatures increased from locations at the head of the fjord ($\delta^{15}N \sim 2$), which is exposed to high amounts of freshwater inputs and associated runoff, to sites nearest the ocean ($\delta^{15}N \sim 8$). Both C and N signatures at high irradiance sites were significantly enriched (by ~ 2) compared to signatures at low irradiance sites. Results suggest that irradiance, which largely controls photosynthesis and processing of nutrients, and proximity to terrestrial versus oceanic nutrient pools are the primary mechanisms driving isotopic variation in *U. lactuca* throughout Doubtful Sound. In order to confirm these results and isolate the effects of irradiance, salinity, and nutrient source pool on isotope signatures, a series of controlled laboratory experiments were completed at the Portobello Marine Lab. Knowledge of mechanisms controlling isotope signatures will assist in understanding inorganic C and N uptake by macroalgae along physical gradients and improve the application of isotopes as tracers in food web studies throughout Fiordland and other estuarine ecosystems.

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A novel marine dietary indicator utilising compound-specific bone collagen amino acid $\delta^{13}C$ measurements of ancient humans

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The application of bone collagen stable carbon and nitrogen isotope analysis for the identification of marine food consumption in particularly arid environments may be hindered by two factors: (i) the overlap in C_4 and high marine protein (HMP) consumer bulk collagen $\delta^{13}C$ values, and (ii) the unreliability of bulk collagen $\delta^{15}N$ values in regions of extreme aridity (<400 mm of rain per annum). Hence, the identification of HMP consumption among archaeological human populations in arid environments such as the Cape region of South Africa can be problematic. In an endeavour to identify a substitute marine palaeodietary indicator, a range of Cape region archaeological faunal and human bone collagens (n = 14 and 26, respectively), representing a spectrum of C_3, C_4 and HMP diets, were subjected to compound-specific stable carbon isotope analysis of their constituent amino acids as trifluoroacetyl-isopropyl (TFA-IP) esters via gas chromatography-combustion-isotope ratio mass spectrometry (GC-C-IRMS).

While human C_4 and HMP consumers were indistinguishable with respect to bulk collagen $\delta^{13}C$ values (-10.9 ± 3.7‰ and -11.7 ± 1.5‰, respectively) they were shown to be readily distinguished based on $\Delta^{13}C_{Glycine\text{-}Phenylalanine}$ values (+4.0 ± 1.6‰ and +12.0 ± 1.9‰, respectively). The relationship between HMP consumption and elevated $\Delta^{13}C_{Glycine\text{-}Phenylalanine}$ values was verified by: (i) the similarly elevated values exhibited by marine species when compared to terrestrial faunal species (+12.5 ± 0.9‰ and +3.2 ± 4.2‰, respectively), and (ii) the strong correlation observed between human $\Delta^{13}C_{Glycine\text{-}Phenylalanine}$ and bulk collagen $\delta^{15}N$ values ($R^2 = 0.83$, $p < 0.001$; $n = 26$), the latter being a well-documented marine dietary indicator. The validity of utilising $\Delta^{13}C_{Glycine\text{-}Phenylalanine}$ values for identifying HMP consumption was further demonstrated in the similarly elevated values associated with both a known HMP consuming iceman (Kwaday Dän Sinchi; +15.6 ± 1.2‰) and a marine faunal assemblage (Dionisio Point, n = 7; +14.4 ± 2.2‰, respectively) from British Columbia. It was concluded that the basis for elevated $\Delta^{13}C_{Glycine\text{-}Phenylalanine}$ values observed in HMP consumers resulted from the direct incorporation into bone collagen of dietary glycine, which is both extremely ^{13}C-enriched and present in high abundance in marine foods. Hence, $\Delta^{13}C_{Glycine\text{-}Phenylalanine}$ values offer considerable potential as indicators of HMP consumption and a valuable substitute for bone collagen $\delta^{15}N$ values in arid regions where bulk $\delta^{15}N$ values are unreliable, and also, in bones where collagen preservation is insufficient for bulk collagen $\delta^{15}N$ determinations.

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$^{13}CO_2$ pulse labelling and analysis of microbial communities in soils under two different grassland plant species

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Carbon cycling in soils has received increasing attention due to the importance of soils as a sink for greenhouse gases such as carbon dioxide. Further investigation of the input of carbon into the soils and its cycling by microbes is required in order to evaluate the use of soil as a carbon sink. Many soil bacteria are not amenable to available culturing techniques and therefore methods of in situ analysis are required. One such method is the use of phospholipid fatty acid (PLFA) analysis. PLFAs make up a highly significant proportion of microbial cell membranes and are readily degraded following cell death, hence, they are useful for determining the presence of viable bacteria in environmental samples. Certain PLFAs are specific for certain functional groups of bacteria, however; generally there are few PLFAs that are species specific. The specificity can be improved by combining these analytical techniques with isotopic labelling (Bull et al., 2000).

The aim of this research was to label grassland plants with $^{13}CO_2$ and trace this label into the soil microbial community by GC/C/IRMS analysis of the PLFAs extracted from soils. Plant species (*Nardus stricta* or *Ranunculus* spp.) were selected for pulse labelling while all other species in each incubation chamber were covered with alpine gravel to prevent photosynthesis. Plants were pulsed with $^{13}CO_2$ (98 atm%, 500 ml min^{-1}) for 6 hours in the field using a mobile laboratory as described by Ostle et al., (2000). Following pulsing, samples of roots, shoots and rhizosphere soil were taken at times of 0, 2, 4, 6 and 18 hours after the pulse was terminated.

PLFAs were extracted from rhizosphere soil samples and analysed by GC/C/IRMS in order to determine the quantities of ^{13}C incorporated into individual PLFAs and provide assessments of the microbial communities associated with different plant species. These results were also used to investigate whether changes occurred in the labelled microbial communities with time following the introduction of ^{13}C-labelled exudates components from the plant.

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Stable metal isotopes reveal copper accumulation and loss kinetics in the freshwater bivalve *Corbicula*

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Definition of metal kinetics in aquatic organisms can provide insights that explain why different species and different food webs bioaccumulate a metal differently (Schlekat et al. 2002). The kinetic parameters that determine bioaccumulation characteristics include gross uptake rates from solution, assimilation efficiencies and rate constants of loss. Usually such parameters are determined using radioisotopes, in order to eliminate interferences from stable metal pools within the organisms. Stable isotopes could also be employed in kinetic studies, if isotope discrimination is minimal, if the isotope can be detected in the animal after short exposures, and if conversion of the data to total metal rates (or rate constants) is feasible (e.g. Evans et al. 2002). Here we used a commercially purchased Cu standard isotopically enriched in ^{65}Cu (99.4%) and used ^{65}Cu as a surrogate for total Cu exposure. We exposed the bivalve *Corbicula* to waterborne Cu at an environmentally realistic concentration ([$^{65}Cu^{2+}$] ~ 5 µg l^{-1}) and determined their rates of Cu accumulation and loss. In measuring *Corbicula*'s stable isotopic ratios prior to exposure, we found that field-collected *Corbicula* were significantly enriched in the heavier isotope relative to their environment (i.e., $^{65/63}$Cu isotope ratios of 0.505 ± 0.001 and 0.481 ± 0.006, respectively). We observed similar enrichment of the heavier Cu isotope in numerous invertebrates collected within the same lake, suggesting that all taxa discriminate ^{65}Cu in the same way, regardless of their trophic position or their source of organic carbon. The similarities in discrimination mean that Cu stable isotopes will not be useful in discriminating sources of Cu; but kinetics determined using Cu isotopes would be comparable among the species. Using the Cu isotope ratios determined in field-collected *Corbicula* as a baseline, we converted Cu concentrations measured in our experimental organisms to ^{65}Cu concentrations and fitted our experimental data to a kinetic bioaccumulation model that takes into account *Corbicula*'s aqueous Cu uptake and Cu loss by efflux. Our estimates of uptake rate constant for dissolved Cu (0.45 l g^{-1} d^{-1}) and efflux rate constant (0.049 d^{-1}) are the first to be published for a freshwater organism. Extrapolation of our laboratory results to nature suggests, that uptake from solution alone underestimates *Corbicula* Cu concentrations; this is probably because dietary Cu uptake is important, a subject that should be amendable to study with ^{65}Cu.

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Using stable isotope analysis and biotelemetry to study fish movement and foraging

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Analysis of stable isotope ratios (e.g., $^{13}C/^{12}C$, $^{15}N/^{14}N$) of organic matter has proven useful for differentiating food sources and rearing areas of many terrestrial and aquatic species. Information about animal movements has often been inferred from stable isotope analysis (SIA), but is dependent on study animals assimilating site-specific isotopic signatures via diet. This potential weakness in ecological interpretation can be overcome by using other investigative tools that provide precise information about movement patterns. In this paper, we demonstrate the value of combining SIA with biotelemetry data to study the movement patterns and feeding ecology of stream fishes in different habitats. In a Newfoundland fjord lake system, Atlantic salmon (*Salmo salar*) smolts and anadromous brook trout (*Salvelinus fontinalis*) were found to use river, pond and marine habitats at different times that reflected variable life history strategies. In the Miramichi River system (New Brunswick), SIA provided evidence of foraging habitats that helped to identify the source of tagged fish that had subsequently moved to cool water refugia during high temperature stress events. Finally, the site fidelity and limited mobility of slimy sculpin (*Cottus cognatus*), a small benthic stream fish, was clearly established based on results of SIA and the recapture of fish tagged with passive integrated transponder (PIT) tags. Technological improvements in recent years have permitted the non-destructive sampling of tissues of wild fishes for SIA and the tagging and remote detection of much smaller animals than was previously possible. These advancements and the combination of investigative tools have yielded new insights in animal ecology.

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Review the use of $\delta^{18}O$ in atmospheric CO_2 to separate photosynthesis from respiration

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The oxygen isotopic composition in atmospheric CO_2 ($\delta^{18}O$-CO_2) can potentially be used to quantify assimilation and respiration separately. But we showed that our knowledge about the $\delta^{18}O$-CO_2 cycle is not yet sufficient to apply the isotopic approach with adequate accuracy (Cuntz et al. 2003a,b). Recent new findings and theoretical work on processes promise now a much better description of $\delta^{18}O$ in atmospheric CO_2. New research on $\delta^{18}O$-CO_2 includes a more accurate description of discrimination of night-time leaf respiration (Cernusak 2004), formulations of leaf water enrichment that include non-steady-state effects (Cernusak et al. 2002, Ogée et al. 2003), a more complete description of soil water isotopes and isotopes in soil CO_2 (Riley et al. 2002) and the inclusion of incomplete isotopic equilibrium of CO_2 with leaf water (Gillon and Yakir 2001). Also the way photosynthesis and respiration are deduced from $\delta^{18}O$-CO_2 was reformulated recently in order to include a proper description of errors on input isotope parameters (Ogée et al. 2004, Ciais et al. 2003).

We will first review the cycle of $\delta^{18}O$ in atmospheric CO_2 and describe the current understanding. We will then present the new research in the field and outline its predicted consequences. We will further point out the assumptions made in the different approaches and theoretical descriptions and show where missing observational evidence is limiting for a precise description of $\delta^{18}O$-CO_2. Finally, we will demonstrate why the traditional way to separate assimilation from respiration leads to great errors and we will present a method to avoid erroneous results.

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Facts from faeces; stable isotope analysis of survivors and non-survivors under the risk of predation

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Predation affects life history strategies of prey and is usually non-random, influencing the distribution of trait values in a population. Typically, investigations of natural selection examine morphological or behavioural traits of prey. Isotopic "traits" represent integrated information about how consumers interact with their environment (i.e. niche) and may provide insight into trade-offs facing prey while foraging under the risk of predation. Sampling individuals that die, however, presents a logistical challenge. Hair within faeces, though, is a readily available material and sampled by the depositing consumer without potential biases of human sampling. In a wolf-deer system, we first examined how digestion affects stable isotope signatures (d15N and d13C) of hair from prey. In feeding trials, digestion slightly but predictably enriched both d15N and d13C. We then compared signatures of surviving (hair moult in spring) and non-surviving individuals (hair in scat) in the wild over two years. Correcting for digestion effects, deer that were predated (non-survivors) showed marginally different isotopic signatures than survivors. Finally, we evaluated the relative contributions of dietary niche and physiological condition in influencing isotopic ratios of free ranging deer. We analysed isotopic variability in deer faeces and in tissues of wild deer with known physiological condition. If isotopic signatures are primarily related to niche, this approach may provide a novel tool to evaluate trade offs faced by prey. Understanding the ecological context of niche space provides insight into genetic, morphological, and behavioural variability within populations.

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Temporal evolution of $\delta^{15}N$ in submerged leaf-biofilm complex: Importance of ambient river N as an N-source for decomposers

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Anthropogenic disturbance of estuaries often result in large inputs of organic material and nutrients supporting high heterotrophic activity. We report on the effect of microbial heterotrophic activity induced by large inputs of untreated waste water on the $\delta^{15}N$ signature and nitrogen content of decaying organic matter in the Scheldt estuary, Belgium.

A long-term *in situ* decomposition experiment was designed to record variation in $\delta^{15}N$ of microbial decomposers of riparian vegetation litter. Therefore, litterbags containing willow leaves were incubated at a freshwater station for a period of 18 months. During the decay process $\delta^{15}N$ composition of willow leaves (original signal = +7.1±0.8‰) and associated decomposers showed considerable seasonal variation with lowest values found during winter-early spring (+11.4 ± 2.2‰) and highest values found just after the summer spring bloom (+18.1 ± 1.1‰). The increase in $\delta^{15}N$ coincided with a decrease in C/N ratio from 24 to values as low as 12. Analysis of biofilm associated with the leaves and attached to the litter bags indicated that microbial decomposers immobilized ^{15}N-enriched ambient N (NH_4^+ and/or DON) to fulfil their N-demand. A mixing model was successful in reproducing the observed evolution of the isotopic signature of the leaf-biofilm complex.

These observations emphasize the importance of microbial alteration of $\delta^{15}N$ signatures of decaying organic matter in nutrient rich estuaries. External N additions to nutrient poor organic matter increase the nutritional quality and render it more attractive to invertebrate feeders. This stresses the need to use microbiologically altered $\delta^{15}N$ signatures instead of original $\delta^{15}N$ values as a baseline $\delta^{15}N$ signature for food web research.

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Using Natural Abundance $\delta^{13}C$ to Investigate Dung Carbon Input to Soils and Soil Microbial Population Dynamics

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Establishing the potential of terrestrial environments to sequester carbon requires detailed knowledge of the cycling of organic compounds in soils. Dungs and manures constitute a large pool of carbon that contribute to soil organic matter. The aim of this work was to: quantify the bulk contribution of dung carbon to the surface horizons of temperate grassland soil, explore the fate of the major compounds from dung in the soil, and, assess the impact of dung carbon on the soil biota.

C_4 dung ($\delta^{13}C$ -12.6 ‰) from maize fed cows was applied to a temperate grassland surface ($\delta^{13}C$ -29.95 ‰) at IGER–North Wyke (Devon, UK), and dung remains and soil cores beneath the treatments collected at t = 7, 14, 28, 56, 112, 224 and 372 days. An increase in the quantity and diversity of PLFAs extracted from the soil coincides with a peak in movement of dung carbon into the soil following a period of rainfall. We hypothesise that an initial stimulation of the soil microbial population by soluble components in the dung should be followed by a sustained incorporation of dung carbon from more recalcitrant organic compounds. A combination of analytical chemical techniques, including continuous flow - isotope ratio mass spectrometry (CF-IRMS), gas chromatography (GC), gas chromatography - mass spectrometry (GC/MS) and gas chromatography-combustion-isotope ratio mass spectrometry (GC/C/IRMS) are used to investigate the routing of ^{13}C-label into the microbial biomass, and to assess the response of soil microbial population to dung carbon using PLFA analysis based on their distributions, concentrations and compound-specific stable isotope values.

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Forensic applications of stable isotope analyses

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Stable isotope concentrations at natural abundance levels may prove useful in several areas related to forensics and to domestic and international terrorism. Today stable isotopes of carbon ($^{13}C/^{12}C$), nitrogen ($^{15}N/^{14}N$), oxygen ($^{18}O/^{16}O$), and hydrogen ($^{2}H/^{1}H$) in both organic and inorganic compounds can be determined routinely using an isotope ratio mass spectrometer. These analyses can be performed on both bulk materials (organic and inorganic) or materials that have been purified from a mixture. Of particular interest in forensic science are applications of stable isotope ratio analyses where the information can be used to determine region-of-origin, authenticity, or relatedness of two or more materials of identical chemical composition. Stable isotope analyses have a rich history of application in the geochemical and biological; they are just now being more extensively applied to forensic sciences. In this talk I explore the utility of stable isotopes to determining region-of-origin in several key areas of forensic interest: (a) documents and counterfeit currencies, (b) illicit drugs and other controlled substances, (c) commercial and military explosives, (d) reconstructing terrorist movements, and (e) pathogenic microbes. For documents and currencies where cotton is the predominant fiber component, there can be significant variations in the carbon, hydrogen, and oxygen isotopes of cellulose fibers that provide region-of-origin information. These applications include determining the authenticity of currency papers and reconstructing paper sources used in false document and currencies. In illicit drugs, such as cocaine, the carbon, nitrogen, oxygen, and hydrogen isotopes of the cocaine molecule provide key information about geographic location. Using a combination of stable isotopes, it is possible to predict the region-of-origin of cocaine in South America with a probability exceeding 90 %. Chemical fractionations occur during synthesis of explosives that impart distinctive combinations of carbon, nitrogen, and oxygen isotope ratios to particular explosives, such as PETN, RDX, and TNT. Regional source water and dietary information are recorded in the hair and fingernails of humans providing a chronological record of an individual's movements over the previous 4-12 months. Lastly, the growth regime (water environment) of bacteria is recorded in spores, providing important information about the geographical region in which those bacteria were cultured. Each of these cases highlight situations in which key environmental information is recorded permanently in the stable isotopic composition of organic molecules that are of forensic interest.

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Tracing carbon flow into individual soil invertebrates by $^{13}CO_2$ pulse labelling

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Soil invertebrates are an integral part of the cycling of nutrients, and their involvement in critical processes, such as primary production, decomposition and mineralisation, directly and indirectly influence the physical and chemical terrestrial environment. Nevertheless, the extent to which plant, microbial and faunal biodiversity interact to control the fate of carbon in the soil is poorly understood. Impacts of global change on the environment have recently increased the awareness of the role of soil as a globally significant and potentially vulnerable store of carbon; in particular the functional role of biodiversity in soil systems and investigations to track and model energy and nutrient flow in soil systems have become progressively more important. Stable isotope approaches are proving to be extremely valuable for such studies and are also helping to improve our understanding of trophic interactions and dietary preferences of soil organisms. However, variations in natural abundance stable isotope values often make interpretations difficult; this can be overcome by introducing isotopically enriched substrates and undertaking stable isotope analysis of individual organisms.

In situ $^{13}CO_2$ pulse labelling (98 atm %, 350 ppm) was carried out using a mobile Stable Isotope Delivery system (Ostle *et al.*, 2000) on the NERC Soil Biodiversity experimental site, Sourhope, Scotland. Pulse labelling of four individual plant species (*Anthoxanthum odoratum*, *Ranunculus repens*, *Trifolium repens* and *Nardus stricta*) was performed to trace the carbon flow into individual species of soil invertebrates. Soil cores were collected at 1, 7, 14 and 28 days after the pulse labelling, and samples of soil, shoots, roots, and soil invertebrates were collected for isotopic determination. We hypothesised that the long term fate of carbon inputs will differ according to which plant species and decomposer communities are involved in the initial assimilation and consumption of the incoming carbon. Furthermore, that not all plants are equal in terms of their inputs of carbon to soil organisms.

Individual collembola species, *Protaphorura armata* (*P. armata*) were probed directly for their fatty acid stable isotope signature by pyrolysis-GC/C/IRMS (Evans *et al.,* 2003). The fatty acid $\delta^{13}C$ values determined show the greatest ^{13}C enrichment from the *P. armata* extracted from the *A. odoratum.* This suggests that there is a feeding specificity for certain plant species by different species and groups of soil fauna. Enrichment was also found to be greatest 14 days after the pulse, except for *N. stricta* where greatest enrichment was seen after 7 days, indictaing different carbon residence times between different plant species.

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Identifying South American wintering sites of migratory shorebirds: a case for multiple stable isotope and trace element analyses

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We are evaluating the use of stable isotopes and trace elements to identify the location of winter sites in Argentina for several shorebird species that breed in North America. The objectives are: 1) to determine the best combination of stable isotopes and trace elements for identifying the location of a shorebird's winter site; 2) to evaluate the degree of interspecies differences in feather isotope/trace element signatures within sites; and 3) to measure inter-annual variation in feather isotope/trace element signatures within sites.

In January and February 2002, 2003, and 2004 we collected newly grown flight feathers from shorebirds captured at 23 wintering sites distributed across 7 provinces in Argentina. The feathers were analyzed for $\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$, $\delta^{18}O$ and δD by continuous flow methods. Additionally, the concentrations of 29 chemical elements were analyzed from the feather shafts using laser ablation ICP-MS.

δD and corresponding $\delta^{18}O$ values ranged from –107 to 57 and 1.7 to 20.7 ‰ respectively and fell along a band displaced about 21‰ from the local meteoric water line. In spite of the wide geographic spread of study sites, δD values alone did not provide a strong ability to predict a shorebird's winter origin. Discriminant analyses predicted a shorebird's province of origin very poorly, ranging from 8% correct (Santiago del Estero) to 80 % correct (Santa Cruz). When other isotopes were included in the analysis, prediction accuracy increased (to values from 56 % in Buenos Aires to 100% in Tucumán). The improvement in accuracy was due to the inclusion of C/N isotope data, which separated D-depleted sites in the west (orographic effect) from those in the far south (latitude effect), and the inclusion of S isotope data, which separated sites with respect to their distance from seawater sulfate. The addition of trace elements including strontium, boron, and copper improved predictions based solely on stable isotopes. No interspecific or inter-annual differences in isotopic signatures were detected within sites.

Stable isotope and trace element data reliably identify shorebird wintering sites in Argentina at a provincial level. Prediction accuracy is constrained by a high degree of intra- and inter-bird variability, especially in the Pampas region, where there is wide variety of wetland/water conditions. These results are the first step in linking wintering shorebird populations with specific migration corridors and breeding sites, an important conservation objective.

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Hydrogen and Oxygen isotopes of water from trees on the west coast of Africa reflect an east coast origin.

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The Cedarberg Mountains are situated approximately 200 km north of Cape Town on the west coast of South Africa. *Widdringtonia cedarbergensis* is a rare and endangered tree endemic to these mountains. Hydrogen and oxygen isotope analysis of the water cryogenically extracted from twigs of six of these trees on a monthly basis from October 1999 to February 2002 are comparable with isotopic values for rainfall for that month (R^2 = 0.53, $P < 0.0001$). Very negative oxygen and hydrogen isotope values for February (δO^{18} -20‰ and δD -168‰) and March (δO^{18} -11.5‰ and δD -99.7‰) 2000 in both twigs and rain can be related to cyclones Eline and Gloria occurring on the east coast of Southern Africa. Rainfall for the Cedarberg is predominantly of west coast origin. It is only unusual cyclonic events on the east coast that move across the entire continent. This suggests that hydrogen and oxygen isotope values of the wood from well-dated rings of trees from the Cedarberg may be used to plot cyclonic events in Kwazulu Natal and Mozambique some 2500kms away.

Isotopic ratios of body lipids, lipid-free matter and chitin of the beetle *Tribolium castaneum* reared on different culture media

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In 1979, Teeri & Schoeller published a study on the $\delta^{13}C$ values of beetles (*Tribolium castaneum*) reared on media with different ratios of wheat and corn. They concluded that there was a constant trophic shift between diet and beetles, regardless of ratio C_3 plant/C_4 plant and growth of the beetles. This has been considered as experimental verification of the back-calculation of the diet of animals based on stable isotope studies, thus Teeri & Schoeller (1979) have been cited in more than 50 other publications so far.

However, in more recent studies different values for the trophic shift in animals fed either C_3 or C_4 plant material have been reported as well as important differences in the isotopic composition of tissues or chemical fractions (e.g. Tieszen et al. 1983, Webb et al. 1998) and effects of food quality (which is reflected in growth) on stable isotope ratios (e.g. Oelbermann & Scheu 2001). In order to investigate the effects of culture media on stable isotopic values of different chemical fractions of the body, beetles were reared on corn meal (C_4-plant), wheat meal (C_3-plant) and 3 mixtures thereof (25%W/75%C, 50%W/50%C, 75%W/25%C), either as flour only, flour inoculated with yeast (*Saccharomyces cerevisiae*) or with addition of dry yeast. Beetles were collected within 2 days after molting to adult stage. Pooled samples were freeze-dried and defatted (modified Smedes-method, Schlechtriem et al. 2003). Chitin was prepared according to Webb et al. (1998). Isotopic ratios of carbon (lipids, lipid-free matter, chitin) and nitrogen (lipid-free matter, chitin) were analysed by EA-IRMS. As can be seen e.g. from the $\delta^{15}N$ of chitin (Fig. 1), both ratio of C_3 to C_4 plant and presence of yeast have effects on the isotopic ratios of the chemical fractions of the beetle, which may have implications on the application of the back-calculation method.

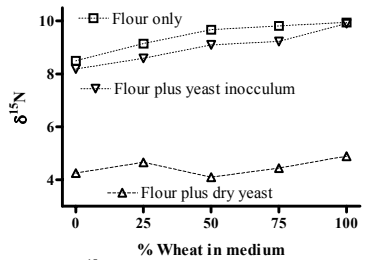

Figure 1: $\delta^{15}N$ of chitin extracted from beetles reared on media with different ratio of C_3 plant/C_4 plant, without yeast, with yeast inocculum or addition of dry yeast (12%). $\delta^{15}N$ corn flour: 5.3 ‰; $\delta^{15}N$ wheat flour: 6.4 ‰; $\delta^{15}N$ dry yeast: -0.76‰

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Dietary niches within the Rancho La Brea raptor and vulture guilds

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The late Pleistocene avifauna from the La Brea tarpits has highly diverse raptor (20 species) and vulture (9 species) guilds. In this study we use stable isotope biogeochemistry to characterize the dietary niches of some of the largest raptor and vulture species that were scavenging and hunting in the La Brea ecosystem. The study includes two regionally extinct species (*Teratornis merriami* - teratorn; *Coragyps occidentalis* - western black vulture) and two extant species (*Haliaeetus leucocephalus* - bald eagle; *Gymnogyps californianus* - California condor). The dietary ecology of the extinct versus extant raptors provides us with interesting insight into species survival across the Pleistocene-Holocene (P-H) boundary. This boundary in North America coincides with a mammalian and avian mass extinction event that heavily depleted terrestrial communities at the generic level, whereas marine communities were unaffected at the species or generic level.

We analyzed the $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios of collagen from bones collected from tarpits that range in age from 32,000 to 11,000 years before present. All of the teratorn (n=10) and vulture (n=10) individuals have $\delta^{13}C$ and $\delta^{15}N$ values that indicate they were feeding on terrestrial resources. Known isotope values for several abundant La Brea herbivores (horse, bison, camel, mammoth) are all within this range of values, and may have been scavenged by both raptors species. The majority of the bald eagles (n=8) have isotope values that show they were feeding on marine resources. Modern coastal bald eagles are piscivores that feed in estuarine or nearshore environments, and the La Brea bald eagles may have had a similar life-history. The condors (n=9) are split into two groups of individuals; those with terrestrial $\delta^{13}C$ and $\delta^{15}N$ values and those with marine values. These finding suggest that raptor species survival into the Holocene was more strongly correlated to a marine (biomass constant across P-H boundary) versus terrestrial (biomass decline across P-H boundary) diet.

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Habitat-specific nitrogen dynamics in New Zealand streams containing native or invasive fish

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Streams are important sites of nutrient transport and transformation in the landscape but little is known about the way in which individual taxa or individual habitats (riffles and pools) influence nutrient dynamics within stream reaches. We used 5-week additions of a stable isotope ($^{15}NH_4Cl$) tracer to investigate nitrogen dynamics in pools and riffles of two New Zealand streams, one with native fish (*Galaxias depressiceps*) and the other with invasive brown trout (*Salmo trutta*). In New Zealand, brown trout initiate a trophic cascade leading to increased algal biomass that we predicted would lead to higher N uptake and retention. Uptake of NO_3^-, but not ammonium, was greater in the trout stream. Rather than causing a large increase in N demand, trout may induce a re-allocation of N uptake and retention among food web compartments in different habitats. The largest differences between streams were apparent in riffles, where most uptake and retention of N occurred. In the trout stream, uptake rate by epilithon in riffles was more than six times greater than uptake rates of any other compartment. In the *Galaxias* stream, several compartments in both habitats had similar uptake rates. Epilithon also accounted for a larger percentage of the ^{15}N retained in the study reach in the trout stream (51%) than the *Galaxias* stream (34%). Our results show that an individual predatory taxon (in our case an invader) can influence N dynamics in streams, but that the magnitude and location of the impact depend on a range of abiotic and biotic factors involved in N dynamics in streams.

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Measuring enzyme activities can improve estimates for the trophic shift

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The use of stable isotope techniques for the reconstruction of diets has increased tremendously over the last decade. However, isotopic ratios in an animal are not only affected by the composition of the feed, but also by the amount of feed consumed. Starvation leads to an increase in the heavier isotope (Hobson et al. 1993). The influence of feeding level on trophic shift not only covers the range below maintenance but also that at which the animal is gaining weight (Focken 2001, Gaye-Siessegger et al. 2003). Gaye-Siessegger et al. (2003) observed an uncertainty of up to 1‰ for both $\delta^{13}C$ and $\delta^{15}N$ values when the feeding level is unknown. This may have substantial effects on the results of back-calculation. As the feeding level of animals is unknown in nature, additional indicators for their nutritional status are needed. High feeding levels and a consequent surfeit of dietary energy lead to the synthesis of lipids. In order to test whether the level of lipogenesis could be used as an indicator, Nile tilapia (*Oreochromis niloticus*) were fed semi-synthetic diets differing in their lipid contents. The extent to which the $\delta^{13}C$ values of the whole body were related to the activity of two lipogenic enzymes in the liver was investigated under controlled laboratory conditions.

The diets used for the experiment were isoenergetic and isonitrogenous semi-synthetic diets with different lipid contents and were made of casein, wheat starch, corn germ oil supplemented with vitamins, minerals and L-arginine. Thirty-five tilapia were reared individually. After a prefeeding phase, seven tilapia were sacrificed to determine the initial composition of the fish. The remaining tilapia were randomly assigned to four groups and fed at the same level for nine weeks. Proximate composition, gross energy and $\delta^{13}C$ values in the lipid and lipid-free fractions were determined in diets and fish bodies. The livers of three fish per group were assayed for the activity of the ATP-citrate lyase and the malic enzyme.

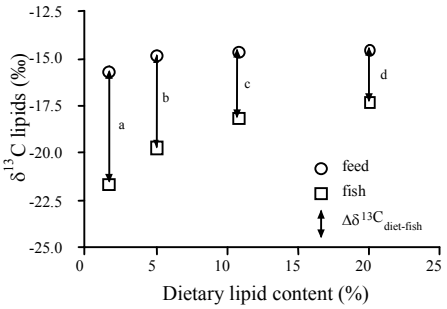

Fig. 1: $\delta^{13}C$ values in the lipids of fish and diets.

$\Delta\delta^{13}C_{diet\text{-}fish}$ in the lipids decreased significantly with increasing lipid content in the diet (Fig. 1). The specific activity of lipogenic enzymes increased significantly with increasing $\Delta\delta^{13}C_{diet\text{-}fish}$ values in the lipids. Therefore the measurement of enzyme activities can help to obtain estimates for the trophic shift. More experiments will be necessary to validate this result in situations where the feeding level is not known.

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Chironomids as conduits of chemosynthetic production through aquatic food webs

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Chironomid (midge) larvae often represent substantial biomass in benthic ecosystems and play an important role in the recycling of organic matter between the benthos and the pelagic of lakes. Non-predatory larvae typically select bacteria, phytoplankton and detritus settling from the water column, or from the surrounding sediments. These putative dietary sources generally exhibit $\delta^{13}C$ values from –35 to –20‰ in freshwater systems. Yet recent studies of larval $\delta^{13}C$ include values considerably lighter (-55 to -64‰: e.g. Jones & Grey 2004). Assimilation of carbon derived from methanogenesis in anoxic sediments and mediated via methanotrophic bacteria at the oxic boundary has been postulated as the likely cause (Bunn & Boon 1993) because methane is markedly ^{13}C-depleted.

Larvae contribute substantially to the diet of benthic predators, while pelagic predators prey upon the pupal stage during migration to the surface, and the imagos provide a significant energetic subsidy to terrestrial predators. Since the distinctive nature of the methane-derived carbon isotope signature is maintained during the life-cycle of the midge, it is possible to trace benthic production being incorporated into different compartments of both aquatic and terrestrial food webs. I have focussed on chironomid species common to Western Europe and will present both inter- and intraspecific isotope data from different lakes. The degree of larval ^{13}C-depletion is related not only to the species analysed, but also to the physical and chemical characteristics of the lake, and particularly oxygen concentrations. In lakes where larvae ingest more methane-derived biomass, intraspecific isotopic variability is high (up to 35‰ in $\delta^{13}C$ and 15‰ in $\delta^{15}N$). Furthermore, larvae from sites more susceptible to prolonged anoxia exhibit greater intraspecific variability, larger larvae are significantly more ^{13}C-depleted, and $\delta^{13}C$ values for one species within one lake may vary ~30‰ seasonally.

I have tracked the export of methane-derived production, via emerging imagos, into spider biomass in lake riparian zones. Estimates derived from two-source mixing models suggest that at certain times of the year, 20% of spider biomass may stem from methane produced in the lake sediments.

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Discriminative powers of stable isotope analyses to reveal ecological plasticity in a myxohaline population of European eel.

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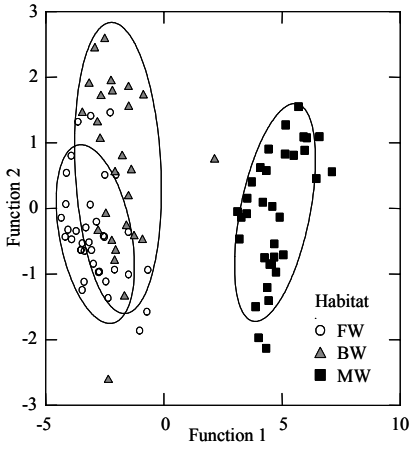
Recent studies have demonstrated that Anguillid eel populations in habitats spanning the marine/freshwater ecotone can display extreme plasticity in the range of catadromy expressed by individual fishes (Tsukamoto *et al*, 1998). We used stable isotope analyses (SIA) to examine levels of plasticity in yellow-phase European eels (*Anguilla anguilla*) in Lough Ahalia, an Atlantic coastal lake that exhibits a marked salinity gradient. During autumn 2003, eels were sampled at a series of sites representing contrasting salinity conditions: freshwater (FW, <1PSU, n=37); brackish (BW, ~10 PSU, n=27); and marine (MW, >25 PSU, n=34). Baseline-corrected $\delta^{15}N$ and defatted $\delta^{13}C$ muscle tissue data were examined using ANOVA to assess how the isotopic signatures of eels varied in relation to capture site. Relationships between individual signatures and various life-history characteristics were examined by correlation. Multiple discriminant analysis (MDA) was used to examine the utility of the derived data ($\delta^{13}C$, $\delta^{15}N$, C:N) to distinguish between eels captured from different habitats.

Eel $\delta^{13}C$ varied significantly ($F_{2,95} = 457$, $P < 0.001$) along the salinity gradient (mean $\delta^{13}C$ ± SE: FW = -23.6 ± 0.17‰, BW = -22.6 ± 0.25‰, MW = -16.3 ± 0.16‰). Thus, $\delta^{13}C$ clearly identified eels from marine habitats, but lack of difference in carbon source signature between brackish and freshwater habitats made discrimination more difficult. The trophic ecology of eels differed markedly along the salinity gradient, with significant differences in relative trophic level (from $\delta^{15}N$: $F_{2,95} = 8.30$, $P < 0.001$). Mean $\delta^{15}N$ values were lowest in BW eels (4.8 ± 0.26‰) compared to MW (5.8 ± 0.17‰) and FW (5.8 ± 0.1‰). Eels in the environmentally-variable, brackish habitat showed wider niche breadth (measured as $\delta^{15}N$ variance). Fish size or age did not show any consistent relationship with increased trophic level but there was a positive correlation between fish condition and $\delta^{15}N$ in each site. The MDA model (Fig . 1) showed increased discriminative power over a simple univariate comparison of $\delta^{13}C$, correctly classifying 85% of the eels to capture site, and highlighting the potential use of SIA in the assessment of fish stock structure.

Fig.1 MDA plot showing function scores and 95% confidence intervals. Function 1 is strongly associated with $\delta^{13}C$, Function 2 with $\delta^{15}N$ and C:N.



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Isotopic investigation of feathers and claws of white-throated sparrows: Delineating catchment areas of a migration monitoring station

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The Canadian Migration Monitoring Network (CMMN) consists of several avian migration monitoring stations (MMS) that apply constant-effort protocols to track changes in the abundance of migratory birds. The CMMN could prove instrumental for the long-term monitoring of songbird populations but one limitation is that the geographical catchment areas of MMS are unknown. Thus, fluctuations in population estimates can not be linked with specific geographical areas. Here, we used stable hydrogen isotope (δD) values of feathers and claws of staging white-throated sparrows (*Zonotrichia albicollis*) to determine wintering and breeding ground catchment areas of a station located in Manitoba, Canada.

The isotopic composition of metabolically inert structures such as feathers and claws reflect the isotopic composition of diet during tissue synthesis. For deuterium, this is controlled by predictable large-scale continental patterns in deuterium in precipitation. White-throated sparrows undergo a complete molt near the end of the breeding season and a partial molt prior to spring migration that is restricted primarily to head feathers. As demonstrated Bearhop et al. (2003. Functional Ecology 17: 270-275), claws of small passerines integrate dietary information over weeks to months, suggesting that their isotopic measurement could also potentially be useful for identifying recent origins of migrating birds. We sampled head feathers, tail feathers, and claws from spring migrants and tail feathers and claws from fall migrants in 2002 and 2003 at the Delta Marsh Bird Observatory in Manitoba.

δD values of tail feathers collected from spring and fall migrants corresponded to latitudes ranging from the very northern to the very southern extent of the western boreal forest. δD values of claws were positively correlated with δD values of head and tail feathers during spring and fall migration, respectively, although a significant amount of claw growth occurred during migration resulting in biased estimates of breeding and wintering origins. δD values of head feathers indicated that white-throated sparrows stopping over at Delta Marsh during spring migration were originating from a broad catchment region in the southeastern United States. However, some head feathers had unusually high δD values that fell outside of the range of δD values of growing-season average rainfall in North America.

Our results suggest that claws in white-throated sparrows grow too quickly to provide unbiased estimates of breeding and wintering origins. The source of the high δD values in certain head feathers will require further investigation. Nevertheless, our findings demonstrate the usefulness of δD measurements of feathers for tracing catchment areas of migration monitoring stations, delineations that are otherwise extremely difficult or impossible to estimate for almost all migratory songbirds.

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Evidence for a precipitation signal in levels of enrichment of ^{15}N in two New Zealand birds

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Levels of enrichment of ^{15}N in mammal bone collagen can be higher in taxa from warm or dry environments. Direct relationships between precipitation and collagen ^{15}N enrichment have been suggested for certain African herbivores, Australian macropod marsupials, and Eurasian mammoths, among other mammals. We report patterns of enrichment in bone collagen ^{15}N in some New Zealand birds that appear to be related to precipitation, especially precipitation in the driest month. In two extinct species, a flightless duck and an owlet-nightjar, whose diets were based on herbs or forbs or (the owlet-nightjar) the insects feeding thereon, there were strong correlations between driest month rainfall and collagen $\delta^{15}N$, but no such relationship existed in an arboreal-feeding, folivorous pigeon. The difference in effect between ground-feeding and arboreal-feeding taxa parallels that reported in Australian marsupials. In addition, a relationship between collagen $\delta^{15}N$ and rainfall in the insectivorous owlet-nightjar suggests that a precipitation signal may also be present in ground-feeding insects. Measurements of collagen $\delta^{15}N$ in duck fossils of Last Glacial Maximum age suggest the potential for quantitative data on rainfall in the North and South Islands of New Zealand at a time when patterns of wind and oceanic circulation differ from those at present.

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Analytical error in stable isotope ecology

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Stable isotope analysis (SIA) has become a popular tool among ecologists in elucidating dietary information from plants and animals collected in the field. The advent of automated analysis in isotope ratio mass spectrometry (IRMS) and the establishment of numerous service labs utilizing the technology, have led to a rapid increase in the number of ecological studies applying SIA as a research tool. As a result, the gap between ecologists and the operators of IRMS equipment is growing ever larger. The end product of this ever-widening gap is a deterioration of an understanding of IRMS methodology and its proper dissemination in the literature.

The purpose of this paper is threefold: 1) to give a brief overview of methodology and terminology in IRMS, 2) to illustrate the disparity that exists in the ecological literature when reporting stable isotope analytical error, and highlight examples of commendable reporting, and 3) to make recommendations for a standardized method of reporting analytical error in future ecological studies using SIA.

We surveyed ecological research papers from selected journals. Of the 304 papers surveyed, 57 (19%) failed to report any form of analytical error associated with IRMS. Of the 247 papers that reported analytical error, there was considerable variation both in the terminology used to describe error and the approach used to quantify it. “Precision” (38%), “reproducibility” (13%), and “error” (12%) were the three most commonly employed terms. Internal laboratory standards, so chosen because they are homogenous and have isotopic signatures that do not vary over time, were typically used to determine the analytical error associated with IRMS. However, we argue that true ecological samples collected in the field often fail to adhere to these two criteria, and hence will produce a greater analytical error than those commonly reported.

Minor protocol adjustments, including the submission of blind replicates by researchers, random assignment of sample repeats within a run by analytical labs, and reporting one standard deviation of a single sample analyzed both within and between runs, will only serve to strengthen the interpretation of true ecological effects by both writers and reviewers.

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Food webs in relation to trophic status and morphometry of the Rotorua lakes: a stable isotope study

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Replicate samples of rainbow trout (*Onchorhynchus mykiss*), common bullies (*Gobiomorphus cotidianus*), common smelt (*Retropinna retropinna*), freshwater crayfish (*Paranephrops planifrons*), plankton, macrophytes, and benthic invertebrates in the 14 lakes of the Rotorua Lakes District were analysed by the Waikato Stable Isotope Unit, University of Waikato, for their ratios of the stable isotopes of carbon and nitrogen. These values were compared to the lake morphometry and nutrient status. In the deep, oligotrophic Lake Rotoma (mean depth 37 m, Secchi depth 11.0 m), common smelt were the most important food of trout. The $\delta^{15}N$ of trout was 4.0‰ greater than that of smelt, and the $\delta^{13}C$ was 1.5‰ greater, which is within the range of previous estimates of isotopic enrichment for a single trophic step. For the shallower, eutrophic Lake Okaro (mean depth 12 m, Secchi depth 2.0 m), stable isotope ratios indicated that smelt were not an important food for trout. However, the $\delta^{15}N$ of trout was 2.8‰ greater than bullies, which approximates the value of a trophic step. $\delta^{13}C$ values suggest, however, that bullies were not the only food of trout. These results broadly agree with previous analyses of trout diet from stomach contents that suggest that trout switch from feeding on smelt in oligotrophic lakes to bullies in eutrophic lakes.

About half of the trout in this study were hatchery releases. Because of the marine fish meal in the hatchery food, trout in the hatchery had enriched isotopic signatures ($\delta^{15}N$ 14.7 to 16.0‰, $\delta^{13}C$ −18.5 to −16.8‰). One to three years after release from the hatchery, the isotopic signatures of hatchery trout were indistinguishable from wild trout from the same lake.

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Dietary plasticity of the apex predators in an Antarctic ecosystem

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Within the Antarctic ecosystem, seabirds are amongst the most abundant consumers. Although some species are heavily dependent on krill (Reid and Croxall 2001), others consume large quantities of fish and cephalopods (Berrow and Croxall 1999). Indeed, even those generally regarded as krill specialists will switch to alternative prey when krill stocks decline (Croxall et al. 1999). Climate change and exploitation of prey species by commercial fisheries may threaten the balance of this ecosystem. It is therefore important to establish the current dietary plasticity of these apex predators and to predict their impact on marine prey resources so that we may assess the likely robustness of the ecosystem in the face of probable future structural changes.

Feather and blood samples were obtained from 25 adults of 19 species from the seabird community breeding on Bird Island, South Georgia. Representative samples from *c*.20 species of prey were obtained from several sources (bird regurgitates and by-caught fish from commercial vessels operating in South Georgia waters). Through a combination of field observations, stable isotope analyses (SIA) and molecular sexing (at Sheffield Molecular Genetics Facility) we are aiming to produce a broadly inclusive model of trophic interactions within this structured marine ecosystem.

Satellite-telemetry and conventional dietary assessment techniques are very useful but can only elucidate part of this marine ecosystem. $\delta^{15}N$ and $\delta^{13}C$ values of seabird feathers and blood and tissue samples from prey items in this study, have provided us with information concerning winter foraging areas; between-season dietary plasticity; and sex-specific differences in feeding areas for both large sexually size-dimorphic species and smaller monomorphic species. Blood and feathers provide contrasting dietary data, with blood representing the recent breeding season diet and feathers, a proxy for the over-wintering diet. SIA has also provided details of the winter diet for several species whose prey base had previously been poorly understood.

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Functional overlap of root systems in an old-growth forest inferred from tracer ^{15}N uptake

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Belowground competition for nutrients and water is considered a key factor affecting spatial organization and productivity of individual stems within forest stands, yet there are few data describing the lateral extent and overlap of competing root systems. We quantified the functional root distribution of different tree size classes in an old-growth forest in the Oregon Cascade Range by measuring aboveground uptake of ^{15}N (99 atom % as NH_4Cl) injected at different radii around target Douglas-fir trees. Tree size classes included sapling, intermediate and dominant trees. Each target tree received 50 evenly-spaced soil injections of ^{15}N at 0.5, 1 or 1.5 times the mean crown radius of each tree-size class. Analysis of the ^{15}N content of aboveground tissues collected 4 months after injection show a monotonic decrease in root function with distance; however, uptake beyond 1 crown radius accounts for over 1/4 of total uptake. These results indicate significant extension of root systems beyond the "dripline" of trees and direct belowground competition among neighboring stems.

We applied the tracer data in combination with spatial data on stem location and size to calculate the functional belowground overlap among neighbors within our 3-ha study plot. Results show that the intensity of belowground overlap (competition) is unevenly distributed within this stand. Growth rates of individual stems are negatively correlated with belowground competition, but only for dominant (emergent) trees ($P<0.03$). Subordinate trees show no correlation between growth and belowground competition, suggesting that these trees are limited primarily by light. Our approach establishes a means for improving the representation of belowground competition in stand models of forest growth, a feature that existing models either lack or treat theoretically.

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Applications of stable isotopes to conservation: assessing threats and setting restoration targets

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Freshwater ecosystems are in peril because they aggregate multiple threats in a watershed. In many systems, managers can identify the prominent threats and implement programs to mitigate them. Most assessment programs, however, monitor physical rather than biological responses, even when conservation of biodiversity is an explicit goal. Biological monitoring is usually limited to a few species of special interest. I used stable isotopes to bring a food web perspective to conservation and restoration of two ecosystems that face similar threats: water extraction and exotic species. In Cuatro Cienegas, Mexico I employed stable isotopes to identify which native species are most likely to be affected by an exotic fish and showed through manipulative experiments that the stable isotope analysis correctly predicted that the exotic would compete with juvenile cichlid fish. In Fossil Creek, AZ a stream slated for two major restoration actions – dam decommissioning and exotic fish removal, I used stable isotopes to compare food web structure in a pristine site, a reach above the dam with full flows and no exotic fish, with disturbed sites. Results indicate that the trophic level of native fish decreases and that there is more resource overlap among functional feeding groups of macroinvertebrates in disturbed relative to pristine sites. This food web analysis will be used to assess the success of the restoration actions.

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Estimating photosynthetic quantum yield from temperature and stable carbon isotope ratio

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Models of photosynthesis at the leaf and canopy levels are often based on the efficiency with which absorbed photosynthetically active radiation (APAR) is converted to photosynthate. Such APAR models are highly sensitive to estimates of the photosynthetic quantum yield, defined as the moles of photosynthate produced per mole of photons absorbed. This trait is driven primarily by the substrate specificity of Rubisco, the enzyme responsible for photosynthesis in C3 plants. This specificity, which controls the proportion of photons used for carbon dioxide reduction rather than photorespiration, is strongly conserved in higher plants. Theory suggests that it should be controlled by temperature and the CO_2 concentration in the chloroplast (C_c). Stable carbon isotope ratios provide an integrated estimate of C_c. We present our model of quantum yield and test it against measured quantum yield across several species growing across a broad range of conditions in the northern Rocky Mountains, USA. This approach offers a simple, inexpensive means of constraining the photosynthetic gas-exchange of canopy photosynthesis models, eliminating one of the empirical tuning variables that have limited their credibility. The approach also provides a means of parameterizing canopy models in the past based on historical temperature records and the isotopic composition of tree rings.

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Do stable isotopes weigh down the nichemetrician's toolbox?

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Ecology has a rich history of designing, modifying, and reinventing tools that measure abstract properties of biological communities. Niche theory has many metrics and indices, each with their own strengths and weaknesses (Hurlbert 1978). When considering diet as the niche axis, all these indices share the problem of temporal consistency in measuring the gut contents of individuals. The emergence of stable isotopes, as a tool to measure a time integrative average of feeding behavior, seems to be an ideal fit for the nichemetrician's (Hurlbert 1978) toolbox. Recently, ecologists have been using intrapopulation isotopic variance to make inferences about dietary variation among individuals, and to estimate a population's niche width. However, inter-individual differences in stable isotope signatures at a given site do not necessarily imply diet variation. We propose a null model approach to generate an expected intrapopulation isotopic variance for a consumer, given isotopic variability among diet categories. Results demonstrate that intrapopulation isotopic variance is not necessarily synonymous with a population's dietary based niche.

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Tracing carbon through the soil microbial community during ryegrass decomposition

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Decomposition of plant materials has been much studied and successional trends in microbial communities during decomposition have also been monitored. We have revisited the linkage between microbial community composition and decomposition by using compound-specific analysis of microbial phosopholipids (PLFAs) during the decomposition of ^{13}C-labeled ryegrass straw. Our specific objectives were: (i) to follow shifts in microbial community structure during decomposition, (ii) to examine the influence of the soluble component of the ryegrass straw on microbial community composition, and (iii) to determine whether different PLFAs, representing different functional groups of microorganisms, were enriched differentially between treatments and through time.

Laboratory microcosms were set up with four treatments, including three types of C amendments (unleached straw, leached straw, and straw leachate) and an unamended control. Added substrates were enriched between 120 and 180‰; the native soil was –26‰. Destructive sampling took place after 0.6, 1.6, 15, 18, 50, and 80 d of incubation. PLFAs were extracted from bulk soil and isolated straw (detritosphere) using a modified Bligh-Dyer method (White at al., 1979) and analyzed by GC-C-IRMS.

Distinct temporal shifts occurred in PLFAs of bulk soil samples. In all amended treatments, early communities were dominated by short, branched-chain PLFAs; later samples contained more complex, longer PLFAs. In particular, 19:0cy was an indicator for late succession communities in the leachate and unleached straw, and 18:2ω6,9 characterized late samples in leached straw. Soluble C affected when the temporal shift among communities occurred, with leachate and leached straw shifting sooner. At all times, more ^{13}C was detected in the 16:0 and 18:2ω6,9 PLFAs of the detritosphere compared to the bulk soil. A fungal biomarker, 18:2ω6,9, was most highly labeled in all treatments.

Combining ^{13}C and PLFA analysis provides a clearer insights into microbial community structure during decomposition and allows us to link different microbial groups with different stages of the decomposition process.

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Using stable isotope analysis to determine the winter moult extent in migratory birds: the complex moult of Savi's Warbler

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The vast majority of birds, and in particular the passerines, replace (moult) their entire plumage at least once a year. In adult resident passerines, the annual complete moult takes place shortly after the breeding season, but this is not always the case for migratory species, which have a large variety of moult strategies. Although the phenology and extent of summer moult is relatively well known for most species, the winter moult of many migratory birds remains little studied. In addition, due to the lack of information about the complementarities of the different moults that take place during the annual cycle, a natural classification of moult types is still lacking. It is possible to describe the winter moult extent of birds when they return to the breeding quarters by observing the wear and bleaching of different sets of feathers (feather generations). In some species, it seems to be relatively straightforward to interpret the patterns of feather wear, simply by assuming that the old feathers grew in the breeding quarters and the new or fresh feathers grew in the winter quarters, although no confirmation of this has ever been done. However, the moult patterns become very difficult or even impossible to interpret as the season progresses, in species with asynchronous moult or long moult periods and in species that show more than two feather generations.

Here, we use stable isotope signatures to determine the origin of different generations of wing feathers and describe the winter moult extent in a long-distance migratory bird with a complex moult. Stable isotope ratios of intrinsic tissues reflect those of local food webs, which, in turn, can vary spatially due to several possible biogeochemical processes. Animals that move between isotopically distinct food web baselines can retain information on the previous feeding location for periods that depend on the elemental turnover rates of the tissue of interest. Keratinous tissues like hair, feather or nail are metabolically inert following synthesis, and so their isotopic signatures reflect those of the location where they were synthesized. Stable isotope analysis has been used successfully to determine the diet and provenance of feeding, trace the origin or migration of wildlife and link winter and summer events. Therefore, this technique might also be useful to distinguish the feathers that grew up in the breeding quarters from those that grew in the wintering quarters of migratory birds, thus allowing the description of the winter moult extent. We found highly significant differences in $\delta^{13}C$, $\delta^{15}N$ and δD between European and African feathers taken from a variety of insectivorous passerines that occur in wetlands. A discriminant analysis, which correctly classified 93.8% of cross-validated cases, was used to determine the origin of feathers taken from each wing feather generation of Savi's Warblers, *Locustella luscinioides*, captured in Portugal during spring 2003. This methodology was compared with the analysis of feather wear, providing a partial confirmation of the general interpretation of moult patterns. In addition, the complex moult of Savi's Warbler was described in detail and novel isotopic data from the European-African bird migration system was provided.

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Historical foraging ecology of northern fur seals (*Callorhinus ursinus*): an annual $\delta^{13}C$ and $\delta^{15}N$ time series derived from canine teeth

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Over the past 30 years the Alaskan stock of northern fur seals (NFS; *Callorhinus ursinus*) have witnessed drastic shifts in population estimates. After a 1968 moratorium on the pelagic harvest of NFS adult females, the Alaskan population recovered to approximately 1.25 million in 1975. The population then began to steadily decline at a rate of ~7% per year into the early 1980's and stabilized by 1985. Many researchers have suggested that the decline is related to shifts in the quantity and/or quality of prey species (2,3). One of the questions yet to be resolved is whether or not such prey fluctuations are due to the 'top down' impacts of commercial over-fishing or natural variations in prey quality due to 'bottom up' climatic forcing. Complicating the issue is the fact that NFS prey on over 60 different kinds of fish and squid (2,4,5). Dietary records of NFS spanning several years are rare, and the little dietary data available, gleaned from gut content or scat analysis, has inherent biases that make it difficult to compile into long-term temporal records (6).

We have constructed a record of average annual $\delta^{13}C$ and $\delta^{15}N$ values of NFS prey by measuring the isotopic composition of NFS canine teeth collected from a single rookery during subsistence and commercial harvests over the past 50 years. Preliminary analysis shows $\delta^{13}C$ values were relatively stable during the period 1960-1975 (-15.2±0.2‰), followed by a significant decrease of ~1.0‰ beginning in the mid- to late 1970's. Time series $\delta^{13}C$ values range from –14.7 to –16.3‰, with the largest inter-annual variability in the early to mid-1980's. Furthermore, the largest intra-annual variation is in 1982, coinciding with one of the most intense El Niño/La Niña (ENSO) events of the past few decades. Overall, this record provides insight into the diversity of NFS forage and/or foraging location on historical timescales, which can be linked to known climatic events (i.e., ENSO, PDO) and population estimates. In addition, this record can be compared to isotopically derived foraging records of other northeast Pacific marine mammals, which have been used to argue for a regional decline in primary production that is roughly in phase with the decline of NFS and other pinniped populations.

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Insights into the vertical transfer of organic matter by zooplankton in subtropical and subantarctic waters using stable isotopes

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Mesozooplankton (>200 μm) samples were collected in spring, October 2001 from the subtropical and subantarctic zones, east of New Zealand, using day and night tows of a 1 m^2-MOCNESS over discrete depth intervals (15-200 m) in the upper 800 m. Biomass estimates, species identification and stable isotope analyses for $\delta^{15}N$ and $\delta^{13}C$ of three different size classes of the zooplankton community (200-500, 500-1000, >1000 μm) were undertaken to investigate the trophic relationships and energy transfer efficiency of the plankton community in these contrasting water masses. Nitrogen stable isotope compositions ranged from 2-10‰ across all depths and water masses, while $\delta^{13}C$ values varied from -19 to -24‰. In general, $\delta^{15}N$ values at water depths >500 m were 2-3‰ higher in subtropical, compared to subantarctic waters, especially in the >1000 μm size class. Differences were less marked over the upper 500 m for the smaller size classes, suggesting a higher degree of zooplankton carnivory in the warmer subtropical waters. No discernible differences were observed in the $\delta^{13}C$ signatures, except in the largest size class, which was enriched in $\delta^{13}C$ by up to 2‰ in subtropical waters deeper than 500 m. Zooplankton assemblages in both water masses were similar with *Clausocalanus* and *Oithona* species dominating the smaller size classes (200-1000 μm) in surface waters and *Oncaea* and *Pleuromamma* spp. in waters deeper than 350-400 m. *Neocalanus tonsus*, *Pleuromamma* spp., chaetognaths and ostracods prominent in the size class >1000 μm.

Typically $\delta^{15}N$ values in all size classes increased with increasing water depth, from 2-4‰ in the upper 300 m during day-light hours to ~6-8‰ at 600-800 m in both subtropical and subantarctic waters. A preponderance of omnivores and detritivores in surface waters and at least one trophic shift (~3-4‰) between surface and deep zooplankton communities are suggested by these data. These relationships were less pronounced at night, while in day-time subtropical waters the >1000 μm size class had a slightly higher $\delta^{15}N$ composition, indicating carnivores were prominent in this size-fraction, as confirmed by a numerical dominance of chaetognaths and euphausiids. In subtropical waters, the >1000 μm size class had variable although approximately constant $\delta^{13}C$ values whereas the isotopic ratios became more positive with increasing water depth from -23‰ in surface waters to -21‰ at 600-800 m. In subantarctic waters, the day-time $\delta^{13}C$ values exhibited similar trends for the >1000 and 200-500 μm size-fractions while the 500-1000 μm size class did not vary substantially with water depth. In contrast, the night-time tow in subantarctic waters indicated an increase in $\delta^{13}C$ over all three size classes, especially for those zooplankton >500 μm.

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Patterns of egg and tissue $\delta^{15}N$ enrichment during reproduction in insects with little adult protein intake: a model for starvation?

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Butterflies and moths have been a productive model for investigating carbon turnover and incorporation into reproduction. Most species rely on nectar as their sole adult food source, a diet which is rich in sugars but depauperate in amino acids and other nitrogen-bearing compounds. In previous work on five species of nectar-feeding Lepidoptera, the nonessential amino acids used in egg manufacture were found to be largely synthesized from the carbon in nectar sugars. For a female butterfly to provision her eggs with newly-synthesized amino acids, she must transfer amine groups from endogenous protein to new carbon skeletons. Because trans-amination reactions prefer ^{14}N, both body protein reserves and egg $\delta^{15}N$ may exhibit patterns of nitrogen enrichment or depletion. We have measured egg $\delta^{15}N$ data over time in six species of nectar feeding butterflies: three exhibited significant N enrichment over time, and three exhibited significant N depletion. In this study, we investigated tissue $\delta^{15}N$ in one of those species, *Speyeria mormonia*. We dissected laying females at 0, 5, 10, 15, and 20 days of adult life, and measured $\delta^{13}C$, $\delta^{15}N$ and C/N in eggs and in samples of flight muscle, ovary, and fat body (the primary storage organ for both fat and protein in Lepidoptera). We also analyzed samples of wing, which are metabolically inactive and served as a control. Ovary and fat body C/N increased dramatically over the course of oviposition, suggesting that these two tissues were providing the amine groups used in egg production. Flight muscle C/N did not change with age. Individual $\delta^{15}N$ at emergence from pupation was variable (wing $\delta^{15}N$ ranged from 3.1 to 6.6‰), reflecting significant variation in N among larval hostplants. This variation obscured tissue level variation in $\delta^{15}N$ with age, thus, we standardized tissue $\delta^{15}N$ to a mean wing $\delta^{15}N$ of 4.1‰ to control for individual level differences. At the time of adult emergence, fat body $\delta^{15}N$ was almost 2‰ lighter than other body tissues. Over the first week of oviposition, ovary nitrogen decreased significantly in $\delta^{15}N$, suggesting a transfer of stored N from the fat body to the ovary. Egg $\delta^{15}N$ followed that of the ovary, decreasing over the first week of egg laying. However, after about 10 days of oviposition, ovary, fat body and eggs all showed significant enrichment (~1.5 - 2‰) as ^{14}N was lost from the butterfly. Flight muscle also shows a modest enrichment during the last week of oviposition (< 1‰). These data support the idea that ^{14}N is preferentially lost over the course of oviposition, and show how nitrogen is transferred from storage to synthetic tissues during egg manufacture. They also help to explain why different species might show different trends in egg $\delta^{15}N$, as the pattern of egg nitrogen change varies with the total extent of N loss from the system. These butterflies are a potentially useful model of nitrogen storage and recruitment, as animals which are protein starved during their reproductive phase.

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Evaluation of primary production and community respiration in aquatic environments based on $\delta^{18}O$-O_2 and $\delta O_2/Ar$

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Evaluation of gross primary production within aquatic environments is one of the most fundamental measures of ecosystem health and ecology. While the use of ^{14}C labeled bicarbonate in incubation has been the standard for quantifying rates of primary production for the past 50 years, recent studies based on incubations with ^{18}O labeled water have shown rates of primary production 2-10 times greater (Luz et al., 2002). Consequently, we conducted simultaneous estimates of primary production in Lake Erie in July and August of 2003 using ^{14}C labeled bicarbonate, light-dark bottle incubations, and ^{18}O labeled water. Following incubation with $H_2{}^{18}O$, determination of $\delta^{18}O$-O_2 and $\delta O_2/Ar$ enables determination of gross and net primary production and respiration. All three approaches were initiated using a common reservoir of water and incubated simultaneously at the same light level. Three stations in Lake Erie were sampled that ranged from 1 to 20 μg-Chl/L. Rates of epilemnetic primary production in July based on ^{14}C, light-dark bottles, and ^{18}O labeled water for the eastern basin (5.33, 4.23, and 5.65 $mgC/m^3 \cdot hr$, respectively), central basin (4.47, 1.59 and 4.26, respectively) and Sandusky Bay (332.0, 520.61, and 402.8, respectively) were of comparable magnitudes and did not show consistent differences between stations.

To better understand differences in primary production rates obtained by the three techniques we calculated the photosynthetic quotient (PQ) defined as the ratio of carbon uptake to O_2 production. Slight differences in PQ were evident in July between the light-dark bottle (0.36 to 1.57) and $H_2{}^{18}O$ (0.95 to 1.21) methods that may reflect the difficulty of measuring small changes in O_2 concentrations over the course of incubation. Values of PQ based on incubation of $H_2{}^{18}O$ in August ranged between 0.36 and 4.6. Low values of PQ occurred in the epiliminion and highest values in the metalimnion and this pattern of variation is consistent with observations of Fahnenstiel and Carrick (1988) who also reported a PQ for the deep chlorophyll layer of Lake Huron of 4.90. Such variation in PQ is a reflection of variation in the source of N utilized by phytoplankton, the products of photosynthesis, distinctions in O and C fluxes during photosynthesis (ex. Mehler reaction, photorespiration, and dark respiration), the potential loss of ^{14}C due to respiration, and the release of $DO^{14}C$ during the course of incubation. These comparative productivity assays allow us to conclude that the assumption of a single value for PQ when converting O to C flux is not appropriate and variation in PQ is an important consideration in explaining differences in productivity estimates based on $^{14}CO_3$ and $H_2{}^{18}O$.

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Are you what you eat… all year long ?

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«You are what you eat» is the maxim that best summaries the principle of stable isotope analysis (S. I. A.) of trophic relationships. The isotopic composition of the consumer in C and N reflects, with a known enrichment rate in heavier isotope, the isotopic composition of its food. The basis of S. I. A. have been established for 20 years from lab experiments where captive animals have been fed with a diet of known isotopic composition until its tissues reached an isotopic equilibrium. The isotopic equilibrium between tissue and food results from the progressive integration of the diet nutrient in proteins and lipids of tissues through growth and metabolic tissue replacement. The strength of S. I. A. is that it provides reliable information about assimilated food (and not only ingested) but this assimilation requires a delay. S. I. A. had numerous applications in field studies. However, in the field, carbon sources isotopic composition exhibits strong seasonal variations and we assume they could prevent tissue from reaching equilibrium. Moreover, organisms undergo different metabolic phases during the year with a phase of somatic growth, a phase of gonadic growth and a phase of maintenance metabolism, during which the somatic growth is null. The isotopic turnover of tissue may then strongly depend on the metabolic phase. Our second assumption is that food isotopic signature may consequently not be integrated in tissues at the same rate, all year long.

To address these assumptions, we compared the evolution of the ^{13}C and ^{15}N composition of Whitefish (*Coregonus lavaretus*) food and tissues (liver and muscle) in a natural environment, the lake Leman (Geneva). Whitefish is known to be mainly zooplanktivorous in this lake. During a 2-year survey, Whitefish were sampled monthly and muscle and liver were collected for stable isotope measures. Zooplanktonic taxa abundance in gut contents were also determined in order to estimate stable isotope composition of diet from stable isotope composition of fresh zooplankton. As a result, zooplankton taxa underwent strong seasonal variations in its isotopic composition, spanning over a 10‰ range for both $\delta^{13}C$ and $\delta^{15}N$. As we assumed, Whitefish muscle was never in equilibrium with its food isotopic composition, contrary to liver, a fast turnover tissue. Moreover, kinetics of food isotopic composition integration in muscle depended strongly on the season while liver isotopic composition represented a nearly continuous record of food isotopic composition.

This study provides some precisions about integration kinetics of the food isotopic signal that point out the signification of tissue isotopic composition and could avoid some misunderstanding in future S. I. A. of food webs.

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Source aggregation in stable isotope mixing models: lump it or leave it?

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A common situation when stable isotope mixing models are used to estimate source contributions to a mixture is that there are too many sources to allow a unique solution. To resolve this problem one option is to combine sources with similar signatures such that the number of sources is small enough to provide a unique solution. However, this will tend to increase the uncertainty of the combined end-member and consequently the source contribution estimates, an effect that can be quantified using the IsoError model described by Phillips & Gregg (2001). Common and reasonable practices are to consider lumping only when the isotopic signatures of clustered sources are not significantly different, and when sources are related such that the combined source group has some functional significance. For example, lumping several species within a trophic guild will allow more interpretable results in a dietary analysis than lumping disparate food sources (elephants and amoebae), even if they have similar isotopic signatures. As an alternative to lumping sources before a mixing analysis, the IsoSource mixing model (Phillips and Gregg 2003) can be used to find all feasible solutions of source contributions that are consistent with isotopic mass balance. While the range of feasible contributions for each source can often be quite broad, functionally related groups of sources can be combined *post hoc,* producing a range of solutions for the aggregate source that may be considerably narrower than that of each individual source. A paleohuman dietary analysis example is given to illustrate this point, which involves a terrestrial meat food source, a combination of three terrestrial plant foods, and a combination of three marine foods. In this case, *post hoc* aggregation of sources allowed strong conclusions about temporal shifts in marine vs. terrestrial diets that would not have otherwise been discerned.

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Controls on carbon isotopic fractionation in marine microalgae

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Comparison of the isotope ecology of different marine ecosystems requires knowledge of the isotopic composition of the base of the food web from which the organisms derive their carbon and nitrogen. In many environments, however it is impractical to measure the carbon isotopic composition of marine microalgae. Therefore, considerable effort over the last decade has been directed towards understanding controls on the carbon isotopic composition of oceanic phytoplankton. Laboratory studies have demonstrated that $CO_2(aq)$ concentration, microalgal growth rates, cell geometry and the degree to which phytoplankton utilize inorganic carbon concentrating mechanisms affect carbon isotopic fractionation in marine microalgae and thus provide the foundation for predictive models. Unfortunately, laboratory experimental data indicate that the degree to which these factors influence carbon isotopic fractionation is dependent on culture technique. For example, the magnitude of carbon isotopic fractionation is quite different for the same alga grown under identical conditions (same growth rate and $[CO_2(aq)]$) in batch and continuous (chemostat) cultures. Additionally, the extent to which variability in fractionation noted in culture studies represents that found in the field is still unknown. Clearly, independent field evidence is required to evaluate whether results from any of the laboratory culture methods represent natural systems.

To field-test laboratory-based microalgal fractionation hypotheses, we developed and laboratory-tested a method to measure the growth rate of alkenone-producing algae in field samples and have applied this method to determine the depth of alkenone export production and the growth rate of alkenone-producing algae at Station ALOHA, a well-studied subtropical oligotrophic North Pacific time-series site. Alkenones are long-chain ketones known to be biosynthesized in the open ocean by only two different algae, the most notable example being the cosmopolitan alga *Emiliania huxleyi*. This biomarker therefore limits isotopic analyses to only very select algae of similar physiological characteristics, and thus provides a model organism for investigating relationships between oceanic $CO_2(aq)$ levels, microalgal growth rates and carbon isotopic fractionation in marine environments.

Our method uses 24-hour *in situ* incubations with ^{13}C-labeled bicarbonate and irmGC/MS analysis to determine the rate of ^{13}C incorporation in alkenones. Laboratory studies confirm the utility of this method. We have found that the depth of alkenone export production at Station ALOHA varies throughout the year but is restricted to the surface mixed layer or just below the surface mixed layer strongly suggesting that growth of these algae are nutrient-limited rather than light-limited. These experiments combined with analyses of the $\delta^{13}C$ of $CO_2(aq)$ and alkenones throughout the euphotic zone allow field evaluation of laboratory-based carbon isotopic fractionation models for alkenone-containing algae. Preliminary results from Station ALOHA indicate that neither laboratory batch nor chemostat culture results using *E. huxleyi* faithfully reproduce how this phytoplankton fractionates carbon isotopes at this site.

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Tracking carbon transfer from decomposing invertebrate faeces; the fate of faecal carbon in the soil

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The formation and dynamics of soil organic matter (SOM) are subjects of global relevance at present due to increasing anthropogenic emissions and climate change. The enhancement of carbon sinks in the soil is an option for increasing the removal of carbon dioxide from the atmosphere (Royal Society, 2001) but the processes by which carbon is sequested in the terrestrial environment is poorly understood.

Soil macrofauna and mesofauna play an essential role in the initial breakdown and processing of organic matter entering the terrestrial environment and previous studies have shown animal faeces to constitute almost the entire H layer (Kubiena 1953). Soil fauna enhance the biodegradation and humification of organic residues by: (i) by commuting organic residues and increasing the surface area for subsequent microbial attack; (ii) Producing enzymes which break down the complex biomolecules such as proteins and carbohydrates; (iii) Utilising simple components for their own biochemical requirements for growth and reproduction such as sterols, fatty acids, amino acids and simple sugars. Comparatively little is understood about the impact of the activities of soil fauna on plant derived organic matter at the molecular level. This has important ramifications for carbon cycling, particularly in relation to the production of stable forms and long term fate of carbon in the terrestrial environment. The chemical basis of these differences need to be elucidated if the long term accumulation of carbon in soils is to be understood.

A faeces decomposition experiment has been established with an isotopic ($\delta^{13}C$) difference between the soil (C_3, –29.0‰) and collembola faeces (ca. 1000‰) derived from different ^{13}C labelled diets fed to collembola. The $\delta^{13}C$ values of the respired CO_2 were analysed to determine the rates of respiration and proportions of CO_2 derived from the soil and faeces. The isotopic label allows us to study the fate of faecal carbon in the soil using compound specific carbon isotope analysis. The result of the respiration experiments will be correlated with the chemical composition of the faeces of collembola raised on different diets in order to provide a molecular base for understanding carbon sequestration and cycling.

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Carbon flow interactions between bacteria and microphytobenthos on a temperate mudflat – insights from bulk and compound-specific isotope analysis

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The sources of organic matter were studied on the upper and lower regions of a mudflat in the upper euryhaline part of the Huon Estuary, south east Tasmania over four seasons. The aim of the study was to develop a detailed conceptual understanding of sedimentary carbon and nitrogen cycling processes in relation to the activity of microphytobenthos (MPB) (a complex community consisting of diatoms, cyanobacteria, green microalgae and other algae) in this system.

Sediments for analysis of C and N contents and stable C and N isotopes were dried, ground, treated to remove carbonate and analysed *via* a Carlo Erba elemental analyser coupled to a Finnigan Mat Delta S isotope ratio mass spectrometer. Fatty acids were isolated from sediment samples using solvent extraction techniques. These were then converted to methyl esters and analysed using a Hewlett Packard 5890 gas chromatograph coupled to the same instrument.

Bulk isotope analysis indicated that the organic matter pool was primarily composed of terrestrially derived material, with organic matter from microphytobenthos representing a smaller proportion of the total organic carbon (as indicated by a more enriched $\delta^{13}C$ value), although this did vary seasonally .Given that sediments at this site exhibited significant rates of respiration and that terrestrial organic matter is generally regarded to be refractory, as indicated by high C:N ratios (>10), the question remains as to the source of the carbon being consumed by the bacteria. A more likely supply of organic carbon to bacteria within the sediment is that derived from MPB, which has a lower C:N ratio (*ca*. 7) and contains high proportions of labile forms of organic carbon. MPB are known to secrete large amounts of extracellular organic carbon (EOC), consisting predominantly of sugars and carbohydrates which can be directly measured in sediments, and it is this material that may be used for microbial respiration. Sugars are generally isotopically enriched in primary producers compared to the bulk cellular material, and it has also been reported that sugars and carbohydrates are enriched compared to the bulk cellular material in cyanobacterial mats. In this study we have investigated the potential substrates consumed by the bacteria utilising compound specific isotopic signatures of bacterially derived fatty acids.

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Estimating seasonal energy flow for two bivalve species: a carbon stable isotope diet switching experiment

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Stable carbon isotope ratios were measured in gonad, adductor muscle, digestive gland and gills of scallops (*Pecten maximus*) and oysters (*Crassostrea gigas*) held on a constant diet of phytoplankton of known isotopic composition for 15 days.

The aim was to determine how quickly stable isotopes in tissues would be replaced by isotopes derived from the diet, depending on the organ, the season and the species, after switching the stable carbon isotope composition of the diet. Based on previous hypothesis on annual energy allocation schedule for those species, 4 different experiments were conducted in spring, summer, autumn and winter. Scallops and oysters were reared on a mixed diet of 4 phytoplankton species depleted in ^{13}C. Each experience was conducted at temperature of the period in the field.

Experimental bivalves were switched to the phytoplankton diet on day 0 and three individuals were randomly chosen and sacrificed (after 2 days of starving to empty digestive tract) for isotopic analysis on day 0, 2, 6 and 15.

After switching the diet, isotope values gradually shifted toward expected value in a clear temporal pattern. Interestingly, replacement curves showed differences among the organs but also among seasons and species.

A carbon incorporation index (CII) is calculated to compare metabolic activity of each organ of the two species between day 0 and day 15:

$$CII = [(\delta^{13}C_{d15} - \delta^{13}C_{d0}) / (\delta^{13}C_{diet} - \delta^{13}C_{d0})] * 100$$

For both species, digestive gland presents the most important carbon incorporation and muscle the minimal value, while gonad and gill present intermediate values. Carbon incorporation (CI) is in general much more important for *P. maximus* than for *C. gigas*. For scallops, CI is maximal during the spring and then decreases during the year whereas for *C. gigas*, carbon incorporation is highest in winter. For *P. maximus* digestive gland, an huge change is observed between summer and spring where CI is high, and autumn and winter where CI is low, irrespectively of food availability in the tanks, suggesting an endogen control, environmentally driven or not, of metabolic activity.

Therefore, these experiments revealed different windows of metabolic activity depending on the season, the organ and the species. Stable isotope diet switching experiments seem to be of great value to assess metabolic orientation for bivalves.

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Natural abundance of ^{15}N in different compartments of a spruce forest ecosystem under acid rain and manipulated clean rain field conditions

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We analysed stable isotopes of N in a spruce forest both under ambient rainfall (no further manipulation of the atmospheric input) and the clean rain scenario, i.e. to the status after about 10 years of reduced inorganic N- and acid-constituent input (clean rain application). The objectives of the study were to assess whether or not the natural ^{15}N abundance will function as an indicator for the N-status of our forest ecosystems under above mentioned field conditions. For this purpose, natural 15N abundance values were measured in needles, litter fall, roots, soil, bulk precipitation, thoughfall and soil water of both plots. In the bulk precipitation, $\delta^{15}N$ values of NO_3-N were in the range reported from other studies (–16 to +23‰). In control plot of D2, the pathway of ambient rainfall through the canopy significantly influenced ^{15}N the abundance of nitrate; the throughfall was greatly depleted in ^{15}N compared to the bulk precipitation. The throughfall water after passing through the O-horizon (below 10 cm) and the upper mineral soil layers, the $\delta^{15}N$ abundance of nitrate increased in the 100 cm soil depth from -4.34 ‰ to – 3.22‰ at D2 and from –6.29 ‰ to – 2.04 ‰ at D1, i.e. the ^{15}N enrichment was significantly ($p \leq 0.05$) stronger at the clean rain plot D1. This stronger 15N enrichment in the soil water of clean rain plot of D1 has been explained by the presence of insignificant nitrification in clean rain D1 plot. ^{15}N enrichment of both green needles and litter fall was greater in the N-saturated control plot of D2 than in the clean rain plot of D1. In all soil depths, both living and dead fine roots were depleted in ^{15}N compared to the soil $\delta^{15}N$ in the respective depths. We found further positive correlation between $\delta^{15}N$ in soil and roots, leading us to believe that roots preferentially used N from the soil horizons they were in for their own biomass production. For the clean rain plot D1, a typical vertical gradient of the soil ^{15}N-enrichment was observed, whereas the roof control site D2 differs from the clean rain plot D1 with respect to the ^{15}N abundance trend in organic/humus soil layer. In the organic layer (0-6 cm depth) of plot D2, there is almost a trend of slight soil $\delta^{15}N$ depletion with increasing depth and this is explained by the presence of prominent nitrification at this plot. Our observations have concluded that the $\delta^{15}N$ natural abundance of undisturbed forest soil profiles can provide information about the N-status in forest ecosystems; comparatively low ^{15}N abundance in the surface layer than in the lower layers appears to indicate N limitation and low rates of nitrification, whereas a higher ^{15}N in the surface layer than in the deeper layers appears to indicate high rates of nitrification.

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Tracing pelagic versus benthic food sources to cultivated blue mussel *Mytilud edulis* L. on SW France with stable carbon and nitrogen isotopes

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Mussel cultivation has been started on the Aiguillon Cove (SW coast of France) in the 13th century, and cultures on wooden poles projecting from the substratum between tide marks remain the principal method of cultivation to the present day (Prou & Goulletquer, 2002). However, reduced availability of suitable intertidal areas for mussel culture has led to the development of subsurface long-line culture in the coastal sound located in the vicinity of the Cove. Higher growth performances and yield are excepted due to longer submersion time, lower turbidity and higher renewal of food sources. In that context, seasonal variations of the stable isotopic composition ($\delta^{13}C$ and $\delta^{15}N$) of *Mytilus edulis* reared on long-lines and available food sources to mussels were used to determine the contribution of continental, intertidal and oceanic food to mussel diet during one year.

Terrestrial detritus were depleted in $\delta^{13}C$ (from -30.2 to -26.4 ‰) and mainly advected into the sound by the River Sevre Niortaise. Intertidal benthic diatom inputs via wave and wind-induced resuspension, macroalgae and marine plants had higher $\delta^{13}C$ values ranging between -17 and -13 ‰. Taxonomic determination of phytoplankton samples showed that oceanic inputs were insignificant and that neritic and/or estuarine phytoplankton constitute the main available food sources for mussels. Isotopic values of resuspended organic matter (POM) varied seasonally according to decay and origin of organic matter and phytoplankton bloom. A large seasonal pattern was also observed in isotopic composition of mussels (from -20.3 to -17.3 ‰ for C and from 6.6 to 9.5 ‰ for N). Since isotopic composition of animals reflects the isotopic value of their diets but with an enrichment in heavier isotopes, isotopic fractionation must be considered in the interpretation (i.e. 1 ‰ and 3 ‰ per trophic level for carbon and nitrogen, respectively). Using a two-way mixing model with microphytobenthos and POM as end-members, our results showed that microphytobenthos contribution to the mussel diet was lower in spring (at least 40 % in March) than in summer and autumn (up to 70 % in July) but constitute the major food source utilised by mussels.

This suggested that although studied long-lines were located in the middle of the sound, mussels derive a major part of their diet from resuspended intertidal benthic diatoms coming from adjacent intertidal areas through the River Sevre Niortaise plume. Both satellite SPOT pictures taken at low tide and numerical modelling of coastal currents confirm this result.

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Fish tack wastewater pollution to estuaries

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While discharge of nutrients to estuaries is a waste disposal practice with a history as old as human settlements in the coastal zone, its ecological ramifications continue to remain a significant pollution problem. Whether some of the increases in nitrogen loads caused by the rampant population growth in coastal areas are indeed off-set by sewage treatment processes is debatable; and such debates are politically opportunistic (indeed feasible) because alternative inputs of nitrogen (e.g. fertilisers) can be conveniently argued to override wastewater loads. Thus in catchments with multiple potential sources of nitrogen pollution, it is desirable to develop biological indicators to gauge the potential of wastewater-derived nitrogen to propagate through the receiving food chain. Consequently, we tested whether estuarine fish are suitable as indicators of sewage N-pollution. Fish were analysed from three estuaries on the Australian East Coast that differ markedly in their degree of wastewater loading: 1) the Maroochy Estuary which receives a large fraction of the local shire's treated sewage (via 6 treatment plant outfall points), 2) the Mooloolah Estuary in which no approved discharge of sewage supposedly occurs but some inputs are suspected from marinas/harbours and stormwater, and 3) the Noosa Estuary which neither receives legally approved nor suspected wastewater loads. All three estuaries are located within 100km along the Sunshine Coast (SE-Queensland) and sampling for fish fauna included both high- ('rainy season') and low rainfall ('dry' season) periods. Muscle $\delta^{15}N$ was the variable predicted to respond to sewage loading, exploiting the property of an enriched nitrogen isotope signal in treated fecal material compared with alternative N sources such as fertiliser and natural N inputs (both ^{15}N-depleted). Of 19 fish species occurring in all three estuaries, all have significantly elevated $\delta^{15}N$ values in the Maroochy Estuary compared with the other estuaries. This ^{15}N-enrichment in the Maroochy fish's muscle tissue was substantial, with individual species' values elevated by up to 9.9‰ in the sewage-contaminated estuary (Maroochy) compared with 'control' locations (Noosa Estuary). Furthermore, not only do all fish (irrespective of species) from an estuary in which wastewater loads are known carry a distinctive sewage-N in their tissues, but enriched muscle-$\delta^{15}N$ is also evident for all species from the estuary in which sewage contamination was previously only *suspected* (i.e. fish $\delta^{15}N$ from Mooloolah Estuary are enriched by 0.2-4.8 ‰ compared with conspecifics caught in Noosa Estuary). These spatial contrasts amongst estuaries are consistent across seasons. Thus, fish muscle $\delta^{15}N$ is a suitable indicator of sewage pollution not only in systems that receive relative large volumes of treated wastewater but also for the detection of more subtle inputs. Furthermore, we argue that fish are more meaningful indicators of sewage-N contamination as opposed to the traditional, chemical water variables (e.g. DIN concentration measurements) because they i) integrate nitrogen inputs over longer time periods (c.f. 'spot measures' of water chemistry), ii) have an element of 'ecological relevance' because fish muscle-$\delta^{15}N$ reflect movement of sewage-N through the food chain, and iii) assessments can be built on multiple species evidence. Because sewage is a complex mixture containing more than just N & P (e.g. hormones, various chemicals), a logical next question to be settled is whether such strong patterns in sewage nitrogen uptake by fish transmit to detrimental effects on fish health, which in turn may ultimately impair the structural and functional integrity of estuarine fish populations and assemblages in sewage-affected waters.

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Reconstructing diet among coastal hunter-gatherers: some issues in stable isotope analysis

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Stable isotope analysis is increasingly widely used to investigate diet in fields ranging from archaeology to zoology. Approaches differ in different fields, partly as a result of the kinds of research questions most often asked, but also because of different levels of awareness of methodological issues. For example, linear mixing models continue to find favour with some workers (Phillips & Gregg 2001; Coltrain *et al*. 2004). These models may be useful in some situations, but this paper argues that they are not appropriate for omnivores that eat very varied diets, including humans. In omnivores, the different metabolic pathways involved in the absorption and utilization of different types of foods introduce substantial complexity into the interpretation of isotopic ratios. Over the past few years there have been a number of controlled feeding experiments on laboratory animals, to try to understand this complexity (e.g. Ambrose & Norr 1993; Howland *et al*. 2003). This paper will briefly review these studies, and consider their implications for interpreting stable carbon and nitrogen isotope analyses of coastal people who lived by hunting and gathering. Comparison of $\delta^{13}C$ with $\delta^{15}N$ in collagen, and $\delta^{13}C$ in collagen with that in apatite, allows one to draw limited conclusions about the relative importance of different categories of foods, and their trophic levels. Reliable quantification of the contributions of various foods cannot be achieved at present.

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Site fidelity of tributary caught native and alien fish along the Colorado River in Grand Canyon National Park.

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We used multiple stable isotope analysis to determine if fish caught within six tributaries along the Colorado River in Grand Canyon were resident or not. The main stem Colorado River aquatic community is heavily influenced by Glen Canyon Dam operations for hydro-power and the water quality released from Lake Powell Reservoir. Tributaries however, are relatively pristine in comparison due their remoteness and subsequent limited access for recreation. These tributaries support a dozen species of alien fish and several native fish including the federally listed as endangered humpback chub (Gila cypha).

Collections occurred in June and January 2001. Algae and macroinvertebrates were collected within each tributary and within 1km up stream of the confluence. Fish were caught using backpack electro-shocking methods, with alien fish sacrificed and muscle plugs taken from native fish. These samples were analyzed for $d^{13}C$ and $d^{15}N$ relative abundance. The assumption for this study was that resident fish would be isotopically more aligned with tributary lower trophic levels than with the main stem data

Results indicate that tributary lower trophic levels $d1^{3}C$ and $d^{15}N$ data did vary seasonally and between the main stem. For example, in Kanab Creek invertebrate January $d^{13}C$ and $d^{15}N$ had a mean values of -25.9 ‰ (5.5 sd) and 5.2 ‰ (1.9), respectively. Native speckled dace had $d^{13}C$ and $d^{15}N$ mean values of -22.7 ‰ (0.3) and 6.3 ‰ (0.8), respectively. Main stem invertebrate $d^{13}C$ and $d^{15}N$ mean values were -17.6 ‰ (6.4) and 9.5 ‰ (7.4), respectively. Therefore, it is likely that the dace were winter residents of Kanab Creek. This is provided that you assume a 2-3 ‰ trophic enrichment. These small dace (< 15 cm) are commonly seen in tributaries because of a more stable habitat than the larger fluctuating main stem (Angradi, 1994). However, brown trout caught in Kanab creek had a mean $d^{13}C$ of -27.5 ‰ (1.8) and $d^{15}N$ of 12.4 ‰ (3.19), which indicates that these fish could be feeding in Kanab or the main stem based on the overlapping $d^{13}C$ data. Also, they could have been preying on other fish in the tributary or the main stem because of the depleted $d^{15}N$ values.

These data demonstrate that multiple stable isotope analysis can provide evidence for site fidelity of both alien and native fish along the Colorado River. Understanding which fish taxa are residents or seasonally feed in the tributaries is valuable information for managers with goals to protect native fish.

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Extreme isotopic depletion of ^{15}N in terrestrial algae and lichens implicates diffusive uptake of N.

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The epilithic and epiphytic alga *Trentepohlia* has a $\delta^{15}N$ isotopic signature averaging -21‰ across a wide variety of substrates and habitats. The most extreme depletion of -24‰ was found in algal crusts growing on rocks and tree trunks adjacent to active geothermal fumaroles. Several lichens in the genera *Usnea* and *Ramalina* growing in similar habitats have similar highly depleted ^{15}N values, as do some higher plants growing on extremely N deficient soils. The degree of isotopic depletion is unrelated to substrate type, is not specific to geothermal areas, although is more enhanced there, and is commonly found in species that are in aerial, or otherwise extremely nutrient depleted sites.

The isotopic value of the plants whether epilithic, epiphytic or on other parent material shows no relationship to the isotopic signature of the substrate and there is no evidence that the plants are receiving any nitrogen from those substrates. *Trentepohlia* $\delta^{15}N$ values ranged from -10.08‰ to -18.59‰ on substrates ranging from -2.24‰ to +13.4‰ with no correlation between them.

Atmospheric ammonia levels in the areas sampled range from 12 to 60 µg N L^{-1} in collected rainfall with $\delta^{15}N$ values averaging -0.90‰ to -3.24‰. In order to simulate the absorptive thallus of an alga or lichen, fibreglass strips were impregnated with mild phosphoric acid and left in the field, under protective lids, for three months. The strips absorbed significant ammonia and showed a discrimination of up to -12‰. In addition to this, collection of ammonia diffusing from oxidation ponds, and absorbed from the atmosphere adjacent to high pH ponds, showed that significant isotopic depletion is associated with both the loss of ammonia and its uptake by absorbing sites.

It is hypothesised that those organisms not linked to soil N by roots or are growing on highly depleted sites may obtain significant N from gaseous diffusion with resulting isotopic depletion.

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The effects of altered precipitation patterns on ecosystem responses in a desert rangeland

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Many desert regions are characterized by seasonally dominated rainfall regimes. Global climate change models predict regional changes in average seasonal precipitation, but few models address how changes in the delivery of precipitation within a season will affect ecosystem responses. In the southwestern U.S., summer rainfall is highly unpredictable and punctuated by intervening dry periods of variable lengths. These patterns of precipitation pulses and subsequent dry periods may differentially affect water use and net ecosystem carbon exchange depending on the ability of various ecosystem components (e.g. plants, soil microbes and biological soil crusts) to respond to increases in water availability and maintain function during soil dry-down. The objective of this study was to examine how a 60 mm increase in summer precipitation (46% increase relative to mean summer rainfall), applied in two contrasting treatments affected carbon exchange and water use. Eighteen 4x6 m plots containing both a mesquite shrub (*Prosopis glandulosa*) and scattered black grama grass (*Bouteloua eriopoda*) were assigned to 1 of 3 watering treatments (n=6): ambient precipitation (controls), ambient plus frequent small (5-6 mm) rainfall events applied weekly, and ambient plus infrequent large (20-24 mm) events applied monthly. Treatments were applied for 12 weeks during summer using deuterium-enriched water. Stable isotope ratios of hydrogen (δD) in plant xylem water and carbon ($\delta^{13}C$) in respired CO_2 were used to partition water source use and respiratory CO_2 flux.

The δD of mesquite xylem sap indicated that mesquite was able use water from both small and large precipitation events, while control plants accessed water from deep within the soil profile. Changes in soil moisture, mesquite predawn water potentials and mesquite stem elongation were highest in monthly-watered plots, intermediate in weekly plots and lowest in control plots. Although large events appeared to favor carbon gain of C3 shrubs, biological soil crust communities may have taken advantage of smaller more frequent events. Daytime measurements of net ecosystem carbon exchange on subplots containing soil, plant roots and biological soil crusts indicated control and monthly plots were losing CO_2 to the atmosphere, while weekly plots were acquiring CO_2. After application of 2 mm of water to all plots, net uptake of CO_2 was observed in all plots, but the magnitude of crust photosynthetic response was greatest in weekly-watered plots. Keeling plot analyses of the $\delta^{13}C$ of respired CO_2 during dark hours indicated that the contribution from different sources (roots, microbes and soil crusts) varied with watering treatment. Small frequent rainfall appeared to promote the proportional contribution of microbial respiration or C4 roots to total respiratory flux, while infrequent large rainfall resulted in a greater proportion of CO_2 originating from biological soil crusts or C3 root activity. Control plots, that had received little ambient rainfall, had more negative $\delta^{13}C$ values indicating another potentially deeper C3 dominated source of respired CO_2.

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Modelling stable isotope tracers in the environment

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The delineation of source areas for groundwater recharge is the first step in protecting groundwater resources as a source of water for human consumption and ecological preservation. To accomplish this task, a thorough understanding of water pathways from precipitation to streamflow is required. The rainfall-runoff process can be modelled using hydrological models, in which conservative tracers can be incorporated and used to disaggregate streamflow into its various origins and pathways. The measurement of naturally occurring isotopes in streamflow can then provide a relatively simplistic and inexpensive validation tool by verifying that flow paths and residence times are being correctly modelled. The objective of this paper is to validate groundwater discharge estimation for the Fort Simpson basin by comparing modelled conservative tracers to measured isotopic data.

Detailed field work, daily discharge measurements and an isotopic study were conducted for five wetland-dominated catchments, ranging in size from 202km^2 to 2050km^2 within the lower Liard River Basin near Fort Simpson, Northwest Territories (61º45'N; 121º14'W) from 1997 to 1999. Stable isotopes of oxygen and hydrogen in streamflow are seasonally influenced by the mixing of three isotopically-distinct inputs, primarily snowmelt, surface water and groundwater, allowing streamflow to be separated using the classical two-component mixing model. Major variations in streamflow isotopic composition are therefore controlled by the balance between snowmelt and groundwater during the spring freshet period and by the balance between surface water and groundwater during the late fall and winter. Results from the isotopic partitioning of streamflow have shown that groundwater strongly dominates the annual discharge.

A tracer module has been integrated with the WATFLOOD model; a fully distributed, physically based, meso-scale hydrologic model for watersheds having response times larger than one hour. Conservative tracers are used to track water through the model by quantifying and segregating the various contributions to the total streamflow. Flow separation is accomplished using simplified storage routing of groundwater through the subsurface and into the stream. A specified concentration of tracer is added to the groundwater at its origin and upon reaching the stream, a mass balance is performed to determine the concentration of tracer in the stream, allowing for a separation of groundwater from streamflow.

Once the model is validated in this way, the work will be expanded to include modelling of seasonally sensitive and ecologically important regions, such as the Peace-Athabasca Delta in Northern Canada, which depends on seasonal flooding and groundwater discharge, for climate change research and impact studies.

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Intramolecular distribution of nitrogen isotopes (isotopomers) as a basis for differentiating microbial N_2O production pathways

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The intramolecular distribution of nitrogen isotopes (isotopomers) of N_2O is emerging as a new tool for defining the relative importance of microbial sources of this potent greenhouse gas. In the N_2O molecule, the central nitrogen atom is referred to as alpha (α) and the terminal is referred to as beta (β). The site preference is equal to the difference between $\delta^{15}N^{\alpha}$ and $\delta^{15}N^{\beta}$. Determination of the site preference of N_2O is based on the analysis of the molecular (N_2O^+) and fragment ion (NO^+) formed in the ion source of the mass spectrometer.

Previous field studies suggest that microbial sources of N_2O can be distinguished using isotopomer analysis (Perez *et al*., 2001 and Popp *et al*., 2002). However, the isotopomer composition of N_2O formed by distinct microbial pathways is still undefined. Hence, pure culture studies are necessary to isolate distinct pathways by controlling experimental conditions. Four pathways of N_2O production including methanotroph-nitrification, nitrification, nitrifier-denitrification and denitrification were evaluated in this study. We used concentrated cell suspensions of a nitrifier (*Nitrosomonas europaea*), a methane oxidizer (*Methylococcus capsulatus* Bath) and a denitrifier that lacks N_2O reductase (*Pseudomonas chlororaphis*). The average site preference of N_2O produced by the oxidation of hydroxylamine by *M. capsulatus* Bath and *N. europaea* was significantly different: 32.2 ± 4.1 ‰ and 12.2 ±- 4.0 ‰, respectively. By contrast, N_2O produced by the reduction of nitrite by *N. europaea* and *P. chlororaphis* had a site preference of –3.4 ± 7.8 ‰ and -5.9 ± 5.1 ‰, respectively indicating that nitrifier-denitrification and denitrification cannot be differentiated by using site preference. This study provides the first measured values of site preference for isolated microbial pathways. Although the number of microbes sampled was limited, it nevertheless appears that isotopomer analysis can be used to resolve N_2O derived from methanotroph-nitrification, nitrification and denitrification, suggesting that this technique may provide a basis for apportioning biological processes of N_2O production.

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How does the stage of development of a mouse affect its carbon and nitrogen isotopic composition following a change in diet?

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Stable isotopic analyses of animal tissues are providing information about physiology and diet that is critical for ecological studies of both the past and present. The accuracy and breadth of this information improves with understanding of how isotopes are partitioned when diet is assimilated and this understanding can best be achieved with controlled laboratory experiments (Gannes et al. 1997). We built upon the methods of Tiezen et al. (1983) to address the questions 1) do $\delta^{13}C$ and $\delta^{15}N$ values of unstressed animals change as they mature and 2) how are turnover times of animal tissues affected by the stage of life when a dietary change is imposed? Mouse siblings were given an isotopically homogeneous, diet (control diet) and bred. After the offspring were weaned, some were given the control diet and some were given a **nutritionally equivalent** diet with a different isotopic composition (group 1). Three weeks later, some of the control group of mice were put on the isotopically distinct diet (group 2). The $\delta^{13}C$ and $\delta^{15}N$ values of blood, brains, bone collagen, hair, heart, liver, and gut contents of mice of seven different ages were analyzed. No significant changes in $\delta^{13}C$ values of tissues occurred with age within the group of control mice. Of the carbon analyses, the $\delta^{13}C$ values of hair least accurately reflected those of diet, *per se*, or change of diet. Otherwise, $\delta^{13}C$ values of control mouse tissues were similar to those of diet and approached those of the isotopically distinct diet more quickly in group 1 than in group 2 mice. In contrast, $\delta^{15}N$ values of blood neither changed with mouse age nor with diet. Brain, heart, and liver $\delta^{15}N$ values increased with age, regardless of diet. The $\delta^{15}N$ values of hair did not vary with age, *per se*, but did become significantly different in group 1 mice from the other groups. Collagen $\delta^{15}N$ values of group 1 mice showed more immediate responses to change in diet than did hair and collagen $\delta^{15}N$ values increased in all groups with mouse age. The $\delta^{15}N$ values of all components of all groups, at all ages were higher than that of the control diet but never exceeded that of the second diet in some components.

These results are supportive of assumptions that the carbon isotopic compositions of nutritionally unstressed mammalian tissues reflect those of their diets in accordance with turnover times of those tissues. Even in nutritionally unstressed animals, however, $\delta^{15}N$ values may be more sensitive to developmental changes than to changes in the isotopic compositions of diet in some tissues.

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Experimental test of the effects of omnivory on carbon and nitrogen isotopic labels

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An organism's trophic status can be identified by observing ratios of isotopic forms of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) relative to other components of a food web. Ratios of these isotopes in tissues of plants and animals reflect those in their C and N sources, with a shift toward dominance of the increasingly lighter form of N, but not C, with each trophic level. Omnivores (animals feeding at multiple trophic levels either serially during life or simultaneously) provide a special challenge for interpretation of isotope data. To explore the effect of omnivory on interpretation of isotopes, we raised neonate sailfin mollies (*Poecilia latipinna*) over an 80-day period on diets with three isotopic values (a vegetable diet $\delta^{15}N$ = 2.8, a carnivore diet $\delta^{15}N$ = 8.2, and a mixture of these two $\delta^{15}N$ = 5.4). For some fish, we switched the diets after the first 40-days to test for the persistence of diet shifts on tissue isotopic signatures. We found that diet affected growth rate, with herbivores growing the slowest and carnivores the fastest. The isotopic signatures of fishes sacrificed after 40 days were consistent with those of their diets (vegetable $\delta^{15}N$ = 7.8, mixed $\delta^{15}N$ = 10.1, animal $\delta^{15}N$ = 12.0), though the fractionation varied among the diets. Liver and soma revealed different fractionation, but the difference was consistent across all diets. Forty days after their diet was switched, somatic isotopic values were strongly affected by the second diet, though a residual effect of the initial diet was noted. These results indicate that simultaneous consumption of foods from multiple trophic levels yields tissue isotopic signatures close to a simple mean of the diet components weighted by their relative contribution. However, interpretation of serial diet shifts is more complicated, though residual effects were greatly diminished after 40 days. Consistent with past work, we found little difference between the $\delta^{13}C$ of our fish and their food, though our two diets did not differ enough to track the effects of a diet change on this isotope. We have constructed a model based on our results that illustrates their implications for interpretation of field data.

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Isotopic source partitioning: insight into predation patterns in the Boreal forest of Canada

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Mammalian assemblages and their trophic interactions in the boreal forest are complex and poorly known. Large-scale ecosystem processes, facilitation of natural selection and structuring of communities are affected by ubiquitous trophic interactions like predation. Predators can play a significant role in perpetuating plant and animal diversity. Using $\delta^{13}C$ and $\delta^{15}N$ values measured in hair from 18 mammalian species collected from the boreal forest of Saskatchewan, Canada, we constructed a boreal food web. By expanding our use of the $\delta^{13}C$ and $\delta^{15}N$ values obtained in our trophic investigation we examined predation patterns in the Gray wolf (*Canis lupus*), a predator known to have cascading impacts on entire ecosystems.

We used IsoSource, an isotopic source partitioning model, to quantify the relative proportions of 5 prey items in a 2 isotope system in the diets wolves. The distribution of feasible contributions from each source was dominated by elk (*Cervus elaphus*) (mean: 48%, range:11-75%), followed by white-tailed deer (mean:21%, range:0-54%) (*Odocoileus virginianus*), moose(*Alces alces*) (mean:14%, range:0-41%), beaver (*Castor canadensis*) (mean: 8%, range:0-25%) and snowshoe hare (*Lepus americanus*) (mean: 8%, range:0-24%). We then compared these results to a parallel investigation of gross scat contents where hair identification provided quantification of frequency of occurrence (FO) (33%, 43%, 7%, 5%, and 0% respectively) and percent biomass contribution (50.48%, 33.77%, 14.23%, 1.46% and 0.06% respectively) of prey items in wolf diet. These data generally agree. We also tested FO data for selective predation. White-tailed deer occur in much higher densities than elk and appear more frequently in scats, however, when correcting the p-values to account for prey densities and the number of scats produced by wolves per prey item, we see that elk are preyed upon disproportionately to their abundance (χ^2=141.27, $p<0.0001$). Frequency of occurrence data alone does not give an accurate picture of actual wolf diet whereas our isotope model agrees with % biomass contribution results and tests for selective predation, thus reflecting key patterns of predation in the boreal forest and an unbiased measure of assimilated diet.

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Carbon and hydrogen isotopic signatures of sub-ppb to ppt-level bioavailable waterborne contaminants

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Compound-specific carbon and hydrogen isotope analysis (CSCIA and CSHIA) has been increasingly used to study the source, transport, and bioremediation of organic contaminants such as petroleum hydrocarbons. The isotopic signatures of the contaminants are useful tracers for their sources, while the isotopic fractionation can be used to quantitatively assess the progress of an environmental process such as biodegradation. A wide range of contaminants have been studied, including alkanes in crude oil, BTEX compounds (benzene, toluene, ethylbenzene, *o*-xylene and *p*-xylene) and tert-butyl methyl ether in gasoline, chlorinated solvents trichloroethene and tetrachloroethene, polycyclic aromatic hydrocarbons (PAHs) in soil, and polychlorine biphenyl congeners in animal tissues.

In natural aquatic systems, dissolved or waterborne contaminants represent bioavailable fraction that is of the greatest toxicological significance. However, determining the isotopic signatures of waterborne hydrophobic contaminants in natural waters is very challenging because of their extremely low concentrations (often at sub ppb, or even ppt level). In order to acquire sufficient quantities of PAHs with 1 ppt concentration for CSHIA, more than 10,000 liters of water must be extracted. Conventional liquid-liquid or solid phase extraction is generally not suitable for such large volume extractions.

We have developed a new approach that is capable of efficiently sampling ppt-level waterborne petroleum hydrocarbons for CSIA. We use semipermeable membrane device (SPMD) to accumulate hydrophobic contaminants from polluted waters, and then recover the compounds in the laboratory for CSIA. In this study, we demonstrate, under a variety of experimental conditions (different concentrations, temperatures, and turbulence levels), that SPMD process does not induce C and H isotopic fractionations. The applicability of SPMD-CSIA technology to natural systems is further demonstrated by determining the $\delta^{13}C$ and δD values of contaminants present in Pawtuxet River, Rhode Island. Our results show that the combined SPMD-CSIA is an effective tool to investigate the source and fate of hydrophobic contaminants in the aquatic environments. SPMD-CSIA allows tracking the sources of the dissolved hydrophobic contaminants using both molecular fingerprints and isotopic ratios. Moreover, deploying SPMD in ground water undergoing active or intrinsic bioremediation will allow convenient sampling of low-level contaminants for CSIA and monitoring of biodegradation progress.

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Stable isotope composition of O_2 in the aquatic environment

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Molecular oxygen (O_2) in aquatic systems plays a key role in terms of aquatic community productivity, microbial respiration, biodegradation, metal oxidation, and global oxygen cycling (Dole Effect). In recent years there has been renewed interest in improved analytical techniques and approaches in the application of stable isotopes of O_2 (^{16}O,^{17}O,^{18}O) in environmental and global biogeochemical cycling studies. New continuous-flow IRMS technologies now facilitate research on isotope fractionation and biogeochemical oxygen cycling.

In aquatic systems, biogeochemical processes consume dissolved oxygen (DO). Human impacts like industrial and sewage effluents can stimulate critically low as well as supersaturated O_2 levels. Few studies have employed the isotopic composition of DO as a tracer of the relative importance of heterotrophic and autotrophic state. The isotopic composition of DO will reflect the net effect of re-aeration (+23.5 ‰, SMOW), photosynthesis (0 to –23 ‰), and isotopic fractionation resulting from net community respiration and the predominant respiration pathway. The range in $\delta^{18}O$ values for DO in Canadian rivers and lakes is large, ranging from +6 ‰ to over +45 ‰, reflecting the compromise between re-aeration, photosynthesis and respiration. Diel cycling was found to have small $\delta^{18}O$ isotope effects (2-3 ‰ day / night) in large rivers and lakes, but was dramatic in smaller wetlands. In one wetland near Saskatoon the $\delta^{18}O$ ranged from +12 ‰ (160 % DO daytime) to +37 ‰ (1 % DO nighttime) over a 24-hour period.

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Tracing the Mississippi River floodprint in estuarine consumers in coastal Louisiana

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Due to flood protection, the wetlands adjacent to the Mississippi River in coastal Louisiana have been disconnected from seasonal flooding limiting freshwater, nutrient, and sediment inputs. Recently, as a restoration effort, river diversions have been implemented to re-introduce Mississippi River water into the coastal wetlands. During winter and spring, with discharges up to 200 m^3 sec^{-1}, these diversions deliver large amounts of particulate and dissolved nutrients into the wetlands and can cause widespread flooding. Over a period of two years, we evaluated the impact of the largest diversion at Caernarvon just south of New Orleans on the Breton Sound estuary. Grass shrimp were collected in the upper to mid estuary, and bay anchovies were collected in the mid to lower estuary. Muscle tissue of all specimens was dried, ground to a fine powder, and analyzed for C, N, and S isotopic signatures. Generally, nitrogen isotopes served as a tracer for riverine influence since both particulate organic nitrogen and nitrate in the Mississippi River had significantly elevated values relative to the freshwater end of the estuary. Carbon isotopes helped us to distinguish between productive and unproductive marsh types, while sulfur isotopes were important to follow temporal changes in the salinity gradient throughout the estuary.

For grass shrimp, using C, N, and S isotopes, we could identify four distinct food sources: 1) riverine, 2) marine, 3) productive marsh, and 4) unproductive marsh. The relative importance of riverine influence diminished with distance from the diversion, whereby along the eastern side of the estuary (the major flow path of diversion water) the riverine signal could be traced much further than on the western side. Accordingly, the western side was stronger influenced by unproductive marsh, while a productive marsh signal was more pronounced along the eastern side. As expected, the marine influence increased with distance from the diversion. Seasonally, the riverine influence was especially strong within three months of maximum discharge periods. Bay anchovies, which were collected further down the estuary showed increasing $\delta\ ^{15}N$ values with increasing distance from the diversion. This indicated a significant additional influence of the Mississippi River from the nearby bird foot delta affecting the estuary from the marine end.

This study allowed us to evaluate the temporal and spatial influence of Mississippi River water on consumers in the Breton Sound estuary. According to our results, the influence of the river diversion did not be reach beyond the mid estuary where $\delta\ ^{15}N$ values were minimal. Furthermore, the eastern side of the estuary (as major flow path of riverine water) seemed to be more productive due to increased input of nutrient rich waters.

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Stable oxygen isotope analyses of chironomid head capsules preserved in arctic lake sediments – paleoenvironmental implications

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A paleoenvironmental perspective of temperature change is paramount to understanding the significance of recent warming in the Arctic. Late Quaternary sediments from many arctic lakes provide environmental archives with decadal resolution, but reconstructions are hampered by the relative insensitivity of many traditional proxies to temperature. Here we show that the $\delta^{18}O$ of head capsules of chironomid larvae are equilibrated with the $\delta^{18}O$ of lakewaters in which they live. In suitable lakes, lakewater $\delta^{18}O$ is controlled by the $\delta^{18}O$ of local precipitation, which is strongly correlated to mean annual air temperature (MAT). From this correlation, chironomid $\delta^{18}O$ can be used to examine past changes in MAT. For calibration purposes, chironomid head capsules were manually isolated from the modern sediments of four lakes spanning a wide climate and latitudinal gradient (44˚27'N to 67˚11'N). Modern chironomid $\delta^{18}O$ was highly correlated with both the expected $\delta^{18}O$ of precipitation ($r^2 = 0.96$) and MAT ($r^2 = 0.98$). A biological fractionation factor (BFF) of 1.028, assuming constant fractionation between chironomid head capsules and water, was observed and illustrates that the head capsules were 28.5‰ more positive than the precipitation at each site, which is indistinguishable from that observed between aquatic moss cellulose and lakewater $\delta^{18}O$. We illustrate the potential of this novel paleoclimatic approach with sediment core samples removed and dated from two arctic lake sites (Baffin Island and Greenland), which reveal strong regional paleoclimatic gradients in the early Holocene. In every case, 1 cm slices of sediment were processed, corresponding to between 20 and 100 years of deposition, thus minimizing interannual and within-population variability. Approximately 100μg of chironomid head capsules (representing 300-700 head capsules) from each sample was required for the measurement of $\delta^{18}O$. Duplicate analyses of separate chironomid picks from surface sediments yielded 2σ uncertainties of $\leq \pm 0.17$‰. Measurements of chironomid head capsule $\delta^{18}O$ were made using a Finnigan ThermoQuest TC-EA coupled to a continuous Finnigan MAT Delta Plus XL. Inferred MAT estimates from chironomid head capsule $\delta^{18}O$ were independently assessed by summer water temperature (SWT) reconstructions using chironomid faunal assemblage transfer functions. We also present our initial results applying this approach to sites in Iceland and interior Alaska. For example, preliminary data from measurements of a series of modern lake water samples from a wide geographic area in Iceland show a range of $\delta^{18}O$ values (~ -11.5‰ to -7‰) that are correlated ($r^2 = 0.62$) with MAT and therefore show promise as sites to seek fossil chironomids.

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α-cellulose – lignin linkage in plant material and its implications for dendroclimatological and physiological studies using oxygen isotopes

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α-cellulose is the preferred biochemical component from which a stable oxygen isotopic signature can be extracted for dendroclimatological and ecophysiological studies. Production of pure α-cellulose by the conventional three-step method (delipification with solvents, delignification with CH_3COOH-$NaClO_2$ and hydrolysation with concentrated NaOH) has not been fully verified, despite that the method is widely used. Furthermore, it is commonly assumed that the method causes no oxygen isotopic fractionation. However, phytochemical studies have shown that there are linkages such as ether and ester bonds among the different biochemical fractions (primarily lignin, hemicellulose and á-cellulose) which can lead to isotopic fractionation during isolation due to unavoidable bond cleavage. This study aims to quantify the isotopic fractionation (if any) during the purification of á-cellulose from plant material, and to assess the suitability of the treated cellulose for dendroclimatological and physiological studies.

We first tested the role of CH_3COOH and $NaClO_2$ by bleaching a commercial cellulose standard (Fluka, from spruce) in solutions of contrasting $\delta^{18}O$. We then bleached delipified and dehemicellulosed maize leaf (*Zea mais*, representing C_4 plants) and hoop pine wood (*Araucaria cunningham*, representing C_3 plants) in isotopically contrasted solutions. We compared the $\delta^{18}O$ of the cellulose standard and samples recovered at different bleaching stages and interpreted the material loss and elemental change in terms of the altered chemical composition of the samples. We found that the $\delta^{18}O$ of maize leaf α-cellulose recovered from the enriched bleaching solution ($\delta^{18}O$ = 280.00‰) is 44.59‰, significantly higher than that recovered from unenriched solution (39.66‰) whilst there was no significant $\delta^{18}O$ difference between α-celluloses from hoop pine wood treated in enriched (33.27‰) and unenriched (33.97‰) solutions. We conclude that the "standard" bleaching method can cause significant isotopic effects and distort the $\delta^{18}O$ of α-cellulose. Interpretation of α-cellulose oxygen isotopic compositions should be undertaken carefully and standardisation of this method is necessary for a more sound interpretation of the oxygen isotopic composition for paleoclimatological and ecophysiological studies.

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Section 2:

POSTER PAPERS

Natural abundance of H, C, and O and species distribution and function in Eucalyptus-Nothofagus forests in south-east Australia

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We assessed species distribution and function in *Eucalyptus-Nothofagus* forests in south-east Australia using the natural abundance of stable isotopes of C, H and O as indices of physiological and biosphere-atmosphere processes. Tree ring analysis showed that mean annual growth of *Nothofagus cunninghamii* did not vary between trees growing as an understorey to eucalypts or growing as co-dominants in deeply incised valleys. Growth varied with altitude and was best at higher elevations (>1000m asl) and at the lower limits of the observed range (<500m asl), and was least on steeply sloping and south-facing slopes at intermediate altitudes. Growth and carbon isotope discrimination ($\delta^{13}C$) in late wood were moderately well related but the latter was not related to interpolated annual or summer rainfall. As a co-dominant, *Nothofagus cunninghamii* was significantly more depleted in the heavy isotope ^{13}C than as an understorey and a highly significant altitudinal variation was dominated by the signal obtained from trees on steeply sloping sites. Using the wettest and driest years in the record, in place of all years, we found strong and consistent altitudinal gradients in $\delta^{13}C$ that had a slope of between 4 and 5 $^{0}/_{00}$ km^{-1} of altitude and predicted a $\delta^{13}C$ at sea level of around $31^{0}/_{00}$. When coupled with a lack of any relationship between $\Delta^{13}C$ and $\Delta^{18}O$ of foliage, and strong and consistent patterns in $\Delta^{18}O$ and $\Delta^{2}H$ in rainwater, soilwater, streamwater and plant water, the spatial and temporal patterns in carbon isotopes and growth strongly suggest that water availability plays little role in governing growth of *N. cunninghamii*. Instead throughout much of its current range growth of *N. cunninghamii* is more likely related to the availability of light.

Stable isotopes as tracers of trophic structure and tuna movement in the equatorial Pacific pelagic ecosystem

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The equatorial eastern Pacific Ocean is characterized by an upwelling-induced "cold tongue" of water with high levels of primary production, while the equatorial western Pacific has warmer surface waters ("warm pool") with lower levels of primary production. A larger proportion of the tuna catch in the Pacific originates from the warm pool, despite greater primary production in the cold tongue. We are combining diet and stable isotopic analyses with food web modeling to investigate the relationships between tuna productivity and the cold tongue–warm pool system. The main objectives of the study are: 1) to define the trophic structure of the pelagic ecosystems in the western, central and eastern parts of the tropical Pacific Ocean, 2) to establish an isotope-derived (upwelling-related) biogeography of the equatorial Pacific ecosystems, and 3) to characterize large-scale tuna movements related to upwelling regions along the equator.

To define the trophic structure, stomach content analysis is conducted in conjunction with stable-isotope analysis to assess trophic position of the different functional groups ($\delta^{15}N$) and to trace how different sources of primary production, related to upwelling and other environmental factors, are important in supporting these groups ($\delta^{13}C$). We will use the biodynamic modeling tool, *Ecopath with Ecosim*, to represent the trophic flows between the ecosystem components. To establish an isotope-derived biogeography, an extensive sampling program has been implemented to collect specimens from as many functional groups as possible, from plankton to top predators. Through this isotope cartography, we are characterizing the food webs in the contrasting production regimes of the western, central and eastern Pacific. We are using $\delta^{13}C$ values to identify different sources of primary production, and to distinguish rapid production associated with upwelling. The combination of $\delta^{15}N$ and $\delta^{13}C$ serve to map different regions of primary and secondary production. To characterize large-scale tuna movements, the previously established isotope biogeography will form the basis for identifying natural isotope tags. Isotope ratios will serve as internal chemical tags that are characteristic of areas where they are living. We will attempt to acquire a more refined view of tuna movements by comparing tissues and compounds that have different turnover rates.

Preliminary results are presented for the first year of the three-year study. The study should help define the principal ecosystem linkages underlying tuna production and the effect of climate variability on the systems. This information is important for improving our understanding of fisheries production and ecosystem dynamics of the equatorial Pacific Ocean.

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Growth versus metabolic tissue replacement in mouse tissues determined by stable carbon and nitrogen isotope analysis

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Stable isotope analysis is becoming an extensively used tool in animal ecology. The isotopes most commonly used for analysis in terrestrial systems are those of carbon and nitrogen, due to differential carbon fractionation in C3 and C4 plants, and the approximately 3‰ enrichment in ^{15}N per trophic level. Although isotope signatures in animal tissues presumably reflect the local food web, analysis is often complicated by differential nutrient routing and fractionation by tissues, and by the possibility that large organisms are not in isotopic equilibrium with the foods available in their immediate environment. Additionally, the rate at which organisms incorporate the isotope signature of a food through both growth and metabolic tissue replacement is largely unknown. In this study we have assessed the rate of carbon and nitrogen isotopic turnover in liver, muscle and blood in mice following a diet change. By determining growth rates, we were able to determine the proportion of tissue turnover caused by growth versus that caused by metabolic tissue replacement. Growth was found to account for approximately 10% of observed tissue turnover in sexually mature mice (*Mus musculus*). Blood carbon was found to have the shortest half-life (16.9 days), followed by muscle (24.7 days). Liver carbon turnover was not as well described by the exponential decay equations as other tissues. However, substantial liver carbon turnover was observed by the 28th day after diet change. Surprisingly, these tissues primarily reflect the carbon signature of the protein, rather than the carbohydrate, source in their diet. The nitrogen signature in all tissues was enriched by 3-5‰ over their dietary protein source, depending on tissue type, and the isotopic turnover rates were comparable to those observed in carbon.

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$\delta^{18}O$ of leaf water instantly reflects microclimatic changes

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Photosynthesis is always associated with transpiration. Since ^{16}O is lighter than ^{18}O, water molecules containing the lighter oxygen isotope evaporate more readily than the heavier molecules. Leaf water becomes enriched in ^{18}O relative to the source water in the soil. Since plants respond very sensitively to environmental changes, in particular to the ambient air humidity, these changes are reflected in altered $^{18}O/^{16}O$ isotope ratios of leaf water (Barbour et al, 2000; Craig and Gordon, 1965).

At our CARBOMONT study site, an alpine grassland ecosystem, we investigated five different plant species for their isotopic composition within leaf water. We found that during our measuring campaigns the $\delta^{18}O$ value of leaf water showed a positive correlation to measured diurnal courses of temperature. Air humidity showed a negative correlation with the $\delta^{18}O$ of leaf water. The course of rapid changes in temperature and air humidity were well reflected in the $\delta^{18}O$ of the leaf water

Therefore, we conclude that the stable oxygen isotope ratio in the leaf water of plants is a sensitive indicator for short term climatic variations on the local scale.

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Legacy effects of CO_2 treatments on plant and soil isotopic composition of a Mediterranean type community

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Between 1996 and 2001, field grown plants of two dominant species, *Adenostoma fasciculatum* and *Ceanothus greggii* (perennial woody shrubs), of a Mediterranean type community in northern San Diego, CA were exposed to continuous, but different CO_2 treatments. Plants were grown in enclosed chambers (2×2×2-m, total volume of 8000 liters) where the CO_2 concentration was kept constant at either 250, 350, 450, 550, 650, or 750 ppm. In 2001 (after five full growing seasons of these CO_2 treatments), these experiments were terminated by removing the chambers and exposing the plants to ambient conditions. In March of 2003, during the early parts of the second growing season after terminating the CO_2 treatments, we measured a number of plant and soil_carbon (C) and nitrogen (N) isotope compositions to determine whether there is a legacy effect of CO_2. Surprisingly, we found that almost two years after the termination and exposing the plants to ambient CO_2, leaf $\delta^{13}C$ and $\delta^{15}N$ signatures were significantly and negatively correlated with increased CO_2 concentrations of these plants during the 1996-2001 periods. Furthermore, day and night isotope composition of the respired soil C was inversely correlated with increased CO_2 concentrations of the treatment period. The C isotope signature data from respired soil indicates that a substantial portion of the current C usage for growth and respiration came from storage sources within the plant. Therefore, the current $\delta^{13}C$ signature may be a poor indicator of short-term responses of C use to changes in the environmental factors in general, and CO_2 in particular. The $\delta^{15}N$ data indicate that the rising CO_2 levels, at least in this system, stimulated the plant and soil processes of the N cycle that deplete $\delta^{15}N$ of the foliage. In parallel to the C isotope data, the bulk leaf $\delta^{15}N$ pattern reported here is relatively recalcitrant and remains depleted long (possibly years) after the cessation of the CO_2 treatment. The data presented here offers a new perspective in the interpretation of stable isotope data in ecological studies. Such perspectives may be particularly important in systems where isotope composition of plants have a very low turnover rate.

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Determining trophic niche width - a novel approach using stable isotope analysis

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Although conceptually robust, it has proven difficult to find practical measures of niche width that are simple to obtain, yet provide an adequate descriptor of the ecological position of the population examined. Trophic niche has proven more tractable than other niche dimensions. However, indices used as a proxy for trophic niche width often suffer from the following difficulties. Such indices:

i) rarely lie along a single scale making comparisons between populations or species difficult;
ii) have difficulty in combining dietary prey diversity and evenness in an ecologically meaningful way;
iii) fail to integrate diet over ecological timescales thus usually only comprise single snapshots of niche width.

We propose an alternative novel method for the comparison of trophic niche width: the use of variance of tissue stable isotope ratios, especially those of nitrogen and carbon. This approach is a potentially powerful method of measuring trophic niche width, particularly if combined with conventional approaches, since:

i) it provides a single measure on a continuous axis that is common to all species;
ii) it integrates information on only assimilated prey over time;
iii) the integration period changes with choice of tissue sampled; data production is theoretically fast and testing among populations simple.

Empirical studies are now required to test the benefits of using isotopic variance as a measure of niche width, and in doing so help refine this approach.

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Carbon isotopes discrimination for the estimation of transpiration efficiency of tomato, vegetable soybean and cotton subjected different levels of soil moisture and aeration treatments

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Lack of oxygen in the rhizosphere of crop, which is a common manifestation in heavy soils, compacted soils, saline soils and soil at higher moisture content; limit productivity of many plant species. Application of aerated water (oxygenation) directly to root zone with subsurface drip irrigation significantly improved crop yield and water use efficiency (dry biomass/unit of applied irrigation water) compared to control in a range of crops while maintained at field capacity and at saturation in heavy clay soils. The instantaneous transpiration efficiency (TE) (μmol of CO_2 fixed in carboxylation per mol of water transpired in the leaf) was also significantly improved in all the tested species irrespective of soil moisture content between the field capacity and saturation.

Measuring TE of field grown plant is time-intensive and subject to fluctuate with the changes in environmental parameters. Early studies showed that carbon isotope discrimination (delta) could be used to measure transpiration efficiency of plants indirectly. Study indicted that the overall gain in water use efficiency measure based on dry matter accumulation per unit of water applied for irrigation over season and instantaneous water use efficiency could not be tracked by analysis of carbon isotopes discrimination techniques at least for cotton, vegetable soybean and tomato while irrigated at field capacity and saturated level of soil moisture.

Theoretical and empirical studies have demonstrated that carbon isotopes discrimination is highly correlated with plant water use efficiency. As selection for TE is more relevant for drought prone environments, moisture stress has been the most extensively studied environmental variable. A substantial increase in TE under drought conditions has been demonstrated in tomato, cotton and vegetable soybean compared to soil moisture at field capacity and soil moisture at saturation. So it is concluded that the negative correlation between transpiration efficiency and the carbon discrimination (delta) hold true only under dry soil environment but not in moderate to high soil moisture conditions at least for the same cultivar of the tested species, and is not affected by the aeration treatments while grown under non-limiting soil moisture conditions.

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Identifying potential riparian zones for groundwater NO_3^- retention at plot and catchment scale using plant $\delta^{15}N$ data

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Riparian zones can be used to control diffuse NO_3^- pollution. Their hydric and geomorphic status are the most important characteristics that affect groundwater NO_3^- retention. Identification of efficient riparian areas prior to restoration projects is, however, crucial. Landscape position may influence $\delta^{15}N$ values in plants and the emergence of distinct $\delta^{15}N$ patterns within and among ecosystems illustrates the opportunity for using $\delta^{15}N$ as an integrator of N cycle processes. The aim of this study was, firstly, to investigate the spatial variation of the $\delta^{15}N$ signal in plants at the plot scale in three adjacent riparian sites, showing a clear groundwater NO_3^- retention via denitrification and plant uptake. Secondly, the spatial variation of the $\delta^{15}N$ signature in plants was evaluated as a tool for identifying potential effective riparian zones at the catchment scale.

All riparian zones were located in the Zwalm catchment (Belgium), a basin with a total drainage area of 114 km² and a drainage density of 1.55 km/km². Land use consists mainly of arable crops (40%) and permanent pasture (20%). Only a small part of the catchment is forested (5%). The soil type in the catchment is predominantly silty loam. In the Zwalm catchment three adjacent riparian zones with a different vegetation cover (mixed vegetation, forest and grass) were selected and divided in an uphill, intermediate and downhill zone. For the catchment study all forested and grass riparian zones within a distance of 30 m from a watercourse were selected by an overlay of land use and topographical maps. Twenty riparian sites were randomly selected out of 855 locations and divided into an uphill zone and a downhill zone.

At the plot scale, the average $\delta^{15}N$ value of individual plant species in the mixed vegetation riparian site, that effectively retained groundwater NO_3^-, was significantly higher ($p < 0.05$) in the uphill zone (stinging nettle and great willowherb: 7.6 ± 3.9 and 11.1 ± 3.3‰, respectively) in comparison to the intermediate (stinging nettle and great willowherb: 2.2 ± 1.8 and 4.7 ± 2.8‰, respectively) and the downhill zone (stinging nettle and great willowherb: 2.9 ± 1.2 and 4.5 ± 1.0‰, respectively). For the mixed vegetation and forested riparian zone $\delta^{15}N$ values increased with increasing total N contents. In general, the observed $\delta^{15}N$ – total N trend was consistent with the capacity for groundwater NO_3^- retention. This trend could not be observed in the grass riparian site. Comparing the uphill and downhill zones of twenty randomly selected riparian sites (thus forty zones in total) of the Zwalm catchment, the average $\delta^{15}N$ values were significantly lower ($p < 0.05$) in forested riparian plant samples (0.5 ± 3.3‰, $n = 10$) compared to grass riparian plant samples (4.7 ± 1.6‰, $n = 30$). In general, the $\delta^{15}N$ value decreased with increasing total N contents in the forested zones, while no clear trend was observed in the grass riparian zones. It is suggested that an increase of the $\delta^{15}N$ value in riparian plants with an increasing total N content could be an indicator for groundwater NO_3^- retention in riparian zones.

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Hydraulic Redistribution in the Pacific Northwest: Tweaking the System.

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Hydraulic redistribution (HR) has recently been documented in Pacific Northwest forests, but the controls governing this process and its importance to shallow-rooted species are poorly understood. Our objective in this study was to manipulate the soil-root system to tease apart important components of control over HR. We manipulated the system by irrigation, soil trenching, and tree removal to alter competing water sources and sinks. In the irrigation experiment, we applied 2100 liters of 9000 ‰ deuterated water to a 1 m^2 plot over three weeks, and sampled soil and the surrounding vegetation for 36 days. Deuterated water was immediately taken up by the surrounding dominant 25 yr-old Douglas-fir trees and within 7 days was detected in surface soils in front of those trees, but at least 1 m from the watering site. By the end of the sampling period (36 days) deuterated water was detected in soils behind the target trees, and in Oregon grape, blueberries, and small understory hemlocks as much as 4 m away from the watering source. The amount of HR in the upper soil layers at the watering site was twice that of the control, and the amount of water utilized from the upper soil was also increased. Trenching was hypothesized to decrease HR by separating a section of soil from the trees that had access to ground water; but because a large root in the plot was severed from a nearby dominant Douglas-fir tree, HR and soil moisture actually increased. We assumed that once the large sink of the tree was removed from the root, the soil sink received more water from the root. To confirm this, we measured sapflow on several roots on another tree, and then cut down the tree. In some roots we found a dramatic reversal of flow from up the tree to flow out to the surrounding soil. These manipulations highlighted that the magnitude of HR is governed by a competition between shallow roots in dry soil and the aboveground portion of the tree for water taken up by deep roots. Hydraulically redistributed water can move several meters from the original source where it can be utilized by other vegetation.

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What drives high levels of primary production on the northeastern continental shelf of New Zealand?: stable isotopes expose some myths.

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Dual-labelled ^{13}C and ^{15}N enrichment experiments are a powerful tool for measuring the productivity of a marine ecosystem. By designing simultaneous carbon, nitrate, ammonium and urea uptake experiments it is possible to establish to what extent biological production in a system is driven by inputs of new nutrients (nitrate) versus recycled and regenerated nutrients (ammonium and urea). The degree of regeneration in a system is traditionally represented by the *f* ratio (the proportion of new to regenerated production, where an *f* ratio >0.5 represents a nitrate-driven system with high carbon export). *F* ratios and carbon export values are often derived from nitrate uptake data alone, estimating carbon fluxes using a C:N Redfield conversion ratio of 6.6, or frequently from a ratio of new production to total production. Such *f* ratios overestimate the level of new production (and hence C export) of a system as they do not reflect accurately the proportion of regenerated productivity.

We used ^{13}C and ^{15}N size-fractionated *in situ* incubation experiments and sediment traps to measure total (carbon), new (nitrate-supported) and regenerated (ammonium- and urea-supported) primary production, *f* ratios and carbon export during 4 voyages between early spring (Sept 1996) and late summer (Feb 1997) in the northeastern continental shelf, New Zealand. This area is known to be highly productive with sporadic upwelling events through autumn, winter and spring often introducing deep-water nitrate to the shelf stimulating primary productivity. However, it is not known in detail what drives and controls the levels of primary production or the relative contribution of regenerated nutrients. We expected to observe higher levels of integrated primary productivity in spring than in summer. Contrary to our expectations we measured the highest primary production values in late summer (1.05-1.27 gC $m^{-2}day^{-1}$) with spring values ranging from 0.31–0.85 gC $m^{-2}day^{-1}$. *F* ratios were consistently low (<0.2) for all stations throughout spring-summer indicating that despite the early spring to early summer upwelling of nitrate onto the shelf, primary production in the Gulf, shelf and offshore regions is driven primarily by regenerated nutrients. Low C and N sediment trap export fluxes further support the notion of a regenerated nutrient-driven system.

Using this C and N uptake data set we illustrate the pitfalls in some of the traditional methods of *f* ratio calculations and discuss the implications that this has to marine C flux models. Our data show that C:N ratios are frequently lower than the assumed Redfield ratio of 6.6 and also illustrate that unless direct measurements of regenerated productivity are made, gross errors result in terms of determining what nutrients drive the productivity of an ecosystem and levels of export from the system.

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Genetic variation for carbon isotope composition in clones of *Eucalyptus camaldulensis* hybrids under dryland and irrigated conditions

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Hybridisation of *Eucalyptus camaldulensis* with *E. grandis* and *E. globulus* produces F1 genotypes with wide-ranging rates of growth in dry and irrigated environments. Some hybrid clones reliably outperform both their parents while others within the same family display poor growth. The physiological basis for differences in growth is a potentially useful selection criterion. Physiological markers are particularly important when the target planting environment is drought-prone and water use patterns or efficiency may differ greatly between genotypes.

Measurements of carbon isotope discrimination in leaf tissue were used to assess whether clonal differences in growth rates are linked with water use efficiency in two field trials. One trial is on a dryland site considered marginal for commercial eucalypt establishment and the other is irrigated with good quality water.

Growth from two to three years was measured on 26 *E. camaldulensis x E. grandis* clones, 16 *E. camaldulensis x E. globulus* clones and seedlings of each of the three parent species at the dryland site. Leaves were collected in early summer when trees were about 2½ years old and analysed for carbon isotope composition. Stomatal conductance was measured on seven hybrid clones and the three parent species in early summer the following year. At the irrigated site, growth from 1½ to 2½ years was measured on 14 *E. camaldulensis x E. grandis* clones, four *E. camaldulensis x E. globulus* clones and seedlings of *E. grandis*. Leaves for isotope analysis were collected in early summer when the trees were 2 years old. Clonal differences in growth traits and stomatal conductance were related to leaf carbon isotope composition.

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Oxygen and carbon isotope composition of parasitic plants and their hosts in southwestern Australia

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We measured leaf dry matter $\delta^{18}O$ and $\delta^{13}C$ in parasitic plants and their hosts growing in southwestern Australia. Parasite/host pairs included two mistletoe species, three species of holoparasites, and five species of root hemiparasites. Among these parasite functional types, significant variation was observed in parasite/host isotopic differences for both $\delta^{18}O$ (P<0.0001, n=65) and $\delta^{13}C$ (P<0.0001, n=64). Mistletoes were depleted in both ^{18}O and ^{13}C compared to their hosts; parasite/host differences were -4.0‰ for $\delta^{18}O$ (P<0.0001) and -1.9‰ for $\delta^{13}C$ (P<0.0001). The lower $\delta^{18}O$ in mistletoe leaf dry matter compared to their hosts is consistent with the frequently observed high transpiration rates of these parasites. Root hemiparasites were also depleted in ^{18}O and ^{13}C compared to their hosts, but not to the same extent as mistletoes; parasite/host differences were -1.0‰ for $\delta^{18}O$ (P=0.04) and -1.2‰ for $\delta^{13}C$ (P=0.0006). In contrast to mistletoes and root hemiparasites, holoparasites were enriched in both ^{18}O and ^{13}C compared to their hosts; parasite/host differences were +3.0‰ for $\delta^{18}O$ (P<0.0001) and +1.5‰ for $\delta^{13}C$ (P=0.02). The enrichment in ^{18}O for holoparasite dry matter did not result from more enriched tissue water; holoparasite tissue water $\delta^{18}O$ was less than host leaf water $\delta^{18}O$ by a difference of -3.8‰ when sampled at midday (P=0.0003). Enrichment of holoparasites in ^{13}C compared to their hosts is consistent with a generally observed pattern of enrichment in heterotrophic plant tissues. Results provide insights into the ecology of parasitic plants in southwestern Australia; additionally, they provide a context for the formulation of specific hypotheses aimed at elucidating mechanisms underlying isotopic variations among plants.

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Using δD, $\delta^{13}C$, and $\delta^{15}N$ measurements to link breeding and wintering areas of lesser scaup (*Aythya affinis)* in North America.

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The lesser scaup, a North American diving duck species, winters in the southern United States and northern Mexico and breeds principally in remote boreal forest of western Canada and Alaska. Systematic surveys of key breeding areas indicate that the scaup population has declined by *ca*. 150,000 birds annually during the past 15 years, and declines have been most pronounced in northern regions. Breeding and wintering ground hypotheses have been proposed to explain this trend, but ruling out explanations has been hampered in part because affinities of scaup from specific breeding areas to specific migration and wintering areas are uncertain. Widespread banding of large numbers of scaup in boreal forest is not possible. However, previous studies have demonstrated that stable isotope values in feathers of birds, particularly δD values, can reveal the general geographic location where feathers were grown. Therefore, we used this approach to determine natal origins of juvenile scaup shot at migration and wintering areas in the U.S. We also characterised isotopically feathers collected from flightless scaup at four locations in northwestern North America.

Feather samples were taken from lesser scaup ducklings captured for banding at two northern (Yellowknife, NT; Minto Flats, AK), one inter-montane (Riske Creek, BC) and one southcentral (St. Denis, SK) locations. Strong geographic separation was detected between northern and southern known-source samples using δD values, whereas northern sites were separated further with $\delta^{13}C$ values. Wings of all juvenile (HY) scaup sent to the 2000 U.S. harvest survey were also obtained. Analyses of δD in wing feathers of these HY scaup (n = 450) revealed much spatial overlap in natal origins of birds across the four main flyways (Atlantic, Mississippi, Central, Pacific). However, in 2000, more HY scaup shot in the Mississippi flyway originated from areas farther north than did those shot in other U.S. flyways (MANOVA, $F_{3, 446} = 6.63$, $P < 0.001$); Central flyway birds tended to originate from southern boreal forest and areas of the northern Great Plains. δD values in wing feathers shifted annually in Mississippi scaup, the only flyway for which 1999 samples were analysed. This pattern could arise if more scaup had bred farther north or had higher breeding success there, or if timing of migration and harvest varied annually.

This is the largest study conducted to date on free-ranging game birds, and illustrates the utility of stable isotope analyses to help link natal and wintering areas. Current results suggest that non-breeding season studies and management actions should focus on scaup occupying areas along the Mississippi River and Caribbean Gulf Coast because these birds show comparatively stronger affinity to northern boreal forest breeding grounds, the region where population decline has been most severe.

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Recruitment to mallard populations: identifying natal origin using δD, $\delta^{13}C$ and $\delta^{15}N$ values in feathers

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Reproductive success of mallards (*Anas platyrhynchos*), a common dabbling duck species, apparently is insufficient to maintain local populations in the absence of immigration in many areas of the Canadian prairies. However, population sizes are often stable in these same areas suggesting that populations may be "rescued" demographically by immigrants from other productive regions. This hypothesis has not been tested because of problems in assessing immigrant status of unmarked individuals.

Many immigrant female mallards are probably yearlings because older females show strong fidelity to previous locations and are less likely to emigrate. Thus, using δD, $\delta^{13}C$ and $\delta^{15}N$ values in feathers of yearlings could reliably indicate general natal origin of unmarked birds if isotopic landscapes differ between the origins of recruits and the local population of interest. This technique holds promise because these stable isotopes are permanently incorporated into feathers of ducklings from local food webs and have well-known geographic patterns. The second greater-secondary covert wing feathers grown by ducklings are retained by females until after their first breeding season as yearlings.

Wild female mallards were uniquely marked as ducklings and then recaptured as nesting yearlings over 18 years at St. Denis Wildlife Area, St. Denis, SK, and during 2003 and 2004 at Minnedosa, MB, Canada. This provided feather samples from St. Denis and Minnedosa of known (n = 67 and 15, respectively) and unknown (n = 90 and 61, respectively) origin. Feathers were age-classified and analyzed using CFIRMS to determine δD, $\delta^{15}N$ and $\delta^{13}C$ values.

We found clear separation in δD values for known-origin feather samples among the collection sites but no differences for $\delta^{13}C$ and $\delta^{15}N$ values. Our results for these three isotopes resemble patterns from previous work and suggest that δD and, to a lesser extend, $\delta^{15}N$ values from yearling female wing feathers could be used to delineate natal origin. Comparison of δD and $\delta^{15}N$ feather values for known-origin versus unknown-origin birds indicated that >90% of unknown-origin yearlings had emigrated to these sites from elsewhere (i.e., values for unmarked birds were not contained within the 95% confidence interval for mean feather values of known-origin birds). We plan to link our immigration estimates with mark-recapture and local reproductive data to explain patterns and sources of recruitment in the population of breeding female mallards at Minnedosa, an area where breeding success is generally low but the population is relatively stable.

Relationships between foliar $\delta^{15}N$, %N and %P across a nutrient gradient in a restiad raised bog

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In wetlands and other nutrient deficient environments, the nitrogen isotopic signature of plants has been negatively correlated with nutrient (N and/or P) limitation. We measured plant $\delta^{15}N$, %N, and %P of woody and herbaceous species across a nutrient gradient extending from the margin to 1500 m into the centre of Kopuatai restiad (dominated by Restionaceae) bog, northern New Zealand. The higher nutrient margin is characterised by tall *Leptospermum scoparium* shrubs that rapidly decrease in height and abundance to become a minor component in the nutrient-poor bog centre, dominated by the restiads *Empodisma minus* and *Sporadanthus ferrugineus*. Our aim was to determine whether plant $\delta^{15}N$ became more depleted as N and P levels in plants and in peat decreased across the gradient. *Leptospermum scoparium* $\delta^{15}N$ became rapidly depleted along the transect, averaging 3.71‰ at the very margin and –14.00‰ in the bog centre (500-1500m). In contrast, the two restiads (*E. minus*, *S. ferrugineus*) showed no isotopic gradients and further, these two species were isotopically significantly separated with $\delta^{15}N$ means of –0.81‰ and –4.97‰ respectively. *Leptospermum scoparium* and *E. minus* co-occurred in all plots and site-by-site in the bog centre, their $\delta^{15}N$ differed by an average of 13.2‰.

Foliar $\delta^{15}N$ of *L. scoparium* was strongly positively correlated with P levels in foliage and peat, and negatively correlated with plant N:P ratios. In contrast, no significant relationships were found between foliar $\delta^{15}N$ and foliage %N, or peat total N. For *Empodisma minus* and *Sporadanthus ferrugineus*, there were no significant correlations between plant $\delta^{15}N$ and peat or plant nutrients.

Plants with ectomycorrhizal (ECM) associations have been shown to have more depleted $\delta^{15}N$ than nonmycorrhizal (NM) plants. *Leptospermum scoparium* can have ECMs and we observed abundant ECMs in roots of *L. scoparium* at the bog margin. However, ECMs were not detected at 300 m into the bog or in the bog centre. The depleted $\delta^{15}N$ values, low levels of P, and high N:P ratios (>16) in *L. scoparium* plants within the bog suggest they could be P-limited, which may be partly attributed to the absence or marked reduction of ECMs. Restiad species are normally NM, having cluster roots (a dense network of fine roots and root hairs) that enhance nutrient acquisition (especially P) in nutrient-poor environments. Therefore, they were probably less limited by P. Restiad species also had lower concentrations of nutrients than *L. scoparium*, suggesting restiads are better adapted to the low nutrient raised bog conditions.

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The $\delta^{15}N$ and $\delta^{18}O$ values of N_2O emitted from a New Zealand pasture soil amended with lactose-depleted dairy factory effluent and urea – preliminary results

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Lactose extraction at New Zealand dairy factories has resulted in dairy factory effluent that is lactose (carbon)- depleted (LD-DFE). More information is currently required on the fate of nitrogen in LD-DFE irrigated on land with respect to decisions in environmental management and future resource consent hearings. This specific study reports on the preliminary data of the influence of LD-DFE on the $\delta^{18}O$ and $\delta^{15}N$ values of emitted N_2O from a New Zealand pasture soil. This data was collected in December 2003 from the dairy factory effluent lysimeter experiment at Lincoln University (Lincoln, New Zealand). The study is part of a larger project examining the fate of N in LD-DFE in a "grazed" pasture system (i.e. with urine inputs), through measurements of system losses through denitrification, leaching, plant uptake and immobilisation in the soil. Headspace gas samples were collected using a closed chamber technique. Samples were then analysed to determine the $\delta^{18}O$ and $\delta^{15}N$ value of the soil emitted N_2O. The initial results indicated that the isotopic signatures were comparable to that of other pasture soils (Bol et al. 2003). The isotopic content of emitted N_2O varied between field samples taken during the first five days after the LD-DFE additions and those collected later (after 14 days), suggesting a shift in the production pathway or source of the emitted N_2O.

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Searching for the earliest evidence for milking in Europe

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The precise nature and timing of the emergence of dairying in prehistory has been hotly debated by archaeologists over the past few decades. For example, one hypothesis suggests that following domestication, ruminant animals were at first only raised for their carcass products (i.e. meat/fat), and then approximately one millennia later their 'secondary products' (e.g. milk, wool, traction) were then also utilised by the early farmers (Sherratt, 1983). This was suggested by a number of 'indirect' lines of evidence such as animal bone profiles at archaeological sites, however, it was only recently that a 'direct' method of detection of dairying has been established.

Pottery vessels are porous by nature, so that in prehistory, lipids may be absorbed into the walls of the pottery vessels during use. These lipids are readily extracted with organic solvent, and quantified and characterised through the use of a suite of techniques including gas chromatography (GC), GC/mass spectrometry and GC-combustion-isotope ratio mass spectrometry (GC-C-IRMS). Crucially, it is the compound-specific $\delta^{13}C$ values of the $C_{16:0}$ and $C_{18:0}$ fatty acids, obtained *via* GC-C-IRMS, that the extracts may be classified to predominant commodity group. This is achieved through differences in the $\delta^{13}C_{16:0}$ and $\delta^{13}C_{18:0}$ values of ruminant adipose fats, ruminant dairy fats and non-ruminant adipose fats which reflect their specific biosynthetic origins. For example, ruminant dairy fats have lower $\delta^{13}C_{18:0}$ values and $\Delta^{13}C$ ($= \delta^{13}C_{18:0} - \delta^{13}C_{16:0}$) values than ruminant adipose fats, thus distinguishing them from other fat-types.

Using these techniques it has previously been possible to detect dairy fats in a large number of pottery vessels from British prehistory (*c.* 4500 – 500BC), therefore suggesting that by the time farming arrived in Britain, dairying was already an important component of farming practices (Copley *et al.*, 2003). We are now extending our analyses geographically and temporally to cover the Neolithic in South-eastern Europe/Asia. To this end, pottery from numerous key archaeological sites from the region have been selected for organic residue analysis. Although the number of sherds yielding significant lipid concentrations is lower in these older sherds, compared to Britain, we provide direct evidence for the processing of dairy products from as early as the 6th Millennia BC in the region.

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^{13}C-labelling of phospholipid fatty acid and hopanoid biomarkers in the determination of methane oxidising bacterial communities in soils

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An important terrestrial sink of methane is its oxidation by bacteria residing in soils, either by oxidising high concentrations of methane produced in the anaerobic environments the soils cover, or removing ambient levels of methane directly from the atmosphere. The bacteria responsible for ambient methane oxidation, termed high affinity methanotrophs, are not amenable to currently available methods of culturing, resulting in the need for a method of in situ analysis. A combination of phospholipid fatty acid (PLFA) analysis and stable isotopic labelling has been employed in this investigation as a means of cultivation-independent bacterial analysis. PLFAs have been used in the chemotaxonomic identification of bacteria with specific PLFAs being indicative of particular classes of bacteria. PLFAs produced by organisms metabolising $^{13}CH_4$ can be used in the identification and comparison of methane oxidising bacterial communities (Bull et al., 2000). Soil samples were incubated in closed chambers with ^{13}C labelled methane in order to label the PLFAs. These compounds were analysed by gas chromatography/combustion/isotope ratio mass spectrometry to determine the quantity of additional ^{13}C incorporated into the individual PLFAs and hence give an isotopically labelled PLFA fingerprint for the methane oxidising bacteria. The development of these techniques provides opportunities for improving our understanding of the factors that influence populations of methane oxidising bacteria.

In addition to PLFAs another important group of bacterial lipids are the hopanoids. The hopanoid content of various pure cultures of methanotrophic bacteria has previously been investigated and results indicate that in general type II methanotrophs produce mainly pentafunctionalised and some tetrafunctionalised bacteriohopanoids, whereas type I methanotrophs produce mainly hexafunctionalised and some pentafunctionalised bacteriohopanoids. Type X also generate almost exclusively hexafunctionalised bacteriohopanoids. The lack of significantly large amounts of these hexafunctionalised triterpenoids in the majority of common bacterial species can result in their use as an indicator of the presence of type I or type X methanotrophs in environmental samples. Hopanoids were extracted from soil samples following incubation with $^{13}CH_4$ and analysed by GC/C/IRMS. The relative abundance of labelled hopanoids was calculated from the δ^{13}C values of the individual hopanoids (Crossman et al., 2001). The results were in agreement with those obtained by PLFA analysis.

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Feeding ecology of wolverines in northwestern Alaska

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Seasonal and annual changes in food abundance are important ecological factors, requiring animals to develop various strategies for coping with temporal variability. Mammalian carnivores often adopt generalist foraging strategies and change their dietary preferences relative to prey availability. Knowledge of animals' ability to respond to seasonal fluctuations in food has important implications for predicting demographic responses of animal populations to changes in food abundance. We studied feeding ecology of wolverines (*Gulo gulo*) in relation to seasonal and annual abundance of caribou (*Rangifer tarandus*) in northwestern Alaska. Little is known about feeding ecology and prey selection of wolverines. The little quantitative work that has been done has exclusively focused on winter diets, and relied on analyses of stomach content or collected feces. Isotope analysis provides a yet untried alternative to assess seasonal feeding ecology of wolverines. We combined analyses of stomach and colon content with analyses of stable carbon and nitrogen isotope ratios in wolverine skeletal muscle and femur collagen. The study was part of a research project conducted from 1996 through 2002, aimed at providing ecological information to be used for management of this and similar species in northern Alaska national parklands. The study area lies within the migratory range of the Western Arctic Caribou Herd (WACH), which consists of approximately 400 000 animals. We predicted that the diet of wolverines was strongly influenced by the seasonal presence of caribou. Visual identification of stomach and colon content from wolverines harvested during December through April confirmed this for diets during winter and spring, with caribou comprising 75% of stomach contents and moose comprising 24%. Estimates of $\delta^{13}C$ and $\delta^{15}N$ signatures for summer and winter diets, estimated from wolverine skeletal muscle and recalculated femur collagen, indicated a prey shift during summer, with summer diets depleted in ^{13}C and enriched in ^{15}N compared to winter diets. The observed difference does not appear to be a shift toward any prey with a marine isotopic signature, such as salmon or migratory geese and waterfowl. Instead, wolverines primarily seem to utilize prey with a terrestrial isotopic signature (e.g. moose, microtine rodents, and arctic ground squirrels) when availability of caribou is low. The profound seasonal abundance of caribou is apparently not great enough to carry wolverines through the summer and fluctuations in abundance of prey other than caribou may have important implications for wolverine demographics.

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Trophic dynamics in an Australian arid woodland: Effects of patchiness on vegetation and arthropods.

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In semi-arid and arid ecosystems, vegetation patches are critical to the capture and retention of water, nutrients and carbon. Runoff of water is greater from interpatch areas with nutrients transferred to the richer perennial patches. In Australia, understanding the functioning and maintenance of these patches is critical to ecosystem maintenance and restoration in the face of extensive pastoral management. In the present study we used carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotope ratios to examine landscape functioning and trophic relationships in a banded mulga *Acacia aneura* system in central Australia.

We compared $\delta^{13}C$ and $\delta^{15}N$ values of soils, vegetation and invertebrates (termites, ants, spiders) and their diets, collected from each of three mulga groves and three intergroves on each of two soil types. $\delta^{13}C$ and $\delta^{15}N$ values of *A. aneura* trees in groves indicated that they have a lower water use efficiency and greater reliance on recently fixed nitrogen than trees in intergroves. In contrast, grasses did not show this difference. $\delta^{13}C$ and $\delta^{15}N$ vegetation values were affected by landscape position; consistent with existing previously described sink-source relationships in the groves and intergroves of banded mulga. For termites, the similarity in $\delta^{15}N$ values of *Drepanotermes perniger* and *Tumulitermes tumuli* and their likely food sources suggests these termites are fixing nitrogen. C4 grasses dominated the diets of *D. rubriceps* and *Tumulitermes* sp. 'd', whereas *D. perniger* and *Amitermes vitiosus* had more polyphagous diets. The diets of *Schedorhinotermes actuosus*, *T. tumuli* and the ant *Polyrhachis macropa* were dominated by C3 plants.

Overall the observed variations in stable isotope signatures support existing knowledge about the functioning of banded mulga systems. We suggest stable isotope techniques have the potential to be used in other similar systems to investigate patch-interpatch functioning and that they can also be used to deepen our understanding of trophic relationships and the functional roles of invertebrates.

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Multiple stable isotopic analyses (C, N, O, H) of a high arctic kelp community: A trophic and temporal perspective

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The Boulder Patch, located in the coastal Beaufort Sea, is a high arctic marine ecosystem dominated by the kelp *Laminaria solidungula.* This isolated community is ecologically distinct from the surrounding area, which typically has a sandy or muddy substrate. Stable isotope ratios of organisms from this ecosystem are allowing the structure of the food web to be resolved. To date over 200 samples have been analyzed for $\delta^{13}C$ and $\delta^{15}N$. Isotopic signatures for the primary producers and a variety of invertebrates have been obtained. Distinct carbon and nitrogen signatures have been shown for items at the base of the food web, these being kelp ($\delta^{13}C$ = -20.01‰ ± 2.25, $\delta^{15}N$= 5.82‰ ± 1.37) and POM ($\delta^{13}C$= -25.1‰ ± 0.39, $\delta^{15}N$= 9.95 ‰ ±0.62). Due to the number of species encountered red algae signatures are more variable. The organic $\delta^{18}O$ and δD data from samples are also being used to exam the scope of variation in this high arctic ecosystem, and the degree to which components are related to each other. $\delta^{18}O$ and δD values show distinct differences for the possible water sources (salt water $\delta^{18}O$ = -3.39‰ ± 1.61, δD= -26.52‰ ± 12.14 fresh water from a local river mouth – Sag River $\delta^{18}O$ = -20.09‰ ± 0.70, δD= -153.29‰ ± 0.92). Archived samples collected from Boulder Patch between 1979 and 1984 allow temporal changes in the isotopic composition of the kelp community to be examined. Historical data are aiding determination of long-term food web changes in this high arctic marine ecosystem.

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A multi-organismal isotopic study of north Pacific and Bering Sea marine mammals: responses to a changing environment

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Whales may be shifting their current over-wintering habitat due to decreased productivity and/or sea ice extent in the Bering Sea. Simultaneously, the unexplained rapid decline of pinniped populations in the Bering Sea and the Gulf of Alaska has become a recent topic of both concern and debate in the United States. In spite of apparent strong correlations between the rapid rise of fisheries in these regions and commercial pressure on herring and pollock stocks, no definitive links or cause and effect relationships have been established; a shift in overall productivity in the Bering Sea could play a major role in both of these species' survival. A multi-organismal approach is being applied in order to determine the impact of shifts in nutritional limitation, where biogeochemical data is being used to reconstruct the biology of Steller sea lions (SSL-*Eumetopias jubatus*) and bowhead whales (*Balaena mysticetus)*. Previous examinations of the stable isotope ratios of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) along baleen plates from bowheads illustrate their annual migration, as well as their related nutritional ecology. Given the more negative oxygen ($\delta^{18}O$) and hydrogen (δD) values for freshwater sources (e.g. Mackenzie River, $\delta^{18}O \approx$-19 ‰, $\delta D \approx$ -142 ‰ based on GMWL) versus the fairly constant $\delta^{18}O$ and δD values for ocean water (SMOW ≈0 ‰ for both isotopes), it is hypothesized that oxygen and hydrogen isotope analyses may further enhance studies of migratory behavior. To investigate this hypothesis, baleen samples from archived Alaskan *B. mysticetus* were taken at 2 - 5cm intervals and analyzed for their stable oxygen and hydrogen isotope composition. Both the $\delta^{18}O$ and δD values were found to vary greatly along the length of the baleen ($\delta^{18}O$ = 8 to 18 ‰; δD = - 180 to -80) and appear to correspond to the whales' annual migration, with signatures likely indicative of their seasonally resident area. Lower $\delta^{18}O$ values ($\delta^{18}O$ < 12 ‰) and δD (δD < -122‰) in the baleen are likely the result of whales assimilating material during summer feeding near points of freshwater discharge in the region (e.g. Mackenzie River). Oxygen and hydrogen isotope analyses of organic samples are a novel and promising technique for ecological studies of baleen whales and may provide accurate resolution for tracking shifts in long-term migration patterns. Additionally, we have begun to determine the stable carbon and nitrogen isotopic ($\delta^{13}C$ and $\delta^{15}N$) composition of SSL populations to elucidate shifts in diet, diversity in trophic inputs, as well as any regional differences that correlate with their population decline. Thus far, we have found that carbon and nitrogen isotopes in SSL populations vary greatly according to the location and year of the animal ($\delta^{15}N$ = 14 to 22 ‰; $\delta^{13}C$ = - 17 to -11 ‰). These results will supply the necessary information from which the role of nutritional limitation may be evaluated, while providing unique historical information about the long-term intra-specific interactions that may be used to help direct the recovery of the SSL populations throughout the north Pacific and Bering Sea.

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Enhancing the capabilities of IRMS: overcoming the CO_2 "mind set"

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Sample preparation and introduction for isotope ratio mass spectrometry have been undergoing significant changes and enhancements. The capabilities of IRMS have been extended from just being able to measure the isotope ratios of CNOSH (Si, Cl, Br) to:

a. **Molecular ratios** (*e.g.* $N_2/O_2/CO_2$)
b. Quantitation of electron impact fragmentation patterns (*e.g.* $NO^+/N2O^+$, SO^+/SO_2^+) allows measurement of **isotopomer ratios** (*e.g.* $^{15}N^{14}NO/^{14}N^{15}NO$) and correction for interfering isobaric species (*e.g.* ^{18}O in SO_2, ^{17}O in CO_2)
c. **Species concentrations** (*e.g.* [CO_2] in air)
d. **Elemental concentrations and ratios** (wt% C, N, C/N; wt% O, H, O/H)
e. **Isotopologue ratios** (*e.g.* $^{13}C^{18}O^{16}O/CO_2$)
g. **Non-mass-dependent-fractionation** in O isotopes (^{17}O) and S (^{33}S and ^{36}S)

The developments that have allowed these enhancements to the measuring capabilities of IRMS include:

1. **incorporation of capillary GC technology into the inlet system** allowed the development of "continuous flow" applications, including compound specific isotope analysis (GC-IRMS), bulk stable isotope analysis (EA-IRMS), gas handling (air, dissolved gases, and headspace and membrane inlets), and laser ablation (combustion/decarbonation) inlets. The largest implications for ecological studies come from the new capabilities for analysis of $^2H/^1H$ and $^{18}O/^{16}O$ in both bulk (high T quantitative carbon reduction-EA) and compound specific analysis (GC-pyrolysis).
2. Significant **extension of the IRMS focal plane**, allowing simultaneous collection of ion beams for which peak jumping was previously required (*e.g.* 28-32-44)
3. Enhancements to the IRMS electronics to allow **static multicollection** of up to nine ion beams. These capabilities are significantly enhanced by computer-controlled high ohmic resistor switches and extension of the upper signal size to 50V, which together significantly **enhances the dynamic range of accessible signal size**.
4. Major developments in instrument control and data handling software, which allow the **formation of any set of ratios from the raw data**, the use of new ion corrections, and significant improvements to background stripping algorithms.

Significant new measurement capabilities include

a. Sequential measurement of CO_2 and N_2 without magnet jump
b. Static/sequential measurement of CO and CO_2 without magnet jump
c. Static/sequential measurement of N_2 , NO and N_2O without magnet jump
d. Static measurement of SO and SO_2 without magnet jump
e. Sequential measurement of N_2, O_2, Ar, CO_2 without magnet jump
f. Sequential measurement of H_2 and CO without magnet jump
g. Static measurement of Ne and Xe without magnet jump

These capabilities have arisen as a result of rethinking the strictures inherited from the two collector instruments used in the 1950's for measuring $^{18}O/^{16}O$ of carbonates.

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Basin-scale responses to major episodic sediment resuspension events in Lake Michigan

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In southern Lake Michigan, intense winter and early spring storms massively resuspend materials with characteristics of sediment from depositional regions. Estimates show that individual large events can resuspend over one million MT of particulate matter, more than the annual external input. This material is transported both alongshore and offshore into the center of the lake. Sediment traps were used to record the offshore passage of this material. Several years of trap collection at offshore sites show a range in mass and nutrient fluxes that span a factor of ten. Carbon isotope analysis of trapped material indicates a correlation between the size of the annual event and primary productivity, hypothesized to be due to the recycling of the large inventory of sediment-associated phosphorus. The implication is that large events are crucial in the cycling of particle-associated constituents and basin-scale ecology.

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Variations in enamel $\delta^{13}C$ and $\delta^{18}O$ values of modern Australian marsupial wombat incisors: evidence for seasonal changes in C_3 and C_4 plants. Palaeoecological implications.

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The continuously growing teeth of grazing wombats present a unique opportunity for examining seasonal isotopic variations in $\delta^{13}C$, which records the dietary preference of C_3 and C_4 plants of the animal, and $\delta^{18}O$, possibly a proxy for rainfall. A series of enamel micro-samples were measured along the longitudinal growth axis of the lower incisors of modern wombats from temperate, alpine, subtropical and semiarid regions within eastern Australia. Ultimately, these modern baseline data are required for the interpretation of isotopic values found in Australian fossil faunas.

The lower incisors (average length of 70 millimetres) were longitudinally cut in half and the enamel cleaned of adhering dentine with a diamond drill. Sequentially, one millimetre subsections were cut, and each ground to a fine powder. Isotopic measurements were undertaken on a Finnigan MAT 251 coupled to a Kiel Device, where samples reacted for 12 minutes in 105% phosphoric acid at 90° C.

The range of $\delta^{13}C$ values within a tooth series varied between habitats. The largest intra-tooth variation was 7.98‰ in *Vombatus* from temperate New South Wales. The mean $\delta^{13}C$ value of -13.04‰ indicates a diet of C_3 grasses, but the changes in the isotopic values indicate input of C_4 grasses at certain stages of tooth growth. Wombats inhabiting semiarid regions in South Australia and Queensland had average intra-tooth variations of only 1.50‰. Their mean $\delta^{13}C$ values were -13.66‰ and -1.54‰, respectively, also reflecting their different C_3 and C_4 grass diets. However, the small variability in $\delta^{13}C$ values at these sites shows that the dietary preference, and most probably plant distribution, was relatively constant during the year. The $\delta^{18}O$ values in all animals did not show consistent patterns. The most common trend was the enrichment of $\delta^{18}O$ in animals from inland semiarid sites compared to the cooler temperate areas.

These carbonate microsamples represent a time series of enamel growth over approximately 1.5 years. Isotopic changes along the teeth are likely to reflect seasonal changes in C_3/C_4 plant distributions within different habitats. As this distribution is predominantly determined by temperature and season of rainfall, the isotopic series provided by wombat teeth may become a valuable indicator for climatic and plant seasonality at that location.

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Precipitation pulse use by an invasive woody legume: the role of soil texture and pulse size

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The dynamics of plant metabolic activity in arid and semiarid regions is regulated by episodic precipitation events or "pulses". In these water-limited ecosystems the ability of plants to take up and utilize rain pulses during their growing season is in part determined by pulse timing, intensity and size and by hydrological properties of the soil.

We assessed the sensitivity of an invasive woody plant (velvet mesquite, *Prosopis velutina* Woot.) to large (35 mm) and small (10 mm) irrigation pulses on two contrasting soil textures (sandy loam *versus* loamy clay). Pre-dawn leaf water potential (Ψ_{pd}), the isotopic abundance of deuterium in stem water (δD) the abundance of ^{13}C in soluble leaf sugar ($\delta^{13}C$) and percent volumetric soil water content (θ_v) were measured prior to irrigation and periodically for two weeks following irrigation. Mesquite water potential and the percent of pulse water present in the stem xylem indicated that mesquite trees on both soils responded equally to the large irrigation pulse, reaching a maximum of pulse water use four days after irrigation. Conversely, only mesquite trees on sandy loam soils showed a significant response to the 10-mm pulse. Regression lines between predawn water potential and $\delta^{13}C$ revealed that differences between the two sites in photosynthetic response of mesquite trees to irrigation pulses was a function of soil water availability in the rooting zone rather than biochemical or physiological constraints of the canopy. These results suggest that the response of woody species to pulses of growing season precipitation is not homogeneous at the landscape level, but varies across different soil textures. A better understanding of how the interaction between soil and precipitation pulses affects plant water availability and photosynthetic response is needed to realistically predict how plant community structure and ecosystem function will respond to climate change at the landscape level.

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Trophic variability in sharks off Baja California Sur, Mexico using $\delta^{13}C$ and $\delta^{15}N$ stable isotopes

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Sharks are top predators in marine ecosystems, and are vulnerable to overfishing due to slow growth, delayed ages at maturity, low fecundity, and long gestation periods. These considerations underscore the urgency to understand the trophic role of exploited sharks in coastal and pelagic ecosystems of Mexico.

Stomach contents and stable isotope ratios of four shark species, scalloped hammerhead (*Sphyrna lewini*), silky (*Carcharhinus falciformis*), angel (*Squatina californica*), and blue (*Prionace glauca*), caught in the lower Gulf of California and southwestern coast of Baja California Sur, Mexico were analyzed to study the influence of sex, size, and migration on their trophic ecology. Their stomach contents were analyzed at CICIMAR in La Paz, Mexico and the stable carbon and nitrogen isotopes at the University of California at Davis.

Scalloped hammerhead juveniles preyed mainly on demersal coastal fishes (muraenids, synodontids) and some epipelagic squids (*Dosidicus gigas*); whereas adult scalloped hammerheads fed mainly on oceanic cephalopods (e.g. *D. gigas*, *Ancistrocheirus lesseuri*, *Stenoteuthis banksii*). The silky and blue sharks of both sexes and all sizes preyed mainly on oceanic squids and red crabs (*Pleuroncodes planipes*); and the angel sharks consumed benthic fishes (e.g. Batrachoididae, Synodontidae).

The isotopes ratios and average of the sharks analyzed were: scalloped hammerhead ($\delta^{13}C$ = -15.5 to -17.5‰ X= -16.4‰; $\delta^{15}N$ = 19 to 21.8‰ X=20.9); silky ($\delta^{13}C$ = -17.1 to -18.3‰ X=-17.8‰; $\delta^{15}N$ =15 to 17.6‰ X= 16.2‰); angel ($\delta^{13}C$ = -15.1 to -16.5 ‰ X= -15.9 ‰; $\delta^{15}N$ = 18.3 to 19.5‰ X= 18.9‰), and blue ($\delta^{13}C$ =-17.2 to -19.3 ‰ X= -18.5‰; $\delta^{15}N$ = 15.2 to 18.8 ‰ X= 16.5).

The scalloped hammerhead juveniles of both sexes were found only during the winter in the lower Gulf of California, whereas the adults are present in the summer and fall. Our $\delta^{13}C$ and $\delta^{15}N$ values indicated that the juvenile hammerhead sharks utilized prey of coastal origin, and the adults consumed prey of oceanic origin. Silky sharks inhabit coastal areas off Baja California Sur only during the summer, migrating from the waters off southwest Mexico. The isotopes values of the silky sharks indicated oceanic feeding habits during their migration. Angel sharks stay in shallow coastal areas off the lower Gulf of California during the winter; whereas they change their habitat to deep coastal areas during the spring to fall. The isotopes values of angel sharks indicated that they consume prey of benthic origin. Blue sharks are caught off Baja California Sur all year long, and the stable isotope data indicate that they utilize prey of oceanic origin year round.

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Stable isotope analysis of yellowfin tuna, spotted, and spinner dolphins in polyspecific aggregations in the eastern tropical Pacific Ocean

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Aggregations of yellowfin tuna (*Thunnus albacares*), spotted dolphins (*Stenella attenuata*), and spinner dolphins (*Stenella longirostris*) are common in the eastern tropical Pacific Ocean. Despite considerable interest, the bond between yellowfin tuna and the dolphins is not fully understood. The hypothesis that the association is food-based has been explored through stomach-contents analysis, which is limited to describe only short-term feeding behavior. Stable isotope ratios, $\delta^{13}C$ and $\delta^{15}N$, represent ecological tracers to evaluate nutrient flow in ecosystems and to study long-term diet and trophic relationships. In our study, we combine diet analysis and stable isotope analysis of muscle to obtain more information on the trophic relationships among tunas and dolphins caught in polyspecific aggregations during purse-seine fishing operations.

Although we found some variations in stable isotope ratios, food habits and isotope data yielded consistent results. According to the index of relative importance, there were significant differences in the taxonomic composition of the stomach contents among the three species (ANOSIM, Permutation test: $R=0.42$, $p<0.01$). However, the R values indicated that yellowfin tuna and spotted dolphins shared a more similar diet than yellowfin and spinner dolphins. The yellowfin tuna fed mainly on *Auxis* spp. and epipelagic cephalopods, the spotted dolphins on mesopelagic myctophid fishes and epipelagic cephalopods, and the spinner dolphins preyed mainly on mesopelagic fishes. Isotopic signatures in the muscle samples of these three species showed significant differences in $\delta^{15}N$ (ANOVA: d.f.=2, $H=20.71$, $p<0.05$), and therefore a clear separation in trophic level. Spinner dolphins had the lowest $\delta^{15}N$ (mean±1 SD 13.33±0.57) and yellowfin the highest $\delta^{15}N$ (mean±1 SD 14.33±0.59), whereas we found intermediate values (mean±1 SD; 13.86±0.68) for spotted dolphins. $\delta^{13}C$ values were also different among the three species (ANOVA: d.f.=2, $F=5.82$, $p=0.044$): yellowfin tuna had a mean±1 SD of -16.40±0.46; spotted dolphin -16.88±0.52, and spinner dolphin -16.86±0.44. Multiple comparison, showed differences between yellowfin tuna and spinner dolphins ($p<0.01$), probably by result of trophic fractionation during feeding on different food resource at different trophic levels, and not by different primary energy sources incorporated into the oceanic food web.

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Stable isotopic evidence for uptake of fish farming induced organic pollutants by filter-feeding mussels (*Perna viridis*) in a polyculture system

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Previous studies demonstrated that fish farming activities could result in pollution effects owing to the release of organic and inorganic wastes from uneaten feed, faeces and dissolved excretory products (Karakassis et al, 2000). Different from salmonid fish culture predominating in Western Europe and North America, where artificial feed is commonly used, non-salmonid species (e.g. groupers, sea breams and seabass) are traditionally cultured in Asian-Pacific waters, including Hong Kong, and fed with trash fish. Filter-feeding bivalves can take up particulate matter in the water column in a considerable efficiency owing to the nature of their high filtration rate and large population density (Dame, 1996). In an integrated mariculture system combining fish and filter-feeding mussels, mussels can utilize the organic wastes from the culture cages as food sources. Compared with modern pelletised fishmeal, trash fish is inherently wasteful as a consequence of their lower digestibility and tendency to break up and shed small unconsumed particles during feeding (Leung et al, 1999). However, the high remaining nutrition content and breakup make the feed residue accessible and ingestible by filter-feeding bivalves.

To evaluate the availability of organic wastage derived from fish farming as food sources to filter-feeding bivalves, mussels from a single population were transplanted to a fish culture cage and a reference site without effects of fish farming activities. After 3-month acclimation, samples of mussels, particulate matter, fish feed and fish faeces were collected for measurement of carbon and nitrogen isotopic ratios. Enrichment of ^{13}C and ^{15}N in mussel tissue living inside the fish cage relative to those at the reference site indicated the uptake and assimilation of isotopically heavier fish feed and fish faeces. Based on isotope mixing model, the contributions of particulate organic matter, fish feed and fish feces to mussel food were 56.7%, 34.2% and 9.2%, respectively.

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Stable isotopic comparison in otoliths of juvenile sablefish (*Anoplopoma fimbria*) from waters off the Washington and Oregon coast

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Genetic differentiation and techniques have been extensively used in examination of fish population composition and structure (e.g., Grant et al. 1987; Ward et al. 1994; Shaklee and Bentzen 1998). However, it is generally not easy to determine the stock structure of a marine fish using the genetic markers, particularly given the large population size and wide spread distribution of the long-lived fish. As an alternative, sagittal otoliths of juvenile sablefish (*Anoplopoma fimbria*) along the Washington and Oregon coast were collected and analyzed for stable oxygen and carbon isotope ratio ($^{18}O/^{16}O$ or $\delta^{18}O$, and $^{13}C/^{12}C$ or $\delta^{13}C$) analyses. $\delta^{18}O$ values of the otolith nuclei ranged from -1.3 to +0.1 ‰ VPDB, whereas $\delta^{13}C$ of the same otoliths ranged from -8.7 to -6.2 ‰ VPDB. In contrast with the lower isotopic values in early life, $\delta^{18}O$ and $\delta^{13}C$ values of sablefish otoliths at adult stage were levelled at about 2.0 ‰ VPDB and -1.0 ‰ VPDB, respectively. From north to south three different spawning stocks or sub-populations were recognized, with a gradual decrease in otolith $\delta^{13}C$ and trophic level changes in sablefish's diet. Therefore, stable isotopic records of otoliths may be a potential supplement for genetic markers, particularly when little or no genetic differentiation has been detected between stocks of marine fishes.

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Are small estuarine plumes traceable on exposed open coasts?

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Coastal seas trap much of the material exported from land, with most of the matter transported by rivers to the sea being deposited fairly close to shore. This riverine-and estuarine conduit may be visually observable in the form of prominent plumes which push from estuaries into the nearshore zone. Such plumes and their interaction with the nearshore marine environment are mostly documented for large systems (e.g. Mississipi, Congo, Amazon), but on the Australian East Coast the setting is different: rivers are much smaller, estuaries discharge directly onto an exposed coast, and distinct plumes are only evident after substantial rainfall events.

The pathways (both physical transport and trophic channelling into nearshore food webs) of organic matter and nutrients carried in these small plumes are largely unknown. Thus, our principal goal was to measure some fundamental characteristics (e.g. spatial extent in the nearshore zone, physico-chemical characteristics, provenance of suspended carbon, etc.) for two such small plumes originating from the Mooloolah and Maroochy Estuaries on Australia's East Coast. Estuarine out-welling is evident in the nearshore zone after rainfall with substantially elevated nutrient levels (DIN increases by ~330%, Si by ~530%), increased phytoplankton stocks (chlorophyll *a* up by 20%) and a less saline and more turbid water column. These changes to water chemistry and algal production are, however, often confined to surface layers: the plumes are 'buoyant' and less clear differences between plume and non-plume waters are observable below the top 2-3m of the water column. Carbon of suspended particulates in the visible plume shifts from approximately marine source-dominance during dry weather ($\delta^{13}C$ ~ -21.5‰) to a stronger influence of estuarine, out-welled material following rain ($\delta^{13}C$ ~ –24.3‰).

To estimate the spatial range over which plumes deposit river/estuarine in the nearshore zone we also used seafloor mapping of sediment properties. This approach encompassed both 'standard' sedimentary variables (e.g. granulometry, organic matter content) as well as tracers (e.g. copper concentration – from marinas, and stable carbon and nitrogen isotopes of organic matter) to indicate whether material of estuarine/riverine provenance can influence the nearshore sedimentary organic pool. The $\delta^{13}C$ value of organic material in the sediments below plumes is depleted (~ 4.5‰) relative to marine (reference, ~ 3km offshore) areas indicating some contribution of estuarine/terrestrial carbon to nearshore seafloor areas. Plume material may also enter nearshore food webs. We started to assess whether such trophic subsidy of nearshore food webs by plumes is measurable by comparing carbon isotope signals in key consumers distributed both in areas of direct plume influence and in offshore/reference areas. 3-spot sand crabs (*Portunus sanguinolentus*) from plume areas have significantly depleted $\delta^{13}C$ values (-17.7 ± 0.2‰) compared with conspecifics caught offshore (–16.8 ± 0.1‰), indicating that some carbon carried by the plume is incorporated by marine consumers. We currently continue this theme of 'hypothesised trophic subsidy' by encompassing zooplankton responses to plumes and the possible fate of plume-material in entering littoral consumers via onshore advection of previously outwelled material.

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Laser ablation micro-analysis of $\delta^{15}N$ in Antarctic scallop (Adamussium colbecki) shells reveals changing food source with age

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Stable isotopic analysis of ^{13}C and ^{18}O in shells and bones have often been used to provide detailed time-series information about the environment in which the animal lived – $\delta^{13}C$ indicating the movement between regions, and $\delta^{18}O$ indicating water temperature and hence seasonality (e.g. Best & Schell 1996; Devereux 1967; Dufour et al. 1998; Lenanton et al. 2003). Because it is present in very low levels, the ^{15}N component has not been examined in such detail.

Investigation of climate change effects in McMurdo Sound, Ross Sea, Antarctica, such as changes in ice melt and cross-shelf advection of off-shore water masses, requires long term records of water temperature and movement. As the Antarctic scallop, *Adamussium colbecki*, is a long-lived animal with a life-span of up to 100 years, and is abundant on the floor of McMurdo Sound, their shells should contain a long-term record of environmental change.

As part of this study, I developed a method for the micro-analysis of ^{15}N in the ultra thin (~5 μm) nacreous protein layers between growth rings of the scallop shells. The technique uses laser ablation to volatilise the shell nitrogen and cryogenic focusing of the N_2 gas before introduction to a Delta Plus-XL isotopic ratio mass spectrometer in continuous flow mode. Preliminary results show a marked change in $\delta^{15}N$ signatures for the animal age across the shell, from 0.9 ± 0.5 ‰ as the spat, to 10.9 ± 1.5 ‰ as the adult. This difference in $\delta^{15}N$ signatures is consistent with a shift from primary production with atmospheric nitrogen fixation to secondary production using re-cycled nitrogen from microbial decomposition in the sediments. The shell $\delta^{15}N$ record indicates that spat are most likely to be feeding on pelagic phytoplankton while the adults tend to feed on microphytobenthos and detritus that they have suspended by "puffing" their shells.

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Intra- and Inter- specific spatial and temporal dolphin diet investigation by stable isotope ratio analysis of Museum specimens

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Spencer Gulf, South Australia is utilized for a variety of human uses, whose effects are poorly understood. For example, if dolphins and other apex predators associate fish farms with food, there may be ecosystem effects due to changes in habitat use and nutrient flow in local foodwebs. A preliminary study of gut contents from South Australian dolphin carcasses suggested that dolphins do vary their diet to opportunistically feed near fish farms(1) and this may be a result of enhanced productivity arising from associated nutrient inputs.

While gut contents show short-term feeding behavior, stable isotope ratio analysis may be a better indicator of long-term changes in diet because nutrients that are assimilated are recorded(2). To enhance gut content analysis and further examine feeding habitat, nutrient flows and trophic levels, we propose to conduct $\delta^{13}C$ and $\delta^{15}N$ stable isotope ratio analysis using teeth from South Australian Museum dolphin specimens. These provide an opportunity to study variation in diet and nutrient flow over time.

Our aims are to compare variation by spatial distribution within and between the three species of dolphin that inhabit Spencer Gulf (*Tursiops truncatus, T. aduncus* and *Delphinus delphis*) with specimens that were collected contemporaneously. Temporal variation within and between species will be examined using museum specimens collected over the past 100 years.

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Sources of primary production in suspension-feeding mussels (*Mytilus californianus*) from urban southern California shores

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Mussels can obtain food directly and indirectly from phytoplankton and benthic macrophyte (algae and seagrass) detritus. Carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotope values have been successfully used to differentiate and track sources of production through mussel diets in estuarine habitats and in colder seas characterized by large algal beds. Here, we used stable isotopes to analyze mussel (*Mytilus californianus*) diets in urban coastal and offshore island habitats in southern California. On urban coasts, added organic inputs from estuarine vegetation and storm drainage may also contribute carbon to the diets of benthic intertidal organisms. $\delta^{13}C$ and $\delta^{15}N$ tissue compositions from these suspension-feeding mussels were determined from animals collected across a gradient of sites characterized by variable macrophyte standing stocks. Coastal and estuarine POM, benthic macrophytes, and other potential $\delta^{13}C$ and $\delta^{15}N$ sources were obtained from several southern California sites and used to analyze dietary inputs into mussel populations. Our results indicate that the mean $\delta^{13}C$ of macrophytes was −15.5 and averaged > 50 % of the carbon in mussel diets at all of our mainland sites, even in the absence of large macrophyte standing stocks. Mussel $\delta^{15}N$ values remained similar between sites with a mean of 10.9‰ and were enriched by 1.5‰ over macrophyte sources. Mussels from Santa Catalina Island, an offshore, oligotrophic site 22 km from the urban southern California mainland, had distinct and more depleted $\delta^{13}C$ and $\delta^{15}N$ values than mainland populations, indicating greater contributions of planktonic POM in their diets.

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Tuna trophic dynamics in Hawaiian waters: Are there differences in the $\delta^{15}N$ of mesopelagic and epipelagic food webs?

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Inter-specific differences in the trophic dynamics of yellowfin and bigeye tuna and the possible impact of their association with man-made and natural structures in Hawaiian waters are being explored using carbon (C) and nitrogen (N) stable isotope analyses. Initial results suggest that yellowfin and bigeye tunas, ranging from 25 to 45cm in forklength, captured at fish aggregating devices (FADs) around the Island of Oahu have similar C and N isotope values. Interestingly, individuals over 45cm also have similar C and N isotope values, but are approximately 1.5 to 2 trophic levels higher than smaller tuna. In terms of body mass, a large increase in $\delta^{15}N$ occurs between 1kg and 3kg, reaching an asymptote with a $\delta^{15}N$ value near 12‰. These results suggest that a distinct dietary shift occurs around 45cm FL or from 1 to 3kg. Extensive data from tuna stomach analyses also indicate a dietary shift from predominately stomatapod larvae to oplophorid shrimp around 40 to 50cm forklength. Isotope values for these prey are 3 to 4‰ lower than the respective sized tuna which reflect the typical isotope trophic enrichment. Stomatapod larvae are epipelagic filter-feeders whereas oplophorid shrimp are migrating mesopelagic omnivores. Accordingly, oplophorids should be enriched in ^{15}N relative to stomatapods by no more than one trophic level (~3‰). We observed a difference between the epipelagic and mesopelagic shrimp of 4.5 to 5‰. These data suggest that the $\delta^{15}N$ at the base of the mesopelagic food web is fundamentally different from that in the epipelagic food web, wherein the mesopelagic food web is enriched in ^{15}N. Stable isotope analysis, therefore, could indicate niche separation by predators and their prey in the pelagic environment.

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Free air respiratory carbon isotope enrichment experiment

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Recycling of respiratory carbon in canopies is difficult to measure. Other than a steady state model, there are currently no direct methods of measuring recycling. Respiratory carbon recycling will affect the carbon isotope ratio signature of the canopy air, therefore is also important in partitioning gross photosynthesis and respiration from next ecosystem exchange (NEE). Often environmental conditions affect P and R differently, therefore improving estimation of these parameters as individual components of NEE will allow better prediction of how ecosystems will respond under climate change scenarios. In order to better understand recycling, we empirically derived an integrated measure of recycling in a cover crop (*Crotalaria juncea* L.) and compared the results with those derived by the steady state model. In the empirical experiment, a measured dose of nitrogen gas having CO_2 with high Carbon-13 abundance (41%) was applied at ground level (~10cm above the soil surface) with a system of hoses in a 9 by 9 m plot embedded in a 30 by 30 m plot of *C. juncea*. We adjusted the flux rate of this enriched gas weekly to correspond to weekly measurements of soil respiration rates. The gas application rates at ground level were sufficiently low so as to not affect the carbon dynamics of the treatment plot relative to that of a control plot with only nitrogen (no CO_2) applied. The isotopic composition of the applied gas, however, was high enough to significantly increase the isotopic composition of respired CO_2.

To calculate the results of the empirical experiment we used the carbon isotopic composition of respiration and biomass from the control and treatment plots, in conjunction with mass balance principles, and calculated that 71% of the total respired CO_2 is recycled by this crop. In comparison, 48% recycled CO_2 was derived by the theoretical, steady state model. Methods of measuring respiration in the empirical experiment may have caused an elevated estimation of recycling and account for the difference between the values calculated by empirical and theoretical methods.

Partitioning of gross photosynthesis and respiration by isotopic methods usually assumes no recycling. Recycling, however, will have an effect on the isotopic composition of respired CO_2. After correcting for the recycling effects on the carbon isotope rations of respired CO_2 leaving the canopy, we calculated that average gross photosynthesis for this crop was 43.7 μmoles/m^2s and gross respiration was in the order of 10.6 μmoles/m^2s. These values are similar to those previously observed in agricultural crops. Photosynthesis and Respiration calculated without correcting for recycling effects were 180.8 μmoles/m^2s and 79.1 μmoles/m^2s, respectively, indicating a likely probability of overestimation.

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Stable isotopic differentiation of two congeneric chironomids from W. Europe: the role of tube morphology and larval physiology

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Chironomid (midge) larvae have been postulated as key species in mediating methane-derived production into higher trophic levels (Grey 2002). However, different species are likely to exhibit different efficiencies in such a transfer. The stable isotope ratios ($\delta^{13}C$ and $\delta^{15}N$) of two common, sympatric species of tubiculous chironomids, *Chironomus plumosus* and *C. anthracinus*, from the profundal sediments of European lakes were examined for consistent interspecific variation.

There was between-lake variation in both $\delta^{13}C$ (-29.8 to -56.3‰) and $\delta^{15}N$ (-7.8 to 14.7‰) in the 2 species. *Chironomus plumosus* was consistently depleted in both ^{13}C and ^{15}N relative to its congener. After correcting for differing baseline $\delta^{15}N$ between lakes, the slopes of the regressions (representing the $\delta^{15}N/\delta^{13}C$ relationship) appeared to differ between species (*C. plumosus*: slope = 0.6, $r^2 = 0.96$; *C. anthracinus*: slope = 0.82, $r^2 = 0.95$), suggestive of some interaction between $\delta^{15}N$ and species, but the difference was not statistically significant ($F_{1,11} = 3.52$, $p = 0.097$).

Furthermore, the $\delta^{15}N/\delta^{13}C$ relationship between individuals of the same species within each lake exhibited similar slopes to the pooled data for that same species from differing lakes. The interspecific differences cannot be explained by alternative feeding modes that are generally attributed to the two species, namely 'filter-feeding' versus 'deposit-feeding'. Rather, we explain the differences between the species in relation to tube morphology and/or larval physiology. *Chironomus plumosus* is the larger species, creating deeper tubes of a U-shaped nature, and being more capable of functioning under low oxygen conditions. *Chironomus anthracinus* builds J-shaped tubes sealed at the distal end. We postulate that the differing slopes of the $\delta^{15}N/\delta^{13}C$ relationship between the species is a function of the greater capability of *C. plumosus* to flush excreted ammonium out of its tubes.

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Marine-derived N and C in juvenile coho salmon rearing in the Copper River Delta, southeastern Alaska

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Pacific salmon of the genus *Oncorhynchus* rear to adulthood at sea, but return to freshwater to spawn. After spawning they die, contributing marine-derived nutrients to the early rearing habitat of their offspring and associated food web. This nutrient subsidy has the potential to increase the productivity of the aquatic food webs and the adjacent riparian ecosystem. We studied stable isotopes in beaver ponds of the Eighteen-Mile drainage of the west Copper River Delta, Cordova, Alaska, in response to spawning coho salmon (*O. kisutch*). Three types of pond treatment were sampled between 1999 and 2001, i.e., 1) naturally enriched by spawning salmon, 2) artificially enriched via the addition of salmon carcasses and eggs, and 3) control ponds with no salmon enrichment. In the naturally enriched ponds, adult salmon entered the ponds directly, and spawned in the stream channel immediately upstream. The control and artificially-enriched ponds were located in side channels and were inaccessible to adult salmon. All ponds supported juvenile coho salmon.

Salmon carcasses and eggs were added to the artificially-enriched ponds between September and November, 2000. Selected food web components (vascular plants, invertebrates, juvenile coho, and three-spined stickleback (*Gasterosteus aculeatus*)) were collected seasonally and analysed for stable isotopes (N and C) by the Waikato Stable Isotope Unit, University of Waikato. We detected marine N and C enrichment of juvenile coho salmon, stickleback, and chironomids, and marine N-enrichment of riparian and aquatic vascular plants, and terrestrial herbivorous invertebrates in the habitats that received spawners naturally. In the ponds that received artificial additions of carcasses and eggs, marine N and C were incorporated into juvenile coho salmon following these additions. From these results, returning salmon appear to play a vital role in the aquatic-riparian systems in the Copper River Delta likely through increasing productivity. Understanding the ecological role of marine-derived biomass in these freshwater systems will help advance fisheries management in the delta and other coastal ecosystems, as well as in the adjacent marine environments.

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Using marine isotopic gradients to link breeding and wintering grounds of North American diving ducks.

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In North America, several species of diving duck breed in terrestrial locations, primarily in Arctic regions, and winter in coastal marine habitats in either sub-polar regions of the Bering Sea to the west or western Greenland to the east or temperate regions further south along both coasts. Recent declines in populations of these birds has underlined the need to link breeding and wintering populations in order to better understand possible causes. Conventional means of linking populations using band returns or genetics have generally been unsuccessful in providing such linkages. However, since foodwebs of the Bering/Chuckchi seas are relatively enriched in ^{13}C and ^{15}N compared to more eastern seas of the Arctic, we investigated the potential to use naturally occurring isotope abundance of these elements in feathers to provide information on general wintering location.

In the first phase of this study, we used $\delta^{15}N$ and $\delta^{13}C$ measurements of king eider (*Somateria spectabilis*) feathers from several tracts to successfully assign individuals to eastern and western Arctic winter populations. Strong differences in isotopic ratios of head feathers, grown on the wintering grounds, enabled us to correctly classify 99% and 94% of king eiders as having wintered in western or eastern North American seas, respectively. Differences in $\delta^{15}N$ values provided the greatest discrimination between populations. Use of $\delta^{13}C$ measurements increased classification accuracy by an additional 4%. In the second phase of the study, we investigated the possible consequences of wintering to the east or west sides of North America. We investigated the occurrence of winter site philopatry among nesting females, and examined potential cross-seasonal effects of wintering area on subsequent breeding. Isotopic data suggested that 66-73% of this central arctic breeding population wintered to the west (i.e. Bering and North Pacific) and the remaining 24-37% wintered to the east (i.e. west Greenland, northwest Atlantic). In contrast, limited band recoveries from hunter-killed King Eiders marked at the same breeding location suggested that about 56% of individuals were shot in eastern wintering areas. These differences likely reflect stronger hunting pressures along the coast of Greenland. Our results suggest that female King Eiders did not exhibit strong philopatry to wintering areas among years. Our results have important implications for gene flow and for potentially associating wintering ground conditions on overall population demography and individual fitness of King Eiders.

Finally, our current investigations of other species of diving duck that winter further south on both coasts support our findings on eiders and suggest consistent isotopic differences in marine foodwebs between east and west sides of North America that can be used to investigate breeding and wintering ground linkages.

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Use of stable isotope and contaminant profiles to identify populations of long-tailed duck in the Canadian Arctic

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In North America, western populations of long-tailed duck are in decline and, although populations appear stable in the east, the long-tailed duck is considered to be a *Species of Continental Conservation Concern*. This species nests in greater numbers in the Canadian High Arctic than any other duck. The birds nesting in the eastern Canadian Arctic likely overwinter along the Atlantic coast whereas the western Arctic birds overwinter in the Bering Sea and along the Pacific coast of Alaska and British Columbia. Considerable numbers also winter on the Great Lakes, a freshwater system known to be contaminated. It is unknown where the dividing line occurs between the two breeding populations. Since stable isotopes of carbon ($\delta^{13}C$), nitrogen ($\delta^{15}N$), sulfur ($\delta^{34}S$) and hydrogen (δD) are typically enriched in marine vs terrestrial/freshwater systems, measurements of these naturally-occurring stable isotopes in foodwebs can be used to delineate relative inputs from freshwater and marine biomes. Upon arrival on the breeding grounds, metabolically active tissues of birds that wintered in freshwater environments can be distinguished isotopically from those that wintered in marine areas. The connection between risk of contaminant exposure and overwintering area can then be investigated.

Wing bone samples from 101 long-tailed ducks collected from across the Canadian Arctic were analyzed for stable isotopes ($^{13}C/^{12}C$; $^{15}N/^{14}N$, and $^{34}S/^{32}S$) and tissue samples from those same birds were analyzed for Hg, Cd, Se, Pb and Cu. Selected samples were also screened for a wider range of trace elements in liver. There was a large range in the stable-nitrogen isotope ratios which differed significantly between males and females suggesting that the males feed on different prey items than females for at least part of the year. The $\delta^{13}C$ values varied significantly with collection location and longitude. The birds from the western Arctic had a stronger freshwater signal compared with birds from the eastern Arctic which had a stronger marine signal. The marine overwintering areas for North American long-tailed ducks include both the Atlantic and Pacific coasts whereas the only major freshwater overwintering region is in the Great Lakes. This suggests that many of the birds breeding in the western Arctic overwinter in the Great Lakes whereas many of the birds breeding in the eastern Arctic overwinter along the Atlantic coast. Concentrations of As, Cd, Fe, Mn, Mo, Se and Zn were higher in the western birds compared with the eastern birds. It is not known whether or not the birds are more exposed to metal contamination on the arctic breeding grounds or on their overwintering grounds.

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Stable isotopic analysis of feeding niche in extinct populations of an endangered duck (New Zealand brown teal: *Anas chlorotis*)

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The New Zealand brown teal (*Anas chlorotis*) is an endangered duck, whose remaining populations are restricted primarily to streams on farmland adjacent to estuaries. At the time of European settlement, the species was found in inland areas and on seacoasts. Fossils suggest that it formerly occupied a range of habitats, including dense forests away from permanent waterways or open water. Conservation of brown teal is predicated on the belief that it is a typical dabbling duck and primarily an occupant of open water habitats. However its former range suggests a wider niche. Management of the two remaining, declining populations would benefit from an understanding of the teal's prehistoric feeding ecology and habitats instead of this being derived solely from the restricted and much-altered environment occupied at present.

We present preliminary data on $\delta^{13}C$ and $\delta^{15}N$ values measured on bone protein extracted from fossil brown teal bones of Holocene age from a range of sites in both North and South Islands. In successive phases of our project, these will be compared with data from bones from living populations and from other living waterfowl. The preliminary data show that the isotopic signature, in comparison to that of the extinct Finsch's duck (*Chenonetta finschi*), a herbivore, and the living blue duck (*Hymenolaimus malacorhynchos*), an insectivore, is that of a generalist. These early results suggest that the brown duck could survive on a range of animal and vegetable foods, and was not therefore a "conventional" dabbling duck. Conservation management plans for its rehabilitation must take into account the species evolutionary history as a forest-dwelling generalist that is now confined to an atypical environment.

Comment [GNS1]: A wider niche was what?

Nitrogen isotope composition of the soil microbial biomass: integrator of ecosystem N cycling?

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Examining the natural abundances of stable isotopes of biologically important elements has substantially advanced ecosystem science, but the advances have been more striking for some element cycles than for others. Use and clear interpretation of natural isotopic signatures of nitrogen (N), for example, has been impeded by the complexity of the N cycle, consisting of several pools that mix in various ways and numerous transformations with different degrees of isotopic fractionations.

We submit that comparing the ^{15}N natural abundances in the soil microbial biomass with other soil pools provides both mechanistic and integrative information about ecosystem N cycling. We have measured soil microbial ^{15}N isotope signatures in several ecosystems and experiments, and have developed a conceptual model to explain its variation. Our model holds that the microbial biomass becomes increasingly enriched as the proportion of N exported from microbial cells increases, due to fractionation during N dissimilation. We predict that microbial ^{15}N enrichment is negligible with N limitation of microbial growth, but increases with C limitation. If our model is correct, microbial ^{15}N enrichment reflects the elemental controls over microbial metabolism, as well as the functioning of the soil microbial biomass as a source and sink for N.

The elucidation of the controls on microbial metabolism is paramount to understanding the regulation of ecosystem N cycling. This is because microbial metabolism largely determines the flux of N through inorganic pools, and it is these pools that serve as the crossroads among plant N uptake, N retention in soil organic matter, and N loss via leaching or denitrification. Thus, measurement of microbial ^{15}N enrichment may prove to be a powerful probe and integrator of ecosystem-level N cycling.

Variation in the carbon isotope ratio of ecosystem respiration in New Zealand forests

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Applying the Keeling plot approach (Keeling 1958) to the carbon isotope ratio of CO_2 respired by an ecosystem ($\delta^{13}C_R$), is a valuable tool to investigate ecosystem-scale photosynthetic and respiratory processes. Recent work has shown that $\delta^{13}C_R$ reflects site-to-site variation in precipitation, and seasonal variation in vapour pressure deficit (Bowling et al. 2002). The mechanistic link between $\delta^{13}C_R$ and environmental conditions seems clear. C_3 plants discriminate against $^{13}CO_2$ during photosynthesis. The level of discrimination depends on CO_2 supply and demand, so that $\delta^{13}C$ of plant tissue ($\delta^{13}C_l$) reflects both stomatal conductance and photosynthetic capacity at the time of carbon fixation. Respiration releases this depleted carbon back into the atmosphere. Therefore, environmental conditions that effect stomatal conductance and photosynthesis (and hence $\delta^{13}C_l$) are expected to be expressed in $\delta^{13}C_R$.

Models of ^{13}C discrimination during photosynthesis (Farquhar et al. 1989) point to the ratio of leaf intercellular to atmospheric CO_2 partial pressure (p_i/p_a) as the most important variable driving variation in $\delta^{13}C$ of plant tissue. We proposed that $\delta^{13}C_R$ should reflect variation in canopy p_i/p_a for several native New Zealand forests. $\delta^{13}C_l$ of sunlit canopy leaves was measured in 5 podocarp/hardwood forests in South Westland, and beech forests in the Southern Alps and on the east coast of the South Island. Flask samples of nighttime within-canopy air were taken and the Keeling plots were used to calculate $\delta^{13}C_R$. $\delta^{13}C_l$ values allowed estimation of p_i/p_a from models, and revealed large differences in canopy p_i/p_a related to soil water availability, vapour pressure deficit and photosynthetic capacity. $\delta^{13}C_R$ was negatively related to p_i/p_a, consistent with models.

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Application of radiocarbon analysis to the ecological studies on termites.

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Termites (Isoptera) are superabundant detritivorous animals in the tropical terrestrial ecosystems, and play an important role in decomposition processes through utilizing a various stages of organic matter ranging from fresh plant materials to humified material (soil organic matter). These abilities largely lie in the sociality and the symbiosis with microorganisms.

Stable carbon and nitrogen isotope ratios have been utilized to evaluate feeding habits of termites. These studies indicate that soil-feeding termites are more enriched in ^{15}N compared to wood- and grass- feeding termites and propose ^{15}N as an indicator of the functional position of the feeding habits in the humification process.

In this study, we applied radiocarbon analysis to the ecological studies on termites as an indicator of feeding habits, which should reflect the average age of carbon (C) the termites utilize. We collected termites from grass savanna and gallery forest in Lamto Ecological Research Station, Ivory Coast and measured the ^{14}C content by accelerator mass spectrometry. A grass-feeding termite, *Trinervitermes geminatus*, resembled the current year grass in ^{14}C. Wood-feeding termites, *Microcerotermes parvus* and *Cryptotermes brevis* showed different ^{14}C content and the former species had lower ^{14}C content, which indicate an average age of C at least 5 years old. A soil-feeding termite *Noditermes aburiensis* was similar in ^{14}C to the wood-feeder termite, *M. parvus*, but had higher ^{14}C content compared to a soil-feeding termite, *Amitermes evuncifer*. These results suggest that ^{14}C could characterize the termites along with the C age gradient, independent of the known feeding habits, indicated by ^{15}N

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Choosing appropriate tissues for stable isotope analysis of fishes, and potential factors influencing variability

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A common question surrounding stable isotope research in fishes is the selection of an appropriate tissue for analysis. Conservation concerns may limit lethal sampling, and budgetary constraints may further limit analysis of multiple tissues. We report here stable carbon and nitrogen ratios in different fish tissues including liver, muscle, fin clips, and gonad, from three freshwater species: Atlantic salmon (*Salmo salar*), slimy sculpin (*Cottus cognatus*), and brook trout (*Salvelinus fontinalis*).

Stable nitrogen ratios showed consistent differences among tissues, possibly caused by fractionation during protein recycling. For example, during fall sampling, muscle tissue of both male and female slimy sculpin had enriched nitrogen-15 levels relative to liver. Following the winter months, male sculpin showed the opposite pattern, with liver tissue enriched in nitrogen-15 relative to muscle, a difference that was not observed in females. These results point to sex differences in overwinter resource accumulation and allocation prior to spring spawning by sculpin, and suggest that tissue specific stable nitrogen ratios may be used as a measure of the nutritional status of wild caught fish species.

Stable carbon ratios were generally equivalent among tissues, with minor variations presumably associated with lipid content. However, lipid correction using a commonly applied formula failed to account for these differences. Further investigation into the mechanism governing the disparity is warranted.

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Can stable isotopes of carbon and nitrogen be used as biomarkers for discards in the diets of scavenging seabirds?

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Discards from commercial fisheries are an important food source for scavenging seabirds such as the great skua (*Stercorarius skua*). However, determining the relative contribution of discards to the diet is difficult, particularly in winter since these birds are exclusively pelagic, rendering conventional techniques such as direct observation or examination of regurgitates or pellets impractical. Stable isotope analysis of carbon and nitrogen in seabird tissues offers an alternative method of assessing the diet. Blood and feathers are commonly used since these tissues can be sampled non-destructively and since feathers are moulted and re-grown at different times of the year, the isotopic signatures reflect the diet over different temporal and spatial scales. In this study, we assess the potential of stable isotopes as biomarkers for fishery discards in the diet of great skuas at Foula, Shetland Islands.

In great skuas the moult starts shortly after the breeding season with the inner-most primary (P1) and is completed in the wintering area with the outer most primaries (P9-10) before re-migration to the breeding colony. By measuring the isotopic signatures of P1 and P9 we can consider differences in diet at the time of feather growth with P1 and P9 reflecting the diet at the breeding colony and the wintering area respectively. With knowledge of the isotopic signatures of the most common discard species from the breeding and wintering areas, it is possible to calculate the relative contribution of discards (demersal fish) to the diet of these birds at different times of the year.

Feathers and blood were sampled from adult great skuas and chicks, during the breeding seasons of 2002-3. We also conducted a survey of isotopic signatures of demersal fish (discards) and pelagic fish from the breeding and wintering areas. We found significant differences in both $\delta^{13}C$ and $\delta^{15}N$ between P1 and P9 indicating that the birds feed on isotopically distinct diets between the breeding and wintering grounds and this was consistent across years. At the breeding grounds, although there was no significant difference in blood $\delta^{13}C$ or $\delta^{15}N$ between adults and chicks, adults showed greater variance than chicks. The simplest explanation for this is that adults fed on a wider range of prey species than they fed to the chicks. However, dietary specialisation of individual adults may offer an alternative explanation. In the survey of isotopic signatures of demersal and pelagic fish, preliminary data suggest that 66% of demersal fish differ significantly in both $\delta^{13}C$ and $\delta^{15}N$ from pelagic fish. We believe that our data indicate that stable isotopes may provide useful information on the contribution of discards to the diet of scavenging seabirds.

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Diet-tissue fractionation of carbon and nitrogen isotopes in captive herring gulls *Larus argentatus* following diet switches between demersal and pelagic fish

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We measured diet-tissue fractionation and equilibration times of carbon and nitrogen stable isotopes in red blood cells and feathers of three groups of captive herring gulls (*Larus argentatus*) fed on isotopically constant diets of demersal or pelagic marine fish. The diet of group A was switched sequentially from a pelagic species to a demersal species every two weeks, starting with sardine then switching to haddock, herring, flatfish sp., mackerel and whiting. The diets of groups B and C were switched from demersal to pelagic (haddock to herring) and pelagic to demersal (sardine to flatfish sp.), after six weeks, respectively. Blood was sampled from group A every three days and every five days from groups B and C. Tail feathers were plucked from birds in all groups on day one and then subsequently on days 42 and 84.

The results indicate that in group A, the blood had not yet reached isotopic equilibrium at the time of the next diet switch. However, over the course of the experiment, blood isotopic values exhibited a 4.5 and 9.8‰ range for $\delta^{13}C$ and $\delta^{15}N$, respectively. Measurements of $\Delta^{13}C_{diet\text{-}feather}$ and $\Delta^{15}N_{diet\text{-}feather}$ in groups B and C ranged from 1.9 - 3.4‰ and 2.5 - 4.9‰, respectively, while $\Delta^{13}C_{diet\text{-}blood}$ and $\Delta^{15}N_{diet\text{-}blood}$ ranged from 0.4-1.6‰ and 1.1-2.8‰, respectively. We believe these results emphasise the need for laboratory calibration of diet-tissue fractionation and isotopic equilibration times prior to commencement of field studies.

We also present the results of a survey of isotopic signatures of pelagic and demersal fish from the North Sea, Atlantic Ocean and Mediterranean Sea. These data will be used to calibrate field studies of scavenging seabirds and to quantify the extent to which these birds feed on discards (demersal fish) from commercial fisheries in the North Sea and the Mediterranean.

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Changes in littoral food web dynamics along depth-dependant habitat zonations

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Gradients of light, temperature and nutrients with depth contribute to large vertical changes in habitat over the littoral zone in clear oligotrophic lakes in New Zealand. These gradients affect both the species composition and physical structure of benthic plant habitats and can influence the quality and quantity of carbon pools available to consumers, thus carbon flow to higher trophic levels. We report results of an examination of benthic food-web dynamics and carbon flow among shallow, mid-water, and deep-water littoral zone habitats at three sites in Lake Wanaka using stable isotopes of carbon. Shallow-water habitats (0 – 3 m) consisted of patchy low-growing macrophyte communities (e.g., *Isoetes sp., Glossostigma sp.*) amongst cobbles, with the lowest density (2300 m^{-2}) and richness of invertebrates. Mid-water habitats (3 – 7 m) were comprised of a mix of taller vascular plant beds (e.g., *Potamogeton sp.*, *Myriophyllum sp.*), and had intermediate invertebrate densities (3600 m^{-2}) and richness, dominated by snails, chironmoids, and oligochaetes. Deep-water habitats (9 - 16 m) were mainly characean meadows (*Chara spp., Nitella spp.*), and had the highest density (9600 m^{-2}) and richness of benthic invertebrates, dominated by snails, caddisflies, chironomids, and bivalves. We were able to separate primary producers by C isotope signatures; epiphytes had a mean $\delta^{13}C$ of -22.2‰, phytoplankton -27.9‰ and macrophytes ranged from -10.6 – -17.5‰.

Our results support the findings of previous lentic studies in New Zealand that most littoral primary consumers predominantly feed on epiphytes and phytoplankton. For example caddisfly larvae, with a mean $\delta^{13}C$ of -21.4‰, and chironomids -21.8‰ both appear to be primarily epiphyte feeders. There appeared to be very little transfer of macrophyte carbon to higher trophic levels, with the exception of snails (mean $\delta^{13}C$ –18.0‰), which appear to directly or indirectly gain some of their energetic requirements from macrophytes. An increasing contribution of phytoplankton carbon to benthic food-webs was apparent with increasing lake depth. Primary consumers from deep habitats showed, on average, 2.8‰ less enriched $\delta^{13}C$ signatures than shallow habitats, despite having unchanged macrophyte and epiphyte $\delta^{13}C$ signatures. The greater contribution of phytoplankton to stable isotope carbon signatures in deep-water habitats may indicate an important energy link between pelagic and benthic food-webs in New Zealand lakes.

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Water mass and isotopic aging in a large impounded river-bay system

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Recent estimates have shown that the mean age of river water reaching the coastal ocean has likely tripled historically as a consequence of reservoir-induced aging of continental runoff. This aging is hypothesized to have a significant biogeochemical and ecological impact on land-margin systems by altering flow regimes, net water balances and residence times, reaeration of surface waters, carbon cycling processes, and sediment storage and transport. In a large watershed system within the Laurentian Great Lakes, a series of main stem reservoirs, impoundments and lakes act to retard flow, retain particles, and significantly attenuate the flux of materials into sequential downstream pools. These pools serve as biogeochemical reactors that both process and repackage nutrients and organic carbon via tightly coupled benthic-pelagic biotic interactions. The net result is that both the quantity and quality of the material transported through the system are significantly altered. We have hypothesized that much of the control on this processing is related to water residence times and the age of river water and its associated constituents within the system. Because impoundments serve as sites of intense and prolonged processing, the nutrient stoichiometry and isotopic composition of organic matter could be expected to reflect the combination of source functions, biogeochemical alterations and remineralization reactions, and food web structure in a coherent manner, e.g. an aquatic system might shift more toward a heterotrophic state if transport shifts to a larger proportion of nutrients being transported downstream via previously fixed organic forms.

Variations in both carbon and nitrogen isotopic signatures of particulate organic matter within this system range by ~10 ‰. Variations across major impoundments appear initially to be consistent with the hypothesis that reservoirs behave as nutrient sinks with losses both to the sediments and to the atmosphere. dN-15 values show the largest shift - up to +5 ‰. The questions to be examined are whether variations in isotopic signatures, nutrient stoichiometry and composition provide insights into biogeochemical cycling either at the level of the individual aquatic reservoir or at the level of the entire land-margin system, and whether the residence time and aging within successive impoundments plays an important role in the biogeochemical structure of this ecosystem.

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Carbon dioxide recycling by understory vegetation in a cool-temperate deciduous forest

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Three main factors govern dynamics of CO_2 within a forest canopy: turbulent mixing with the atmosphere above canopy, photosynthesis and respiration. Canopy CO_2 concentrations ($[CO_2]$) and their carbon isotope ratios ($\delta^{13}C$) are also influenced by stand structure, such as leaf area index and presence of understory vegetation (Buchamann et al. 1998). $[CO_2]$ generally increases near the soil surface because of plant and soil respiration. Therefore, the understory vegetation is capable of fixing respired CO_2 through photosynthesis (CO_2 recycling), and influences carbon dynamics within the forest.

In this study, we examined how $[CO_2]$ and $\delta^{13}C$ of canopy profile change daily and seasonally in a cool-temperate deciduous forest at Takayama Experimental Site (36˚8'N, 137˚6'E, 1420m a.s.l.) in Japan. We also estimated the percentage of respired CO_2 recycled by understory vegetation (dominated by bamboo grass, *Sasa senanensis*) using a model developed by Sternberg (1989).

There were clear diurnal changes in $[CO_2]$ within the forest, especially on the soil surface, in summer. $[CO_2]$ and $\delta^{13}C$ were vertically stratified within the forest. $[CO_2]$ increased near the soil surface, while $\delta^{13}C$ depleted. The higher values of $[CO_2]$ were found within the bamboo grass stand between the nighttime and the early morning, but $[CO_2]$ of the upper canopy was stable during the daytime. $[CO_2]$ and $\delta^{13}C$ showed small diurnal variation and small vertical difference in autumn, reflecting reduced biological activities due to tree leaves fall. The percentage of respired CO_2 recycled by *S. senanensis* ranged from 5 to 20%.

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Carbon isotope composition of anthropogenic methane flux from the Upper and Lower Silesian Coal Basins, southern Poland

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Methane emissions associated with coal production (mining, transportation and storage on the surface) are responsible for about 50% of the global anthropogenic methane flux to the atmosphere. In regions of active coal mining these emissions may lead to elevated atmospheric CH_4 levels. Restructuring of coal mining industry in some countries and shifts of major coal production centres results in transient character of this global flux.

The paper is focussed on emissions of CH_4 from two major coal basins in Poland: the Upper Silesian Coal Basin (USCB) and Lower Silesian Coal Basin (LSCB), both located in the south of the country. Estimates of regional methane flux from these two areas over the past decade are presented, together with the assessment of the importance of various factors controlling spatial and temporal variability of this flux. The emissions of methane through ventilation shafts of mines in the USCB decreased from $1{,}159 \times 10^6$ m^3 in 1988 to about 624×10^6 m^3 in 2001. For LSCB, the corresponding flux was equal 27×10^6 m^3 in 1992, 13×10^6 m^3 in 1996 to about 2.5×10^6 m^3 in 1998. By the end of 1998 all mines in LSCB were closed and their remediation is currently under way. Extensive studies of the transient flux of methane to the atmosphere associated with closing of mines in LSCB and restoring the regional Carboniferous water table have been carried out. Flux measurements using static chamber method were performed on 56 areas. The forced influx of methane into the near-surface zone of the affected region varied between 0.1 and 620 $dm^3m^{-2}h^{-1}$.

The $\delta^{13}C$ values of methane collected at different depths in mines of LSCB vary between -66 and -24‰, the average value being around –36.9‰. For USCB, the corresponding values vary between –80‰ to –44‰, with the average value equal to -64.3‰. Carbon isotope composition of coalbed gases in both basins reveal presence of three genetic types of these gases: thermogenic (methane, higher gaseous hydrocarbons, and carbon dioxide), endogenic carbon dioxide, and microbial methane and carbon dioxide. Carbon isotope signature of the regional methane flux entering the atmosphere in LSCB has been derived from diurnal variability of methane concentration and its carbon isotope composition measured at the ground level using the Keeling-type plots. Two sets of measurements carried out in 1999 and 2001 yield the $\delta^{13}C$ values of this flux between -47‰ and -46‰. Direct, local measurements of $\delta^{13}C(CH_4)$ in the areas of intense CH_4 emissions gave the values between -47‰ and -31‰. Spot measurements of atmospheric methane carried out at different localities of USCB (samples collected close to ground level at periods of most intense vertical mixing) gave the range of $\delta^{13}C$ values between -56.2 ‰ and -45.1 ‰. This apparent range suggest that in the USCB both isotopically depleted and isotopically enriched methane, when compared to regional background atmospheric level, is being released to the atmosphere.

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The role of meiofauna at deep sea hydrothermal vents

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In many aquatic and marine habitats, meiofauna, (small invertebrates passing through 1mm or 500µm sieve and retained on 63 or 40µm sieve) represent an important food source for higher trophic levels such as crustaceans and fish. This may also be the case at deep sea hydrothermal vents. So far mainly taxonomic and biogeographic studies of meiobenthic species from hydrothermal vents are found in the literature and little is known about their feeding strategies and trophic position in the vent community.

In this study, the stable carbon and nitrogen isotopic composition of macrobenthic and meiobenthic invertebrates from Juan de Fuca Ridge and Explorer ridge, Northeast Pacific, was investigated. Results show that the dominant meiobenthic species close to hot vent sites, sulphide copepods (*Stygiopontius quadrispinosus*), may constitute a part of the diet of the sulphide worm, *Paralvinella sulfincola*. The stable nitrogen isotopic composition of ostracods, which are abundant on less active sites, showed to be relatively enriched indicating that it is occupying a higher trophic position in the vent community compared to copepods. In conclusion, meiobenthic species at vents occupy different trophic levels and at least one of the dominant species may be used as a food item for macrofauna.

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Seasonal variations on $\delta^{13}C$ and $\delta^{15}N$ signature among the oyster *Crassostrea gigas* tissues for several culture conditions: implication for nutrition and reproduction physiology

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The use of natural abundance of stable carbon and nitrogen isotopes in coastal ecosystems has received increasing interest during the last decades in order to identify trophic food web pathways and links. However, very few studies have documented seasonally changes in carbon and nitrogen stable isotopes of separate organs of a marine bivalve following its transplantation in new growing conditions. Such transplantations are used to occur within the shellfish farming Marennes-Oléron Bay, in which oyster are traditionally grown on intertidal areas and refined on coastal oyster ponds. Accordingly, Japanese oysters *Crassostrea gigas* previously stored in oyster ponds were replaced into the Marennes-Oléron Bay under two growing intertidal conditions (on-bottom and off-bottom culture) and in oyster ponds. The sampling period was from March 2002 to April 2003 with an increased sampling effort during the summer reproductive period. Diploids and triploids oysters were used to bring up the implication of reproduction physiology on isotopic variations. This research was conducted under the project MOREST of IFREMER, which the main goal is to explain and control summer oyster mortality. Three organs (i.e. digestive gland, muscle and gonad) of the bivalve were used to followed seasonal changes of both stable isotopes ratios ($\delta^{13}C$ and $\delta^{15}N$) and bulk biochemical composition together with isotopic composition of potential food sources.

Storage in coastal oyster ponds during winter time allowed oysters to acquire a continental signature ($\delta^{13}C$ = -27.3 ‰ for digestive gland and -24.9 ‰ for muscle). This explains the initial signature of oysters at the beginning of the experiment. Results on diploid oysters showed a great seasonal isotopic variability, which indicated a shift in assimilated food sources. From March to July, the isotopic carbon value for the on-bottom culture was -22.2 ‰ for the digestive gland and -21.7 ‰ for muscle and reached -19.4 ‰ and -19.7 ‰, respectively during the autumn. The spring signature was acquired during a period of low growth rate and intense reproduction activity. High mortality occurred in June and early July. Summer and early autumn growth phase was characterised by the acquisition of a more enriched signature which indicated a major contribution of benthic food sources to oyster growth. The reproduction period was characterised by considerable isotopic variability on diploid oysters compared with low variability on triploids. Moreover, the oysters on on-bottom culture exhibited an enriched signature, which indicated an higher contribution of benthic microalgae to their diet. Strong differences were found between digestive gland and muscle. The digestive gland carbon signature reflected the available food source signatures without fractionation. Carbon isotopic fractionation between muscle and digestive gland was ca. 1-1.5 ‰ for oysters in intertidal conditions and seems to be higher ca. 2.5 ‰ in oyster ponds. Nitrogen isotopic fractionation was ca. 3 ‰ whatever the rearing conditions and trophic food sources.

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Isotopic assessment of the hydrological importance of fog deposition on snow tussocks on the upland rangelands of southern New Zealand.

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The stable isotopic composition of rain, fog, and groundwater was determined for three sites on the Otago upland snow tussock lands of south-eastern South Island, New Zealand for the snow-free period of November to June in 1996-1998. The rain and fog were collected concurrently, but the rain was consistently isotopically more depleted than the fog. The compositions of the rain form a line of the equation $\delta D = 8.31\delta\ ^{18}O + 13.6$, which is similar to the line of $\delta D = 8.11\delta\ ^{18}O + 13.6$ formed by fog. The groundwater has compositions usually between those of the fog and the rain, shows no isotopic evidence of evaporation, and is assumed to be a mixture of the two in sub-equal proportions. This pattern has been observed previously only in forested regions where the tree crowns act as collection mechanisms for fog water. On the Otago uplands, the only fog collection mechanism is interception gains through fog deposition on the relatively short (0.8-1.2 m) fine wispy foliage of the native tall snow tussock grasses (*Chionochloa* spp.). These results support the earlier but debated claims that interception of fog by the foliage of the dominant tall snow tussock grasses makes a substantial contribution to the substantial water yield from these uplands.

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Swallows as sentinels of environmental pollution of the Rio Grande: predicting trends of DDE and Se with stable isotopes

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Cave (*Petrochelidon fulva*) and cliff (*P. pyrrhonota*) swallows nest in numerous colonies throughout the Texas portion of the Rio Grande, along the U.S. border with Mexico. To investigate the potential use of stable isotopes ($\delta\ ^{15}N$ and $\delta\ ^{13}C$) for predicting concentrations of DDE and Se in birds from the Rio Grande, we collected swallows during 1999 and 2000 from eight locations from Brownsville to El Paso, Texas.

We analyzed stable isotopes of nitrogen and carbon in liver and muscle, and DDE and Se in swallow carcasses. We found significant differences among locations in $\delta\ ^{15}N$ and $\delta\ ^{13}C$ values in liver and muscle of both species. Cave swallows from three locations in the lower Rio Grande Valley were more enriched in $\delta\ ^{15}N$ than swallows from other sites. $\delta\ ^{15}N$ was more enriched in liver than in muscle of both species by an average of 1.34 ‰, whereas $\delta\ ^{13}C$ was 0.145 ‰ less depleted in muscle than in liver. In general, swallows nesting in more northern latitudes along the Rio Grande had lower $\delta\ ^{15}N$ and $\delta\ ^{13}C$ values than those nesting farther south.

Concentrations of DDE were significantly greater in swallows from El Paso than in those from most locations. All swallows (N=21) from El Paso, Llano Grande and Pharr had DDE concentrations ≥ 3 μg/g ww, a value three times greater than the estimated threshold value in diet of some raptors at which some reproductive failures could be observed. Concentrations of Se also were significantly greater in El Paso and Del Rio than at other locations. Most Se concentrations were not of concern for direct effects on birds or their predators.

$\delta\ ^{15}N$ in liver and muscle of swallows was significantly and positively correlated with DDE ($P < 0.01$, $r^2=0.16$ and 0.37 for liver and muscle, respectively). Also $\delta\ ^{13}C$ in liver was significantly and positively correlated with DDE ($P < 0.05$, $r^2=0.13$). Concentrations of Se were negatively and highly significantly correlated with $\delta\ ^{15}N$ ($P < 0.01$, $r^2=0.17$) and $\delta\ ^{13}C$ ($P < 0.001$, $r^2=0.35$) in liver. The significant correlations between $\delta\ ^{15}N$ and $\delta\ ^{13}C$ and DDE and Se suggest that swallows could be good long-time indicators of geographic trends of p,p'-DDE and Se in the Rio Grande.

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Statistical estimates of variance for ^{15}N isotope dilution measurements of gross rates of nitrogen cycle processes

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It has been fifty years since Kirkham and Bartholmew (1954) presented the conceptual framework and derived the mathematical equations that formed the basis of the now commonly employed method of ^{15}N isotope dilution. Although many advances in methodology and analysis have been made in the intervening years, researchers still face several questions in calculating gross rates. We provide guidelines for the choice and proper use of the different gross rate equations and a means of estimating the variance associated with using these equations.

^{15}N isotope dilution experiments require the destructive sampling of soils at two, or more, time points. The requirement for destructive sampling means that paired (i.e., before and after) samples are not available. The mathematical equations for calculating gross rates must, therefore, use mean values of pool sizes and isotopic abundances for the two time points. These means, of course, have variances associated with them, but this information is typically not utilized, limiting the ability to make statistical comparisons of gross rates. We provide a comparison of three methods for estimating the variances of gross rates using modeled data: (i) all possible pairs, (ii) bootstrapping, and (iii) error propagation. Our results show that all approaches give similar estimates of the variances of gross production rates but the error propagation method gives larger estimates of the variance of gross consumption rates. An advantage of the error propagation method, however, is that simple algebraic formulae can be used to calculate the variance.

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The shifting baseline of northern fur seal (*Callorhinus ursinus*) ecology in the northeast Pacific Ocean

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Archaeofaunal analyses suggest northern fur seals (NFS; *Callorhinus ursinus*) are the dominant pinniped species in late Holocene coastal archaeological sites from southern CA to British Colombia (BC), whereas today, NFS breed almost exclusively on offshore islands at high latitudes (>50ºN). We've used natural variations in stable isotopes to investigate feeding and migratory behavior of Holocene NFS and constructed modern bone regressions to accurately age NFS individuals, allowing us to create young-of-the-year (YOY) harvest profiles for mid-latitude archaeological sites. Isotopic work revealed two conclusions about NFS. First, $\delta^{13}C$ values for NFS are always more negative than values for associated harbor seals, showing that NFS foraged offshore across their entire range. Second, NFS cluster into three subgroups: a southern group with high values, a northern group with intermediate values, and a western Aleutian group with low values. NFS harvest profiles from mid-latitude sites (30-50°N) sites contain a surprising number of YOY specimens, especially pre-weaned pups, confirming the presence of local rookeries. Overall, the data imply that there were several NFS rookeries along the mid-latitude coast of North America in the late Holocene, and that local extirpation of these breeding populations was asynchronous across this range.

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Determinants of Red Knot fitness; a stable isotope investigation

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The spring migration of shorebirds through Delaware Bay is one of the most spectacular migration sights. Hundreds of thousands of shorebirds from wintering sites further south pass through Delaware Bay each year to feed on eggs laid by spawning Horseshoe Crabs *Limulus polyphemus*. The Red Knot *Calidris canutus* is one of the flagship species among long-distance shorebirds as some birds travel the length of the American continents each year. In the Nearctic, populations winter in the south-eastern United States (*C.c. roselaari*), in northern Brazil (*C.c. rufa*) and perhaps the majority (also *C.c. rufa*) migrate 30,000 km annually between over-wintering sites in Tierra del Fuego and breeding sites in the Canadian Arctic (Harrington 2001). Delaware Bay is the last major stopover site for several populations of Red Knot, and after fattening up on Horseshoe Crab eggs for two weeks fly non-stop to the breeding areas.

To forecast the possible impacts of global climate change, reduction in food supplies, habitat destruction and disturbance at migratory stopover sites, we need to understand the demographic impacts of these factors on the different populations as these are likely to vary. Using stable isotopes we will identify the wintering area of 500 of these individual birds each year over three field seasons enabling key parameters of each wintering population to be assessed through resighting these birds in Delaware Bay and other places in the flyway. Our objective is to determine the fitness consequences, in terms of survival, of multiple wintering and migratory strategies of Red Knots, i.e. (a) occupying different wintering areas, (b) timing of arrival at migratory stopover sites and (c) mass gain in the last major stopover site (Delaware Bay), before reaching the Arctic breeding areas. As a precursor to the first objective, a pilot run of 20 Red Knot feathers were analysed for $\delta^{13}C$, $\delta^{15}N$ $\delta^{2}H$ and $\delta^{18}O$ to determine whether stable isotope analysis would be useful in identifying the populations of Red Knot that pass through the Bay in spring. Preliminary data revealed that $\delta^{13}C$ and $\delta^{15}N$ appear to be most useful to delineate the wintering areas: birds moulting in Florida were clearly separated from birds moulting at two sites in Tierra del Fuego. All three sites could be identified in feathers obtained in the Bay during spring migration. An additional group of birds, of unknown provenance, was distinguished and efforts are underway to identify these.

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Traffic exhaust related alteration of $^{13}C/^{12}C$ ratios in tree leaves

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Plants are a temporal sink for urban pollutants. Traffic is a main pollution source in cities not only for toxic elements such as Ni, Pb or Cd but also for CO_2. It is widely accepted today that the increase of CO_2 content in the atmosphere contributes essentially to the global climate change. Ratios of stable carbon isotopes played a key role in identifying anthropogenic combustion processes as an important source of atmospheric CO_2. The combustion of fossil and recent organic fuels decreased the natural $\delta^{13}C$-value of atmospheric CO_2 from about -6‰ to -8‰. Along streets, atmospheric CO_2 is even more depleted in ^{12}C. For example, in streets of Paris values as low as -15‰ were registered(1). Plants act as a temporal sink for CO_2 by taking up CO_2 from atmosphere to produce organic matter. Depending on their assimilation pathways plants fractionate C-isotopes to different degrees which leads to specific ranges for $\delta^{13}C$-values of organic matter. First results indicate that the isotopic composition of tree leaves along streets is influenced by the isotopic composition of vehicle emitted CO_2(2). Results reported here derive from an investigation of the alteration of carbon isotope ratios and changes in trace element patterns in the leaves of a deciduous tree in consequence to vehicle emissions.

A typical street tree of the city of Karlsruhe, *Acer platanoides*, was chosen for investigation. The selected tree grows near to one of the most frequented streets of Karlsruhe. Leaves from the tree crown were sampled in 2001 on May 30th (15 sites) and October 31st (28 sites). The leaves were washed with double distilled water, dried, and milled. $\delta^{13}C$-values were measured with an isotope ratio mass spectrometer coupled on-line with an element analyser. Acid digested leaves were analysed for trace elements with ICP-MS.

Samples from May 2001 show $\delta^{13}C$-values between -28.7‰ and -31.0‰ with a mean of -30.1‰, while leaves sampled in October have values between -27.9‰ and -30.7‰ (mean: -29.4‰). In May and October, the lowest values were observed in sampling sites next to the street, indicating a relatively higher share of assimilated CO_2 emitted from vehicles. Higher values occurred in leaves from the opposite site of the tree crown. The street runs east of the tree in N-S direction. The main wind direction is SW. Thus, the street runs partly in the leeside of the tree. Consequently, $\delta^{13}C$-values are higher in leaves from the windward side of the tree crown. Additionally, $\delta^{13}C$-values increase with sampling height, reflecting an increase in the share of atmospheric (background) CO_2 assimilated by the leaves. The spatial pattern of isotope ratios changes between May and October. Especially the leaves at the windward tree site show higher $\delta^{13}C$-values in October. This reflects a decreasing influence of vehicle emitted CO_2. The seasonal increase in leave density (average leave size in May was 71.2 cm² compared to 87.1 cm² in October) seems to hinder the free transport of air through the tree crown and to contribute to increased contrasts of higher and lower $\delta^{13}C$-values in windward and leeward leaves. Concentrations of some trace elements like Al, As, Cd, Pb and V increased in the leaves from May till October. The spatial distribution of the concentrations of those elements shows some relationship to traffic as well. The possibility will be discussed to use $\delta^{13}C$-values as indicator for the pollution of the vegetation with toxic trace elements by traffic via the atmospheric pathway.

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Use of stable isotope ratios to identify landfill leachate contamination in an estuarine environment: Green Island, Dunedin, New Zealand

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A study was undertaken to determine whether the isotopic ratios of C and N could be used to uniquely identify landfill leachate as a source of ecological contamination in the Kaikorai Estuary, Dunedin, New Zealand. Leachate and ground water samples were taken from the adjacent Green Island Landfill, and surface water samples were collected over an eight-month period from the stream and estuary, upstream and downstream of the landfill. Samples were analysed for $\delta^{13}C$ – DIC (dissolved inorganic carbon), $\delta^{15}N$ – NO_3^-, $\delta^{15}N$ – NH_4^+, and particulate organic matter (POM) $\delta^{13}C$ and $\delta^{15}N$. Plant material collected along the Kaikorai Stream was analysed for POM $\delta^{13}C$ and $\delta^{15}N$. Leachate was found to have a distinct isotopic signature characterised by a highly enriched $\delta^{13}C$ – DIC (as much as 16.11 ± 0.23‰) and highly enriched $\delta^{15}N$ – NH_4^+ (as much as 27.42 ± 0.90‰). Evidence of leachate contamination was found in the isotopic signatures of downstream $\delta^{13}C$ – DIC, $\delta^{15}N$ – NH_4^+, and $\delta^{13}C$ and $\delta^{15}N$ of plant material POM. Other potential sources of enriched $\delta^{13}C$ – DIC and $\delta^{15}N$ – NH_4^+ present in the study area, such as estuarine waters and livestock effluent, were eliminated on the basis of their isotopic characteristics.

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Compound-specific isotope analysis of phytol to predict $\delta^{13}C$ of bulk benthic microalgae

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Benthic microalgae (BMA) are abundant in a variety of estuarine habitats. This abundance, combined with rapid turnover rates, suggests that BMA may potentially be a significant source of nutrition for consumers. Stable isotope analysis is often used to trace carbon movement through foodwebs. In studies involving BMA, however, application of stable isotope analysis is limited by problems with the separation of biomass from sediments preventing the determination of a pure isotopic signature for BMA. Through compound-specific isotope analysis using GC-IRMS we investigated the potential for using $\delta^{13}C$ of phytol to predict $\delta^{13}C$ of the BMA total biomass from which it was extracted. Phytol is an ester-linked side chain of chlorophyll-*a* synthesised by all green photosynthetic plants. As phytol in the water column is rapidly degraded by exposure to sunlight during plant senescence, the majority of phytol within sediments is expected to originate from BMA. The relationship between $\delta^{13}C$ values of phytol and BMA total biomass was linear ($R^2 = 0.97$) with a slope of almost unity. Phytol values were depleted relative to biomass by 1‰ or less. This relationship may be applied to predict $\delta^{13}C$ values of BMA biomass in the environment using measured $\delta^{13}C$ values of phytol extracted from sediments. This is expected to enable more effective use of stable isotope analysis to ultimately evaluate the contribution of carbon from BMA to the nutrition of estuarine consumers, and hence the importance of conserving different estuarine environments.

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Nitrogen and carbon isotopic determinates of upper-level trophic structure in the pelagic eastern tropical Pacific Ocean

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The pelagic eastern tropical Pacific Ocean (ETP) is the site of a dynamic ecosystem that supports productive fisheries for tunas and other large fishes. Recent calls for a more holistic approach to fisheries management have motivated efforts to develop models of the ecosystems that underlie fisheries production (NRC 1999). Trophic mass-balance models, for example, use diet data to make continuous estimates of the trophic positions of component populations and functional groups. However, stomach contents represent only the most-recent meal, and the food of mobile, highly-active fishes in tropical habitats is digested quickly. Nitrogen stable isotope ratios ($\delta^{15}N$) offer an alternate measure of trophic position, and, together with carbon stable isotope ratios ($\delta^{13}C$), improve on diet analysis by integrating dietary variability over time.

Samples of stomach contents, dorsal muscle, and liver were taken from pelagic predatory fishes and mammals captured by tuna purse-seine vessels in the ETP during 1992-1994. We measured $\delta^{13}C$ and $\delta^{15}N$ of the muscle and liver in 27 yellowfin tuna (*Thunnus albacares*), 7 bigeye tuna (*T. obesus*), 6 skipjack tuna (*Katsuwonus pelamis*), 6 spotted dolphins (*Stenella attenuata*), 5 spinner dolphins (*S. longirostris*), 1 black marlin (*Makaira indica*), 3 silky sharks (*Carcharhinus falciformis*), 11 oceanic whitetip sharks (*C. longimanus*), 4 dolphinfish (*Coryphaena hippurus*), 4 wahoo (*Acanthocybium solandri*), 6 frigate or bullet tuna (*Auxis* spp.), and 6 rainbow runners (*Elagatis bippinulata*). The $\delta^{15}N$ and $\delta^{13}C$ of liver are assumed to reflect the diet integrated over shorter, more recent intervals than those of muscle due to faster turnover rates in liver.

We compared estimated trophic positions derived from $\delta^{15}N$ with estimates derived from 7,900 stomach samples from the same suite of predators taken during the same time period (Olson and Watters 2003). We examined the variability of the $\delta^{15}N$ and $\delta^{13}C$ measurements in relation to sampling location (productivity gradients, oxygen minimum zone), predator size and life history, and epipelagic *versus* mesopelagic food sources.

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Twentieth Century hydrogen and oxygen isotopic fluctuations from a coastal Antarctic ice core, Victoria Land, Antarctica

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We present new hydrogen and oxygen stable isotope data for the upper 50 metres of an ice core recovered from Victoria Lower Glacier (VLG) in the Dry Valleys region of Antarctica. This small coastal valley glacier is independent of the East Antarctic Ice Sheet, proximal to seasonally-open water in western Ross Sea, and affords a unique opportunity to investigate tropospheric climatic variability during the Twentieth Century on inter-annual to decadal timescales. Moreover, as a consequence of the relatively low albedo of the surrounding Dry Valleys, which amplifies solar radiation, the VLG ice core is an extremely sensitive recorder of regional climatic change. From our results we address the role of radiative forcing mechanisms (anthropogenic greenhouse gases, solar irradiance, and volcanic sulfate emissions) and atmospheric and oceanic circulation (El Nino-Southern Oscillation, Antarctic Oscillation, and Antarctic Circumpolar Wave) in modulating regional climate dynamics. A number of these processes behave quasi-periodically with characteristic frequencies around the decadal/intradecadal-scale, and we employ spectral analyses to identify these frequency components within the isotope time series.

Approximately 1900 hydrogen and oxygen measurement were made on the upper 50 metres of the ice core providing a resolution between 0.4 and 1 year. Our age model, based on decompaction modeling combined with the recognition of annual cycles, is calibrated by tritium dating. The isotopic record exhibits pronounced oscillations throughout the early and mid- to late-Twentieth century with a relatively stable period dominating the interval in between. The largest isotopic oscillation in our data occurs in the early Twentieth Century. It is characterized by a rapid depletion in heavy isotopes (~60 $^{0}/_{00}$ in $\delta^{2}H$ and ~6 $^{0}/_{00}$ in $\delta^{18}O$) over period of one to two years. Hydrogen isotope data from the Talos Dome Ice Core (Stenni et al., 2002), located approximately 400 km northward of the Victoria Lower Glacier site, display a coincident depletion in heavy isotopes with a virtually identical signature. We interpret this as indicating a cooling, driven by an increase in stratospheric volcanic emissions, which simultaneously affected the central and northern regions of Victoria Land.

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Seasonal variations in fish $\delta^{13}C$ and $\delta^{15}N$ in two African reservoirs, Sélingué and Manantali (Mali) : trophic links modifications with water level.

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High biodiversity and frequent diet plasticity of fish are responsible for intricate trophic relationships in tropical freshwater ecosystems. Moreover, those ecosystems are noticeable by high hydrologic variations, that may model trophic relationships. Consequently, tropical freshwater food webs are thought to be complex and strongly variable with seasonality. They are actually poorly known because difficult to study from classical techniques as gut contents. However, stable isotope studies of trophic relationships offer a new field of investigation for complex food webs. As far as we know, there is no study concerning West African lakes and reservoirs although some knowledge about food webs and carbon sources for fish are required for sustainable management strategies of those ecosystems, especially in regions where fish is the main animal protein source for local populations.

Our research took place in the Sélingué and Manantali reservoirs in Mali. In each reservoir, ten fish species, selected according to their abundances in local fisheries species, were sampled in low and high water period in order to measure their isotopic compositions. Potential carbon sources were also sampled and their isotopic composition determined. In order to detect trophic modifications, shifts in the mean isotopic positions and variations in the isotopic dispersion of fish species between low and high water periods were pointed out, using non parametrical methods. As a result, trophic relationships were similar between reservoirs but underwent marked modifications between low and high water periods. Both carbon sources and food web structure were altered with hydrogical seasonality. Although the fish species were shown to exploit mainly pelagic carbon at low water period, deep methanogenetic carbon (in the Sélingué reservoir) and C_4-plants derived carbon were used as alternative carbon sources by some species. The flooding of the grassy fringes at high water season provided a large support for littoral periphytic colonization. At high water period, some fish species shifted towards the newly formed periphytic carbon, at the detriment of the pelagic or allochthonous one. Piscivorous fishes, however, may not benefit from the diversification of the available food sources.

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Seasonal variability of oxygen stable isotopes across the Gulf of Mexico's hypoxic zone

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The northern Gulf of Mexico is site of the largest coastal hypoxic (<2 mg O_2 L^{-1}) area in the western Atlantic Ocean. The main causes responsible for the development of hypoxia are linked to nutrient inputs from the Mississippi River. Large nutrient flux strongly enhances shelf primary productivity, leading to increased algal biomass that sinks to bottom waters. Decomposition of this material in the lower stratified water column and in bottom sediments increases oxygen consumption and decreases oxygen concentrations. We are developing two oxygen budgets, based separately on oxygen concentrations and on stable isotopes, to compare and cross-check results, with the intent to combine the budgets into box model simulations.

Atmospheric oxygen has a $\delta^{18}O$ value of 23.5 ‰, oxygen entering the water column leads to dissolved oxygen with a $\delta^{18}O$ value of 24.2 ‰, due to an equilibrium isotope effect. However, photosynthesis and respiration change oxygen isotopes. Oxygen derived from primary production has an isotopic value of 0 ‰, and will lower the $\delta^{18}O$ of dissolved oxygen in the water. Respiration on the other hand, can significantly increase $\delta^{18}O$ values to up to 50 ‰. From July 2002 to July 2003 we participated in monthly monitoring cruises, right off the Mississippi River Delta. Water samples were collected at 4 stations (10 – 30 m deep) at 5 m intervals. Using headspace equilibration technique, samples were analyzed for oxygen concentration and $\delta^{18}O$ values. Monthly sampling showed a strong seasonal variability of oxygen concentrations and isotopic signatures throughout the development and dissipation of hypoxia. For example, in October, due to wintertime conditions, which favor relatively low primary productivity and high aeration of the shallow (>100 m) Gulf waters, oxygen isotopes across the entire water column were very close to 24.2 ‰, the value for air-equilibrated seawater. On the other hand, in July, summertime development of surface phytoplankton blooms and accompanying bottom water hypoxia resulted in different oxygen isotope patterns across the different depths sampled. Since photosynthesis produces low $\delta^{18}O$ oxygen (near -2 ‰ in this system) that decreases oxygen isotopes values, surface waters showed reduced $\delta^{18}O$ values, between 16 – 23 ‰. Furthermore, intense respiration in bottom waters, lead to hypoxic waters with $\delta^{18}O$ values markedly higher (25 – 40 ‰) than the 24.2 ‰ air-equilibrated value.

During the period of summer stratification, oxygen dynamics were predominantly controlled by biological processes. Nevertheless, physical mixing was more important during the fall and winter months. Using stable isotopes, we can better understand the underlying physical and biological processes that control oxygen dynamics, its sources and sinks. In bottom waters we can partition oxygen dynamics between two sinks, benthic and water column respiration, while in surface waters we can discriminate among two sources, increased primary production and wave action. Intermediate waters depend on mixing dynamics of surface and bottom waters.

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Changes in carbon and nitrogen isotope ratios in Steller sea lion (*Eumetopias jubatus*) vibrissae as a biochemical marker for weaning

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Past and current declines in the western Steller sea lion (*Eumetopias jubatus*) population in Alaska have been hypothesized to be a result of low juvenile survival, potentially related to inadequate quality or quantity of prey available in this region. This decrease in survival may be due to the inability of adult females to deliver adequate milk energy to their large, late lactation pups, or the inability of newly weaned pups or juvenile sea lions to gain sufficient resources while foraging. If adult females are unable to support the energetic demands of their late lactation pups due to low food availability, one would expect those pups to be forced to supplement their milk diet with intake of prey, or to undergo complete weaning at an earlier age. There appears to be a wide range of ages at which weaning occurs in this species, ranging from approximately 9 to >36 months based on behavioral observations. This study uses $\delta^{13}C$ and $\delta^{15}N$ signatures measured in Steller sea lion vibrissae to monitor changes in diet associated with weaning and to determine if there is a difference in timing of these diet changes between regions of population growth and decline. Young Steller sea lions between the ages of 1 and 27 months were captured in the stable eastern population (Southeast Alaska, n= 18) and the declining western population (Prince William Sound, n= 15; Gulf of Alaska, n= 11; and Aleutian Islands, n= 3) using an underwater capture technique. The longest vibrissae was pulled from the left cheek and $\delta^{13}C$ and $\delta^{15}N$ values were measured in 3.0 to 0.1 cm (2.00-2.40 mg) sections along the length of the vibrissae.

Steller sea lion pups are born with developed vibrissae, thus the tip of the vibrissae represents tissue grown in-utero. The vibrissae exhibited a gradual decrease of both $\delta^{13}C$ and $\delta^{15}N$ of 1.5$^{o}/_{oo}$ and 1.6$^{o}/_{oo}$, respectively, with maximum depletion occurring at approximately 5.6 cm from the tip. This pattern was then reversed in most vibrissae with gradual increases towards the root in both $\delta^{13}C$ and $\delta^{15}N$ (2.0$^{o}/_{oo}$ and 2.9 $^{o}/_{oo}$ respectively), suggesting the occurrence of nursing. In all pups less than 12 months of age, $\delta^{13}C$ and $\delta^{15}N$ values increased until the root insertion or remained stable after peak $\delta^{15}N$ values of 20.0 ± 0.1 $^{o}/_{oo}$ were reached. No significant differences were seen between populations. The equilibrium $\delta^{15}N$ value (20.0‰) represents a nursing signature that is one trophic level higher than the mean $\delta^{15}N$ value of milk (15.3 ± 1.1 $^{o}/_{oo}$). In juveniles (14 to 27 months of age) from both populations peak $\delta^{15}N$ values were followed by decreases of 2.3 to 5.0 $^{o}/_{oo,}$ suggesting the ingestion of prey which isotopically represents a lower trophic level than ingested milk. Subsequent $\delta^{15}N$ increases and decreases in the older animals may reflect age related or seasonal changes in the diet.

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How organic are your vegetables? Can we tell?

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Adding value to food products is an important niche market for many producers. The organic produce market is based on an honesty system where the only fertilisers used are purely organic based. But could the tomatoes you are eating have sewage sludge used as the organic fertiliser, or has a handful of inorganic fertiliser been added to give the vegetables the boost they needed before harvesting?

We have taken a variety of fruit and vegetables, both certified organic and non-certified to characterise the carbon and nitrogen signatures for authenticity. In theory it is then possible to detect 'cheating' where non-traditional fertilisers are being used instead of the recommended products. Large isotopic signature differences between inorganic (0 per mil) and organic (up to 30 per mil) nitrogen pathways, combined with precise nitrogen detection levels (as small as 0.2 per mil) can be traced.

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Isotopic effects of changing landuse on the Pahatanui Estuary and Porirua Harbour, Wellington, New Zealand

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A comprehensive ecological study is currently underway in conjunction with Regional Council authorities on the Pahatanui Estuary and adjacent Porirua Harbour. The study will monitor the health and effects of changing land use on the flora and fauna, as well as understanding the anthropogenic contributions to the sediments from urban and rural run-off. During the next year 2004-05, several studies will co-ordinate to form a larger integral part of understanding source contributions from the past and present, in order to monitor and predict future changes within the area. These studies include carbon and nitrogen isotopes of sediments, flora and fauna; trace and heavy metal studies of the sediments and shellfish; and numerous shallow cores taken from within the harbour and estuary to study sedimentation accumulation rates.

Previous marine monitoring studies by Rogers (1999, 2003) and Kurata et al. (2001) have isotopically investigated nutrient sources for flora and fauna in contaminated and non-contaminated sites. This presentation will present the initial findings of fieldwork conducted in January 2004, and forms the basis of the long term study which is expected to span up to 20 years.

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New Zealand unifloral honey and isotopic tests for adulteration : A sticky situation

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Isotopic analyses of 45 honey samples from Waikato University's honey databank and supplementary pollen work has been done at the GNS Rafter Laboratory. The purpose of the research was to determine if the honeys were unifloral and if the honeys showed adulteration of sugars based on isotopic analyses of the whole honey and the protein of the honey, using the AOAC sugar test (AOAC Official Method 998).

Honey is produced when the nectar from flowers of a plant is collected, modified and concentrated by bees. Honey contains about 75% of a mixture of glucose and fructose and about 2% or more sucrose. Cane sugar may be added to honey as adulterants, and particularly much cheaper HFCS (high fructose corn syrup). HFCS is a liquid sugar which can be added to honey with impunity because it cannot be easily detected reliably by conventional chemical techniques. Carbon stable isotope ratio mass spectrometry is used for detecting the presence of cane and/or corn sugars in honey.

Pollen analyses indicated that about 10% of the honeys did not qualify as unifloral. Isotopic results based on the international AOAC sugar test suggest that on the basis of comparisons between whole honey and the honey protein, 75% of the Manuka and 50% of Kanuka samples studied were adulterated with sugar. These are known to be bona-fide honeys with no adulteration, yet manuka/kanuka florals are particularly prone to failing the sugar test. If these honeys are tested based on isotopes alone, they would fail the international export requirements. All other honey types studied did not display this anomaly, so if a failed sugar test arose, this could be attributed to genuine adulteration.

The ranges of C_4 content vary between honeys and within species of honey. The main factor influencing the C_4 content appears to be contributions of manuka pollen of similar (ie kanuka, rata etc). Virtually all the manuka samples fail the AOC test for adulterated honey, although it is highly unlikely that these honeys have been adulterated. It is unlikely with the range of locations of the manuka honeys that 'bee feeding' (the practise of placing sugar and water mixtures near crops to encourage the presence of bees for pollination) has taken place, and based on similar unpublished data (Rogers and Lyon, 2001-2002) manuka honey has a strong tendance to fail the AOC honey adulteration test.

The tendance for manuka honey in particular to fail suggests that there is a photosynthetic pathway which fractionates the carbon located in the honey protein relative to the whole honey, thus rendering it more depleted that the whole honey. This characteristic does occur from time to time in other honeys seen in this study, although in many cases, the pollen contributions suggested manuka influence on the honey. For those honeys with more than 7% C_4 content, that are not manuka influenced, it is possibly either an analytical anomaly, bee feeding or an unusual occurrence within the honey species.

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AOAC Official Method 998.12, C-4 Plant Sugars in Honey, Internal Standard Stable Carbon Isotope Ratio Method.

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Transport of marine nutrients into terrestrial ecosystems by burrowing petrels: evidence from ^{15}N in wood

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Burrow-nesting petrels (Aves: Procellariiformes) bred abundantly on islands in the southwest Pacific until predation by humans and introduced mammals began in recent prehistory and continued into the European era. Research on surviving petrel populations elsewhere has demonstrated that during their breeding seasons, petrels can make a significant contribution to the terrestrial nutrient flux by their guano and other body tissues, which are derived from the marine food chain. Nitrogen sourced from the marine food chain has a much greater level of enrichment in ^{15}N than terrestrial sources and so can be traced in terrestrial systems. The level of ^{15}N enrichment in wood has been shown to indicate the historical presence of marine nutrient inputs mediated by salmon-bear interactions and burrow-nesting auks (Aves: Alcidae). We report the presence of marine levels of ^{15}N enrichment in wood from a pohutukawa (*Metrosideros excelsa*) tree growing within a multi-species petrel colony on Aorangi Island, Poor Knights group, New Zealand, and preliminary results of a search for stable isotopic evidence in wood for known historical extinctions of petrels on Norfolk Island and Raoul Island (Kermadec group).

Origin of carbon and nitrogen in the diet of suspension-feeding bivalve *Cerastoderma glaucum* (L.) in a shallow habitat (southern Mediterranean): a stable isotope study

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We estimated the contribution of natural food sources to the assimilated fraction of the cockle *Cerastoderma edule* (L.) living in a saltworks of southern Mediterranean (Stagnone di Marsala, Italy). Adults specimens of *C. glaucum* and all organic sources (particulate [POM] and sedimentary [SOM] organic matter, seagrass, macroalgae and bivalve biodeposition material) potentially available for it were collected in the field season 1999-2000 and analysed for their natural stable isotopic carbon ($\delta^{13}C$) and nitrogen compositions ($\delta^{15}N$). Pooled POM and SOM showed annual mean of $\delta^{13}C$ –16.6 ± 0.8‰ and $\delta^{15}N$ of 4.9 ± 0.7‰, seagrass detritus (*Cymodocea nodosa*) –8.4 ± 1.5‰ and 6.4 ± 1.4‰ respectively for $\delta^{13}C$ and $\delta^{15}N$; macroalgae –18.8± 0.8‰ and 7.3 ± 0.9‰ respectively for $\delta^{13}C$ and $\delta^{15}N$ and lastly total biodeposition material (bivalve faecal and pseudofaecal organic matter) –18 ± 1.1 and 3.0 ± ‰ 1.3, respectively for $\delta^{13}C$ and $\delta^{15}N$. Isotopic composition of the assimilated fraction of *C. glaucum* was on annual mean -14.7 ± 0.6‰ and 8.1 ± 1.2 ‰ respectively for carbon and nitrogen. Mixing models allowed us to calculate the percentage contribution of each potential source to the *C. glaucum* diet. Organic matter from biodeposition material represented on annual basis the first item in its diet with about 33% of the total, POM/SOM as an expression of pelagic and benthic microalgae participated for about 31%, whereas organic matter coming from *C. nodosa* and macroalgae represented each about 18% in its assimilated. Saltworks cockles experienced a highly variable trophic environment throughout the year in which several organic sources seasonally participated to its diet both of primary and secondary origins. Results from the present study show that *C. glaucum* living in an organic rich environment responde to the saltworks hyper-trophic conditions and is able to maintain stable populations over time.

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Use of stable isotopes ($\delta^{13}C$ and $\delta^{15}N$) for detecting the dispersion effect of fish farming waste: a study case from southern Tyrrhenian Sea (MED)

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We used stable isotopes ($\delta^{13}C$ and $\delta^{15}N$) to detect waste carbon and nitrogen coming from fish farming activities in southern Tyrrhenian Sea (Mediterranean). The study was carried out in two areas off the coasts of Sicily seasonally constrained by constant terrigenous-continental inputs coming from nearby streams. In all areas sediments are generally unvegetated. The hydrodynamic regime of the two cage locations was characterised by different mean velocities: the first (Gulf of Castellammare) was characterised by low velocities (12.0 ± 7.5 cm s^{-1}), while the second (Capo D'Orlando Bay) by very high water currents (41.4 ± 18.4 cm s^{-1}). Water columns and sediments in all locations were almost oligotrophic according to a typical Mediterranean trophodynamics. In each area, a marine fish farm was positioned within two km off the coast and moored on the bottom at about 26-30 m of depth. Farm fish cages were filled with specimens of *Dicentrarchus labrax* and *Sparus aurata*. Samples of particulate (POM) and sedimentary (SOM) organic matter were collected in sampling sites located along the main axis of water current at different distances apart cages downstream. Water and sediments as well as fish faeces and pellets were analyzed to measure their carbon and nitrogen isotope ratios.

In the Gulf of Castellammare significant differences (ANOVA, $p<0.05$) were detected in POM carbon and nitrogen values between 0 m sites and 500 m sites, 500 m and 1000 m and 0 m and 1000 sites. Mixing model outcomes showed a decreasing contribution of waste signals to POM moving from 0 m sites to 1000 sites. In the SOM, nitrogen gradient was similar to that of POM while the carbon did not show difference among distance.

In the Capo D'Orlando Bay, $\delta^{13}C_{POM}$ values were constantly similar in all area (ANOVA, $p>0.05$), while $\delta^{15}N_{POM}$ showed a clear significant enriching gradient (ANOVA, $p<0.05$) moving from cages ($\delta^{15}N_{POM}$ = 1.8 ‰) toward the farther sites ($\delta^{15}N_{POM}$ = 4.6 ‰). The contribution of total waste increased significantly from cages to 1000 m sites. $\delta^{13}C_{SOM}$ values showed significant differences among distances as also $\delta^{15}N_{SOM,}$ which, as a main difference, showed a well-defined enriching gradient moving from cages. The waste contribution to SOM increased moving from cages to farther sites. Natural isotopic composition seems to be a good tool to trace organic matter in organic enriched marine ecosystems. They revealed a good level of sensitivity, accuracy and reliability to trace signals of allochthonous over natural inputs not to mention the "spatial impact threshold" in fish farming. Finally, in our case, the stable isotopic tool individuated effectively the contribution of different organic sources in organic enriched marine environments and did not fail in ecological situations whereas some end-members, considered in the mixing model, partially overlapped.

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Isotope studies to the sorption behaviour of atmospheric sulfate in humus layers of Scots pine ecosystems

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The results of stable isotope data ($\delta^{34}S$ and $\delta^{18}O$ values of sulfate) from long-term studies in formerly polluted Scots pine ecosystems in eastern Germany have shown that atmospheric SO_4^{2-} is retained in humus layers. Sulfate accumulation is increased when organic surface layers are enriched with base-rich material in the form of alkaline dust and high concentrations of Al or Fe. Both elements probably form with organic matter metal-humus complexes, which serve as active binding sites for atmospheric SO_4^{2-}. Furthermore, the $\delta^{34}S$ values of H_2O-soluble SO_4^{2-} in all humus layers were similar to the mean $\delta^{34}S$ values of throughfall at the sites. A depletion of ^{34}S in SO_4^{2-} was not observed. This indicates that the mineralization of organic sulfur is not a potential source for the inorganic S pool in humus layers. On the other hand, the $\delta^{18}O$ values of H_2O-soluble SO_4^{2-} in the humus layer had been depleted by several per mil, indicating that a considerable proportion of atmospheric SO_4^{2-} was immobilized by microorganisms. However, the $\delta^{18}O$ values of SO_4^{2-} in humus layers and in pine needles have significantly increased between 1993 and 2001, suggesting that Scots pines take up more and more sulfate with isotopically positive $\delta^{18}O$ values. Therefore, sorption/desorption and not SO_4^{2-} immobilization by microorganisms is probably the preferred process in the humus layers. It seems to be that atmospheric SO_4^{2-} was reversibly bound in the humus layer at times of higher SO_4^{2-} depositions and will now be increasingly released under conditions of decreasing atmospheric S inputs.

In order to examine the above described hypothesis, the sorption capacity for SO_4^{2-} in humus samples from the investigation sites was determined. The sorption/desorption isotherms were determined experimentally in batch experiments. Additionally, the natural isotope variations of sulfur and oxygen in SO_4^{2-} were analysed as an indication of the sorption of added SO_4^{2-}. The patterns of $\delta^{34}S$ and $\delta^{18}O$ values for SO_4^{2-} in all equilibrium solutions are consistent with findings from sorption/desorption isotherms, which show a close relationship between the SO_4^{2-} concentration in soil solutions and the amount of SO_4^{2-} sorbed onto the humus layer matrix. Furthermore, it can be confirmed that a SO_4^{2-} sorption potential exists in humus samples from the sites. Thus, SO_4^{2-} sorption is a concentration-dependent process, which is influenced by the concentration of SO_4^{2-} in throughfall at which the organic surface layer was previously equilibrated. The isotherms indicate that SO_4^{2-} is reversibly bound in the organic surface layer, as long as soil solution concentrations remain above 38 to 45 mg SO_4^{2-} L^{-1}. In consequence, the stored atmospheric SO_4^{2-} in humus layers being released since 1993, because the sulfate concentration in throughfall has remained under the limit of 38 mg SO_4^{2-} L^{-1} at all sites. Finally, the batch experiments have shown that the concentrations of soluble Al, Fe and organic carbon decreased with increasing sulfate sorption. These results substantiate the important role of active Al and Fe. Both elements can stabilise the reactive surface of metal-humus complexes and have a positive influence on the SO_4^{2-} sorption.

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Stable isotope comparisons of Alaska Steller sea lion (*Eumetopias jubatus*) milk, serum, and vibrissae: investigating nutritional independence and trophic dynamics

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Nutritional stress has been hypothesized as a likely cause behind the decline of the endangered western stock of Steller sea lions (SSL's). The eastern stock is stable. The potential effects of nutritional stress may be expressed as shorter nursing times, changes in weaning time, and related foraging shifts by young. It is important to first determine how isotopic signatures of pup tissues reflect those of ingested milk before determining if differences can be identified between nursing and weaned animals. If such characteristic isotopic signatures can be identified, then comparisons of the declining and stable populations of SSL's can be made to determine possible differences in weaning times. Finally, trophic relationships within and between populations can be investigated by comparing SSL stable isotope values with those reported in the literature for forage fish (Hobson et. al. 1997 and Hirons 2001).

The use of stable isotope data to identify weaning of SSL's first requires that distinct isotopic differences exist between nursing pups and pups that ingest live prey. This study compared $\delta^{13}C$ and $\delta^{15}N$ in matching serum and vibrissae roots to isotope ratios in ingested milk (n=52). Isotopic signatures from free-ranging SSL pups (1-11 months) and yearlings (14-22 months) captured in the eastern (southeast Alaska) and western (Prince William Sound and western Aleutians) stocks were compared to determine if weaning was earlier in one region versus another. Stable isotope values should change with diet such that milk represents a higher trophic level diet than forage fish due to metabolic fractionation by the mother. Mean milk $\delta^{13}C$ and $\delta^{15}N$ values were similar between populations, although values from the eastern population showed a change with age of the pup ($p=0.001$ and $p=0.005$, respectively). Mean $\delta^{13}C$ and $\delta^{15}N$ enrichment between tissue samples [milk to serum (5.0±0.6‰ and 2.0±0.6‰, respectively) and serum to vibrissae (2.4±0.4‰ and 0.2±0.3‰, respectively)] was significantly different in animals within each area (paired T-test $p<0.05$), however those enrichments did not vary between animals in different regions (repeated measures $p>0.05$).

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Environmental and short-term climatic influences on red deer (*Cervus elaphus*) isotope values: Implications for studies of trophic level ecology, palaeodiet and palaeoenvironment

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Carbon and nitrogen stable isotope ratios of red deer (*Cervus elaphus)* bone collagen (154 individuals) and hair keratin (628 individuals) were analysed to determine the potential of isotopic analysis of faunal remains as a palaeoenvironmental and palaeoclimatic proxy. Hair samples from a single population on the Isle of Rum were sampled over the last 27 years. Hair isotope values were compared to individual specific data such as age and sex, and to weather records. Age of individual, sex, along with temperature and rainfall of the 12 months prior to sampling were shown to be significant factors affecting the deer hair isotope values. It is clear that climatic variables do affect faunal isotope values, but these variations are easier to recognise in hair than bone due to the short time period it represents.

Analysis of bone collagen isotope values from 5 different deer populations occupying different habitats showed that intra-population isotopic variability does not appear to be standard for different populations. . The potential influence of several parameters on deer isotope values resulting in intra-population variability were considered (weaning, sex, age, size of area occupied and diversity of habitat, consumption of foodstuffs with marine isotopic signatures, soil development, altitude, and salinity). A weak but significant positive correlation was observed between deer age and collagen $\delta^{13}C$ values from the Isle of Rum deer populations. Significant differences were observed between the mean $\delta^{13}C$ value of each of the populations with the exception of Duror Forest and Sutherland, and between the mean $\delta^{15}N$ values with the exception of Sutherland and Slowinski National Park. Parameters that could potentially result in inter-population isotopic variability were considered including precipitation, temperature, soil acidity, canopy effect and intake of marine foodstuffs. A negative correlation was observed between population mean $\delta^{13}C$ values and site temperature.

Deer isotope values can be highly variable in some populations, whereas in others they can be extremely similar. In modern environments we can attempt to account for this variability based on our knowledge of habitat, but in an archaeological context it is difficult to infer environmental differences from the isotope values as several parameters can result in similar isotopic results. We can not determine from our data whether the isotopic variability within a population is purely due to differential dietary intake and environment, or is due to varying amount of enrichment at each trophic level? If the amount of trophic level enrichment is not standard, it would seriously complicate the mapping of modern and archaeological food webs. The intra population variability also creates problems when using stable isotope mixing models. Due to intra-population variability, significant numbers of individuals from each species are required for modern food web studies, for palaeodietary baseline data, and for palaeoecological studies.

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Experimental improvements in oxygen and hydrogen pyrolysis for stable isotopic analysis

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There are perhaps four main places where the precision and accuracy of continuous-flow isotopic analyses of oxygen and hydrogen in organic matter could be improved: the supplied gases, the pyrolysis, the chromatography and the mass spectrometry. We have not examined mass spectrometric problems as they are specific to machines and locations. We do not feel that we can greatly improve our gas chromatography, although other workers have attempted to do so, for example Paul Brooks at Berkeley back-flushes between samples.

We have not attributed any problems to carrier gas impurities, although several workers have claimed to improve their results by scrubbing the helium carrier, either with a purification cartridge or by chemical means. Note that while oxygen can be removed by chemical means, that the removal of nitrogen is very difficult. The GC cannot separate a continuous background input of nitrogen, so if you are measuring CO, the atmosphere will be represented by an isobaric CO and N_2 background. We are testing an argon filled sample-dropper enclosure and helium purged double-walled reaction tubes to address this problem.

The overwhelming presence of nitrogen in the environment increases the difficulty of measuring the isotopic composition of CO, but must we measure CO? CO is the preferred output from pyrolysis as at low temperatures a mixed CO_2/CO gas is produced whilst at higher temperatures the equilibrium is strongly toward the production of pure CO: it is difficult to produce pure CO_2. Can CO be converted to another gas, for example CO_2? There are some significant advantages in using CO_2 - it is not particularly toxic (reduced risks with the reference gas supply), is not isobaric with nitrogen and can be separated from N_2 with low memory polymers such as Porapak, rather than using a molecular sieve. CO can be converted to CO_2 either by the reaction 2CO 6 CO_2 + C or CO + O 6 CO_2. The former reaction retains 100% of the original oxygen, but no rapid and repeatable mechanism has been tested by us. We will continue to examine it, perhaps trying a catalyst. CO can be converted to CO_2 by some heated oxides: e.g. copper and silver oxides. Ag_2O achieves a 100% conversion of CO to CO_2 even at low temperatures, but more than 50% of the oxygen in the product CO_2 is not from the CO! The reaction is not CO + Ag_2O to Ag + CO_2 as expected. This reaction will at least halve the analytical response to sample variation, but may still offer enough advantages.

The pyrolysis reaction is the ultimate control of the chemical and isotopic purity of the output gases, so we have been examining this in detail. Emphasis is on reducing the cost, improving precision and nullifying the memory effects. For hydrogen in particular, carbon is a major cause of system memory so we have been testing other materials (mainly metals) that may be used in a ceramic column instead of carbon. As the equilibrium between CO and CO_2 is temperature dependent, we have examined the temperature distribution within the pyrolysis furnace, as well as the influence of changing carbon availability.

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Intramolecular site preference of ^{15}N in nitrous oxide isotopomers produced by denitrifying bacteria

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The strength and variability of nitrous oxide (N_2O) emissions from soil is the least understood component in the atmospheric budget of this important greenhouse gas. Recent analytical developments (e.g. Brenninkmeier and Rockmann 1999) allow for the measurement of the intramolecular distribution of ^{15}N in N_2O, i.e. $^{14}N^{15}NO$ ($\delta^{15}N^{\alpha}$) and $^{15}N^{14}NO$ ($\delta^{15}N^{\beta}$),. Significant variability of $\delta^{15}N^{\alpha}$ and $\delta^{15}N^{\beta}$ is observed in field N_2O soil emissions and from soil cultures (e.g. Perez et al. 2001) indicating the potential of these parameters in understanding N_2O emissions. However, net N_2O production in soil is too complex to be unambiguous without the study of pure bacterial cultures.

Measurements of site specific $\delta^{15}N$ values of N_2O produced by isolated concentrated cell cultures of two common soil denitrifying bacteria from nitrate at natural ^{15}N abundance levels are presented. Pseudomonas aureofaciens (Cu-nitrite reductase) and Pseudomonas fluorescens (cytochrome nitrite reductase) cultures were carried out under both limited and excess nitrate conditions.

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Penguins in peril? Isotopic insights into diet, marine productivity and sea temperature changes

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Breeding populations of rockhopper penguins *Eudyptes chrysocome* in New Zealand's sub-Antarctic have declined dramatically over the past 60 years. For example, at Campbell Island, Cunningham & Moors (1994) estimated that between the period 1940s-1980s, rockhopper penguins suffered a 94% reduction in numbers. The reasons for this marked population crash were not clear but Cunningham & Moors (1994) suggested that changes in penguin diet, either a switch in prey species taken or a reduction in prey availability, perhaps the result of changes in sea surface temperatures, were a likely explanation. However, hypotheses involving changes in penguin diet were not tested, and sea temperature data were limited.

This poster presents isotopic data to address the following specific questions: 1. Have rockhopper penguins switched diet over the period when populations crashed? 2. Is there any evidence to indicate that there has been a shift in the level of productivity within the sub-Antarctic marine ecosystem upon which rockhopper penguins depend?

Additionally, the issue of sea temperature change is considered by presenting a time series of $\delta^{18}O$ signatures from shell carbonate of the brachiopod *Neothyris lenticularis*, and relating these data to corresponding trends in the strength of prevailing westerly winds over the sub-Antarctic region.

Finally, we comment on the current state of rockhopper penguin populations at Campbell Island with respect to their interactions with avian and mammalian predators.

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Using stable nitrogen isotopes to identify losses of nitrogen from catchments with different land-use practices

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The Rotorua Land Treatment System, a 192 ha production conifer forest, has experienced the application of over 900 metric tonnes (t) of domestic effluent-derived nitrogen (N) over the past 11 years. The effluent N has a natural $\delta^{15}N$ isotopic signature (+14.12‰), generated by the treatment process, allowing it to be traced into various components of the system.

- Forest storage accounts for 50% of the applied N with a considerable proportion of that immobilised in wood and soil.
- The wetland, although not intensively sampled, retains 115 t, (13%) of the applied N.
- Denitrification, including that occurring within the wetland, accounts for 23 t (3%) of the applied N.
- Nitrogen concentrations in the stream exiting the catchment have risen at least 10 fold over the past 11 years. Nitrogen isotope data confirm that the rise is directly attributable to effluent N. Currently 88% of NO_3-N in the stream is effluent-derived. Using current N isotope values for the stream (+12.88‰), and extrapolating over the discharge period, export of effluent N is estimated as 263 t (29%) of the applied N.
- Overall the forest and wetland ecosystem has intercepted or denitrified 65% of applied N, with 29% lost to the stream, and 50 t (5%) unaccounted for.

Here we apply stable isotope technology to a catchment exposed to domestic effluent irrigation and have generated a realistic approximation of the likely fate of the applied effluent N. Following the successful application of stable isotope technology to this catchment, this method is currently being applied to determine N export from catchments experiencing a wider range of land-use management practices within New Zealand.

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Dietary shifts in the California condor population, Pleistocene to present

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Stable isotopic compositions of feather keratin and bone collagen reveal marked changes in the diet of the California condor (*Gymnogyps californianus*) through ancient, historic and modern periods. The California condor, the largest North American terrestrial avian, has been the subject of intensive conservation efforts since the species nearly went extinct in the late twentieth century. Combined carbon and nitrogen isotope analyses of condor tissues from the Pleistocene La Brea tar pits, historic museum collections dating back to the 18th century, and modern individuals allow reconstruction of the population's diet through time. As large scavengers, the abundant marine mammal populations of the Pacific coast were a likely (though not exclusive) food source in prehistoric times. Our results suggest that California condors had adopted a lower-net-trophic-level, possibly more terrestrial, diet by the mid-19th century. This shift can be related to the industrial whaling and sealing activities in the eastern Pacific from the 1600s on. Analysis of modern condors indicates an increasing agriculturally-derived component of the population's food source. Such dietary information has important conservation ramifications, particularly as metal toxicity has been implicated in mortality of present-day California condors.

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Stable carbon isotope ratios of C_3 plants in China

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The stable carbon isotope of terrestrial plant remains in natural archives as a climate proxy is widely used for past climate reconstruction. The relationship between carbon isotope ratios of plant organic matters and climate factors for a given region forms the basis for such an endeavour and thus requires a systematic investigation. Here we report $\delta^{13}C$ ratios of leaves of more than one thousand C_3 plant samples collected from different regions in China. It is found that the mean $\delta^{13}C$ for all C_3 plant is –27.3‰. The $\delta^{13}C$ of C_3 plant shows nonlinear relationship with temperature; the $\delta^{13}C$ ratios increases from tropical zone to warm-temperate zone, then decreases to frigid-temperate zone with increasing latitude. The $\delta^{13}C$ ratios of C_3 plants are also found to increase from southeastern to northwestern regions in China with decreasing humidity, displaying a significant negative relationship between $\delta^{13}C$ ratios of C_3 plants and mean annual precipitation.

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Natural ^{15}N abundance as a tracer in waste management studies

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Of many options available to manage municipal and industrial wastes, land application of organic wastes appears to be an environmentally friendly solution. When wastes, especially those rich in N, are applied to land, nitrate is often identified as one of the potential contaminants, affecting groundwater quality. Waste derived N may also cause off-site effects, which are difficult to trace their source using conventional means of chemical analyses.

Natural abundance of ^{15}N can be used as a technique to trace the fate of N in a ecosystem. Biological treatment of wastewater can result in the enrichment of ^{15}N in the effluent and biosolids. For example, significant ^{15}N enrichment has been found in municipal sewage effluent ($\delta^{15}N$ +13‰), municipal biosolids ($\delta^{15}N$ +4 to +13‰), dairy and meat processing wastewater sludge ($\delta^{15}N$ +7 to +14‰), and other wastes that are commonly applied to land. In contrast, N deficient coniferous forest soils are generally $\delta^{15}N$ depleted. This provides a good opportunity to trace the waste-derived N by using its distinctive $\delta^{15}N$ signature to understand and manage the movement of its N in the ecosystem.

We will provide an overview on using natural ^{15}N abundance in waste management studies, and explore the potential to apply this technique to help best management practice in land application of wastes. We will also report data from our research trials that have been receiving sewage effluent and biosolids application.

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Carbon-13, nitrogen-15 and deuterium as tracers of caribou (*Rangifer tarandus*) ecology

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Reindeer (*Rangifer tarandus*) is a species of high importance in the subsistence of prehistoric humans in North-western Europe. Numerous bone remains of this animal have been found in the archaeological sites, and hunting strategies of first anatomically modern humans are believed to be overridden by the seeking of this prey. As a result, the ethology of reindeer should have greatly influenced the hunting activity and organisation of the prehistoric hunter-gatherer. This ecological behaviour of the prehistoric reindeer have been deduced from the modern specimens of *Rangifer tarandus* of North America (caribou). But the ecological features of the modern caribou are more diversified than what have been considered by archaeologists, and direct actualistic analogy has proven to be sometimes too simplistic. In this context, stable isotope signatures could be of great help to decipher the actual ecology of ancient reindeer since they are natural tracers of the diet and the environment of the living individual.

The presented study aims at examining how isotopic signatures of reindeer can express their ecological behaviour. To address this question, we have studied the ^{13}C, ^{15}N and D amounts of hair samples from well-monitored modern caribou herd. The studied caribou come from different location in Canada, mainly from Quebec, Saskatchewan, Northwest Territories and Nunavut. Both woodland and barren-ground ecotypes are represented with different migratory behaviour. The $\delta^{13}C$ and $\delta^{15}N$ values of herbivore tissues reflect those of the consumed plants. The $\delta^{13}C$ values of plants vary according to several parameters. For plants in temperate and peri arctic context, one of these parameters is the conditions of the photosynthesis which differ relatively to the density of the vegetal cover. The $\delta^{15}N$ values of plants are influenced by the characteristic of the nitrogen cycle of the local soil which can be influenced by climatic parameters. The δD values in plants classically derived from the δD value of rain during the growing season.

As expected, more negative $\delta^{13}C$ values are found for woodland caribou compared to barren-ground caribou. The $\delta^{15}N$ values of the different herds are similar with the notable exception of the herd from Saskatchewan. The general pattern of δD values of precipitation established for North America continent is reflected in the hair of the different herds. To sum up, Carbon and Deuterium isotopes are fully consistent with both the environment and climatic surroundings. Nitrogen isotopes seem to be more locally controlled.

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Biofilm and trophic differences between regulated and unregulated rivers, evidence from stable isotopes and C:N ratios

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Stable isotopes ($\delta^{13}C$ & $\delta^{15}N$) in biofilms and non-predatory macroinvertebrates and C:N ratios of biofilms were compared over a 21-month period among two regulated and two unregulated rivers in the Murrumbidgee River basin, New South Wales, Australia. Biofilms had much higher $\delta^{15}N$ values and lower C:N ratios in the regulated rivers than in the unregulated rivers. Differences in $\delta^{13}C$ were less pronounced, but the regulated rivers were on average more ^{13}C depleted than the two unregulated rivers. Linear regressions were used to assess trophic relationships between biofilms and non-predatory macroinvertebrates (detritivores and filter feeders) at the broad spatial and temporal scales. Findings suggested that consumers were obtaining a significant amount of their food source from the organic components of biofilm. Biofilm $\delta^{13}C$ signatures overlapped the reported ranges given for allochthonous matter making it difficult to determine if the rivers were autotrophically or heterotrophically driven. Distinct differences between regulated and unregulated rivers based on biofilms ^{15}N signatures appears to be related to anthropogenic impacts such as dams and catchment clearing resulting in deteriorated water quality rather than flow regulation per se. This was supported by significant differences in water quality nutrient samples (oxidised nitrogen) that existed between the regulated and unregulated rivers over the course of this study.

Foodweb structure and carbon flux in the New Zealand fjords

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Fiordland is a globally unique environment that contains exceptional marine biodiversity. Complex physical morphology of the region produces a highly subdivided and diverse array of marine habitats in close proximity to native forests. Because of this there are particularly strong links between terrestrial and marine systems. We used stable isotopes of carbon and nitrogen to identify carbon source pools from benthic, terrestrial and pelagic environments and were able to quantify the input of carbon from these pools to subtidal assemblages of invertebrates and fishes across several environmental gradients in the fjords. Our results highlight the importance of recycled terrestrial production in this system, and the importance of spatial structure in bottom-up influences on metapopulations. In one fjord, Doubtful Sound, we have found evidence for significant changes to the subtidal assemblages, including local extinction of the bivalve *Austrovenus stutchburyi*, wrought by anthropogenic habitat conversion. These results were used to analyze the consequences of changes in community structure and functional diversity (feeding mode) to pathways of the available carbon sources in the system. Together these data give us a unique view of the biotic and abiotic influences on energy fractionation in this system.

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Hydrology and primary production influence food-web patterns in the Florida Everglades

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Energy exchange, nutrient flow, and food-chain length are emergent properties of community structure that exhibit complex relationships with abiotic factors, notably primary production and disturbance. Theory predicts that food-chain length will shorten along gradients of disturbance or lengthen along gradients of increasing productivity; however, in natural systems, these two environmental factors may vary simultaneously. In the Everglades, hydroperiod represents a gradient of disturbance because drying events yield high mortality of fishes and macroinvertebrates. Though oligotrophic, local nutrient availability and primary productivity also vary by a fact or two within the Everglades. In recent years, stable isotopes have become a widespread and reliable method in the examination of trophic feeding relationships over time and space. We characterized food webs at 20 freshwater sites over three regions of the Florida Everglades. Sites were sampled in both the wet and dry seasons and encompass a wide range of productivity and hydroperiod conditions. Indicators of productivity were measured by analysis of soil, floc, and periphyton total phosphorus at each site, as well as by primary production of local periphyton samples using light/dark bottle incubations. A total of 702 samples of taxa representing basal, intermediate, and top trophic levels were analyzed for $\delta^{15}N$ and $\delta^{13}C$. Due to the highly variable nature of primary producer isotopic analysis, primary consumers were used as the baseline values for food-web analysis. Amphipods and Seminole ramshorn snails were selected as the primary baseline detritivore and herbivore, respectively. Eastern mosquitofish and riverine grass shrimp represented the intermediate trophic levels, and Florida gar were the top carnivores at each study site. We measured food-chain length using $\delta^{15}N$ and estimated an index of detritivory (I_D) with $\delta^{13}C$ to describe food webs.

More variation in food-chain length could be explained by interactions of environmental parameters than by single factors. Both productivity and hydroperiod disturbance were correlated with food-chain length, but these results were dependent on season and top consumer. After adjusting for spatial variation in $\delta^{13}C$ of baseline consumers, variation in our I_D indicated a possible shift along environmental gradients of the relative role of detrital and algal carbon at the base of the food web. In the wet season, 9 of 20 sites were best explained by a detritus-based model and the remainder by a two-source, or detritus-algae model. Dry seasons data indicated that 10 of 19 study sites were consistent with a detritus-based model and the remainder with the detritus-algae model; only one site fit a purely algae-based model. The I_D decreased with increasing productivity and lengthening hydroperiod in both seasons, although results were stronger in the dry season. In the dry season, the interaction of productivity and hydroperiod had a greater influence on basal carbon patterns than either of these two factors alone. Our results indicate that detritus is particularly important as an energy source in the Everglades, and suggest that the role of algae may be overestimated in this ecosystem. In the Everglades, hydroperiod disturbance may play a larger role in food-web structure over time and space than variation in productivity in areas not receiving anthropogenic nutrient enrichment, though productivity also influenced our results. Our results support multiple factors influencing ecosystem processes.

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Oxygen isotopes in cellulose identify source water for archeological maize in the American Southwest

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Maize (*Zea mays*) was the staple food source for aboriginal societies in the arid American Southwest. Maize is a summer crop and requires substantial inputs of growing season moisture for successful production. Moisture for maize production in these societies came either from irrigation supplied by perennial water sources fed by snowmelt or from summer monsoon precipitation. Consequently, variation in the intensity and geographical extent of the monsoon system over the past millennium may have had substantial economic and social impact on aboriginal settlements of the region. Agricultural practices used for maize production, and particularly the sources of water used for irrigation, are still in question. Maize cobs are plentiful in the archaeological record throughout the American Southwest and can be easily dated. Such samples may hold isotopic information on the source(s) of water used for their production, and thus would provide a spatial and temporal record of irrigation practices used in aboriginal agriculture. Summer monsoon precipitation on the Colorado Plateau has a $\delta^{18}O$ value (~ -6‰) that is easily distinguishable from moisture derived from winter snowmelt (~ -17‰). We investigated whether the $\delta^{18}O$ signature of irrigation water is preserved in the cellulose of maize cobs. We then examined the cellulose $\delta^{18}O$ value of modern field-grown and archeological maize samples collected on the Colorado Plateau to estimate the importance of monsoonal precipitation in their production.

Experiments using one modern and four aboriginal maize varieties were conducted in field plots in Salt Lake City, Utah and Tucson, Arizona. Potted plants were irrigated with water of known $\delta^{18}O$ value (-15.8 and -8.2‰ in SLC; -8.6‰ in TUC) and grown to maturity. The oxygen isotope values of alpha cellulose ($\delta^{18}O_{cellulose}$) extracted from cobs on experimental plants ranged from 26.8 to 36.4‰ (averaged within varieties) and were highly correlated with values for source irrigation water. However, $\delta^{18}O_{cellulose}$ of two aboriginal varieties were 2.4 to 3.4‰ lower compared to modern hybrid maize and the other two aboriginal varieties, suggesting the potential for genetic variation in biochemical and physiological traits that influence isotopic variation in $\delta^{18}O_{cellulose}$. Cob $\delta^{18}O_{cellulose}$ from five modern fields on Navajo lands and five archaeological sites on the Colorado Plateau ranged from 27.3 to 34.6‰, closely matching the range of values observed in potted plants. An empirical model developed from the pot experiments indicated that monsoonal precipitation accounted for 12 to 82% of the moisture supplied to the archeological maize. Archeological maize from sites within close proximity to a perennial water source reflected greater input of winter snowmelt. These results suggest that transgressions in the North American Monsoon system would have greatly impacted maize agriculture, but this impact may have been mitigated by the availability of perennial water sources in some locations. The $\delta^{18}O$ value of cob cellulose may provide further insight into agricultural and trading networks among aboriginal settlements in a physiographically heterogeneous landscape.

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Studying the Arctic 'migration' of two university professors using stable oxygen and hydrogen isotope analyses

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Stable oxygen and hydrogen isotope analyses of organic samples from organisms are increasingly being used to investigate latitudinal animal migration patterns. Few studies have been conducted on the movements of humans and these types of analyses could find forensic applications to establish the likelihood of reported travel. We used the frequent 'migration' of two university professors (the Authors 1 and 2) between Massachusetts (MA) and Alaska (AK) to investigate whether their travels resulted in an isotopic record of their movements in their organic components (hair and nail). As predicted by the GNIP $\delta^{18}O$ and δD databases (IAEA 2002), Alaskan tap water was depleted in the heavier stable isotopes of O and H ($\delta^{18}O$ = -19.9 ± 0.4‰, n = 23; δD = -156.2 ±1.7‰, n = 23) relative to tap water from MA ($\delta^{18}O$ = -8.8 ± 0.9‰, n = 6; δD = -54.8 ± 7.4‰, n = 6). Facial hair of Author 1, who resides in AK (Fairbanks), was shaved on a daily basis ~14 days prior to a 7 day visit to MA. Sampling continued for a few days after the individual returned to AK. Fingernail samples were taken from Author 2, who resides in MA (Wellesley), at 10-30 day intervals over a ~150 day period, which involved a trip to AK at the start of the sampling period. The $\delta^{18}O$ values of hair ($\delta^{18}O$ from 9.0‰ to 6.1‰) and nail ($\delta^{18}O$ from 9.8‰ to 4.1‰) of both individuals were consistently isotopically enriched relative to the AK and MA tap water. This relationship seems to be consistent with data from the analyses of complex biochemicals (e.g. chitin and cellulose) in other organisms (e.g. chironomids and aquatic plants) relative to habitat water. Within 2 days of author 1's arrival in MA, his beard hair began to rapidly enrich in ^{18}O. This enrichment continued linearly for the next 11 days, at a rate of 0.21‰/day. The duration of this preliminary experiment was not sufficient to allow the response to reach an asymptote; however, we predict it to take somewhere between 15 and 20 days given the expected rate of human body water turnover. Prior to Author 1's arrival in Massachusetts, his beard hair $\delta^{18}O$ showed a shallower, but consistent decrease, likely the residual affect of a previous trip to MA (~30 days prior to the experimental trip to MA). The δD values from the same hair samples revealed changes in the same direction as the $\delta^{18}O$ data, but which were not statistically significant. The $\delta^{18}O$ values of nail samples from Author 2 showed a much slower turnover due to a slower nail growth rate. Nails became isotopically depleted only after 145 days of arriving in AK. Similar to the hair samples, the δD values of the fingernails were more variable, and did not show a systematic alteration over time. One factor that potentially complicates the interpretation of isotopic data in humans is that food may not be grown in the region that provides drinking water, and yet may contribute hair/nail O and H. Although we did not track daily (bulk) diet isotopic composition in this study, preliminary $\delta^{18}O$ and δD analyses of 70 food items bought in AK showed a large range of values ($\delta^{18}O$ = -0.4‰ to 34.9‰; - δD = -198.9‰ to 40.8‰). These preliminary analyses are currently being supplemented with analyses of the individuals' urine (body water) over time and samples covering an extended duration. Our findings show that the $\delta^{18}O$ of facial hair could provide a rapidly responding record of the geographical movements of humans.

Examining the relationships between the $\delta^{18}O$ and δD of invertebrates, diet and water in an aquatic ecosystem

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Stable carbon and nitrogen isotopic measurements of aquatic organisms are frequently used to examine trophic relationships in ecosystems. Stable oxygen and hydrogen isotope measurements could also aid examination of both organism-diet relationships and past environmental conditions provided the relationships between the isotopic compositions of organism, diet and habitat water are understood. Few studies have examined these relationships. We present $\delta^{18}O$ and δD results from zero-water exchange shrimp aquaculture systems. Pacific white shrimp (*Litopenaeus vannamei*) were maintained in 1200 L outdoor mesocosm laboratories at the Oceanic Institute, Waimanalo, Hawaii. Muscle samples were obtained during three experimental periods in which provided formulated feeds differed isotopically (feed 1 = -115‰ and 22.2‰ in δD and $\delta^{18}O$, respectively; feed 2 = -93.3‰ and 26.7‰; and feed 3 = -127.4‰ and 22‰). Measurements of tank suspended particulate organic matter (SPOM) $\delta^{18}O$ and δD were also taken from two of the periods, since carbon and nitrogen isotope data has previously established that tank natural production also contributes to shrimp growth. Shrimp muscle and SPOM samples were taken for stable oxygen and hydrogen isotope measurements using a TC/EA attached to a Finnigan Delta^{plus} XL IRMS. Values for tank water $\delta^{18}O$ and δD were obtained simultaneously with muscle and SPOM samples during the first experimental period. The results showed the shrimp muscle tissue to be distinctly enriched in ^{18}O (~13‰) and depleted in D (~100‰) relative to the habitat water. No consistent pattern existed between the stable isotopic compositions of the shrimp and their diet. For instance, the δD values of shrimp from experiment 2 were ~10‰ more positive than those in experiment 1 and had been fed a diet isotopically heavier than experiment 1. This contrasted with experiment 3 where, although the shrimp had been fed a diet with a δD ~10‰ lower than that in experiment 1, the δD of the shrimp were isotopically heavier (~10‰) than the shrimp in experiment 1. Feeding the shrimp diets of different $\delta^{18}O$ composition did not appear to significantly or consistently alter the $\delta^{18}O$ of the shrimp muscle. Whereas the δD of the algae remained relatively similar between experimental periods and over time, the successional development of the algal community (green algae to diatoms) resulted in a marked change in the $\delta^{18}O$ composition of the algae (+23‰ to 0‰). Our results suggest that shrimp muscle $\delta^{18}O$ and δD signatures are more consistent with tank water $\delta^{18}O$ and δD than values from the feed or tank SPOM.

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Avian migration: promises and pitfalls

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Understanding seasonal movements of migratory birds is a substantial challenge that obscures strong inference in studies of natural history and conservation. Traditional banding techniques rarely yield reliable data about movements of wild populations. Recent advances in the use of stable isotope ecology to study seasonal movements in small migratory birds are promising. Mountain Plovers are insectivorous birds that are sparsely distributed across relatively remote regions of North America. The breeding range spans gradients in $\delta^{13}C$, and δD, and includes no coastal influences. The winter distribution is not as well documented. Flight feathers of Mountain Plovers molt just prior to leaving the breeding grounds and body feathers molt just prior to departure from the wintering grounds, providing opportunity to study the full migration cycle with isotopes.

We studied isotopic patterns in newly developed feathers from plovers captured during summer on the breeding grounds to infer breeding origin of birds captured on the wintering grounds. We incorporated both fixed and random effects into a set of models that we explored using an information-theoretic approach. We also explored a set of hierarchical likelihood-based assignment tests that can incorporate probabilities from relative band recovery rates and abundances.

The variation in isotopic values among sampling sites was four times greater than within-site variance. Thus, descriptive regression models of latitude on isotopes accounted for most of the observed variation (adj. R^2 range 0.75-0.97). Isotope variables were good predictors of latitude in these descriptive models ($P<0.01$ for $\delta^{13}C$ and δD in all models). However, inference from regression models assumes IID residuals, which was violated by every descriptive model. Substantial structure in the errors was associated with δD. Consequently, northern feathers were consistently assigned more southern latitudes and vice versa. Incorporating sample site as a random effect satisfied the assumption and yielded models that were on the order of $9x10^{35}$ times more likely to be the best approximating model than any descriptive model; yet none of the isotopes in these random effects models were good predictors of latitude ($P>0.12$ for all isotopes in all models). The most parsimonious descriptive regression model correctly assigned 79% and 4% of known samples to state and sampling site, respectively. The most parsimonious random effects model correctly assigned 13% and 0% to state and location, respectively, whereas the likelihood-based assignment tests correctly assigned 89% and 77% of known samples to state and site, respectively.

These results for Mountain Plover illustrate that the effective sample size in migration studies is the number of sampling sites, not birds, within the geographic range of interest. The number of birds per site affects only site-level precision, which is apt to be relatively low for most commonly considered isotopes regardless of sample size. Regression models describe specific isotope datasets well. However, likelihood-based assignment tests offer the most flexibility and reliability for studies seeking to link space and time in avian migration. Careful study design and the application of such tests will improve the efficacy of stable isotope techniques in the study of avian migration.

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Potential and limitations of on-line $\delta^{34}S$ measurements on organic samples

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Sulfur (S) isotope techniques have been increasingly used in ecological studies, since they often provide unique information such as distinguishing between continental and marine food sources. However, S isotope measurements on ecological materials are often challenging, because of low S contents and limited sample amounts. Historically, S in a sample was converted to $BaSO_4$ or Ag_2S, and its isotope ratio was determined via dual inlet isotope ratio mass spectrometry (DI-IRMS). The advent of on-line continuous flow (CF-) IRMS has enhanced the application of S isotope techniques in ecological studies by reducing the necessary sample amounts and sample preparation time significantly. Since the early 90's, $BaSO_4$ or Ag_2S generated from ecological materials have been routinely analyzed using CF-IRMS with satisfactory accuracy and precision. However, direct S isotope measurements on ecological materials using CF-IRMS have remained challenging.

Sulfur isotope compositions of various organic materials such as fish tissues, pine needles, tree bark, lichen, and vegetation with S contents ranging from ~1,000 to ~30,000 ppm were successfully measured by direct combustion in an elemental analyzer (EA) coupled to an IRMS in our laboratory. Upon examination of the problems associated with analyzing large amounts of organic materials (up to ~13 mg), close attention was paid to increase the O_2 amount available for combustion using a 10 mL oxygen loop, to reduce the moisture contents in organic materials by oven drying at 70°C for 24 hours before S isotope analysis, and to increase the GC oven temperature to 85°C for better chromatographic results. These adjustments resulted in precisions of ±0.27‰ (n=27) for S isotope analyses on S-containing organic material, which is comparable to that of on-line $BaSO_4$ analysis (±0.18‰, n=12). Samples with as little as 10 μg S can be measured successfully. About 350 organic samples can be analyzed with a single combustion chemical packing, with ash cleaning after every 50 analyses. The total analysis time per sample is 10 minutes.

However, the $\delta^{34}S$ values obtained by CF-IRMS on organic materials were consistently higher than those of CF-IRMS on $BaSO_4$ produced from the same sample ($\Delta\delta^{34}S_{organics\text{-}BaSO4}$ = 0.9-2.9‰). A likely reason for this significant difference between apparent and true $\delta^{34}S$ values ($\Delta\delta^{34}S_{organics\text{-}BaSO4}$) is a higher contribution of $^{32}S^{16}O^{18}O$ to mass 66 during CF-IRMS of organic materials, thus resulting in elevated apparent $\delta^{34}S$ values. The organic samples used in this study had O contents ranging from 25 to 47% and $\delta^{18}O$ values ranging from +9.8 to +23.6 ‰. The difference between apparent and true $\delta^{34}S$ values showed a 2nd degree positive correlation (R^2=0.877) with the O contents and $\delta^{18}O$ values of the organic samples, exhibiting increasing $\Delta\delta^{34}S_{organics\text{-}BaSO4}$ values with increasing O contents and $\delta^{18}O$ values. During combustion of organic materials, oxygen derived from the samples appears to constitute a significant additional oxygen source, which partially controls the oxygen isotope ratios of the generated SO_2 (likely via isotope exchange reactions involving H_2O). Since O contents and $\delta^{18}O$ values in different organic materials are quite variable, it is necessary to correct the apparent $\delta^{34}S$ values obtained by CF-IRMS of different types of organic materials.

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